

Comprehensive  
**Clinical  
Hepatology**

SECOND EDITION

Bruce R. Bacon • John G. O'Grady  
Adrian M. Di Bisceglie • John R. Lake



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## Foreword

Textbooks are snapshots of a specialty at a moment in time and, fortunately, these are exhilarating times for those who are interested in the liver. In many areas of hepatology the pace of progress and understanding has been rapid with the flow of new information outstripping the capacity for the clinician and investigator to evaluate and assimilate emerging concepts into practice. Much of the substantial base of knowledge underpinning the field of hepatology is presented in *Comprehensive Clinical Hepatology* in well written, well edited chapters.

The volume has much to offer to a broad spectrum of readers. Established concepts are fully presented and practical advice is provided. These authoritative discourses allow the construction of reliable scaffolding upon which one can become current and be in a position to integrate further knowledge as it becomes available.

Hepatology as we know it now has been built over the past 100 or so years on the platforms of major advances. The great leaps include the development of clinical chemistry, the introduction and widespread use of percutaneous liver biopsy, the emergence of ever more accurate and progressively less invasive imaging capabilities, the explosion of interest in hepatitis that followed the discovery of hepatitis viruses, the development of effective therapies for liver disease – beginning with corticosteroid therapy of autoimmune hepatitis and continuing through the several therapies available for chronic viral hepatitis – and the surgical revolution that culminated in liver transplantation which has become an accepted procedure. These are but a few of the pillars upon which modern hepatology is built. Already on the horizon are new important areas which promise to expand and reshape our understanding of the liver and its disorders, and will undoubtedly enhance our ability to prevent and treat liver diseases. Increasing attention is being paid to the roles and interactions of the several cells other than hepatocytes and cholangiocytes which are found within the liver. Stellate cells, endothelial cells, macrophages, and immunocytes have all become important areas for investigation. The many actions of chemokines and cytokines and their regulation are becoming clinically relevant. The dynamic processes leading to fibrosis are increasingly understood as are the processes that lead to cell death through apoptosis. Effective therapeutic approaches to modulate hepatic fibrosis will surely soon be available and ways to regulate apoptosis may be forthcoming. Genetic influences on the liver are coming of age. It is widely recognized that individuals are collections of genotypes with genetic control of myriads of hepatic processes that determine the ability to synthesize, transport, and defend.

As is amply discussed and exemplified throughout the volume, hepatologists have benefited from the importation of scientific

advances from other fields. The hepatic effects of alcohol and its multiple interactions are cases in point. Amongst the longest standing concepts in medicine is that excessive use of alcohol is detrimental. The liver is an established target for the damaging effects of alcohol. It clearly induces hepatic changes and injuries to a large extent as a result of the toxic effects of acetaldehyde. The interactions of alcohol and acetaminophen have attracted considerable attention as an example of an unfavorable drug-drug interaction with acetaminophen hepatotoxicity enhanced in patients who are regular (and often excessive) users of alcohol. Furthermore, in an example of alcohol-viral interaction, patients who have chronic hepatitis C and continue to use alcohol have been shown to have increased levels of HCV RNA, and are more likely to accelerate the rate of progression towards cirrhosis and to have impaired the effectiveness of interferon based therapy. Other examples of similar interactions abound and the concept of multiple hits leading to progressive liver injuries has taken traction.

The emerging role and importance of fat in the liver is fully examined in this volume. It is remarkable how in only one generation, concepts have changed rather dramatically regarding the importance of fat in the liver. Once considered a benign hepatic bystander in patients who use moderate amounts of alcohol or who are obese, there has been an evolution in understanding with recognition that the patient with fatty liver may be at risk of developing progressive liver disease and even cirrhosis. In *Comprehensive Clinical Hepatology* the roles of environmental influences including obesity and alcohol, and the interactions between fatty liver and viral induced liver diseases, are fully considered.

The field of hepatology is forward directed with many moving parts. The clinician is far better armed now to precisely diagnose the presence of liver disease, assess the extent of injury, and present informed opinions as to the likely prognosis. New opportunities to apply emerging science to emerging clinical care are all around us. An advance in one area leads to reassessment in other areas. The challenges and opportunities of hepatologists in the 21st century are amply demonstrated throughout *Comprehensive Clinical Hepatology* and the editors and authors are to be saluted for creating such an accessible and complete guide. It is a fine time to be a hepatologist.

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*Jack Lake*

## SECTION I: NORMAL STRUCTURE AND FUNCTION

### CHAPTER

# 1

## Development and Anatomy of the Normal Liver

Bernard C. Portmann

### INTRODUCTION

This introductory chapter illustrates the gross anatomy, blood supply and internal structures of the normal liver. The aim is to emphasize those morphologic aspects that are likely to assist the interpretation of both imaging data and basic pathologic changes observed in the diseased liver. Only succinct reference is made to the morphologic location of the various functions carried out by the liver which are dealt with in Chapters 2 and 3. The discussion is preceded by a brief account of the development of the liver, in particular the development of both the hepatic vasculature and the intrahepatic bile duct, to assist the understanding of congenital anomalies.

### DEVELOPMENT OF THE LIVER

The primitive liver appears in the 3-week-old embryo as an endodermal outgrowth from the ventral wall of the foregut, near the yolk sac, in that part destined to become the duodenum. This liver primordium rapidly develops into a diverticulum whose actively proliferating endodermal cells form solid cords; these invade the mesenchyme of the septum transversum which will later form the capsule and connective tissue framework of the liver. The anastomosing cords of primitive cells spread between the vitelline veins and their capillaries so that they enmesh, penetrate and transform into the primitive sinusoids. Thus in the 4-week-old embryo, an intimate relation between hepatocytes and sinusoids already anticipates the pattern that characterizes the adult liver. The caudal part of the hepatic diverticulum remains independent from the invading sheets of primitive hepatocytes to form the epithelial primordium of the cystic duct and gallbladder.

The liver cell plates of the fetal liver are several cells thick and remain so until after birth. The plate is two cells thick by 5 months after birth, the single cell pattern characteristic of the adult liver being established by about 5 years of age. The production of  $\alpha$ -fetoprotein by the primitive liver cells starts as early as 4 weeks. Hematopoiesis commences within the liver at about 6 weeks and by the 12th week the liver is the main site of hematopoiesis in the body, activity which subsides in the 5th month when the bone marrow starts functioning, and which normally ceases within a few weeks of birth.

#### The hepatic venous system

The vascular arrangement of the fetal liver is given in **Figure 1.1**.

Initially the hepatic sinusoidal plexus is essentially fed by the paired and symmetrically placed vitelline veins which drain into the sinus venosus (Fig. 1.1a). As the liver rapidly grows, the laterally placed right and left umbilical veins which carry the

oxygenated blood and nutrients from the placenta become incorporated to supply the hepatic sinusoidal plexus, a connection which is established by the 5th week (Fig. 1.1b). With the placenta taking over from the yolk sac, the left umbilical vein becomes the principal source of placental blood while the right one disappears. The original vitelline veins give way to a single portal vein, whereas the newly developed ductus venosus provides a bypass channel directly connecting the umbilical vein to the inferior vena cava (Fig. 1.1c). This definitive vascular pattern of the fetal liver is already established in the 7-week embryo (Fig. 1.1d). At birth, blood flow ceases in the umbilical vein, the proximal end of the ductus venosus closes, and the expanding portal vein now takes over the supply of the whole liver, including the left lateral segments (Fig. 1.1e). The obliterated segment of the umbilical vein between the umbilicus and the left portal vein branch regresses to form the ligamentum teres; the ductus venosus becomes a fibrous cord or ligamentum venosum running in and encircling the right side of the caudate lobe.

#### The duct system and hepatic ductal plate

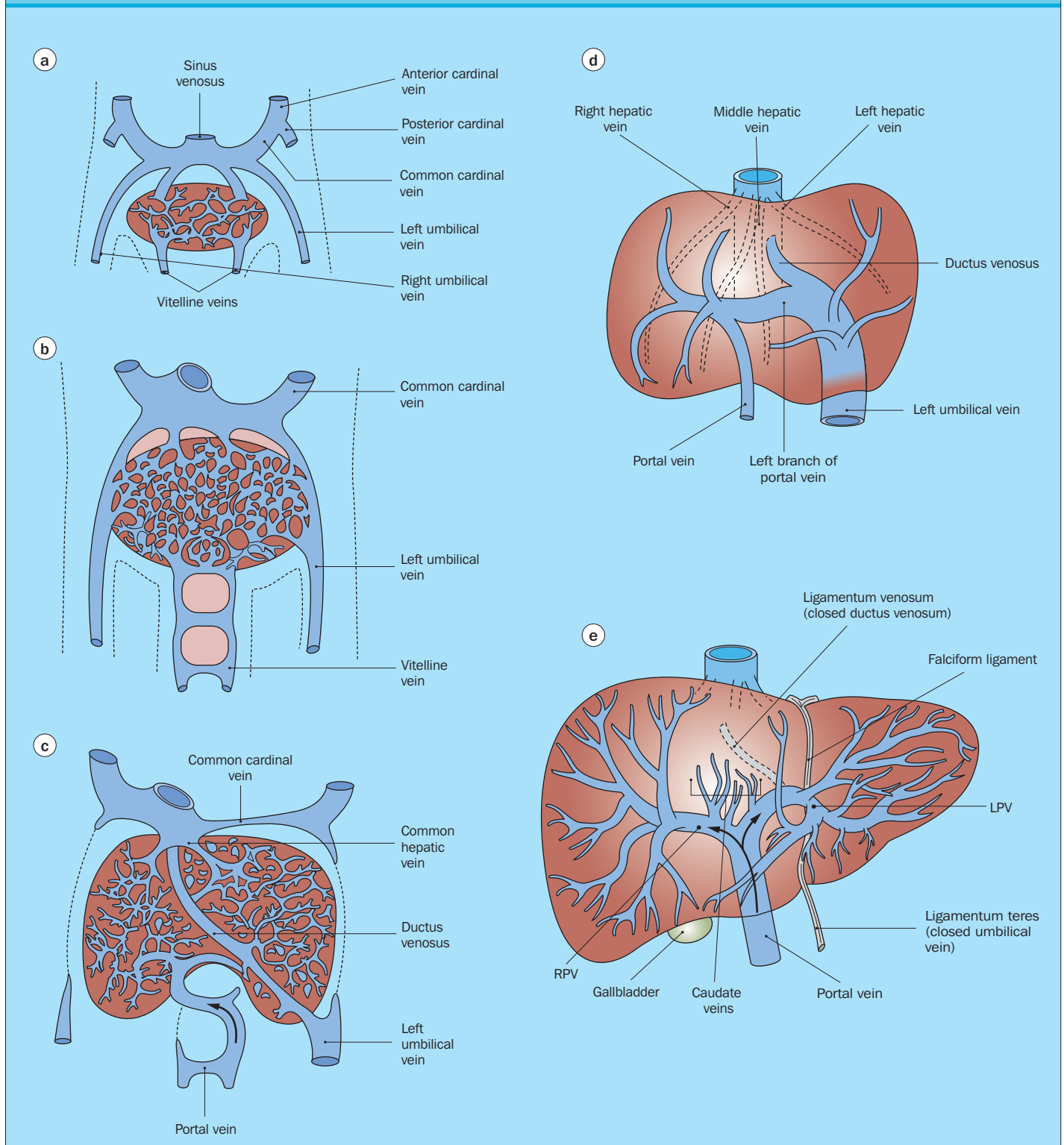
Bile canaliculi are first identified between the liver cell sheets in the embryo at the 6th week, while bile production begins at 12 weeks. The extrahepatic biliary tree arises from elongation of the original liver diverticulum, which initially forms a solid cord of epithelium continuous at its caudal end with the duodenum and at its cephalic end with the primitive hepatic sheets. Vacuolation later produces a lumen first in the common bile duct and subsequently in the hepatic duct, cystic duct and gallbladder.

The intrahepatic ducts in contrast begin to form at about 9–10 weeks, from the hepatocytes in direct contact with the mesenchyme, which surrounds the developing and branching portal veins. These first appear as a single layer of flattened cells ensheathing the primitive portal tracts and strongly expressing cytokeratin 19, similar to adult biliary epithelial cells. A second layer then forms showing similar phenotypic change, and with the development of slit-like lumens from 12 weeks onwards, the ductal plate is formed taking the form of a double-cylinder with a cleft-like lumen surrounding the developing portal tracts (Fig. 1.2).

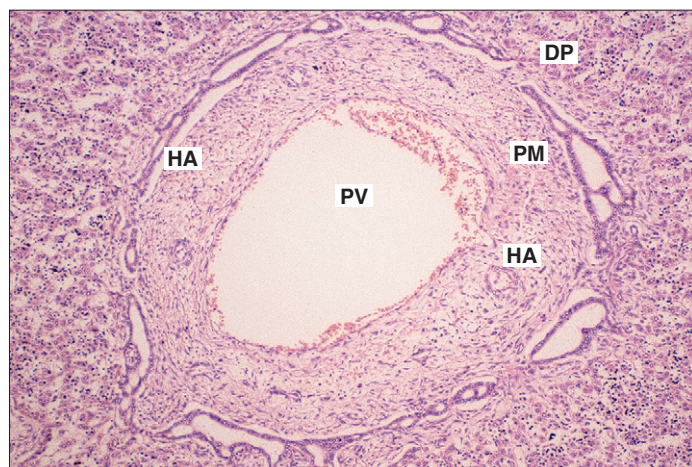
Further remodeling of the plate occurs by a subtle interplay of epithelial resorption and mesenchymal ingrowing; the primitive ductal plate thus changes from a circular structure to a network of tubules which then become separated from the parenchyma and incorporated into the portal mesenchyme. Excessive proliferation and arrest or aberrant remodeling of the embryonal ductal plate are likely explanations for various fibrocystic disorders such as autosomal recessive (infantile) polycystic disease, congenital hepatic fibrosis, Caroli disease and autosomal dominant (adult) polycystic disease.



## Development of the hepatic venous system



**Figure 1.1 Development of the hepatic venous system.** (a) Proliferation of the primitive hepatic plates (brown) into the vitelline vein network to form the primitive sinusoidal plexus which drains into the sinus venosus. (b) Incorporation of the umbilical vessels. (c) Development on the inferior aspect of the liver of a derivation channel – the ductus venosus – that drains directly into the inferior vena cava; partial and total regression of the left and right umbilical veins respectively. (d) The definitive vascular pattern of the fetal liver already established in the 7th week embryo (about 17 mm long). (e) Scheme of the hepatic circulation at birth after both closure of the ductus venosus through a sphincter mechanism and cessation of blood flow in the umbilical vein. LPV, left branch of portal vein; RPV, right branch of portal vein.



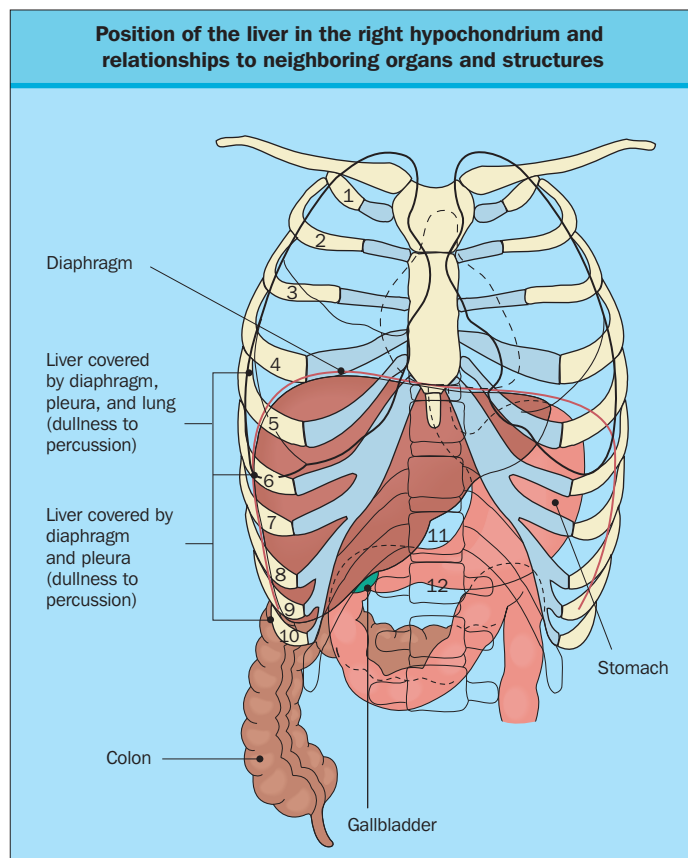
**Figure 1.2** The embryonal ductal plate highlighted in a stillborn fetus with autosomal recessive polycystic kidney disease. Note the persistent ductal plate (DP) encircling the portal mesenchyme (PM) with a centrally placed portal vein branch (PV). HA, hepatic arteriole.

## GROSS ANATOMY OF THE LIVER

The liver is the largest solid organ in the body with a median weight of approximately 1600 g in men and 1400 g in women. It lies in the right upper quadrant of the abdomen essentially under the protection of the rib cage. Its high location and intimate relationship with neighboring organs are of major importance to clinicians examining patients with liver disease and taking percutaneous needle biopsies (Fig. 1.3):

- The right kidney and the right colonic flexure lie in close contact with the inferolateral-lateral surface of the right lobe; the stomach imprints the inferior aspect and margin of the left lobe which extends to variable degrees into the left hypochondrium as far as the left mid-clavicular line.
- The lung and pleural sac overlie the dome of the right lobe for some distance laterally, a disposition which is responsible for dullness to percussion.
- The anterior edge of the liver comes into contact with the anterior abdominal wall below the costal margin and the xiphisternum, where it can be palpated during inspiration.
- The entire liver is covered by the fibrous capsule of Glisson, except posteriorly where it lies in direct contact with the diaphragm, the so-called 'bare area' which is surrounded by reflections of the peritoneum – the coronary and left and right triangular ligaments (Fig. 1.4b, c).

Anatomic landmarks seen on external examination are illustrated in Figure. 1.4. On the upper surface, the falciform ligament running anteroposteriorly attaches the liver to the diaphragm and to the anterior abdominal wall. Visually it divides the organ into two uneven lobes, the right lobe being about six times the size of the left; these have no functional significance (Fig. 1.4a). The anterior portion of the falciform ligament – the round ligament (ligamentum teres) – runs within the fissure of the umbilical vein and connects the left branch of the portal vein to the umbilicus (Fig. 1.4b); it contains small vestigial veins that re-open and even become varicose when intrahepatic portal venous block develops secondary to liver cirrhosis. Posteriorly the falciform ligament merges with the coronary ligaments surrounding the bare area (Fig. 1.4c).



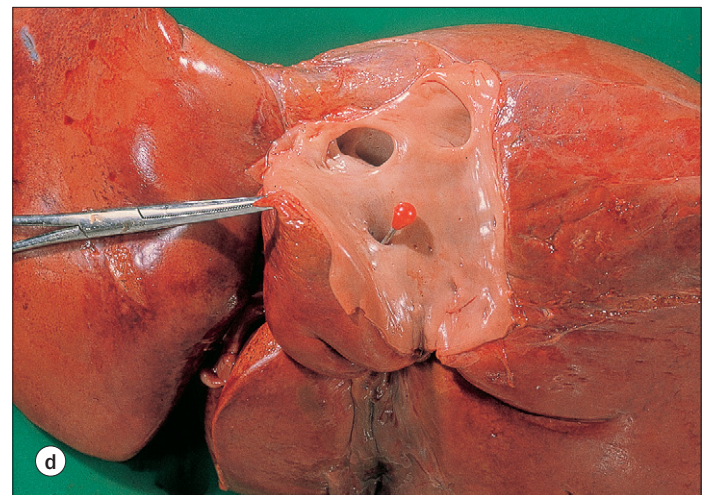
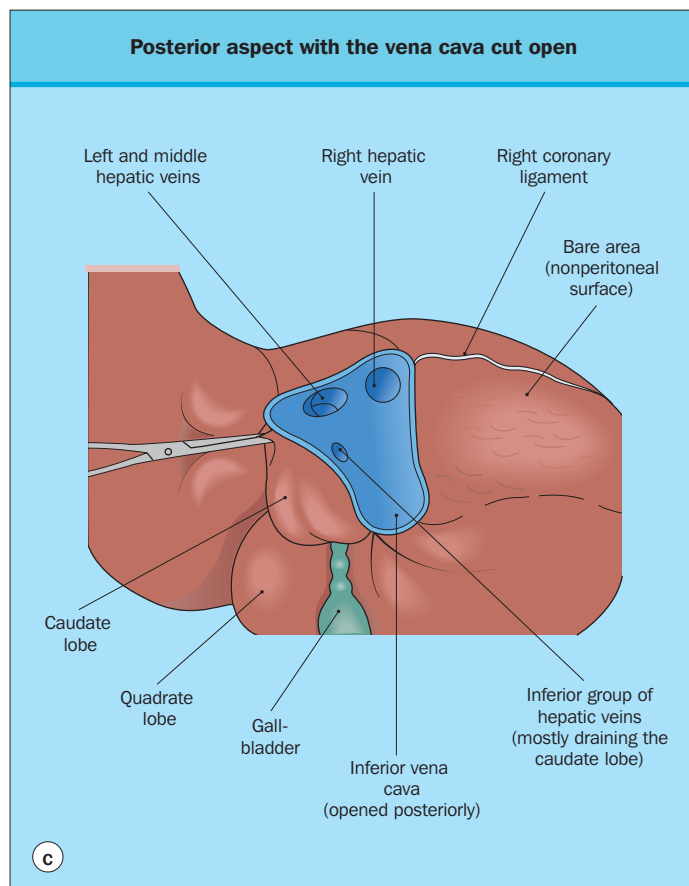
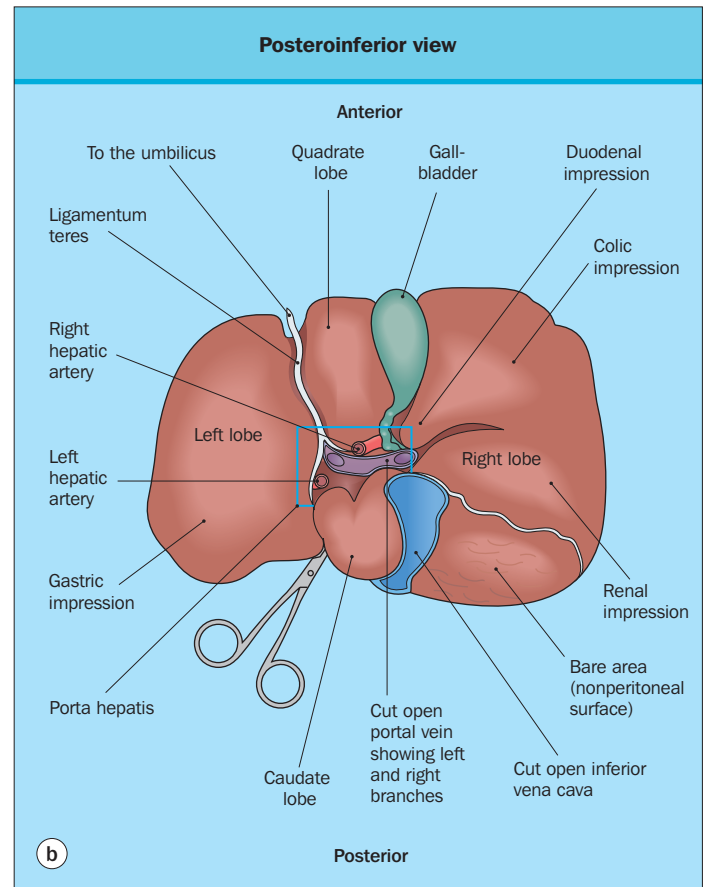
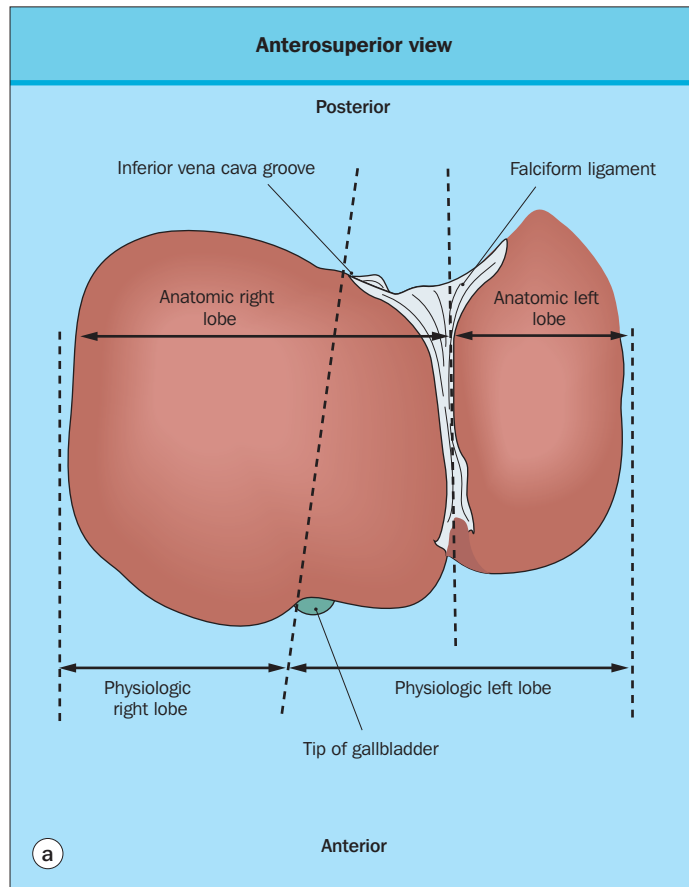
**Figure 1.3** Position of the liver in the right hypochondrium and relationships to neighboring organs and structures.

On the inferior surface, the caudate lobe bulges posteriorly between the fossa for the ductus venosus and the vena cava. The quadrate lobe lies anteriorly, lined by the gallbladder to its right and the fissure of the umbilical vein to its left (Fig. 1.4b).

Between caudate and quadrate lobes is the porta hepatis. In this deep fissure the portal vein and hepatic artery enter, and bile ducts leave, the liver contained in the peritoneal fold of the hepatoduodenal ligament. At that level the Glisson capsule is reflected inwardly to form the fibrous sheaths that invest the portal vessels and ducts throughout the liver, down to their smallest ramifications, the so-called portal tracts.

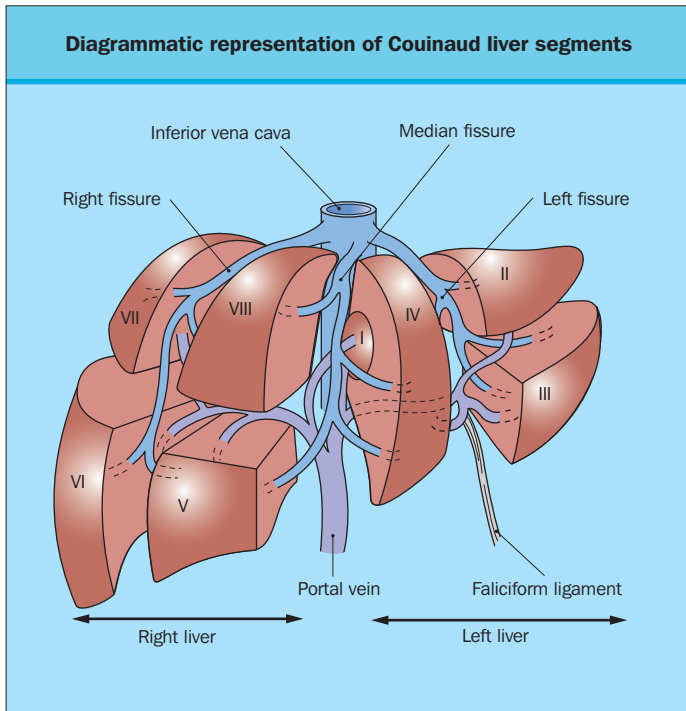
### Vessels and functional anatomy of the liver

The liver receives 75% of its blood through the portal vein that carries blood from the entire capillary system of the digestive tract, spleen, pancreas and gallbladder, and 25% through the hepatic artery, the second major branch of the celiac axis. Functionally, the liver is divided into two roughly equal parts ('true' lobes) on the basis of its blood supply and bile drainage. The line of demarcation between right and left hepatic arterial and portal venous inflow is located along a plane that passes some 4 cm to the right of the falciform ligament, joining the tip of the gallbladder to the groove of the vena cava (Fig. 1.4a). The recognition of this plane of demarcation between true left and right lobes is of major importance in staging primary hepatic tumors. The liver can be further divided into eight functional segments based on vascular distribution as illustrated in



**Figure 1.4 External appearances and surface markings of the liver.** (a) Anterosuperior view. (b) Posteroinferior view. (c and d) Posterior aspect with the inferior vena cava cut open to show the outlets of the hepatic veins.





**Figure 1.5 Diagrammatic representation of Couinaud liver segments.** The anterosuperior view is shown, with divisions of the portal and hepatic veins. The divisions of the hepatic artery and bile duct (not shown) closely follow those of the portal vein. The segments are indicated as: I, caudate lobe; II, III and IV, left lobe; V, VI, VII and VIII, right lobe.

**Figure. 1.5**, which follows the terminology of Couinaud, the one favored by most surgeons and radiologists.

The right lobe is divided into anteromedial and posterolateral sectors, each of which has superior and inferior segments, while the left lobe has medial (segment IV) and lateral sectors, the latter divided into anterior (III) and posterior (II) segments (generally referred to as left lateral segments). The caudate lobe (or segment I) has its own venous supply and drainage and can be regarded as a separate lobe. The segments do not have surface landmarks or intersegmental septa, but the identification of the boundaries (or fissures) is an essential step prior to hepatic resection and during the splitting or reduction of donor livers for transplantation. It is of importance to the surgeon that the left branches of both afferent vessels first supply the two lateral segments before forming a return loop to supply the medial segment IV (see Fig. 1.5). This vascular arcade, which is located at the point of attachment of the obliterated umbilical vein, is liable to injury during resection of the left lateral segments.

The venous drainage from the liver operates through the hepatic veins which emerge from the posterior surface of the liver and open immediately into the inferior vena cava just before it pierces the diaphragm. There are three main hepatic veins: the left, the middle and the right, the first two usually joining to form a short stalk with a common outlet (Fig. 1.4c, d). Inferior vein(s) drain the posterior segment of the right lobe and the caudate lobe directly into the vena cava, allowing the caudate lobe to escape injury and to undergo hypertrophy when the main hepatic veins are occluded (Budd-Chiari syndrome). The hepatic veins are situated in the fissures between the main seg-

ments. The main left vein drains the two lateral segments of the left lobe (segments II and III); the middle vein drains segment IV and the anteromedial sector of the right lobe (segments V and VIII); the right vein drains the remainder of the right lobe (segments VI and VII).

### Intrahepatic organization

The hepatic artery and portal vein enter the liver at the porta hepatis invested by a sheath of connective tissue which incorporates bile duct branches. The vessels run parallel and ramify by dichotomy to form a complex vascular tree in which the terminal branches develop in all directions before emptying into the sinusoids. In addition, small perpendicular branches arise along the course of these vessels. A second system – the hepatic vein – runs in the opposite direction with terminal hepatic venules collecting the blood from the sinusoids and forming, by successive unions, larger and larger channels leading to the main hepatic veins. These two tree-like systems interdigitate in such a way that their thin terminal branches run at right angles to each other and remain constantly separated from each other by a layer of parenchyma of about 0.5 mm in thickness. A three-dimensional representation of this organization is best illustrated by the stereoscopic reconstruction of Hans Elias (Fig. 1.6).

The various vascular relationships are shown with arterioles and portal venules opening into the sinusoids after piercing the parenchymal limiting plate that ensheathes the portal tract. A similar limiting plate surrounds the hepatic venules which contains fenestrations for the sinusoid outlets. Between supplying and draining vessels the sinusoids draw a complex network tunneling the interconnected liver cell plates (liver muralium), an intimate relationship between blood and cell surface that is essential to the high metabolic activity of the liver. Within the thickness of the plates the bile canaliculi form an anastomosing polygonal network, constantly separated by a half-cell thick layer of cytoplasm from the hepatic sinusoids. The canaliculi drain their contents into periportal cholangioles or canals of Hering, which connect canaliculi to the smallest interlobular bile ducts and ductules (see below).

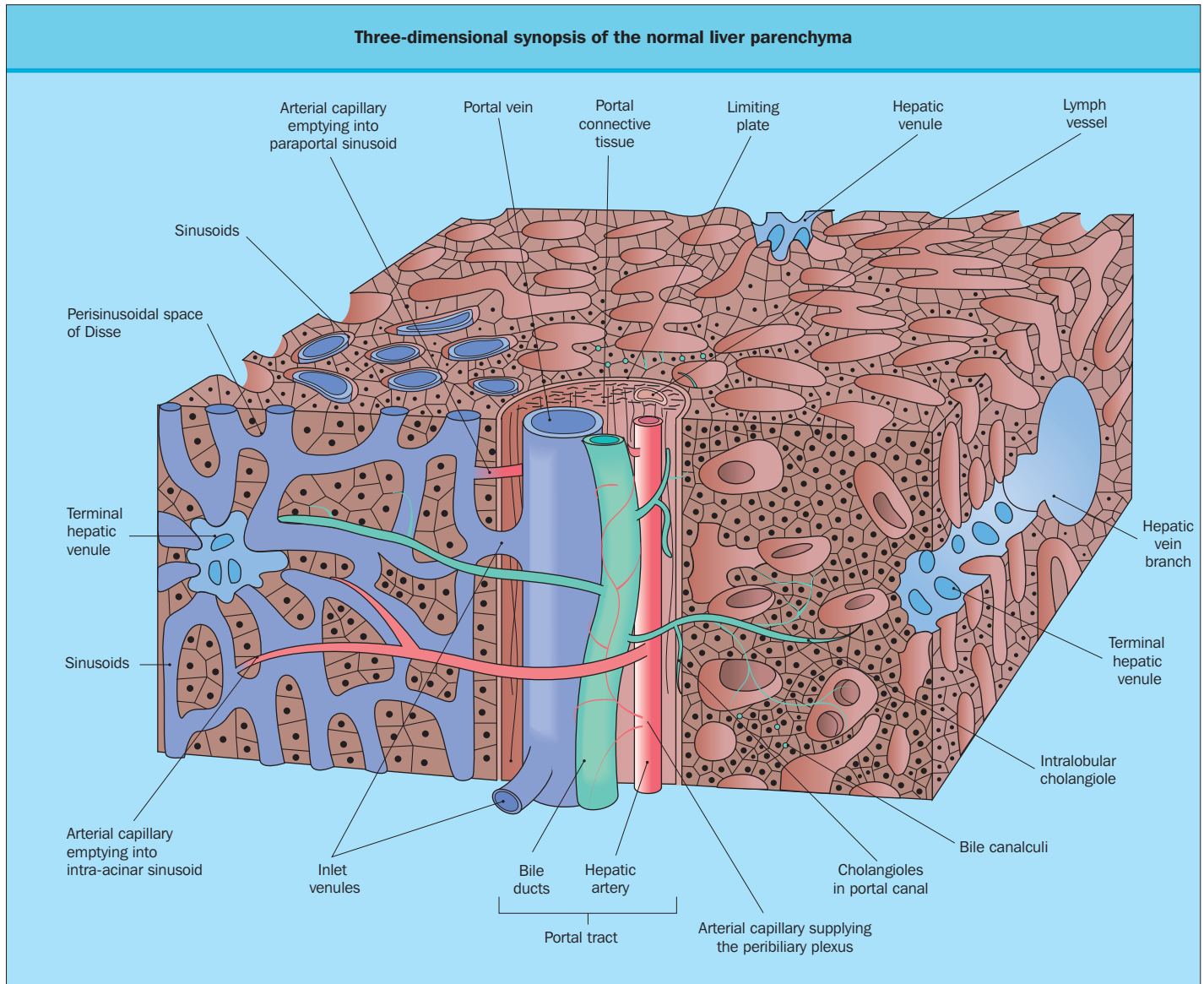
Two aspects are of clinical and diagnostic relevance:

- The hepatic arteries give rise to terminal branches feeding the peribiliary plexi; this explains the development of ischemic cholangitis following arterial compromise e.g. after arterial chemoembolization or with hepatic artery thrombosis after liver transplantation.
- Inlet venules arise at right angles along the course of the portal vein to feed the parenchyma adjacent to the large portal tracts. In cases of peripheral occlusive venopathy (hepatoportal fibrosis), these venules dilate to produce thin-walled periportal collateral channels, a diagnostic feature of non-cirrhotic portal hypertension.

### Functional organization of the liver

Basically there are two different concepts regarding the three-dimensional organization and functional unit of the liver:

- 1. The Kiernan or classic lobule is organized around a central venule, a terminal tributary of the hepatic vein, and is traditionally represented as hexagonal in outline, the boundaries of which are well defined by thin interlobular septa of connective tissue in a few species only (Fig. 1.7). In humans, such



**Figure 1.6 Three-dimensional synopsis of the normal liver parenchyma.** Note the inlet venules piercing the limiting plate to supply portal blood to the parenchyma disposed alongside larger portal tracts and the peribiliary plexus supplied by the hepatic artery. Modified from Elias (49).

lobules are delineated by an imaginary line with the portal tracts lying in between the ‘corners’ of adjacent lobules. This lobular concept is the basis for describing as centrilobular or perilobular (peripheral) the structural alterations occurring around the hepatic venules or portal tracts, respectively. The term mid-zonal is used for changes affecting the intermediate parenchyma. This concept is convenient for descriptive purposes and is still largely used even though it is simplistic to regard the liver lobule as a microcirculatory unit. In reality, each lobule is supplied by several terminal hepatic arterioles and portal venules, which also feed portions of adjacent lobules.

- 2. The liver acinus is arranged around the terminal branches of the afferent vessels as a pear-shaped cuff of parenchyma lying between and draining into terminal hepatic venules, which in this model become peripheral. The diagrammatic representation of the liver simple acinus, the arbitrary zonation of the liver parenchyma within its boundaries, and the relationships with

the classic lobule are illustrated in **Figure 1.8**. Acinar zones 1, 2 and 3 are roughly equivalent to periportal, mid-zonal and centrilobular areas of the lobular concept.

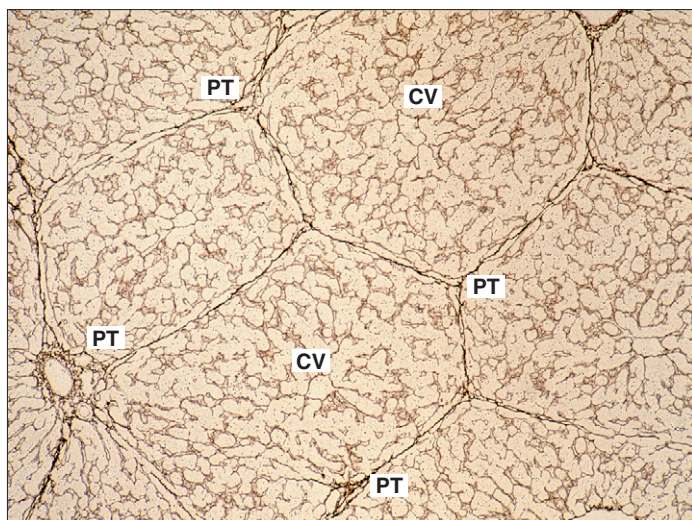
Three or more simple acini form a complex acinus, a sleeve of parenchyma surrounding the preterminal portal vein and hepatic arterial branches. Several complex acini in turn form part of larger units or acinar agglomerates.

## MICROSCOPIC ANATOMY AND ULTRASTRUCTURE

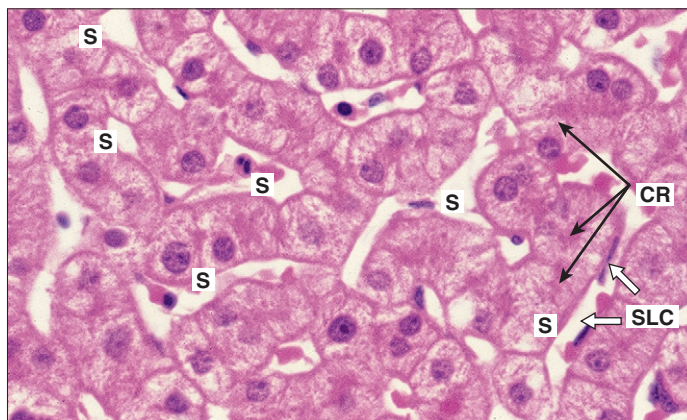
### The hepatocyte

Hepatocytes occupy some 80–88% of the total liver volume in humans. The individual hepatocyte is a polyhedral and highly polarized epithelial cell approximately 30–40  $\mu\text{m}$  in diameter. The cells are arranged in plates, which appear as ‘cords’ when seen in two dimensions under the microscope (**Fig. 1.9**).

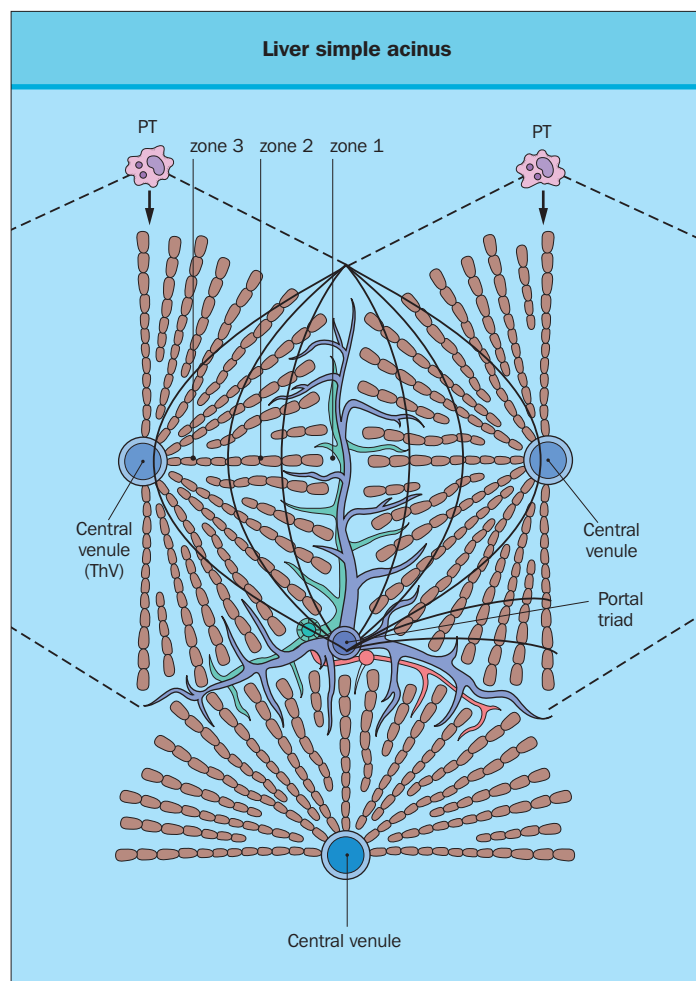




**Figure 1.7** Light microscopy of a pig liver showing the traditional hexagonal lobule (Kiernan) outlined by thin fibrous septa. CV, 'central' venule; PT, portal tracts. (Silver stain for reticulin)



**Figure 1.9** Light microscopy of liver cell plates cut longitudinally. Note the centrally placed nuclei, well demarcated sinusoidal membranes with intimately associated sinusoidal lining cells (SLC), intervening sinusoids (S), and the intercellular membranes with canaliculi regions (CR) outlined by basophilic cytoplasmic condensation.



**Figure 1.8** Liver simple acinus. Diagrammatic representation with the zonal arrangement of the hepatocytes and two neighboring classic lobules outlined by a discontinuous line. Blood becomes progressively poorer in oxygen and nutrients from zone 1 to zone 3, which thus represents the microcirculatory periphery. The most peripheral portions of zone 3 from adjacent acini form the perivenular area, the so-called centrilobular zone of the 'classic lobule'. PT, portal tract; ThV, terminal hepatic venule (central venule of 'classic lobule'); 1, 2, 3: microcirculatory zones; 1', 2', 3': microcirculatory zones of a neighboring acinus.

The basolateral or sinusoidal surface area is considerably increased due to the presence of numerous microvilli which project into the perisinusoidal space of Disse, where they are in direct contact with cell-free blood; this intimate relationship is essential to secure the high absorption and secretory activity of the hepatocyte (Fig. 1.10).

The bile canaliculus is an intercellular space formed by apposition of the margins of a groove half way along the lateral membrane with that of the neighboring cell, the lines of apposition being held together by junctional complexes (Fig. 1.11).

The lateral domain is the flat region of the lateral membrane extending from the canaliculus to the sinusoidal surface, an area specialized in cell attachment and communication. The canaliculi are not seen in conventional histology, but they can be outlined by histochemical ATPase staining (Fig. 1.12a), or by immunostaining using polyclonal carcinoembryonic antigen or antibodies raised against enzymes localized to the canaliculi; accumulation of lipofuscin or hemosiderin at the biliary pole of the hepatocyte also outlines negatively stained canaliculi (Fig. 1.12b).

The nucleus is large, occupying 5–10% of the cell volume, surrounded with one or more prominent nucleoli. About 25% of hepatocytes are binucleated. Hepatocyte nuclei show a variation in size that reflects polyploidy with corresponding increased DNA content. This increases with age and in pathologic situations.

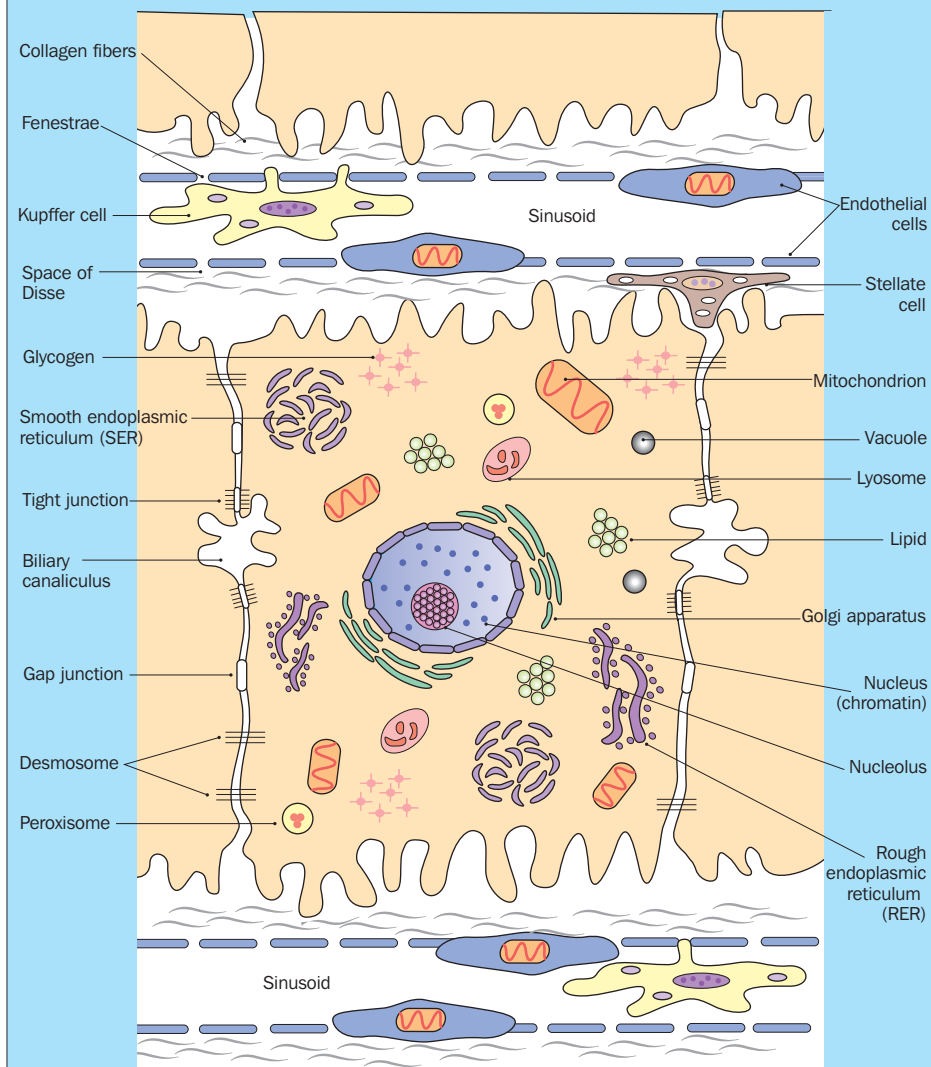
The marked functional diversity is matched by a great variety of cytoplasmic organelles as listed below and illustrated in Figures 1.10 and 1.13.

The endoplasmic reticulum – a complex network of parallel membranes and cisternae – comprises:

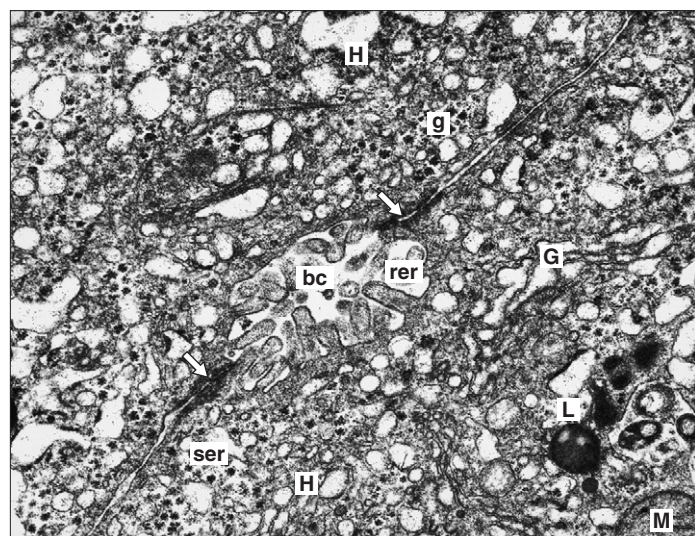
- the rough endoplasmic reticulum (RER), which is more developed in periportal areas, has attached polyribosomes, which are sites of protein synthesis including both cell constituent proteins and secretory plasma proteins;
- the smooth endoplasmic reticulum (SER) of which cisternae are in continuity with those of the RER; here proteins are collected and transported to the Golgi complex, where they are packaged in vesicles prior to their export from the cell; the SER is the site of the metabolism and detoxification of



**Relationship between sinusoid, sinusoid lining cells, and hepatocyte**

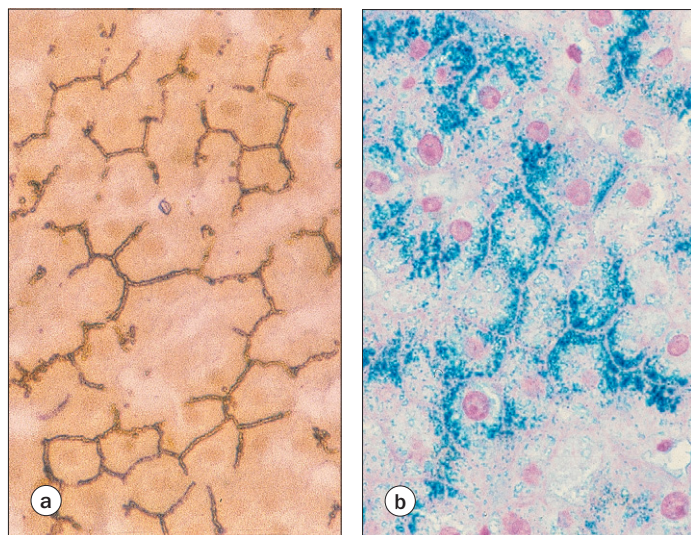


**Figure 1.10 Relationship between sinusoid, sinusoid lining cells and hepatocyte.** The sketch illustrates the various hepatocyte organelles. Note Kupffer cells, the cytoplasmic processes of which are anchored in endothelial fenestrae.

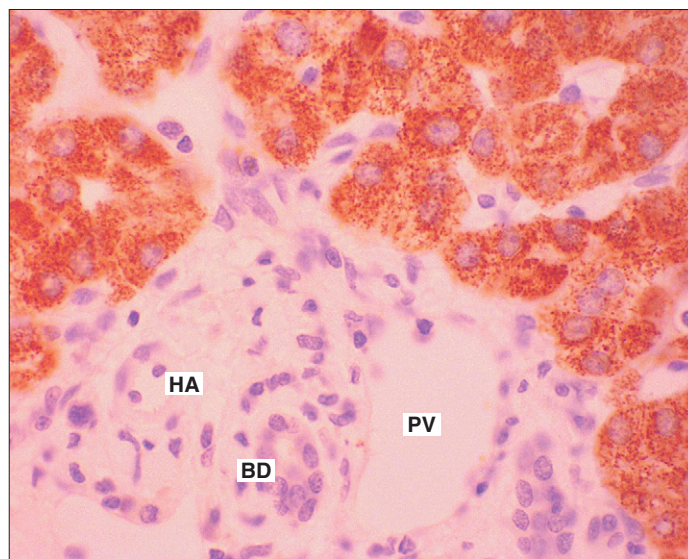


**Figure 1.11 Electron micrograph of bile canaliculi (bc), intercellular membranes and junctional complex.** Note appearances of various organelles within the two adjacent hepatocytes (H). Open arrows, tight junctions; G, golgi apparatus; g, glycogen; ser, smooth endoplasmic reticulum; rer, rough endoplasmic reticulum; M, mitochondrion; L, lysosome ( $\times 18\,000$ ). Courtesy of Professor P Bioulac-Sage.

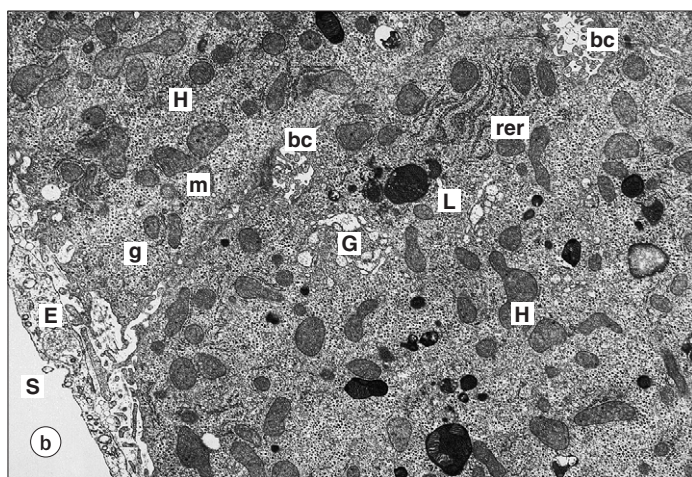
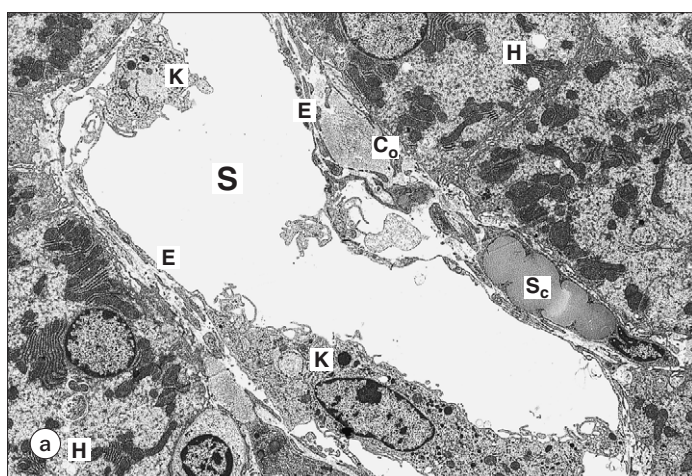




**Figure 1.12 Bile canaliculi in light microscopy.** (a) Histochemical demonstration of ATPase in pericanalicular cytoplasm. (b) Canaliculi outlined in iron overload due to pericanalicular accumulation of hemosiderin (Perls' stain).



**Figure 1.14 Normal hepatocyte cytoplasm shows distinctive brown granules after immunostaining for hepatocyte specific antigen, which appears to detect antigenic material unique to hepatocellular mitochondria.** PV, portal venule; HA = hepatic arteriole; BD, bile duct (HepPar-1, immunoperoxidase).



**Figure 1.13 Electron micrograph of a sinusoid with its lining cells (a), and hepatocytes with organelles (b).** H, hepatocyte; bc, bile canaliculus; m, mitochondrion; L, lysosome; G, golgi apparatus; g, glycogen; rer, rough endoplasmic reticulum; S, sinusoidal lumen; E, endothelial cell; K, Kupffer cell; Sc, hepatic stellate cell; Co, collagen in Disse space. (a) ( $\times 2600$ ). (b) ( $\times 6400$ ). Courtesy of Professor P Bioulac-Sage.

xenobiotics, the SER harboring the cytochrome P450 oxidation system whose induction is expressed by a proliferation of SER membranes (see Ch. 32). Other cell functions associated with SER include metabolism of fatty acids, phospholipids and triglycerides, and synthesis of cholesterol and possibly of bile acid.

The mitochondria are particularly numerous in the hepatocytes, their inner membranes and cristae being concerned with the oxidative phosphorylation and fatty acid oxidation whereas their matrix contains the enzymes involved in the citric acid and urea cycles.

Although not yet fully characterized, a monoclonal antibody referred to as hepatocyte specific antigen or hepatocyte paraffin-1 (HepPar-1) appears to identify an antigen unique to hepatocellular mitochondria. This commercialized antibody is widely used when there is a need to identify hepatocytes or hepatocellular neoplasms (Fig. 1.14).

The lysosomes, first recognized in the liver, are membrane-bound vesicles that are involved in the digestion and catabolism of various exogenous and endogenous substances, a function that is reflected in their heterogeneous content – autophagic vacuoles, storage products, lipofuscin, hemosiderin and copper complexes. They are rich in acid hydrolase, for example acid phosphatase, which can be used for their histochemical identification; they are involved in a number of storage disorders (see Ch. 25).

The peroxisomes (microbodies) are membrane-bound ovoid bodies that contain oxidases and use molecular oxygen for the production of  $H_2O_2$ . This is in turn hydrolyzed by peroxisomal catalase, an enzyme that can be used for the ultrastructural identification of these organelles. Peroxisomes are involved in the metabolism of fatty acid and alcohol; they proliferate following administration of hypolipidemic drugs, such as clofibrate.

The cytoskeleton comprises:

- The microfilaments (6 nm) are composed of filaments of actin and myosin which, associated in bundles, form a three-



dimensional meshwork throughout the cytoplasm; they are attached to the plasma membrane, extend into the microvilli, and are particularly abundant in the pericanalicular ectoplasm, being attached to the junctional complex on either side of the canaliculus; they play a major role in bile secretion and flow regulation, as exemplified by intrahepatic cholestasis produced by drugs that cause depolymerization of the pericanalicular actin belt, in particular cytochalasin B and norethandrolone.

- The intermediate filaments (8–10 nm) are a family of self-assembling protein fibers that act as an intracellular scaffold with a role in integrating cytoplasmic space and organelle movement; like in other epithelial cells in the body they react as cytokeratins, more specifically hepatocyte cytokeratins 8 and 18, in contrast to cytokeratins 7 and 19 which characterize biliary epithelium; intermediate filaments contribute to the formation of Mallory bodies as a result of their depolymerization in alcoholic and other liver diseases.
- The microtubules (20 nm) are hollow, unbranched structures that play a role in cell division (formation of mitotic spindle), in the movement of transport vesicles, and in the transport and export of proteins and lipoproteins.

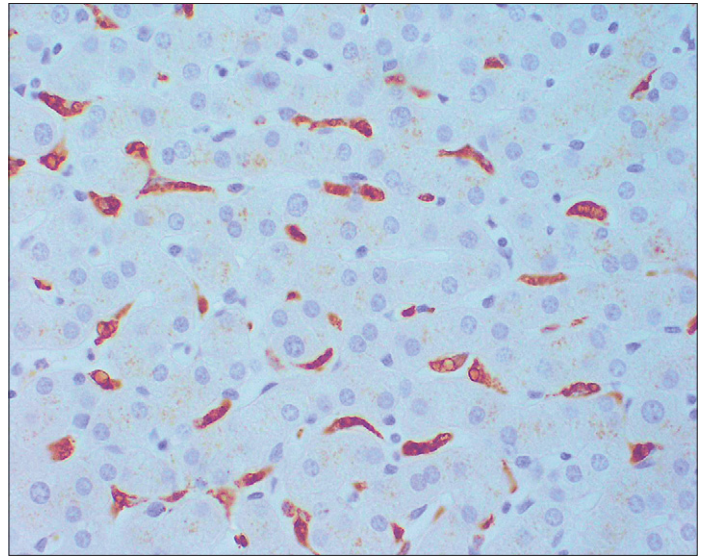
Glycogen is abundant, reflecting a principal role of the liver in the synthesis of glycogen from glucose, or lactic and pyruvic acid, and its breakdown and release as glucose into the circulation. When depletion occurs, glycogen starts disappearing from the perivenular region. In electron microscopy it appears as dense  $\beta$ -particles, 15–30 nm in diameter, and  $\alpha$ -particles, aggregates of the smaller particles arranged in rosettes.

### Cells of the hepatic sinusoid

Sinusoids with an average diameter of about 10  $\mu\text{m}$ , which may distend to about 30  $\mu\text{m}$ , are lined by endothelial cells which delineate the space of Disse running underneath their fenestrated cell process at the sinusoidal surface of the hepatocytes; hepatic stellate cells lie in the space of Disse, the Kupffer cells and liver-associated lymphocytes lie on the luminal aspect of the endothelium (see Figs 1.10 and 1.13).

The endothelial cells form an attenuated cytoplasmic sheet perforated by numerous holes (fenestrae), which constitute the sieve plate. They seem to form no intercellular junctions and do not lie on a basement membrane, a unique configuration that allows free passage of solutes from the sinusoidal lumen into the space of Disse. Unlike vascular endothelium elsewhere in the body, they do not bind *Ulex europaeus*, do not express FVIII-related antigen or CD34 in the normal human liver, but their membrane is immunoreactive for intercellular adhesion molecule-1 (ICAM-1). The natural ligand for this adhesion molecule, leukocyte function-associated antigen-1 (LFA-1), is present on Kupffer cells. In pathologic situations such as capillarization of the sinusoids in the cirrhotic liver and in benign and malignant hepatocellular neoplasms, endothelial cells do acquire CD34 immunoreactivity.

The Kupffer cells are members of the mononuclear-phagocytic system forming the largest part of the fixed tissue macrophages in the body. They account for 2.1% of the non-parenchymal cells on a volume basis and have an increased density, a larger size and are more phagocytic in the periportal areas. Irregularly stellate in shape, they float freely in the lumen of the sinusoids, anchored by their process to the endothelial cells. Their major function is the clearance of particles, immune complexes,



**Figure 1.15** Kupffer cells are clearly identified due to dark brown appearance of their cytoplasm following immunostaining for CD68 antigen.

injured red cells and endotoxins. Their release of mediators, including interleukins 1 and 6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferons and eicosanoids, constitutes part of the host response to infection and explains some of the clinical manifestations of endotoxemia. Kupffer cells are difficult to identify by light microscopy alone and proper identification rests on using their immunoreactivity to CD68 (Fig. 1.15) or their expression of class II histocompatibility antigens, in particular human leukocyte antigen-DR (HLA-DR).

The hepatic stellate cells (perisinusoidal, fat-storing or Ito cells) lie in the space of Disse, their long cytoplasmic processes surrounding the sinusoids. Their nucleus and perikaryon are often embedded within recesses between hepatocytes; their cytoplasm contains many small lipid droplets that are rich in vitamin A. Besides their role in storing vitamin A, they represent resting fibroblasts which are a major source of extracellular matrix in the normal and diseased liver. Their phenotypic transformation into transitional myofibroblasts in acute and chronic liver disease is associated with the acquisition of  $\alpha$ -smooth muscle actin reactivity. Stellate cells are a potential source of hepatocyte growth factor and their contractility has led to a speculative role in controlling sinusoidal blood flow. They are not obvious in conventional light microscopy, but they become evident in semi-thin sections due to their microvesicular fatty cytoplasm (Fig. 1.16).

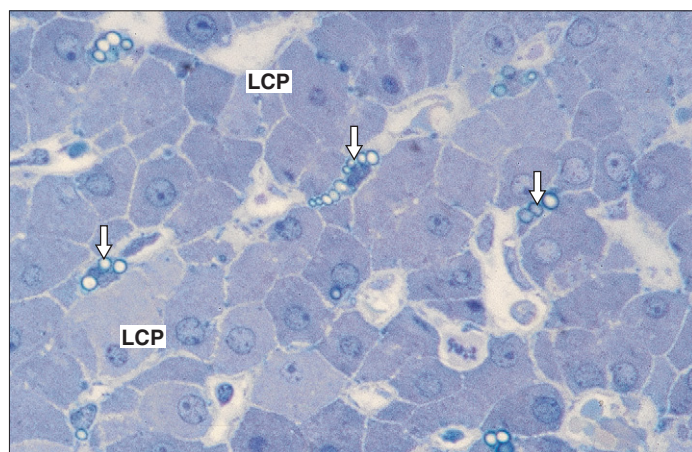
Liver-associated lymphocytes initially described as pit cells in the rat liver are now well recognized in human liver. They seem to be recruited from the peripheral blood to the liver sinusoids where they acquire natural and lymphokine-activated killer cell activity.

### Other constituent tissues

#### Extracellular matrix

The connective tissue represents only 5–10% of the normal liver weight, a figure largely below that of the rest of the body, and 30% of the total protein mass is made of collagen. The other





**Figure 1.16 Hepatic stellate cells.** Semi-thin section of a 2-year-old child's liver showing a two cell-thick plate arrangement with nuclei disposed toward the sinusoidal border of the hepatocytes and easily-identifiable stellate cells due to their microvesicular cytoplasm (arrowed). LCP: liver cell plate.

structural components of the extracellular matrix are glycoproteins and proteoglycans.

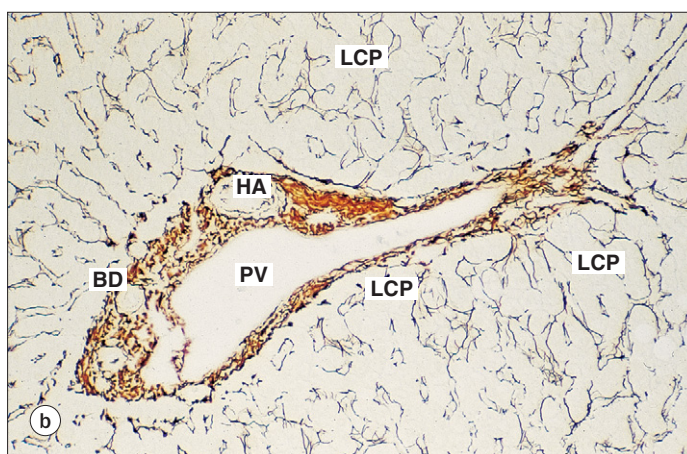
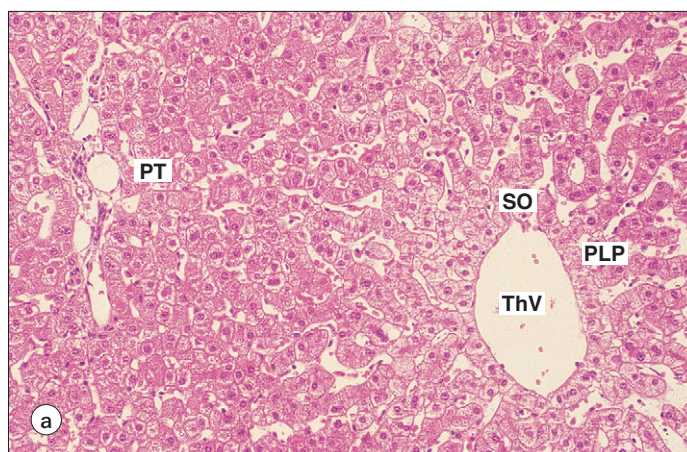
Type I, III, IV, V and VI collagens are found in the normal liver. Type I and III, which represent more than 95% of the total collagen weight, are mainly located in Glisson capsule and its intrahepatic extension supporting the portal vein, hepatic artery and bile-duct branches – the portal tracts. Some extension of the capsular tissue accompanies the major hepatic vein branches, but there is no fibrous coat around the terminal hepatic venules which lie in direct contact with the perivenular hepatocyte plate (Fig. 1.17a).

Discrete strands of collagen are also present in the space of Disse. Type V collagen is closely associated with smooth muscle cells within vessel walls and may also form core fibrils upon which growth of large fibrils of type I collagen is initiated. Type VI forms microfilaments which serve as a flexible network that anchors blood vessels and nerves into the surrounding connective tissue. Type IV differs in that its terminal propeptides are not removed, but serve to cross-link the molecule into a three-dimensional lattice. It is an essential component of the vascular and bile duct basement membranes.

The matrix glycoproteins are highly cross-linked and insoluble. They have well-defined domains that interact with cell surface receptors and other components of the extracellular matrix. Laminin is a major component of basement membranes where it interacts with type IV collagen; both components are normally present in small amounts in the space of Disse, where increased deposition is associated with so-called capillarization of the sinusoids. Fibronectin mediates cell adhesion to collagen.

The proteoglycans are macromolecules that consist of a central protein core to which proteoglycans and oligosaccharides are attached. They are classified according to the type of glycosaminoglycan. In the liver, heparan sulfate is the most abundant and is present in the portal tracts, in basement membrane and on the surface of hepatocytes.

The extracellular matrix in the normal liver is considered of major importance in regulating and modulating hepatocyte function; it serves to provide cohesiveness between cells, induces cell polarization, allows intercellular communication, and affects gene expression and cellular differentiation.



**Figure 1.17 Liver histology at low magnification.** (a) A terminal portal tract (PT) and a terminal hepatic venule (ThV) are shown, the latter in direct contact with the perivenular hepatocyte limiting plate (PLP). Note two sinusoidal outlets (SO). (b) Silver impregnation to show the reticulin network (black) and a small amount of type I collagen (gold brown) supporting portal vessels and bile duct. HA, hepatic arteriole; PV, portal venule; BD, bile duct; LCP, spaces occupied by liver cell plates.

In the lobular parenchyma, the extracellular matrix is confined to the space of Disse. It is of unusually low density and thus invisible ultrastructurally, but visualized in light microscopy after impregnation with a silver method – the so-called reticulin network (Fig. 1.17b).

### Lymphatics

The liver is the largest single source of lymph in the body, producing 15–20% of the total volume. Hepatic lymph has a high protein and cell content and is formed mainly by drainage from the perisinusoidal space of Disse into the first-order lymphatic plexus of the portal tracts. Traced towards the porta hepatis, the plexus, composed of flattened endothelial tubes with a primarily periarterial distribution, progressively enlarges, and in larger tracts becomes associated with portal vein and bile duct tributaries. Large collectors are thickened at the porta hepatis due to the acquisition of a muscle layer. They mainly drain into the hepatic nodes, located along the hepatic artery and celiac nodes. Other efferent routes include:

- via the falciform ligament and epigastric vessels to the para-sternal nodes,

- from the liver surface to the left gastric nodes, and
- from the bare area to the posterior mediastinal nodes.

In conditions such as portal hypertension there is a significant increase in production of hepatic lymph, with a protein content identical to that of the plasma, indicating unrestricted passage of protein into the space of Disse. There ensues a considerable enlargement of the capsular plexus due to important anastomoses between intrahepatic and capsular lymphatics, with lymph exuding from the capsular plexus to form protein-rich ascites.

### Nerve supply

There are two main separate but intercommunicating nerve plexuses around the hepatic artery and portal vein that distribute with the branches of these vessels. These include preganglionic parasympathetic fibers derived from both vagal and sympathetic fibers with cell bodies in the celiac ganglia. Immunohistochemical studies of human liver using antibodies to common neural proteins such as protein gene product (PGP) 9.5 have demonstrated nerve fibers, not only around vessels and bile ducts in the portal tracts, but in the acini, running along the sinusoids. There is evidence that sympathetic nerves play a role in carbohydrate metabolism and possibly in regulating sinusoidal blood flow.

### Light microscopy as seen in biopsy specimens

With reference to the three-dimensional model of Elias (see Fig. 1.6) it is easy to extrapolate that the liver cell plates appear as single cell thick anastomosing 'cords' in conventional light microscopy (see Fig. 1.9). These are two-cell thick up to the age of 5 years (see Fig. 1.16), a pattern that in adults reappears as an indication of liver regeneration, sometimes associated with an acinar or rosette arrangement. Hepatocytes appear as polygonal cells with clearly outlined cell margins and centrally placed nuclei which contain one or two nucleoli. There are occasional binucleated cells. Variation in nuclear size increases with increasing age, a pleomorphism that is more marked in acinar zone 2 (mid-zonal areas). The cytoplasm is granular and eosinophilic, but slightly basophilic aggregates of RER can be identified in a perinuclear and pericanalicular distribution (see Fig. 1.9). At low magnification relatively regularly spaced portal tracts and hepatic venules are expected (see Fig. 1.17a), but the boundaries between acini or acinar zones are not evident. Thus the sleeve of parenchyma surrounding the portal tracts is referred to as periportal parenchyma or acinar zone 1, the one surrounding the hepatic venules as perivenular parenchyma or acinar zone 3, intermediate areas between these zones being considered as acinar zone 2 or mid-zonal. Appearances of the portal areas depend on their size and the plane along which they are cut (Fig. 1.18a–c).

However, at any level the size of the arteriole roughly matches that of the bile duct. The portal venules are of variable shape and size and they are thin-walled, their lumen being generally much larger than that of the corresponding arterial branch. Large tracts (Fig. 1.18c) may show a considerable amount of collagen (Fig. 1.18d), especially around the bile duct, and, when

sampled by the biopsy needle, this can easily be misinterpreted as fibrotic liver.

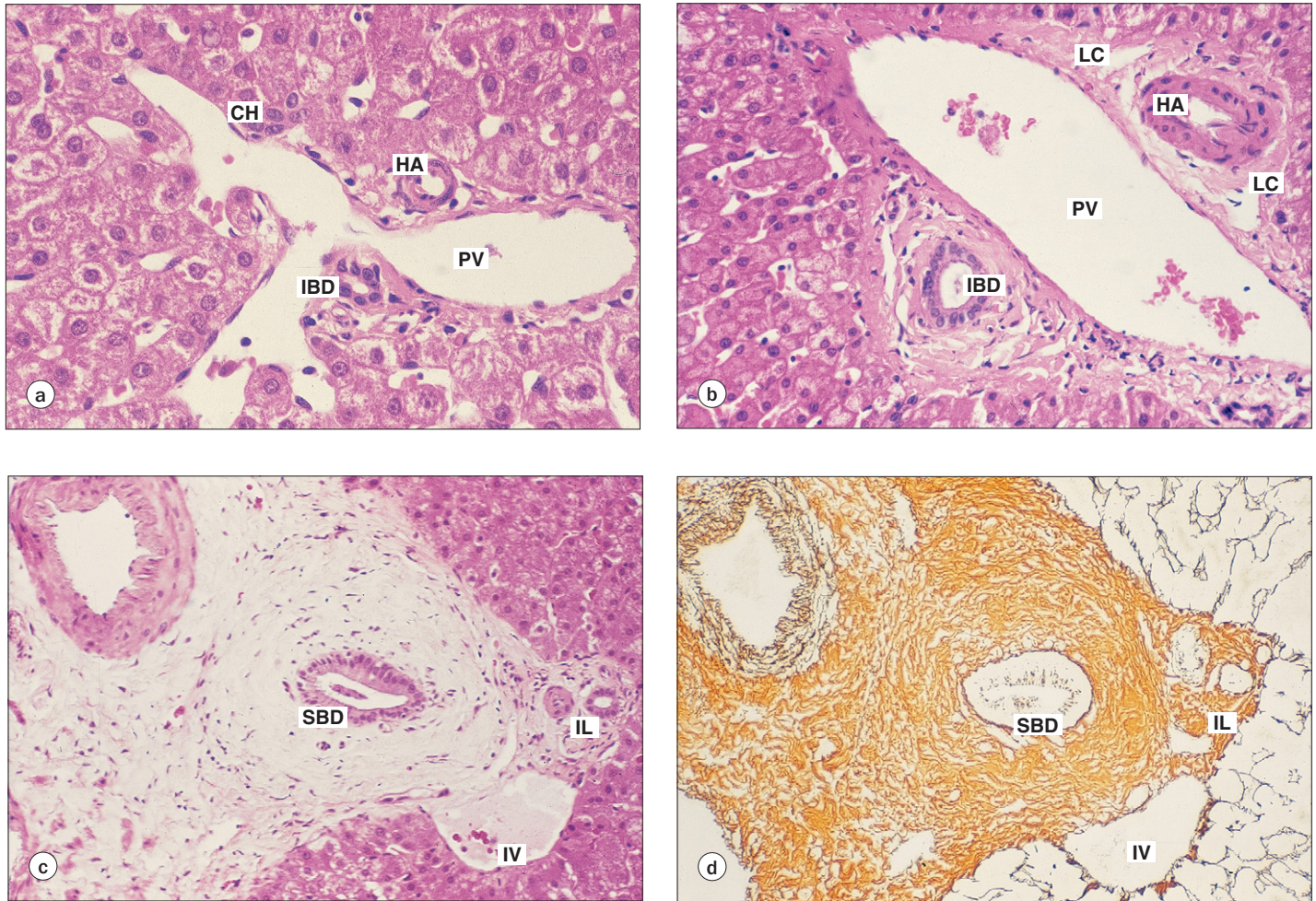
### Intrahepatic and extrahepatic biliary passages

The bile canaliculi, as already described, form a complicated anastomosing network running half way within the thickness of the liver cell plates. They drain into minute periportal channels, also known as canals of Hering, which have a basement membrane and are lined by both hepatocytes and bile duct cells. The canal of Hering is supposed to harbor progenitor cells which, in various pathologic situations, are able to differentiate into bile ducts or liver cells and from which so-called ductular reaction is likely to arise. Bile ductules, lined by flattened cholangiocytes, in which inconstant intralobular and short intraportal segments are recognized, join the canal of Hering to the interlobular bile ducts (Fig. 1.19). Canals of Hering and bile ductules are hardly visible on hematoxylin-eosin stained sections, but clearly revealed by cytokeratin immunostaining (Fig. 1.19b). The interlobular bile ducts, lined by a single layer of low cubic cells (see Fig. 1.18a, b) will by subsequent anastomoses increase in size from 15–20  $\mu\text{m}$  to 100  $\mu\text{m}$  to form septal bile ducts that are lined by tall columnar cells with basally located nuclei (Fig. 1.18c). The portal tract fibrous tissue shows some condensation around these ducts, which through further anastomoses form successively the area and segmental bile ducts. The latter, similar to the extrahepatic bile ducts and neck of gallbladder are invested with intramural mucinous and extramural seromucinous glands, the peribiliary glands (Fig. 1.20). They anastomose further to give rise to the left and right hepatic ducts, which in turn join to form the common hepatic duct, itself joined by the cystic duct of the gallbladder to become the common bile duct or choledochus (Fig. 1.21).

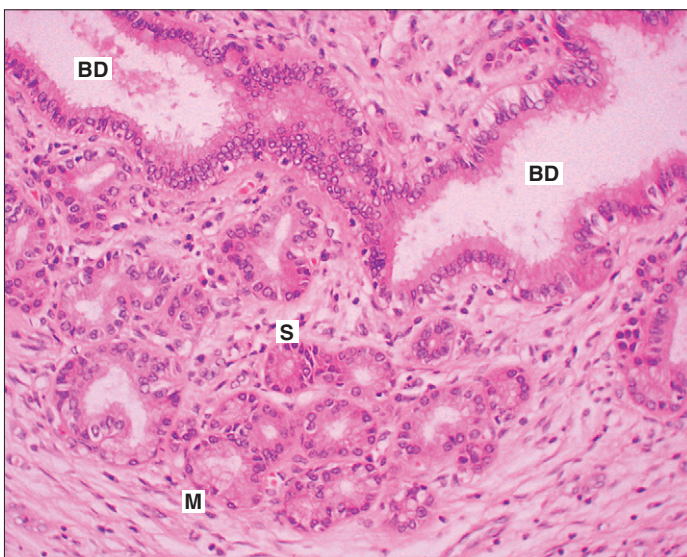
The common bile duct runs between the layers of the lesser omentum, lying anterior to the portal vein and to the right of the hepatic artery. It passes behind the first part of the duodenum in a groove on the back of the head of the pancreas, before entering the second part of the duodenum. The duct runs obliquely through the posteromedial duodenal wall, usually joining the main pancreatic duct to form a variable length common channel, the ampulla of Vater. In about 30% of individuals, the bile and pancreatic ducts open separately into the duodenum. The ampulla of Vater makes the duodenal mucous membrane bulge inwards to form an eminence, the duodenal papilla (Fig. 1.21).

During its passage through this collecting system, canalicular bile is modified by a process of absorption and secretion of water and electrolytes, and addition of seromucinous fluid by the peribiliary glands. There is evidence of immunoglobulin A secretion by the bile duct epithelium which normally expresses HLA class I,  $\gamma$ -glutamyltranspeptidase, carcinoembryonic antigen and epithelial membrane antigen. These represent phenotypic differences between bile duct epithelium and liver cells additional to their different cytokeratin profiles already mentioned.



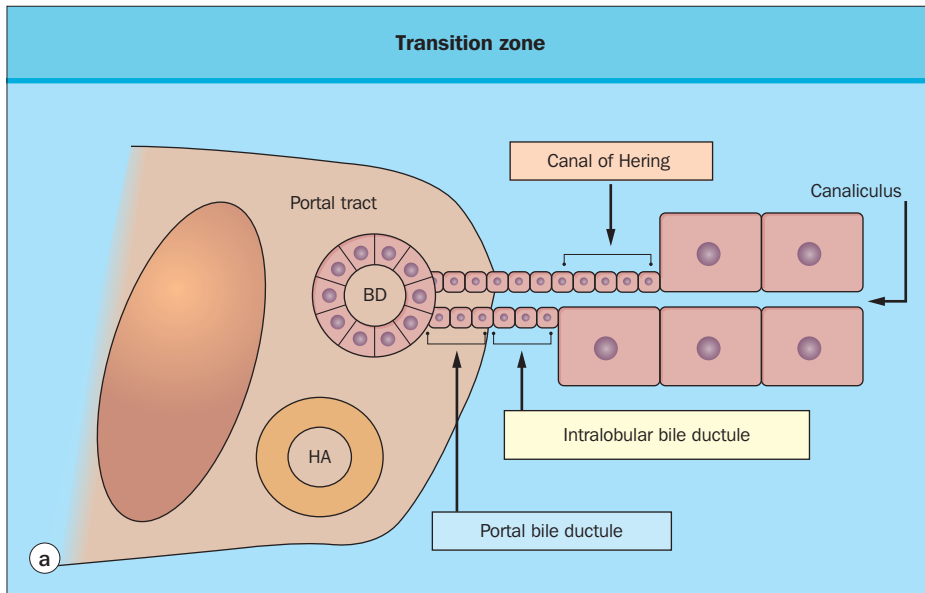


**Figure 1.18 Portal tracts of various sizes.** (a) Small portal tract with interlobular bile duct (IBD), a size matched arterial branch (HA) and a dividing portal venule (PV). A canal of Hering (CH) region is shown. (b) Large interlobular, on the borderline of being a septal bile duct (IBD) with a low columnar epithelial lining. Hepatic arteriole (HA) and lymphatic channel (LC) are seen in the portal area. (c and d) Septal tract giving rise to an interlobular division to the right (IL) and an inlet portal venule (IV). Note the considerable amount of collagen normally surrounding a septal sized bile duct [SBD (d)].

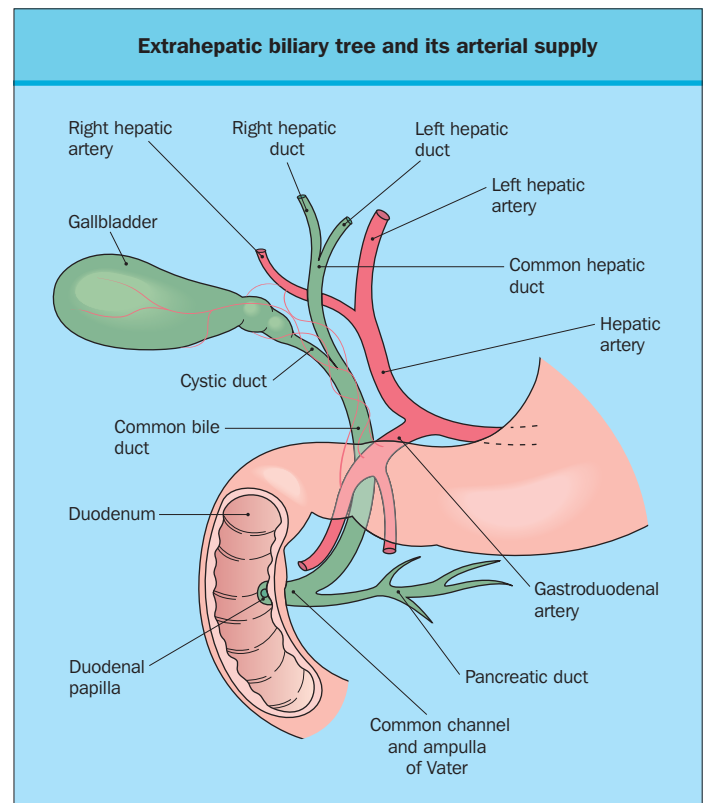
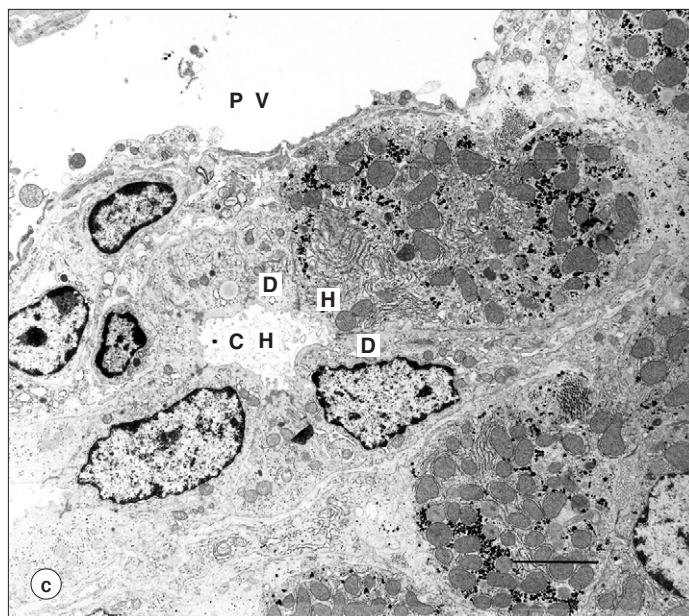


**Figure 1.19 Large intrahepatic bile duct.** The bile duct (BD) is surrounded by peribiliary glands which consist of mixed mucinous (M) and serous (S) acini.





**Figure 1.20 Transition zone.** (a) This sketch shows the transition between canaliculi, canal of Hering, bile ductules and interlobular bile duct (BD). Portal venule (PV), hepatic arteriole (HA). (b) Canal of Hering and ductules, which are difficult to outline in a hematoxylin and eosin stained section, are here highlighted (brown) by immunostaining for cytokeratin 19. Bile ductules (bd); interlobular bile duct (BD); region of canal of Hering (CH). (CK19, immunoperoxidase.) (c) Electron microscopy of a section through the canal of Hering (CH) whose lumen is lined by bile duct cells (D) and a hepatocyte (H). Scale bar – 3.5  $\mu\text{m}$ . Courtesy of Professor R De Vos.



**Figure 1.21 Extrahepatic biliary tree and its arterial supply.** The gallbladder, cystic duct and upper portion of the common hepatic duct and common bile duct derive their arterial supply from the cystic artery and additional small branches that usually arise from the right hepatic artery, although there are several variations recorded. The rest of the common bile duct receives blood from the gastroduodenal artery. There are anastomoses between the two systems, but there may subsist a watershed zone which is important to recognize during the performance of an end-to-end biliary anastomosis at liver transplantation.

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## SECTION I: NORMAL STRUCTURE AND FUNCTION

### CHAPTER

# 2

## Cellular Biology of the Normal Liver

Gary Guangsheng Fan and Clifford J Steer

### INTRODUCTION

More than 50 years ago, EB Wilson believed that ‘the key to every biological problem must finally be sought in the cell’. With the recent dramatic advances in technology and our understanding of cell function, cellular biology of the liver is beginning to take its rightful place as an application to clinical hepatology. In this chapter, we present an overview of how hepatic cells are formed and how they function and cooperate to form the amazing liver. In higher organisms, organ systems are like cellular cities in which groups of cells perform specialized functions and are linked by intricate systems of communication. Cells occupy a halfway point in the scale of biologic complexity (**Fig. 2.1**). A fetal liver has three main cellular compartments, which are:

- parenchymal cells (hepatocytes, bile duct cells);
- sinusoidal cells (endothelial and Kupffer cells); and
- perisinusoidal cells (stellate and hematopoietic cells).

In a human embryo, the hepatic diverticulum consists of a cranial and caudal portion. Hepatocytes develop from the cranial portions, while the caudal bud is the origin of extrahepatic bile duct cells and epithelial cells that form the gallbladder. Generally, the proliferation of hepatic cells occurs by diffusion away from the thickening endoderm, but the formation of the extrahepatic ducts always proceeds from the caudal portion of the diverticulum. Studies in rat embryos suggest that the emerging hepatic tissue is composed of bipotential progenitor epithelial cells that are capable of differentiating either along the hepatocytic or biliary epithelial cell lineage.

The formation of intrahepatic bile ducts is initiated by hepatocytes. It has been suggested that the sinusoidal lining cells in early fetuses may be undifferentiated vascular stem cells. During development, these stem cells diverge, with one group committed to becoming an endothelial cell line and a second to becoming a Kupffer cell line. For mammals, the main function of the fetal liver is hematopoiesis, which begins soon after the appearance of the hepatic diverticulum. The hematopoietic cells are initially scattered throughout the liver, but are ultimately replaced by the developing and differentiating adult hepatic cells. An adult liver is occupied by hepatocytes and non-hepatocytes, in which hepatocytes constitute 78% of the tissue volume, non-hepatocytes account for 6.3% of the tissue volume, and the extracellular space for approximately 16% (**Table 2.1**).

The architecture and ultrastructure of the normal liver are unique to this organ (**Fig. 2.1**). The structure of the muralium simplex consists of polygonal-shaped parenchymal cells or hepatocytes. These cells enclose a chicken wire-like network of bile canaliculi in the central plane and are flanked by sinusoids on both sides of the muralium. The equivalent of an apical pole for the exocrine secretion of bile by the hepatocyte corresponds to

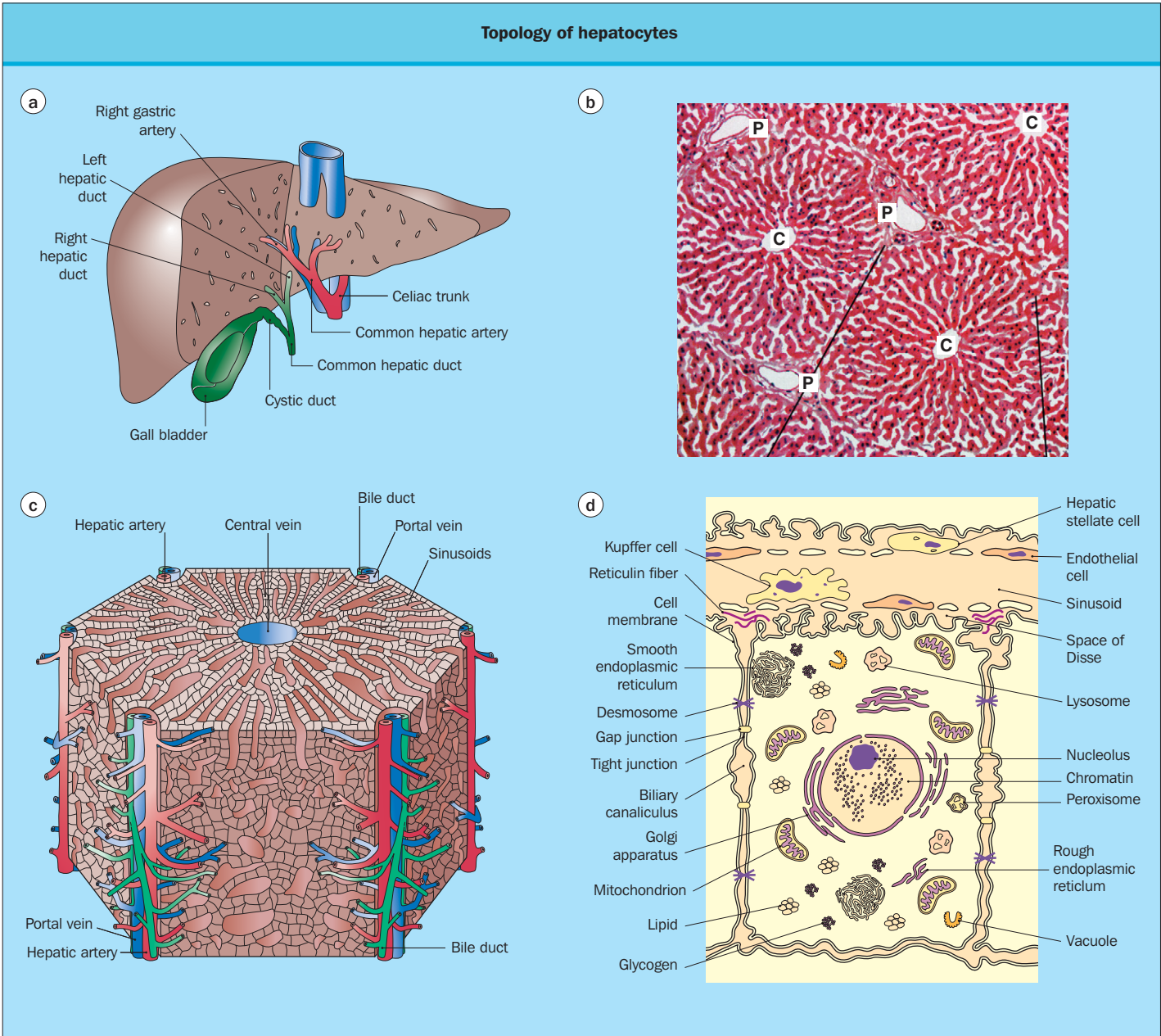
the canalicular membrane, which surrounds the parenchymal cell periphery in a belt-like fashion. This structural organization implies that the biliary secretory polarity of the hepatocyte is oriented towards a peripheral ring around the middle of the parenchymal cell. In contrast, metabolic uptake and secretion occur at, or towards, the surfaces facing the sinusoids, implying bidirectional metabolic secretory activity. This complex secretory polarity requires a well-organized intracellular system for adequate and correct addressing of secretory products to their respective destinations. Hepatocytes are large polyhedral cells of approximately 20–30  $\mu\text{m}$  diameter, which are arrayed in plates one or two cells thick along the hepatic sinusoids. As a metabolically highly active cell, the hepatocyte contains a vast array of organelles (**Table 2.2**). In human hepatocytes, approximately 30% of the cells are binucleate. While adult parenchymal cells display considerable heterogeneity in size, they all share at least one common constituent. The hepatocyte plasma membrane guarantees separation from but, at the same time, links the cell with its environment and matrix.

### HEPATOCELLULAR PLASMA MEMBRANE

The plasma membrane encloses the cell, defines its boundaries and maintains the essential differences between the cytosol and the extracellular environment. All biological membranes have a common general structure which comprises a very thin film of lipid and protein molecules, held together mainly by non-covalent interactions (**Fig. 2.2**). Cell membranes are dynamic, fluid structures, and most of their molecules are able to move about in the plane of the membrane. The lipid molecules are arranged as a continuous double layer which is about 5 nm thick. This lipid bilayer provides the basic structure of the membrane and serves as a relatively impermeable barrier to the passage of most water-soluble molecules. Protein molecules within the lipid bilayer mediate most of the other functions of the membrane, transporting specific molecules across it, or catalyzing membrane-associated reactions such as adenosine triphosphate (ATP) synthesis. In the plasma membrane some proteins serve as structural links that connect the membrane to the cytoskeleton and/or to either the extracellular matrix or an adjacent cell, while others serve as receptors to detect and transduce chemical signals in the cell's environment.

Cell membranes are asymmetric structures. The lipid and protein compositions of the inner and outer faces differ from one another in ways that reflect the different functions performed at the two surfaces. The protein–lipid ratio varies enormously in different type cells. In hepatocytes, plasma membrane contains 54% protein, 36% lipid and 10% carbohydrate (by dry weight) (**Table 2.3**). Hepatocytes express the greatest abundance of





**Figure 2.1 The topology of hepatocytes and liver lobules in a normal liver.** (a) A cross-section of an adult liver. (b) A magnified normal lobular section. The central veins (C) are surrounded by anastomosing cords of block-like hepatocytes. At the periphery of the lobule, typical portal areas (P) consist of branches of the portal vein, the hepatic artery and the bile duct. (c) A schematic view of the relationship of the different liver cells to each other and to the sinusoids. The principal organelles and inclusions in the cytoplasm are shown. (d) Schematic view of a liver lobule.

Relative number and volume of different cell types in the adult liver		
Cell type	Number (%)	Volume (%)
Hepatocytes	60	78
Non-hepatocytes	40	6.3
Endothelial cells	20	2.8
Kupffer cells	14	2.1
Stellate cells	6	1.4
Extracellular space		15.7

**Table 2.1 Relative number and volume of different cell types in liver**

membrane carbohydrates, where they occur almost exclusively on the outer surface.

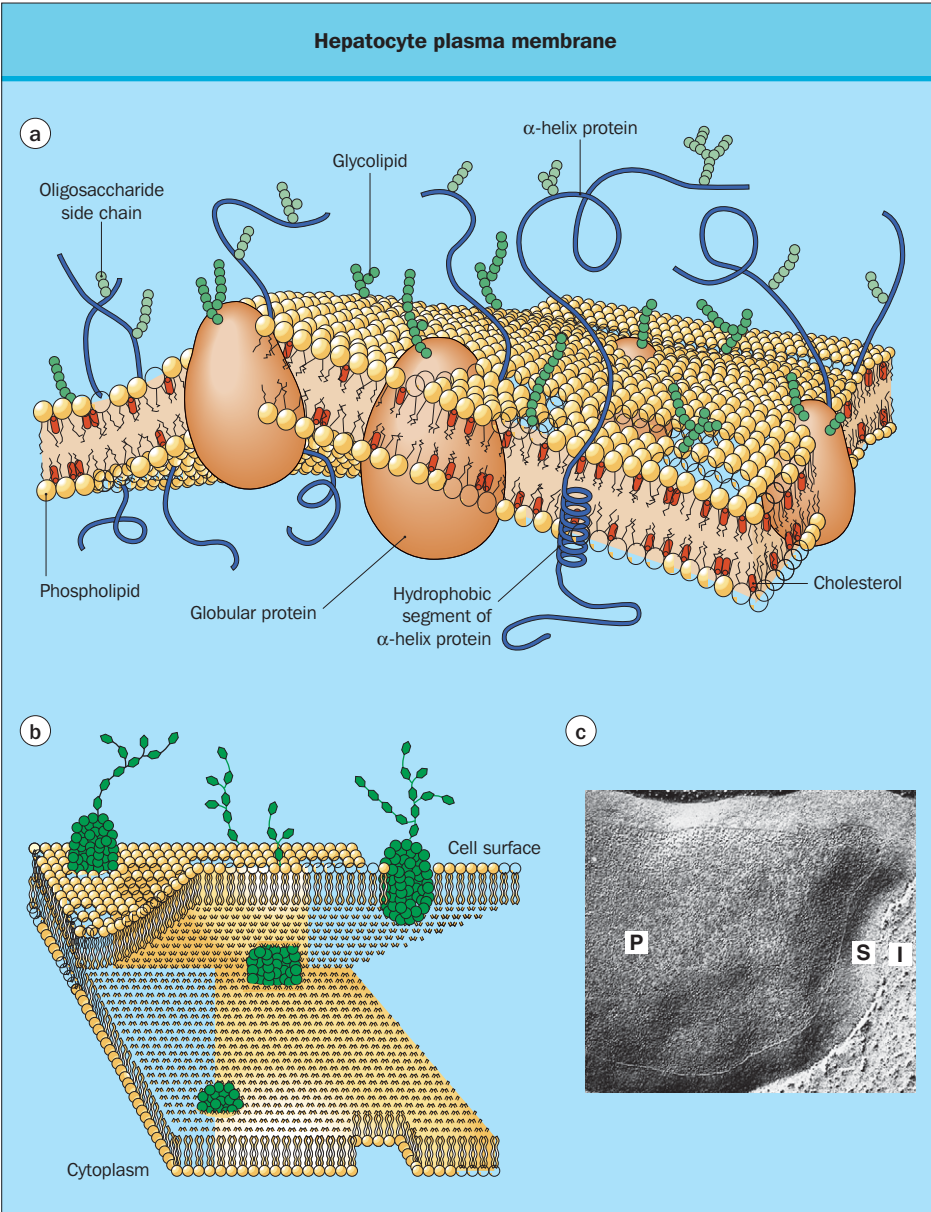
**Membrane lipids**

Lipid contribution to membranes is highly varied depending on the species and cell type. However, in most eukaryotic cells there are three major classes of membrane lipid molecules: glycerophospholipids, cholesterol and sphingolipids (Fig. 2.3). The polar head groups of the phospholipids face the surrounding water and the fatty acyl chains form a continuous hydrophobic interior. In such a lamellar structure, each layer of phospholipid is called a *leaflet*. In addition, the lipid compositions of the inner and outer leaflets are different. Most of the sphingolipids are present in the outer leaflet, while some of the glycerophospholipids are restricted to the inner leaflet. Cholesterol and



Relative volumes occupied by the major intracellular compartments in a hepatocyte		
Intracellular compartment	Cell volume (%)	Approximate number per cell
Cytosol	54	1
Mitochondria	22	1700
Rough ER cisternae	9	1
Smooth ER cisternae plus Golgi cisternae	6	
Nucleus	6	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

**Table 2.2 Relative volumes occupied by the major intracellular compartments in a hepatocyte.** All the cisternae of the rough and smooth endoplasmic reticulum are thought to be joined to form a single large compartment. The Golgi apparatus, in contrast, is organized into a number of discrete sets of stacked cisternae in each cell, and the extent of interconnection between these sets has not been clearly established. ER, endoplasmic reticulum.



**Figure 2.2 The hepatocyte plasma membrane.** (a) Schematic diagram of the membrane. The phospholipid molecules in the top layer, which faces the external medium, are shown as yellow spheres. The bottom layer, which faces the cytoplasm inside the cell, has a different phospholipid composition and is also shown in yellow. In some transmembrane proteins the polypeptide chain crosses the bilayer as a single  $\alpha$  helix (blue); in others the polypeptide chain crosses the bilayer multiple times, either as a series of  $\alpha$  helices or as a  $\beta$  sheet in the form of multipass proteins. Other membrane-associated proteins do not span the bilayer but instead are attached to either side of the membrane. Rigid cholesterol molecules (red) tend to keep the tails of the phospholipids relatively fixed and orderly in the regions closest to the hydrophilic heads; the parts of the tails closer to the core of the membrane move about freely. Side chains of sugar molecules attached to proteins and lipids are shown in green. (b) The path followed by a fracture that splits a membrane into bilayers. (c) The membrane structure visible in a freeze-fracture preparation. P, membrane particles in bilayer; S, external membrane surface; I, ice crystals. Part (a) reproduced from Bretscher (85) by permission of Sci Am. Parts (b) and (c) reproduced from Wolfe (93) by permission of Brooks/Cole.

Lipid, protein, and carbohydrate content in cellular membranes			
Membrane	Protein	Dry mass (%) Lipid	Carbohydrate
Plasma membrane	54	36	10
Nuclear envelope	66	32	2
Endoplasmic reticulum	62	27	10
Golgi complex	64	26	10
Mitochondrion			
outer membrane	55	45	Trace
inner membrane	78	22	

**Table 2.3 Lipid, protein, and carbohydrate content in cellular membranes.**

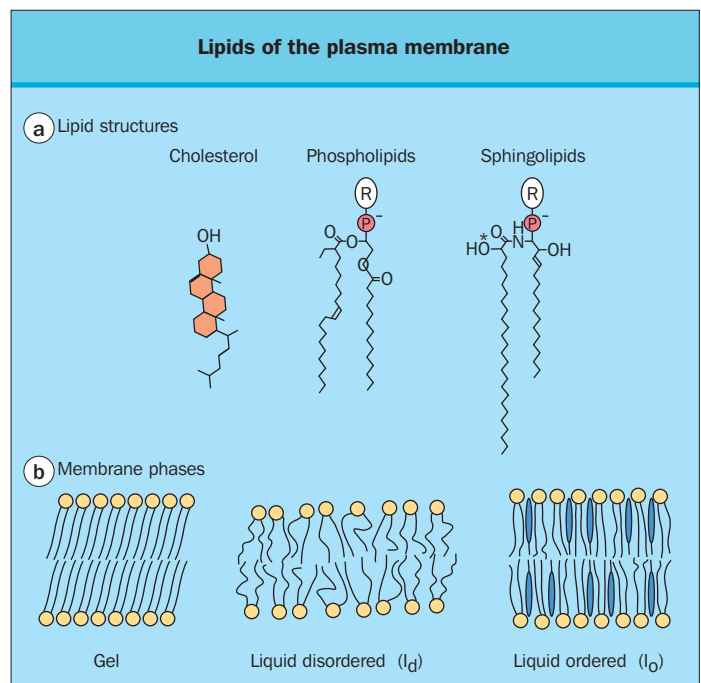
sphingolipids are present at low levels in internal membranes and at high levels in the plasma membrane and endosomes. The high cholesterol bilayer is thus termed 'liquid-ordered' ( $l_o$ ), in contrast to the 'liquid-disordered' state without cholesterol ( $l_d$ ).

The most complex of the glycolipids, gangliosides, contain oligosaccharides with one or more sialic acid residues, which give gangliosides a net negative charge. The glycolipids may serve as receptors for normal extracellular molecules that mediate cell-recognition processes and protection of the membrane from the harsh conditions, and assist cells in binding to the extracellular matrix. Charged glycolipids may be important for their electrical effects. Their presence alters the electrical field across the membrane and the concentrations of ions, especially calcium, at its external surface.

The fluidity of a cell membrane is biologically important. For example, certain membrane transport processes and enzyme activities can be shown to cease when the bilayer viscosity is experimentally increased beyond a threshold level. All cell membranes contain a mixture of different fatty acyl chains and are fluid at the temperature at which the cell is grown and functions. Membrane cholesterol is a major determinant of bilayer fluidity. The net effect of cholesterol on membrane fluidity varies, depending on the lipid composition. Cholesterol restricts the random movement of portions of the fatty acyl chains lying closest to the outer surfaces of the leaflets. However, it separates and disperses the fatty acyl tails and causes the inner regions of the bilayer to become slightly more fluid. At the high concentrations found in eukaryotic plasma membranes, cholesterol tends to restrict fluidity at growth temperatures near 37°C. At temperatures below the *phase transition*, cholesterol maintains the membrane in a fluid state by preventing the hydrocarbon fatty acyl chains of the membrane lipids from binding to each other. This offsets the drastic reduction in fluidity that would otherwise occur at low temperatures.

### The raft model of plasma membrane lipids

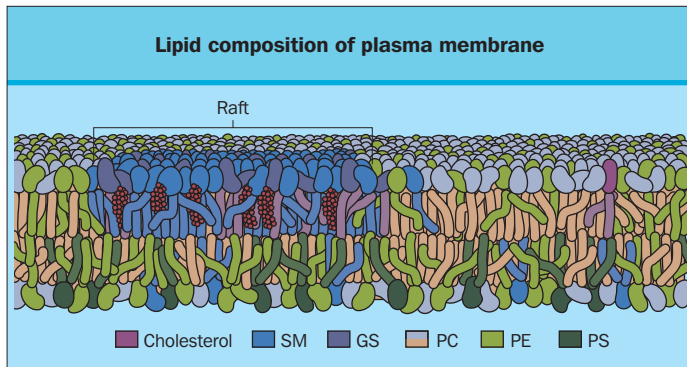
The plasma membrane contains more lipid species than are needed to form a simple bilayer. In plasma membranes, glycosphingolipids lie outside and most phosphatidylserine (PS) is located within the cell. PS asymmetry gives the cytoplasmic



**Figure 2.3 The lipids of the plasma membrane.** (a) Structures of the major lipid classes of eukaryotic cells. Sterols like cholesterol are based on a four-ring structure. Glycerophospholipids are derived from diacylglycerol and typically carry acyl chains of 16–18 carbon atoms, one of which contains a cis double bond. The head group (R) is either neutral (serine or inositol) to give a net acidic charge, or basic (ethanolamine or choline) to give a neutral or zwitterionic lipid. Sphingolipids are based on a ceramide, and in mammals the head group is either choline (sphingomyelin), or in the case of the glycosphingolipids the phosphate is replaced with glucose, which is further elaborated to make a wide range of glycolipids. The acyl chain attached to the sphingoid base is typically saturated, varying in length from 16 to 26 carbons depending on the lipid and tissue (C26 is shown here). (b) Structures of lipid bilayers. Simple phospholipid bilayers below their  $T_m$  form a solidified gel phase, which melts above the  $T_m$  to a fluid phase (liquid-disordered ( $l_d$ ), sometimes referred to as 'liquid-crystalline'). The presence of cholesterol (hatched ovals) orders the acyl chains of the latter phase, and indeed can fluidize the former phase, arriving at an intermediate state for which the term liquid-ordered ( $l_o$ ) was coined. Reproduced from Munro (03) by permission of Elsevier.

surface of the plasma membrane a net negative charge above and beyond that arising from the oriented water dipoles on the surface. In the outer leaflet of the plasma membrane there are microdomains or small islands of cholesterol and a sphingolipid rich  $l_o$  phase that are surrounded by a  $l_d$  phase. They are called *rafts* and are typically about 50 nm in diameter (Fig. 2.4). These small islands of lipid are coupled to cholesterol-rich microdomains in the inner leaflet by an unknown mechanism. Rafts include transmembrane proteins, external glycosylphosphatidylinositol (GPI)-anchored proteins, and fatty acid-anchored proteins (such as Src tyrosine kinase) on the inner face. Raft lipids and associated proteins diffuse together laterally on the membrane surface. Some signaling receptors can move into the rafts upon ligand engagement, or 'cluster' smaller rafts into larger ones.

It is becoming evident that rafts play an important role in a wide range of biological processes, including numerous signal transduction pathways, apoptosis, cell adhesion and migration, synaptic transmission, organization of the cytoskeleton, and protein sorting during both exocytosis and endocytosis. In addition



**Figure 2.4 Lipid composition of a plasma membrane.** The heterogeneity of the lipids and their asymmetrical distribution between the two halves of the bilayer are illustrated. Sphingomyelin (SM) and cholesterol form a small raft in the external leaflet. GS, glycosphingolipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine. Reproduced from Pollard & Earnshaw (02) by permission of Elsevier.

to their roles in normal cellular function, rafts appear to be the point of cellular entry for a wide range of viruses.

### Membrane proteins

Whereas the lipid bilayer determines the basic structure of biologic membranes, proteins are responsible for most membrane functions, including specific receptors, enzymes and transport proteins. Membranes typically contain 10–50 different major protein types with molecular masses ranging from 10 kDa to more than 250 kDa. Proteins that extend or are buried into the hydrophobic interior of the lipid bilayer are called *integral membrane proteins*, or *intrinsic proteins*. They contain amino acid residues with hydrophobic side chains that interact with the fatty acyl groups of the membrane phospholipids. In contrast, *peripheral membrane proteins*, or *extrinsic proteins*, attach by non-covalently binding to other membrane components. Typically, they can be released from the membrane by solutions of high or low ionic strength or extreme pH.

To function correctly, membrane proteins must be properly oriented to the phospholipid bilayer. In contrast to membrane-associated proteins, only those that are transmembrane can function on both sides of the bilayer. The polypeptide chain of transmembrane proteins crosses the bilayer as a single  $\alpha$  helix; in others, the polypeptide chain crosses the bilayer multiple times, either as a series of  $\alpha$  helices or as a  $\beta$  sheet in the form of a closed barrel. Many membrane proteins are able to diffuse rapidly in the plane of the membrane including *rotational diffusion* (rotation about an axis perpendicular to the plane of the bilayer) and *lateral diffusion*. However, most membrane proteins do not tumble (*flip-flop*) across the bilayer.

### Biogenesis and turnover

Most membrane proteins are synthesized on the membrane-bound ribosomes of the rough endoplasmic reticulum (RER), where the sequence of messenger RNA (mRNA) is the basis for translation into polypeptides. The nascent peptides utilize a signal sequence to initiate transport across the endoplasmic reticulum (ER). Once the peptide chains are co-translationally inserted into the ER, an obligatory event for most membrane proteins,

they undergo a variety of chemical modifications, including disulfide bond formation, N-linked oligosaccharide maturation, O-linked oligosaccharide modification, fatty acid acylation and acetylation, addition of GPI anchors, phosphorylation, proteolytic cleavage and subunit assembly. The maturation pathway for plasma membrane proteins continues from the ER through the Golgi network to the cell surface. The concentration of plasma membrane proteins is affected by their biogenesis and degradation. This dynamic balance ultimately determines the resident population of membrane proteins.

Three major pathways exist for the degradation of plasma membrane proteins. The first involves the shedding of intact membrane proteins along with phospholipids as small vesicles. The second pathway is triggered by the release of peptide fragments from within the membrane and can be mediated by proteases in the membrane, extracellular space, or the cytosol, and by phospholipase. The third pathway is probably the most common and involves internalization of the proteins through receptor-mediated endocytosis and/or bulky membrane internalization. The degradation of the proteins can be initiated either in the endosome or within the lysosome. After endosomal breakdown, the released peptide fragments either can be delivered to the lysosome for further degradation or released into the extracellular space through vesicle recycling to the cell surface. Therefore, many plasma membrane proteins are not degraded and simply undergo diacytosis, or recycling back to the membrane. In hepatocytes, receptor recycling is common after release of ligands in the more mature endosomal compartments. Although the ligand is frequently degraded by lysosomal enzymes, the receptor cycles back to the cell surface where it can undergo another round of endocytosis. As an example, the intracellular trafficking of an asialoglycoprotein receptor can require up to 10 min to recycle back to the plasma membrane.

### Plasma membrane domains

The surface membrane of the hepatocyte is a complex structure. It has three specialized domains:

- the bile canalicular domain accounts for 13% of the cell surface, forms the microvilli, and functions in the secretion of bile;
- the intercellular domain makes up 50% and contains desmosomes, tight junctions for cell adhesion, and gap junctions for cell–cell communication; and
- the sinusoidal domain, which accounts for 37% of the membrane, also forms microvilli, and is the site of extensive interaction with the blood, such as secretion of plasma proteins.

The sinusoidal membrane domain represents a cellular frontier with remarkably active trafficking of molecules in and out of the cell. It is the site of numerous processes involved in an array of processes including receptor-mediated endocytosis, channel formation, pinocytosis, as well as phagocytosis. With its highly specific domains, the hepatocellular plasma membrane reflects the polarity of the parenchymal cell.

## CELL SURFACE AND INTERCELLULAR JUNCTIONS

### Cell coat

In eukaryotic cells, the majority of plasma membrane proteins are not entirely exposed on the cell surface. In fact, they are



frequently covered by a vast array of carbohydrates, such as oligosaccharide chains covalently bound to membrane proteins and lipids, or polysaccharide chains of *integral membrane* molecules. They are often described as a *cell coat* or *glycocalyx*. The role of the cell coat is not merely to protect against mechanical and chemical damage, but also to keep foreign objects and other cells at a distance, preventing undesirable protein–protein interactions. Plasma membrane-bound lectins have been identified that recognize specific oligosaccharides on cell-surface glycolipids and glycoproteins, and mediate a variety of specific, transient, cell–cell adhesion events.

### Hepatocyte surface

The hepatocyte is a polyhedral and multipolar cell (see Fig. 2.1). Most hepatocytes have two or three non-contiguous basal surfaces exposed to the perisinusoidal space of Disse. The lateral surface of the hepatocyte is typically divided by grooves of apical surface, which form segments of bile canaliculi. The apical and basal surfaces have irregular microvilli that amplify their areas three- and sixfold, respectively, over their hypothetical smooth counterparts. The basal surface is specialized for exchange of metabolites with the blood. This includes transport of small molecules across the basal membrane, secretion of plasma proteins via fusion of secretory vesicles with the basal membrane, and internalization of circulating macromolecules via clathrin-coated pits and vesicles. The lateral surface shares most of the functions of the sinusoidal front (basal surface) and additionally is specialized for cell attachments and cell–cell communication. The bile canaliculi are sealed off from the remainder of the intercellular space by junctional complexes placed at the boundaries between the lateral and apical surface of the hepatocytes. The junctional complexes contain tight junctions, adherens or intermediate junctions, and desmosomes like those of simple epithelia. Additional desmosomes and plaques of gap junctions can also be found distributed along the lateral surfaces of hepatocytes. The specialized functions of the bile canalicular (apical) membrane include transport of bile acids and products of detoxification across the membrane bilayer into bile, release/transport of lipids at the basal surface, and delivery of secretory immunoglobulin A to bile via fusion of transport vesicles with the apical membrane.

### Cell polarity

Hepatocyte polarity is established at an early stage in the developing liver and the constituents of both the apical and basolateral domains appear at different rates. In contrast, in the regenerating liver, the hepatocyte retains its plasma membrane domains throughout the regenerative process, even during mitosis and cytokinesis when extensive rearrangements in microtubules and microfilaments occur. The membrane comprising the cleavage furrow of dividing hepatocytes is most often basolateral, which avoids potential problems that would arise from the joining of sinusoidal and bile canalicular lumina and the mixing of blood and bile. Moreover, it guarantees that bile canalicular domains are inherited by both daughter cells. Therefore, hepatocyte polarity is maintained with high fidelity during cell division. However, about 30% of hepatocytes appear to divide between basolateral and apical (bile canalicular) domains, which yields acinar arrangements of hepatocytes, like those that have been observed in both regenerating and developing livers. The new

tight junctions form between the daughter cells on the basolateral side at the completion of cytokinesis, which prevents the joining of sinusoidal and bile canalicular lumina. In addition, hepatocyte surface polarity is also maintained in the face of the continued synthesis, delivery, removal, recycling and degradation of the many protein components that make up each plasma membrane domain.

### Cell junction

Many cells in tissues are linked to one another and to the extracellular matrix at specialized contact sites called *cell junctions*. As described above, the plasma membrane of hepatocytes is differentiated into sinusoidal, lateral and bile canalicular domains. The lateral domain contains several junctional structures. In general, cell junctions fall into three functional classes of occluding, anchoring and communicating junctions.

Tight junctions are occluding junctions that play a critical role in forming gasket-like seals as a permeability barrier. They maintain the concentration differences of small hydrophilic molecules across epithelial cell sheets and hepatocytes by sealing the plasma membranes of adjacent cells to create a continuous, impermeable or semipermeable barrier to diffusion across the cell sheet; and by acting as barriers in the lipid bilayer to restrict the diffusion of membrane transport proteins between the apical and the basolateral domains of the plasma membrane in each cell. Desmosomes are specialized membrane structures that anchor intermediate filaments to the plasma membrane and link cells together.

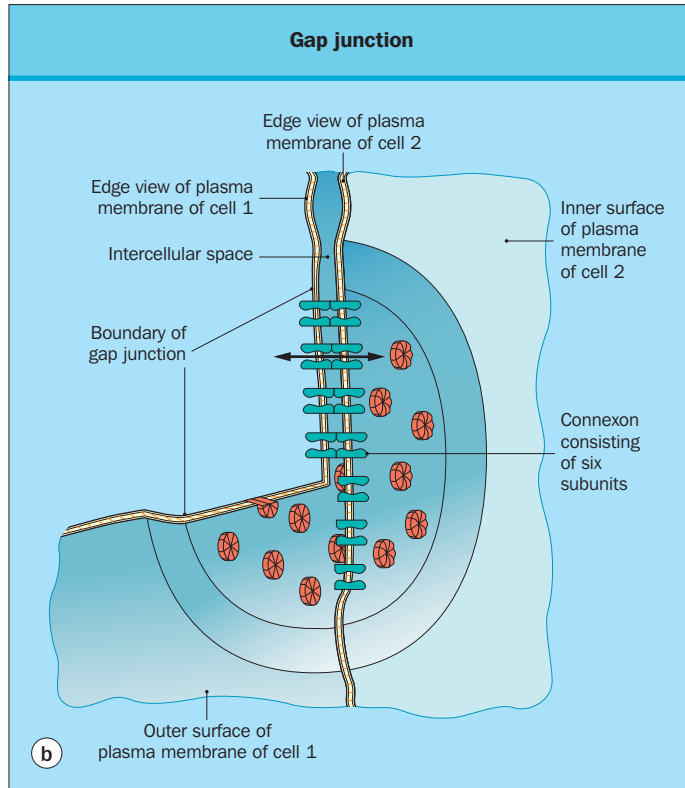
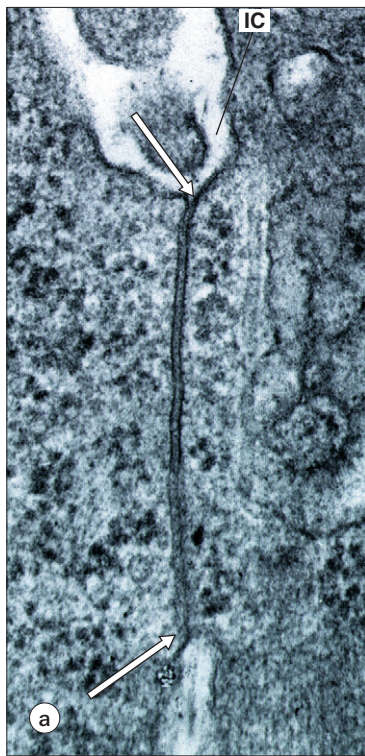
Gap junctions are communicating regions that play an important role in cell–cell communication. They consist of clusters of channel proteins that allow molecules smaller than 1 kDa to pass directly from one cell to another. In the liver, gap junctions occupy as much as 3% of the total membrane surface. Electron micrographs of stained, isolated gap junctions reveal a lattice of hexagonal particles with hollow cores as intercellular channels (Fig. 2.5). Each hexagonal particle consists of 12 *connexin* molecules in which six are formed in a hexagonal cylinder in one plasma membrane joined to six arranged in the same array in the adjacent cell membrane. The assemblage of the six connexin subunits is termed a *connexon*. The subunits make up a single cylinder and are radially symmetric around the central pore, and each is tilted slightly relative to the plane of the membrane. They can interact in two different but related ways, by either opening or closing a channel. It is well established that gap junctions are involved in nutrient exchange, cell regulation, conduction of electrical impulses, development and differentiation, and synchronization and integration of cellular activities.

## CYTOPLASMIC ORGANIZATION

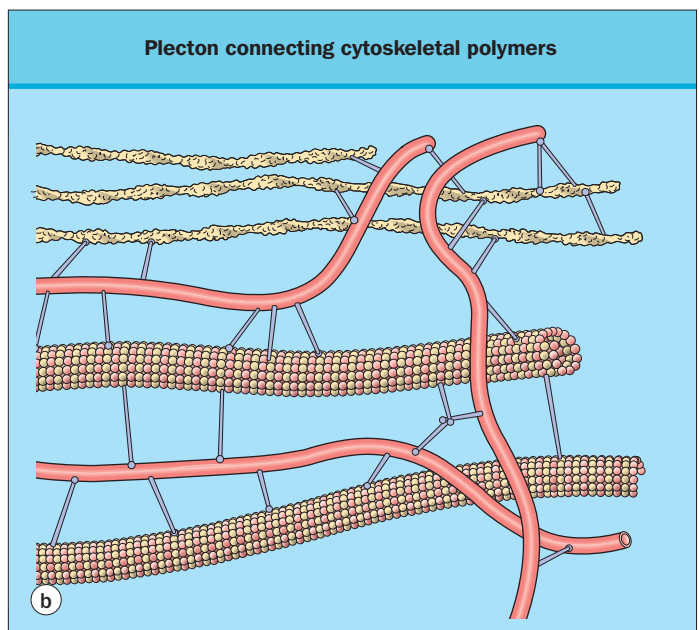
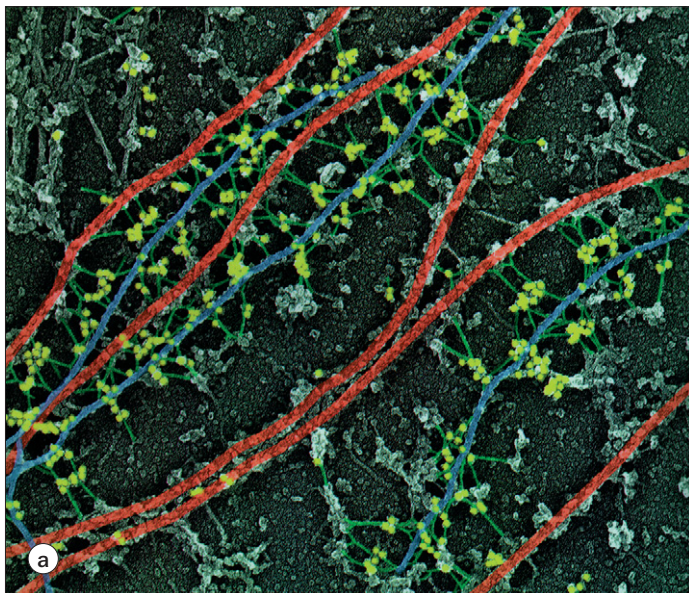
### Cytoskeleton

The cytoskeleton of the liver cell provides an important motor force for maintaining cell polarity, as well as for intracellular movement and transport of molecules. It also supports the organization of subcellular organelles that are located in functional positions within the differentiated hepatocyte. The cytoskeleton of the hepatocyte includes three basic filamentous components (Fig. 2.6) known as microfilaments, microtubules and intermediate filaments, together with cytoskeletal-associated





**Figure 2.5 The gap junction.** (a) Electron micrograph showing the plasma membranes of two adjoining cells forming a gap junction. The unit membranes (arrows) approach one another narrowing the intercellular space to produce a 2 nm-wide gap. IC, intercellular space. (b) Model of a gap junction showing the membranes of adjoining cells creating the 2 nm gap and the structural components of the membrane that form channels or passageways between the two cells (double-headed arrow). The circular array of six subunits forms the basic connexon unit. Reproduced from Staehelin & Hull (78) by permission of Sci Am and author.



**Figure 2.6 The cytoskeleton.** (a) Intermediate filaments interact with microtubules via numerous sidearms made of plectin. Electron micrograph of actin-depleted fibroblast cytoskeleton was digitally colorized to assist visualization of intermediate filaments (blue), microtubules (red), and plectin bridges (green) immunolabeled with 10 nm colloidal gold (lime). Reproduced from Svitkina et al (96) by permission of The Rockefeller University Press. (b) Plectin (blue) connecting cytoskeletal polymers to each other. Reproduced from Pollard & Earnshaw (02) by permission of Elsevier.

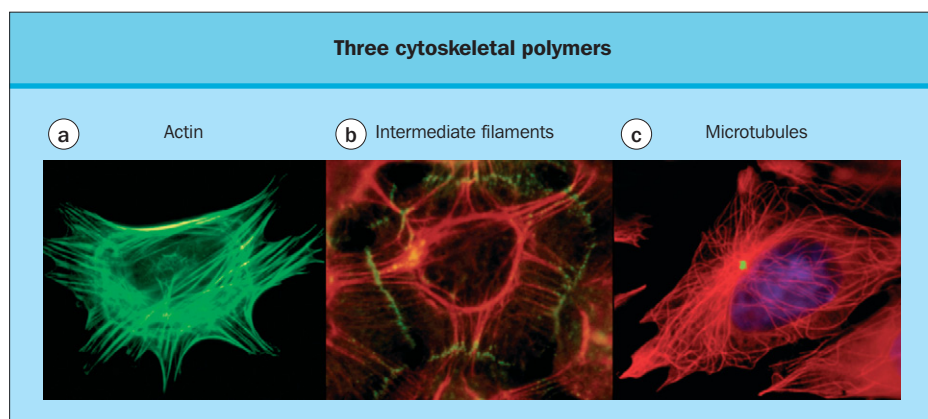
proteins that play important roles in the dynamic movement and interaction of these filaments.

Intermediate filaments are the major components of the cytoskeleton (Fig. 2.7). They are strong, rope-like polymers of fibrous polypeptides that resist stretch and play a structural or tension-bearing role in the cells. The five main types are cytokeratins, vimentin, desmin, lamins, neurofilaments and glial filament acid protein. Liver cells in situ only express the cytokeratins and lamins. However, vimentin can be expressed by hepatocytes in long-term tissue culture, and neurofilament proteins are found in injured hepatocytes and form Mallory bodies. Cytokeratins are heteropolymers that consist of two different coexpressed monomers with either an acidic or neutral-basic isoelectric pH. Hepatocytes express two cytokeratins, CK8 and CK18. Bile duct epithelium expresses not only these two cytokeratins but also CK19 and CK7. The intermediate filaments of hepatocytes interact extensively with each other, the plasma membrane at the desmosomes, the membrane skeleton at the centrosomes, the nuclear lamina, mitochondria, microfilaments and microtubules to form a supramolecular organizational state within the cell. Plectin has been identified as a major linker that binds a number of cytoskeletal ligands. It cross-links intermediate filaments to each other and to the plasma membrane, microtubules and actin filaments. In hepatocytes, cytokeratin filaments appear to maintain cell polarity, adhesion plaques, structure of the canaliculus, localization of microfilaments at the canaliculus, endocytosis, and bile canaliculus secretion of anions.

Microtubules within hepatocytes are found throughout the cytoplasm, including the perinuclear area, the centrosome, and along the plasma membrane. Morphometric analysis of electron micrographs of liver cells indicates that microtubules are more numerous in the vicinity of the Golgi area and close to the sinusoidal plasma membrane. Microtubules consist of polymerized dimers of  $\alpha$  and  $\beta$  tubulins, which are in reversible equilibrium with free tubulin. They are polarized hollow cylindric structures with an outer diameter of 24 nm and a 6 nm thick wall. The cylindric nature of microtubules may confer rigidity, which contributes to cell shape and polarity. Side arms attached to microtubules exist between microtubules and intermediate filaments, actin filaments and plasma membranes. The function of microtubules in the liver has been assessed using drugs that depolymerize microtubules. For instance, colchicine inhibits the

secretion of plasma proteins (e.g. lipoproteins, albumin, retinol-binding protein, secretory component, fibrinogen and other glycoproteins) by the liver without changing the rate of protein synthesis. Microtubules play a role in cellular organization through interaction with the Golgi apparatus and other cytoskeletal elements in the cytoplasmic matrix, including intermediate filaments and F-actin. In addition, they have been demonstrated to maintain the integrity of the canaliculus plasma membrane during canaliculus contraction. The integrity of the microtubular system is necessary for diacytosis, i.e. retroendocytosis. Recent studies revealed that the loss of microtubules in hepatocytes in vivo results in the retention of export proteins as the result of a block in the secretory apparatus. Three motor proteins of cytoplasmic microtubules, which are associated with secretion and vesicle movement, have been identified as kinesin, dynein and dynamin. Kinesin, an adenosine triphosphatase (ATPase) active molecule, regulates gliding to the negative end of microtubules by hydrolyzing ATP at both ends of the molecule. In contrast, cytoplasmic dynein promotes gliding toward the positive end. Dynein also plays a role in retrograde organelle transport, including the endocytic pathway and Golgi apparatus organization. Kinesin and dynein may function as cross-bridges between microtubules and the membranes of organelles and in this way provide the motor force for vesicle transport in hepatocytes.

Microfilaments, composed of F-actin, are associated with the plasma membrane and provide the physical basis for the mechanical properties of the cytoplasmic matrix (Fig. 2.6a). F-actin has been shown by electron microscopy to be attached to isolated bile canaliculus membrane together with intermediate filaments. However, by biochemical analysis most actin is associated with the lateral membrane fraction in the liver. The dynamics of actin-binding proteins include the control of the cellular actin monomer pool, the sequestering, polymerization and/or depolymerization of actin, the cross-linking of actin filaments, the binding of actin to cell membranes or other organelles, and the splicing of actin filaments. In addition, competing actin-binding proteins may provide a means of actin regulation. The roles of actin in the liver include facilitating bile canaliculus contraction, participating in the control of tight junction permeability, and involvement in the integrity, composition and characteristics of the cell matrix. Moreover, a large



**Figure 2.7 Three cytoskeletal polymers.** The figure shows fluorescence light micrographs of cultured cells stained for actin (left), intermediate filaments (middle) and microtubules (right). Reproduced from Pollard & Earnshaw (02) by permission of Elsevier.



number of cellular functions are dependent on actin filaments, including receptor-mediated endocytosis and various transport processes.

### Endoplasmic reticulum

Much of the cellular sorting and distribution system of proteins is through the ER and Golgi complexes. After synthesis and initial processing in the ER, proteins are transported to the Golgi complex where the majority is chemically modified before being sorted into vesicles and targeted to their final destinations. The ER is a collection of membranous tubules, vesicles and flattened sacs that extend throughout the cytoplasm of eukaryotic cells. It is the source of all membranes within the cell and the largest intracellular membrane compartment. ER membranes are continuous and unbroken, and enclose a *lumen* or channel that is separate from the surrounding cytoplasm. The surface membrane of the *RER* is distinguished by the presence of ribosomes facing the surrounding cytoplasm. It is the site of synthesis, folding, assembly and co-translational membrane translocation of proteins. In contrast, the *smooth ER* has no ribosomes and functions in lipid biosynthesis, detoxification and calcium regulation. RER commonly extends into large, flattened sacs called *cisternae*. Smooth ER membranes form primarily tubular sacs that are generally smaller in dimension than RER cisternae. At some points the smooth ER and RER membranes may connect, forming a continuous inner channel enclosed by both systems. In addition to the RER and smooth ER, there are at least four other morphologically distinct domains of the ER, including the nuclear envelope, transitional elements, crystalloid ER and luminal ER bodies containing protein aggregates. Each of these domains is associated with distinct functions (Table 2.4).

### Golgi complex

All newly synthesized proteins that are exported out of the ER system are funneled through the Golgi complex before being sorted to different final destinations within the cell. A Golgi complex consists of a localized stack of flattened, sac-like membranes. Ribosomes are characteristically absent from Golgi membranes as well as the spaces between and immediately surrounding the Golgi sacs. An entire Golgi complex often appears to be cup-shaped in cross-section, giving the structure convex and concave faces. The individual sacs of a Golgi complex, called cisternae, as in the ER, are typically dilated or swollen at their margins. Dispersed around the Golgi cisternae are numerous vesicles of varying sizes. Some of these bud from, or fuse with the edges of the cisternae. Golgi complexes are usually closely associated with the RER, separated only by a layer of small, protein-filled vesicles. Some of these vesicles arise as buds from the RER and others join with the face of the nearest Golgi sac. At least some of these structures are considered to be *transition vesicles* transporting proteins from the ER to the Golgi complex. The side of the Golgi complex facing the ER is known as the *cis* or *forming* face. The opposite *trans* or *maturing* face of the Golgi complex typically contains larger vesicle structures. Sacs in the region between the *cis* and *trans* faces form the medial segment of the Golgi complex. *Shuttle vesicles* are believed to move proteins and glycoproteins between the Golgi sacs.

ER and Golgi compartments	
Compartment	Function
<b>ER</b>	
RER	Protein synthesis and translocation Protein folding and assembly Lipid synthesis
SER	Lipid synthesis Detoxification Ca <sup>2+</sup> storage and release
Nuclear envelope	Compartmentalization of nuclei Nucleocytoplasmic transport Attachment to lamins
Transitional elements	Membrane export out of the ER
Crystalloid ER	Storage of specific membrane proteins
Luminal ER bodies	Storage of luminal protein aggregates
<b>Golgi</b>	
CGN	Receives material from ER
Golgi stacks	Processing of N-linked and O-linked glycoproteins Phosphorylation of glycoproteins Elongation of GAGs and glycolipids Addition of lipid to secretory lipoproteins
TGN	Terminal glycosylation Tyrosine sulfation Proteolytic cleavages Sorting to lysosomes and plasma membrane Concentration of content

**Table 2.4 Endoplasmic reticulum and Golgi compartments.** CGN, cis-Golgi network; TGN, trans-Golgi network; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; GAGs, glycosaminoglycan side chains.

The Golgi complex has three major functions within cells:

- the receipt and sorting of membrane and soluble components arriving from the ER;
- N- and O-linked glycosylation and processing of glycoproteins and glycolipids; and
- sorting of membrane and soluble components that exit the Golgi to different destinations within the cell.

Each of these functions is predominantly associated with specific morphologic domains or subcompartments of the Golgi complex (Table 2.4).

### Lysosomes

All of the proteins that pass through the Golgi apparatus, except those that are retained as permanent residents, are sorted in the trans-Golgi network according to their final destination. The sorting establishes a link for some of the proteins to lysosomes, a ubiquitous cytoplasmic organelle interconnected with other subcellular compartments through selective membrane budding and fusion processes. Lysosomes are membrane-bound sacs containing a combination of hydrolytic enzymes (>50) capable of breaking down most biologic substances. In general, the hydrolytic

reactions catalyzed by lysosomal enzymes occur at pH 4.5–5, which is a typical environment for primary lysosomes. The boundary membranes of lysosomes contain characteristic proteins, among them a  $H^+$ -ATPase active transport pump that continually moves  $H^+$  from the surrounding cytoplasm into the lysosomal interior, thereby maintaining the acidic pH characteristic of these organelles. Newly synthesized lysosomal proteins are transferred into the lumen of the ER, transported through the Golgi apparatus, and then carried from the trans-Golgi network to the lysosomal compartment by vesicular trafficking.

Lysosomal hydrolases contain N-linked oligosaccharides that are covalently modified in the cis-Golgi network so that their mannose residues are phosphorylated. These mannose 6-phosphate (M6P) groups are recognized by the M6P receptor in the trans-Golgi network that segregates the hydrolases and helps to package them into budding transport vesicles, en route to late endosomes as they transform into lysosomes. These transport vesicles act as shuttles that move the M6P receptor to and from the trans-Golgi network and the endosomal compartment. The low pH in the late endosome dissociates lysosomal hydrolases from the M6P receptor, making the transport of the hydrolases unidirectional.

### Exocytosis and endocytosis

Exocytosis provides the route by which proteins that are synthesized within the cell are exported to the cell's exterior. Some of the final steps of the pathway are similar to those exhibited by endocytosis, a route of entry for selected materials from outside the cell. However, endocytosis is more than a simple reversal of exocytosis and utilizes receptors and other elements that are not typically associated with exocytosis.

In the exocytotic pathway the secreting proteins take a route from ER to cis-Golgi, to *medial*-Golgi, from medial- to trans-Golgi, and finally from the trans-Golgi network to the cell surface. This transport is mediated by a novel class of coated vesicles. Unlike the coated vesicles that are involved in endocytosis, these transporting vesicles do not contain clathrin. Liver cells can secrete more than one type of protein in a secretory vesicle. For example, two commonly secreted but unrelated proteins, albumin and transferrin, have been found to be packaged in the same secretory vesicles at the Golgi complex and released together to the extracellular medium.

Endocytosis is defined as the process by which cells bind and internalize macromolecules from the environment (Fig. 2.8). Cells can take up materials from the surrounding medium through three distinct but closely related pathways: pinocytosis, phagocytosis and receptor-mediated endocytosis (RME). Pinocytosis is a constitutive type of process described as the non-selective uptake of extracellular fluid by small smooth-surfaced invaginations in the plasma membrane. Phagocytosis is the ingestion of particles and, not infrequently, internalization of expansive regions of the cell surface. In contrast, RME is the process by which cells specifically bind and internalize a variety of macromolecules or ligands.

Of the three endocytotic pathways, RME has been studied in greatest detail in liver cells. It proceeds in several steps. After binding to specific receptors that are randomly distributed on the hepatocyte sinusoidal plasma membrane, the ligand–receptor complexes are concentrated in distinctive regions of the membrane called *coated pits*. The major building block of the coat

material is the *triskelion*, a three-legged structure composed of three clathrin molecules. Invagination of the coated pits proceeds rapidly until they pinch free from the plasma membrane and sink into the underlying cytoplasm as a coated vesicle. As the vesicle forms, the membrane quickly loses its clathrin coat and becomes an endosome. At this stage, some ligand–receptor complexes may return to the surface membrane by diacytosis, and participate in additional rounds of endocytosis. The endosomal vesicles may be spheric or extend into elongated and variously branched tubules. As they travel along microtubules deeper into the cytoplasm, vesicles often fuse into larger structures.

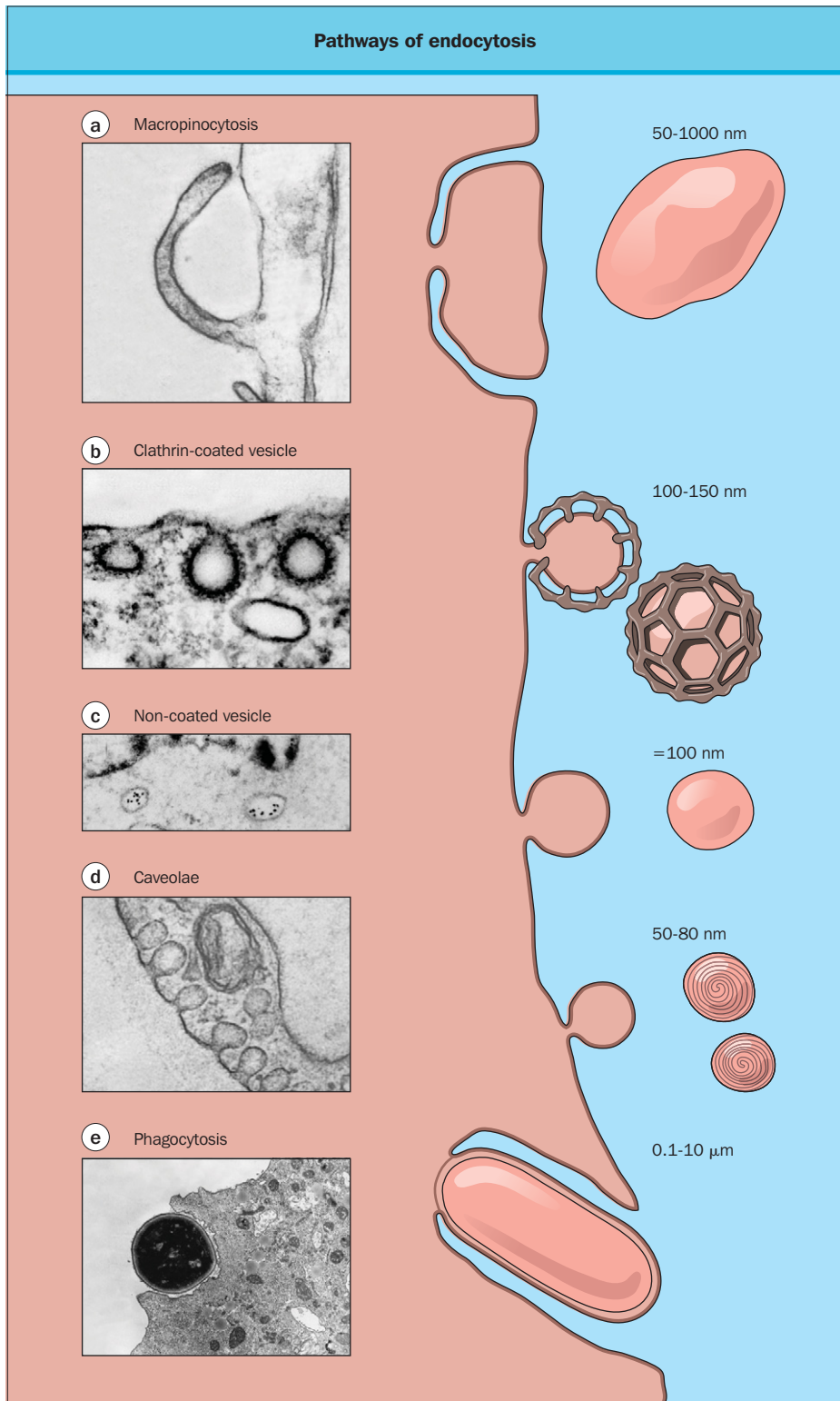
During vesicle movement and fusion, the ligands within the interior space are released from their receptors and begin to be sorted into separate compartments. Hydrogen ions pumped into the intravesicular space typically result in ligand–receptor dissociation. A small fraction of internalized ligand is transported directly to the bile canalicular membrane. The majority of ligands remain within the acidified vesicle interior. Although the basis for sorting is presently unknown, it presumably involves an interaction between recognition sites on the endocytotic vesicles. The sorted molecules may proceed to the Golgi complex as smaller vesicles that pinch off from the endocytic vesicle.

Molecules that are sorted into vesicles that travel to the Golgi complex may remain there or may be distributed further to the ER or perinuclear space. They may also be resorted and secondarily routed in vesicles to the plasma membrane or to lysosomes. Fusion of vesicles with lysosomes exposes their contents to degradation by the lysosomal enzymes. Most hepatocyte receptors, devoid of bound ligand, return to the plasma membrane attached to small vesicles, and are reintroduced by membrane fusion. Not all receptors recycle, however; some undergo lysosomal degradation together with their associated ligands.

In most cells coated pits occupy approximately 2% of the surface membrane area. It has been estimated that about 2500 clathrin-coated vesicles invaginate from the plasma membrane of a cultured fibroblast every minute. In the liver, the coated pit regions are particularly abundant along the hepatocyte sinusoidal membrane base of the microvilli (Fig. 2.9). It is now well-established that the coat structure of both coated pits and coated vesicles consists of a highly ordered array of specific macromolecules organized into polygonal lattices consisting primarily of a single protein, clathrin. Clathrin subunits associate into triskelions. This remarkable structure consists of three molecules of clathrin heavy chain (180 kDa) and three molecules of clathrin light chain (33–36 kDa). Triskelions assemble in an overlapping network to form the lattice lining a coated pit. The structure and assembly of a coat structure is illustrated in and consists of a geometric array of 12 pentagons and a variable number of hexagons depending on the size of the coat.

Caveolae or plasmalemmal vesicles are a characteristic feature of the plasma membrane of many mammalian cell types and especially fibroblasts, endothelial cells and smooth muscle cells (Fig. 2.10). Morphologically, caveolae are 50–60 nm invaginations of the plasma membrane with a characteristic flask shape. Unlike clathrin-coated pits, caveolae exhibit a characteristic spiral coat on the cytoplasmic side of the caveolae, consisting in part of the integral membrane protein caveolin. A number of molecules have now been localized to caveolae including GPI-anchored





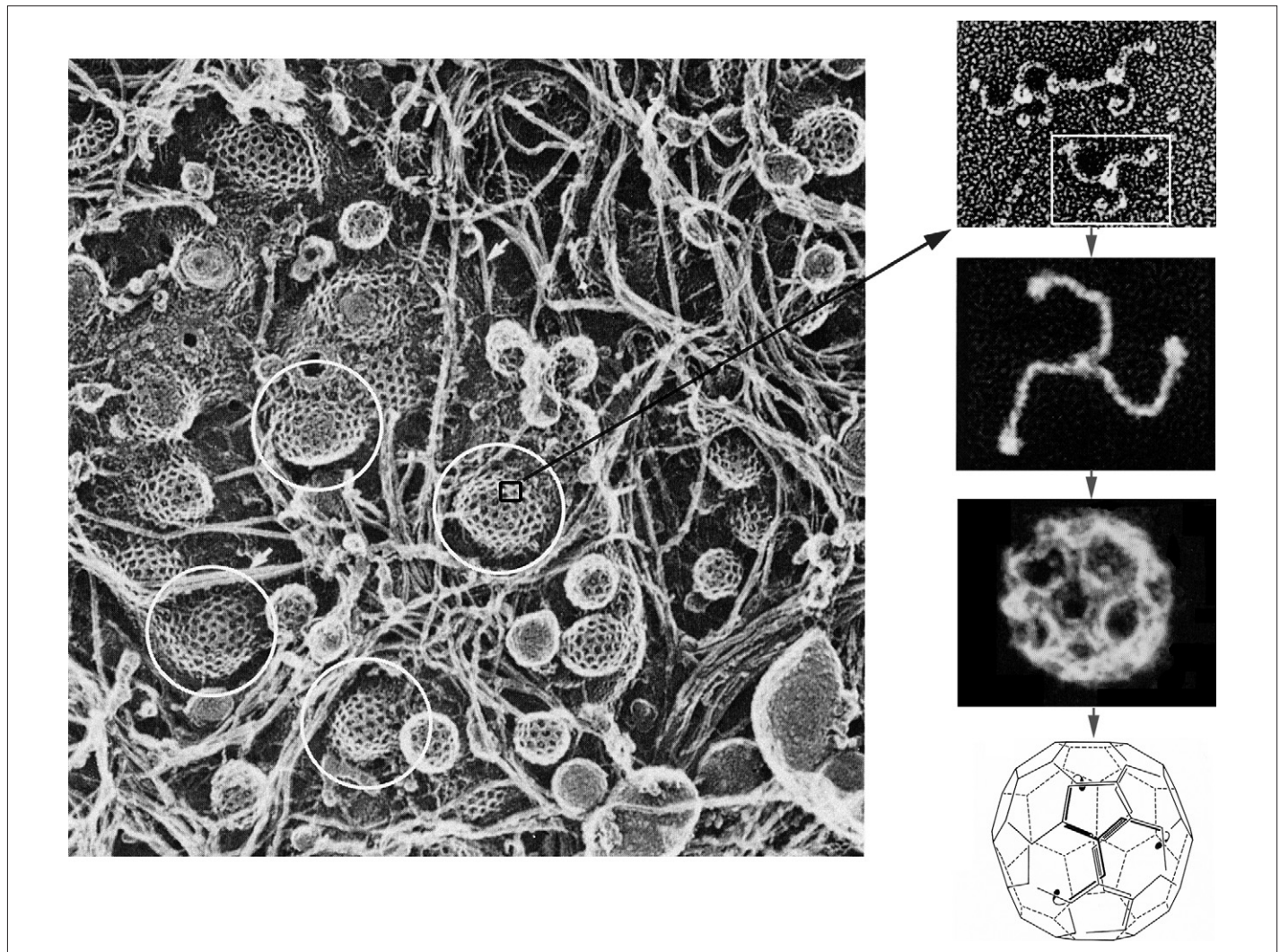
**Figure 2.8 Pathways of endocytosis.** Electron micrographs and diagrams illustrating five structurally and mechanistically distinct pathways for entry into the cell. Receptor-mediated endocytosis has been studied in greatest detail. After ligands bind to the specific receptors, the ligand/receptor complexes are concentrated in coated pits. The pits invaginate and sink into the underlying cytoplasm as endocytotic vesicles. During vesicle movement and fusion, the ligands within the vesicles are sorted and, in many cases, released from their receptors. The sorted molecules may proceed to the Golgi complex or to lysosomes. After release from their ligand, some receptors are recycled to the plasma membrane; others are degraded in the vesicles that fuse with lysosomes. Reproduced from Pollard & Earnshaw (02) by permission of Elsevier.

proteins, the  $\beta$ -adrenergic receptor, as well as tyrosine kinase and substrates. Caveolae exhibit a number of different functions including potocytosis, signal transduction, calcium regulation, and non-clathrin-dependent endocytosis and transcytosis.

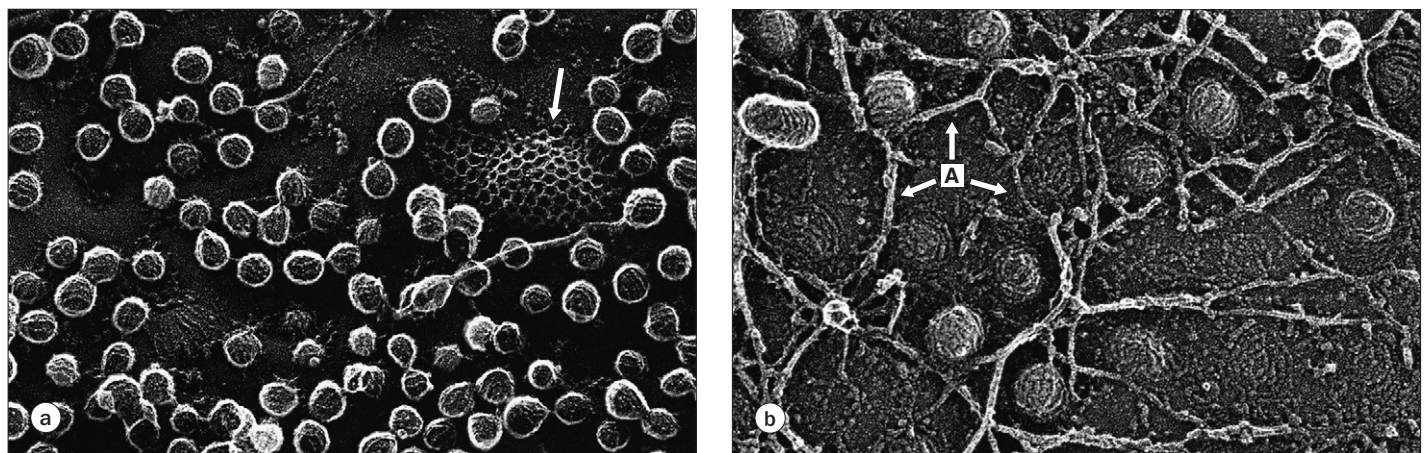
### Mitochondria

Mitochondria are respiratory organelles that constitute about 20% of the cytoplasmic volume of hepatocytes. Their primary

function is to conserve the energy from the oxidation of substrates by oxygen as the high-energy phosphate anhydride bonds of ATP. They contain the enzymes of the tricarboxylic acid cycle, fatty acid oxidation and oxidative phosphorylation. Other functions include parts of the urea cycle, gluconeogenesis, fatty acid synthesis, and regulation of intracellular calcium concentrations. Normal human hepatocytes contain numerous mitochondria throughout the cytoplasm (Fig. 2.11). All mitochondria

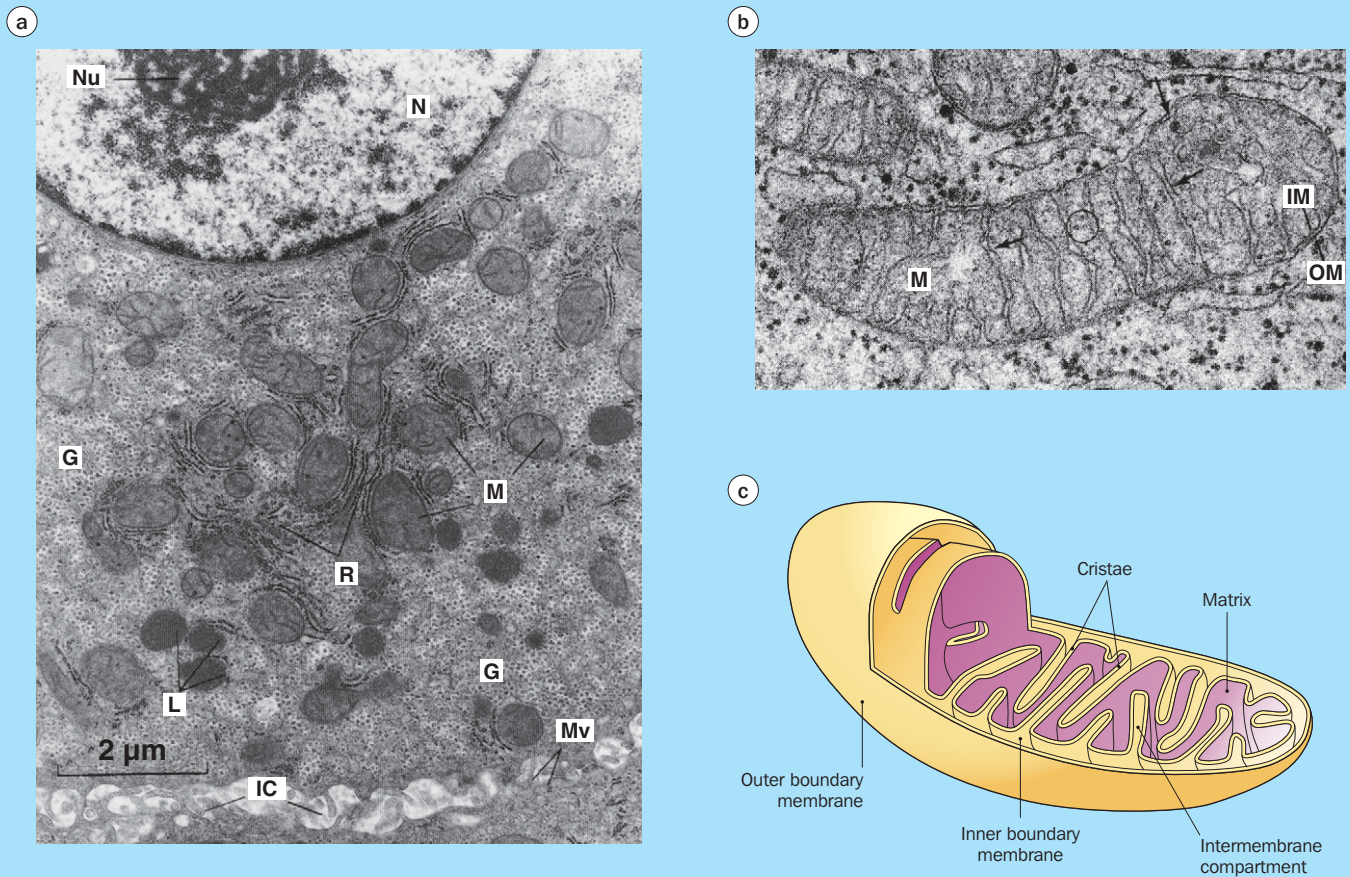


**Figure 2.9 Clathrin-coated cell membrane.** (Left) Schematic view of a coated membrane region from within the cell. Clathrin basketworks (encircled) form coated membranes, pits and vesicles. Smooth-surfaced membranes and intermediate filaments are particularly numerous. (Right) Electron micrographs of clathrin triskelions ( $\times 230\,000$ ), a clathrin shell reformed from triskelions and a schematic representation of the clathrin coat lattice. Each structure is composed of 12 pentagons and a variable number of hexagons forming an outer cage of clathrin, an inner layer of clathrin terminal domains and an innermost shell consisting of an array of proteins that interact directly with receptors in the coated regions. Reproduced from Steer in Zakim & Boyer (96) by permission of Elsevier.



**Figure 2.10 Freeze-etch images of caveolae.** They are smaller and are usually more round than clathrin-coated pits [(a), arrow]. Caveolae are coated with ridges of material on their surface that resemble fingerprints. Typically, they occur in clusters and often in actin filament (A)-rich regions of the cell (b).  $\times 130\,000$  for (a);  $\times 177\,000$  for (b). Reproduced from Steer in Zakim & Boyer (96) by permission of Elsevier.



**Mitochondrial distribution and structure**

**Figure 2.11 Mitochondrial distribution and structure.** (a) Electron microscopic view of mitochondrial distribution in a normal human liver cell. N, nucleus; Nu, nucleolus; M, mitochondria; R, rough endoplasmic reticulum; G, glycogen granules; Mv, microvilli in intracellular space; L, lysosomes; IC, intercellular space. (b) A magnified view of a mitochondrion with (c) a schematic diagram of its structure. Part (a) reproduced from Sherlock & Dooley (97) by permission of Blackwell. Parts (b) and (c) reproduced from Wolfe (93) by permission of Brooks/Cole.

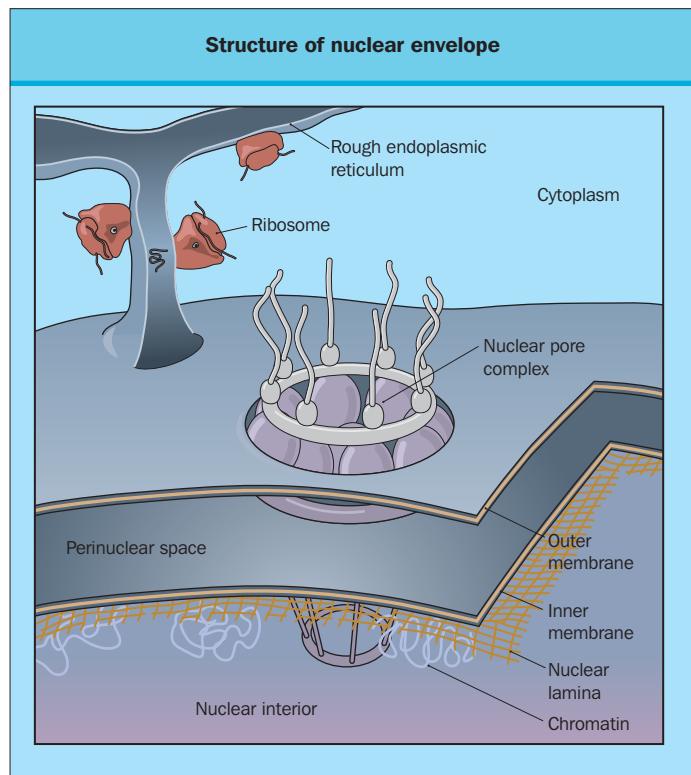
contain two very different membranes both structurally and functionally: the outer membrane defines the smooth surface perimeter of the mitochondrion; and the inner membrane is highly folded. The folds, called cristae, greatly increase the area of the inner membrane. These membrane structures define two submitochondrial compartments, the intermembrane space between the two membranes, and the matrix or central compartment.

Liver mitochondria vary in size and shape, depending on their location in relation to the blood supply. The location of mitochondria in a cell is often controlled so they are positioned near major sites of ATP utilization. This is accomplished by transport of mitochondria along microtubules. The mitochondrial matrix is a concentrated protein solution of enzymes of the tricarboxylic acid cycle, fatty acid oxidation and urea synthesis. The matrix, however, may contain a variety of discrete structures including granules of various sizes, fibrils and crystals. The most common large granule is the so-called *intramitochondrial granule*, a dense, spheric particle that may store calcium and other ions. In contrast, the smaller granules contain the *mitochondrial ribosomes*. Interestingly, mitochondria contain their own DNA,

which is embedded within the matrix and codes for a number of protein constituents. Some of those along with the protein subunits encoded from nuclear genes form the complexes responsible for oxidative phosphorylation for ATP synthesis. The mitochondrion may also be the key organelle in the regulation of programmed cell death, or apoptosis.

Mitochondria receive energy-yielding chemical intermediates from two main metabolic pathways, glycolysis and fatty acid oxidation. Both pathways feed into the citric acid cycle of energy-yielding reactions in the mitochondrial matrix. Acetyl-coenzyme A (acetyl-CoA) is broken down to three molecules of nicotinamide adenine dinucleotide (NADH), one molecule of flavin adenine dinucleotide (FADH<sub>2</sub>) and two molecules CO<sub>2</sub> during a single pass through the citric acid cycle. Energetic electrons donated by NADH and FADH<sub>2</sub> drive an electron transport pathway in the inner mitochondrial membrane that powers a chemiosmotic cycle to produce ATP. Electrons use two routes to pass through three protein complexes in the inner mitochondrial membrane. Starting with NADH, electrons pass through complex I to complex III to complex IV. Along both routes, energy is partitioned off to transfer multiple protons





**Figure 2.12 Structure of the nuclear envelope.** Summary overview of the organization of the nuclear envelope illustrating the association with the endoplasmic reticulum. Reproduced from Pollard & Earnshaw (02) by permission of Elsevier.

across the inner mitochondrial membrane from matrix to the inner membrane space. The resulting electrochemical gradient of protons drives ATP synthesis. The mitochondria also have a key role in cellular response to toxic stimuli from the environment. In response to drugs such as those for cancer chemotherapy, mitochondria release into the cytoplasm a toxic cocktail of enzymes and other proteins that brings about the programmed cell death, or apoptosis (see below).

### Peroxisomes

By electron microscopy, peroxisomes appear as roughly spheric structures that are somewhat smaller than mitochondria. Peroxisomes are enclosed by a single membrane; the material inside the membrane, called the *matrix*, contains a *core* that exhibits a regular lattice or crystalline structure. They are particularly abundant in hepatocytes and provide many important links between the metabolism of carbohydrates, lipids, proteins, fats and nucleic acids. Many of the biochemical reactions of peroxisomes are oxidative and function in catabolic pathways; however, some are anabolic reactions in which the end products serve as building blocks for anabolic reactions elsewhere in the cell. Failure to form peroxisomes during gestation causes death in early infancy and supports the notion that peroxisomal function is essential to human life.

## THE NUCLEUS AND ITS MOLECULAR CONSTITUENTS

### Morphology of the nucleus

Hepatocytes have large, active nuclei, with prominent nucleoli where ribosomal precursors are processed. The nucleus is delimited by a nuclear envelope formed by two concentric membranes (Fig. 2.12). These membranes are punctured at intervals by nuclear pores, which actively transport selected molecules to and from the cytosol. The envelope is directly connected to the extensive membranes of the ER, and it is supported by two networks of intermediate filaments. In one, the nuclear lamina forms a thin shell just inside the nucleus underlying the inner membrane, while the other, less regularly organized, surrounds the outer nuclear membrane. The nucleus contains the nucleolus, a fibrous matrix, and a DNA-protein complex. Both the ribonuclear protein (RNP) network and the perinuclear chromatin radiate from the nucleolus, which is the largest intranuclear structure. The chromocenters are widely distributed in the nucleoplasm and the perichromatin granules are often found associated with the chromocenters. A layer of dense chromatin is often found in close proximity to the nuclear envelope.

### Function of the nucleus

The nucleus is the ultimate control center for cell activities. Within the chromatin, the information required to synthesize cellular proteins is written into the DNA. Each DNA segment encoded for a specific protein molecule constitutes a gene. In a process called *transcription*, the information from a gene is copied into mRNA molecules, which after some processing, move across the nuclear pore complex into the cytoplasm where they become part of a ribosome. The DNA of the human genome contains the codes for many thousands of different proteins. In addition, DNA stores the information for making additional RNA types that carry out accessory roles in protein synthesis and other nucleocytoplasmic functions. For example, ribosomal RNAs (rRNAs) are encoded in DNA regions within the nucleolus and become important participants of the mature ribosome. In addition, transfer RNA (tRNA) binds to amino acids during protein synthesis and provides a necessary link between the nucleic acid code and the amino acid sequence of proteins during protein *translation*. Finally, other RNAs are involved in the processing of mRNA, rRNA and tRNA molecules.

A second major function of the nucleus involves duplication of the chromatin as a part of cell replication. Just before cell division all the components of chromatin, including both DNA and chromosomal proteins, are precisely duplicated and transmitted to the two identical daughter cells. During cell division the two copies of each duplicated chromosome are separated and exactly divided so that the two cells resulting from the division each receive a complete set of genes.

### Nuclear envelope

The nuclear envelope consists of three primary structure elements referred to as the *outer* and *inner membranes*, and the *pore complexes*. The outer membrane, which faces the cytoplasm, is covered with ribosomes on its cytoplasmic side, and at certain points is continuous with the ER. The inner membrane, which faces the nucleoplasm, has no ribosomes. A fibrous nuclear

lamina of intermediate filaments supports the inner nuclear membrane and mediates its interactions with chromatin. The outer and inner nuclear membranes enclose a narrow space about 40 nm wide, the *perinuclear compartment*. The perinuclear compartment encircles the nucleus and, through the connections made by the outer nuclear membrane with the ER, is continuous with the space enclosed in the ER cisternae. Proteins synthesized by the ribosomes on the outer nuclear membrane can enter the perinuclear compartment and eventually make their way to the ER cisternae and other destinations in the cellular-secretion pathways. Nuclear pore complexes bridging both nuclear membranes provide the sole route for communication between the nucleus and cytoplasm.

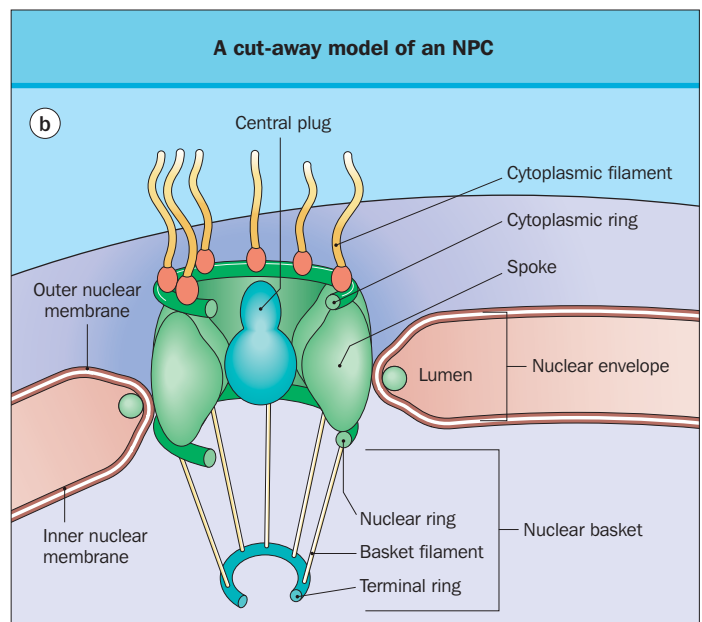
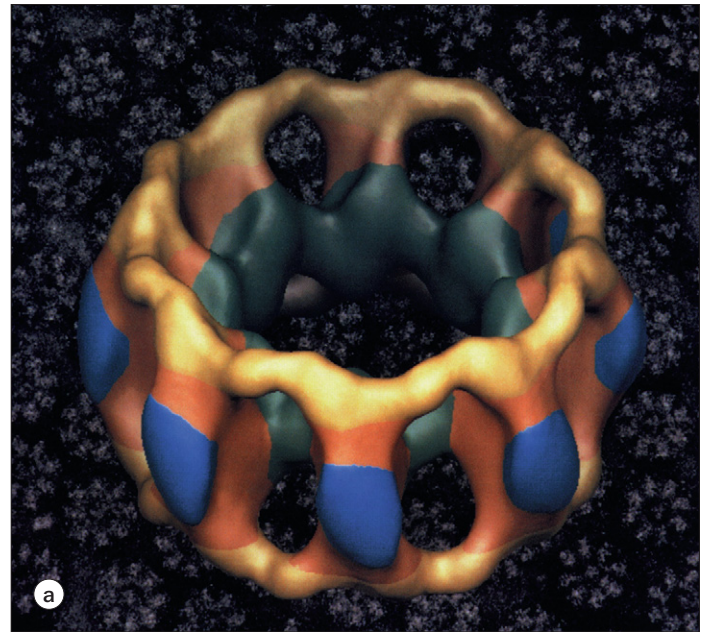
### Nuclear pore complex

The nuclear envelope of a typical mammalian cell contains 3000–4000 pore complexes. However, the number of pore complexes varies with the transcriptional activity of the nucleus. The nuclear pore complex is a large, elaborate structure that is thought to be composed of more than 100 different proteins, arranged with a striking octagonal symmetry. The nuclear pore complex (NPC) is often described as having three parallel rings inserted into and oriented along the same plane of the envelope such that the outer rings face either the cytoplasm or nucleoplasm (Fig. 2.13). The channels in the outer ring appear to control diffusion. The diameter of the opening in the nuclear envelope is roughly 72–94 nm, while the diameter of the pore complex measured along the axis of the envelope is 120–150 nm, depending upon whether the distances contributed by the radial arms are included. The depth of the pore complex is approximately 70 nm. Thus, the pore complex is an extremely large macromolecular assembly and protrudes into both cytoplasm and nucleoplasm.

### Nucleocytoplasmic transport

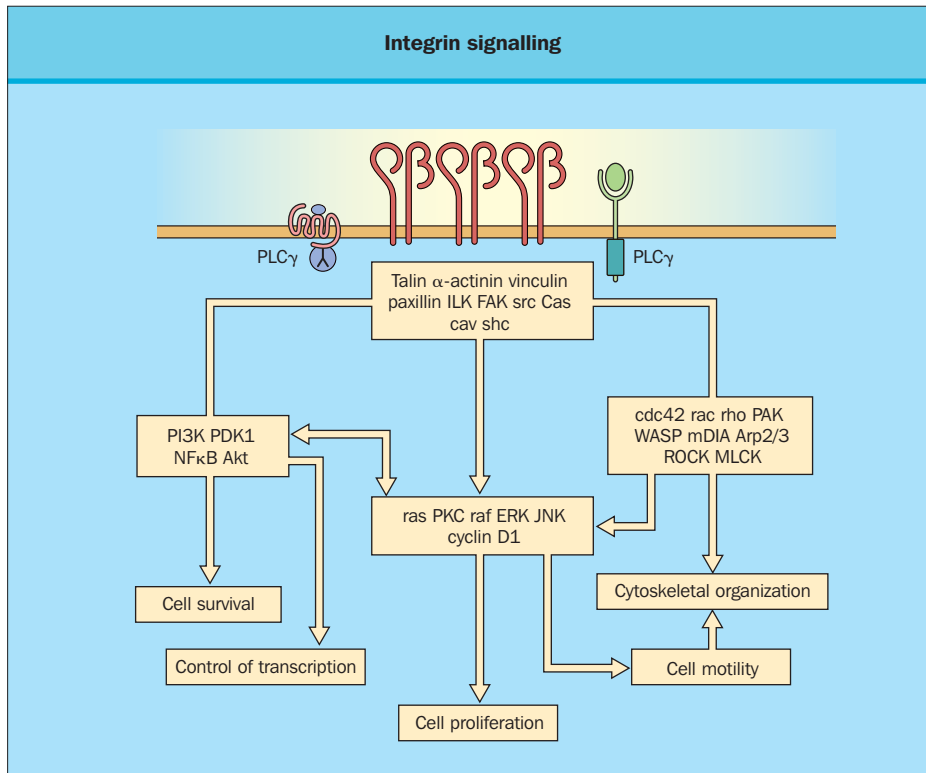
All nucleocytoplasmic transport occurs via the central aqueous channel in NPCs. Bidirectional traffic occurs continuously between the cytosol and the nucleus. The many proteins that function in the nucleus, including histones, DNA and RNA polymerases, gene regulatory proteins and RNA-processing proteins, are selectively imported into the nuclear compartment from the cytosol where they are made. At the same time, tRNAs and mRNAs are synthesized in the nuclear compartment and then exported to the cytosol. Both the export and import processes are selective. mRNAs, for example, are exported only after they have been properly modified by RNA-processing reactions in the nucleus. In some cases, the transport process is complex. For instance, ribosomal proteins are synthesized in the cytosol, imported into the nucleus where they assemble rRNA into particles, and then exported again to the cytosol as part of a ribosomal subunit. Each of these steps involves selective transport across the nuclear envelope.

Macromolecules are actively transported into and out of the nucleus through nuclear pores and appear to require energy supplied by specific ATPase/guanosine triphosphatase (GTPase) enzymes. Two mechanisms have been considered for the uptake of large nuclear proteins. In the first, referred to as *retention*, large molecules diffuse slowly through the NPC and subsequently bind to specific intranuclear sites. In the second mechanism, *signal-mediated* vectorial transport increases the rate of



**Figure 2.13 Nuclear pore complex (NPC).** (a) An oblique view of a three-dimensional map of the NPC calculated from electron micrograph images (shown in background). The nuclear pore is highly symmetric. The arrangement of the subunits within the membrane pore creates a large central channel thought to be involved in active nuclear transport. Eight peripheral channels are also created, which could serve for the diffusion of small ions and proteins. (b) A cut-away model identifying the major structural components of a NPC. Part (a) reproduced from Hinshaw et al (92) and part (b) reproduced from Ohno et al (98) by permission of Elsevier.

movement across the nuclear membrane. The signals are short amino acid sequences and are recognized by receptors in the pore complex. These receptors belong to a family of proteins termed importins or karyopherins. The nuclear envelope is freely permeable to small molecules (<5 kDa), which diffuse rapidly across the pore complex.



**Figure 2.14 Integrin signaling.** Integrins synergize with other cell surface receptors including growth factor receptors to activate the signal transduction pathways. Many of the key players are shown, leading to the major effects on cell behavior mediated by integrins, often acting in concert with G protein-coupled or kinase receptors for soluble factors. The major submembranous, integrin-associated links between integrins and these signal transduction pathways are contained within the rectangle just beneath the clustered integrins. Reproduced from Hynes (02) by permission of Elsevier.

## EXTRACELLULAR MATRIX

### Functions of the extracellular matrix

Animals contain many types of extracellular matrices each specialized for a particular function. Cells bind to extracellular matrices as a means of anchoring themselves, to derive traction for migration, and to receive signals from the matrix and matrix-bound growth factors. The adult liver has been shown to contain many of the known extracellular matrix components. Lipocytes and hepatocytes appear to be the major producers of the extracellular matrix in the liver, although some matrix components are also synthesized by sinusoidal endothelial cells. Matrix components are very important in organizing the liver parenchyma and maintaining the differentiated phenotype of hepatocytes. Excess deposition of connective tissue in the liver is associated with structural alterations in the liver, with changes in hemodynamic properties and ultimately in impairment of liver function.

### Cell-matrix interactions

Cell phenotype appears to be regulated by specific cell-matrix interactions that are modulated by the molecular organization of the extracellular matrix and by the type and availability of distinct matrix receptors on the cell surface. The primary class of these receptors is a family of transmembrane proteins known as *integrins*. The integrin receptors bind to extracellular matrix proteins at specialized cell attachment sites that often have the tripeptide sequence Arg-Gly-Asp as the target sequence for binding. They mediate attachment of the extracellular matrix to the intracellular cytoskeleton network, thereby promoting changes in cell shape, spreading and migration. Moreover, the integrins play important roles in modulating cell proliferation, differentiation, survival/apoptosis and gene expression through

their transmembrane connections and triggering a large variety of signal transduction events (Fig. 2.14).

Non-integrin surface receptors have also been identified in hepatocytes that mediate cell attachment by different mechanisms. Cell-matrix interactions in the liver play important roles in maintaining hepatocyte morphology and proliferation. For example, when plated on collagen, hepatocytes can undergo fourfold higher levels of DNA synthesis than when they are grown on basement membrane gels. Moreover, the level of expression of liver-specific gene products, such as albumin, is also significantly influenced by the type of extracellular matrix used for hepatocyte culture. Cell-cell and cell-matrix interactions in the liver appear to determine the level of synthesis and deposition of extracellular matrix components by different cells, as well as to regulate matrix remodeling by the production of specific enzymes and inhibitors.

### Components of extracellular matrix

All extracellular matrices, including the connective tissues and basement membrane structures, are composed of molecules that fall into three distinct classes: collagens, non-collagenous glycoproteins and proteoglycans. These components, particularly those in the latter two classes, form the *ground substance* which can be visualized in the extracellular space by electron microscopy. In liver, the extracellular matrix is composed of five distinct types of collagen (types I, III, IV, V, VI) (Table 2.5), seven classes of non-collagenous proteins [fibronectin, laminin, entactin/nidogen, tenascin, thrombospondin, secreted protein, acidic and rich in cysteine (SPARC), undulin], and an undetermined number of proteoglycans and glycosaminoglycans, such as membrane-associated syndecan, thrombomodulin, and betaglycan; and the extracellular matrix-associated versican, biglycan, decorin, fibro-



Extracellular matrix proteins in normal liver					
Collagen type	Associated anchorage protein	Associated proteoglycans	Cell-surface receptor	Source	Localization
I	Fibronectin	Chondroitin sulfate	Integrin	Fibroblasts	Vascular space, Glisson capsule, space of Disse
III	Fibronectin	Heparan sulfate Heparin	Integrin	Hepatocytes, fibroblasts	Vascular space, Glisson capsule, space of Disse
IV	Laminin	Heparan sulfate	Laminin	Epithelial cells, hepatocytes	Vascular, lymphatic, canalicular structure, and sinusoids
V	Fibronectin	Heparan sulfate Heparin	Integrin	Fibroblasts	Periportal tissue, basal lamina of bile ducts
VI	Fibronectin	Heparan sulfate	Integrin	Fibroblasts	Periportal spaces, space of Disse

**Table 2.5 Extracellular matrix proteins in normal liver.**

modulin and perlecan. The extracellular matrices are highly structured. The distribution and organization of the extracellular matrix of the liver is unique and during development is produced along the migration path of the hepatocytes. The absence of a continuous basement membrane between the hepatocytes and liver endothelial cells is another unique feature of the liver.

## SIGNAL TRANSDUCTION

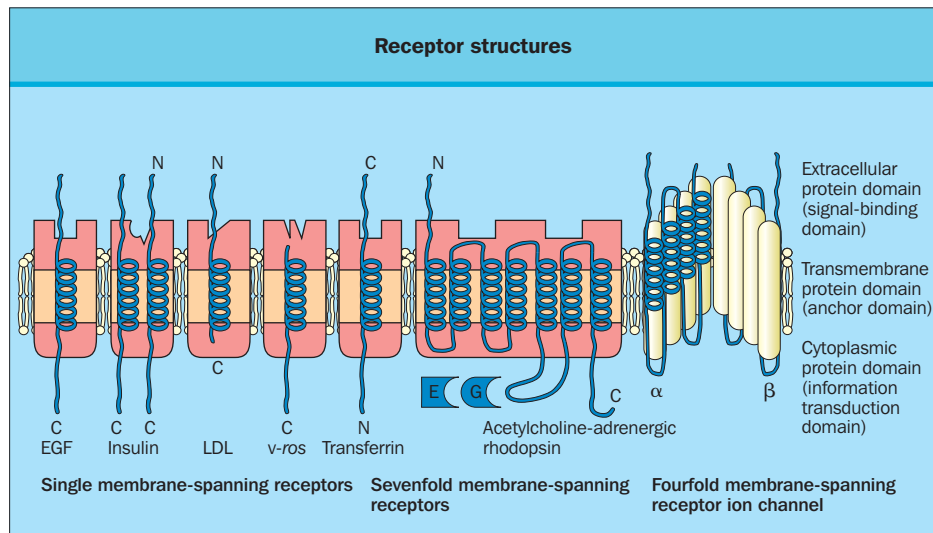
Each cell of the human body is programmed during development to respond to a specific set of signals that act in various combinations to regulate its behavior as well as its life span and rate of replication. Most of these signals are paracrine, in which local mediators are rapidly taken up, destroyed or immobilized, so that they act only on neighboring cells. Centralized control is, in general, exerted by endocrine signaling, in which secreted hormones are carried in the blood to target cells throughout the body. In synaptic signaling, neurotransmitters are secreted by nerve cells which act locally on the postsynaptic cells in contact with their axons.

The liver plays a critical role in the adaptive response to systemic changes such as inflammation and alteration of energy requirements. Hepatocytes are particularly sensitive to signals generated in these environments. Some small hydrophobic signaling molecules, including the steroid and thyroid hormones and the retinoids, diffuse across the plasma membrane of hepatic cells and activate intracellular receptor proteins, which directly regulate the transcription of specific genes. Some dissolved gases such as nitric oxide act as local mediators by diffusing across the plasma membrane of the target cell and activating an intracellular enzyme, usually guanylyl cyclase, which produces cyclic guanosine monophosphate (GMP) in the target cell. But most extracellular signaling molecules are hydrophilic and are able to activate receptor proteins only on the surface of the target cell (**Table 2.6**). These receptors act as signal transducers, converting the extracellular binding event into intracellular signals that alter the behavior of the target cell. Plasma membrane receptors exhibit a variety of topologies and, of course, are res-

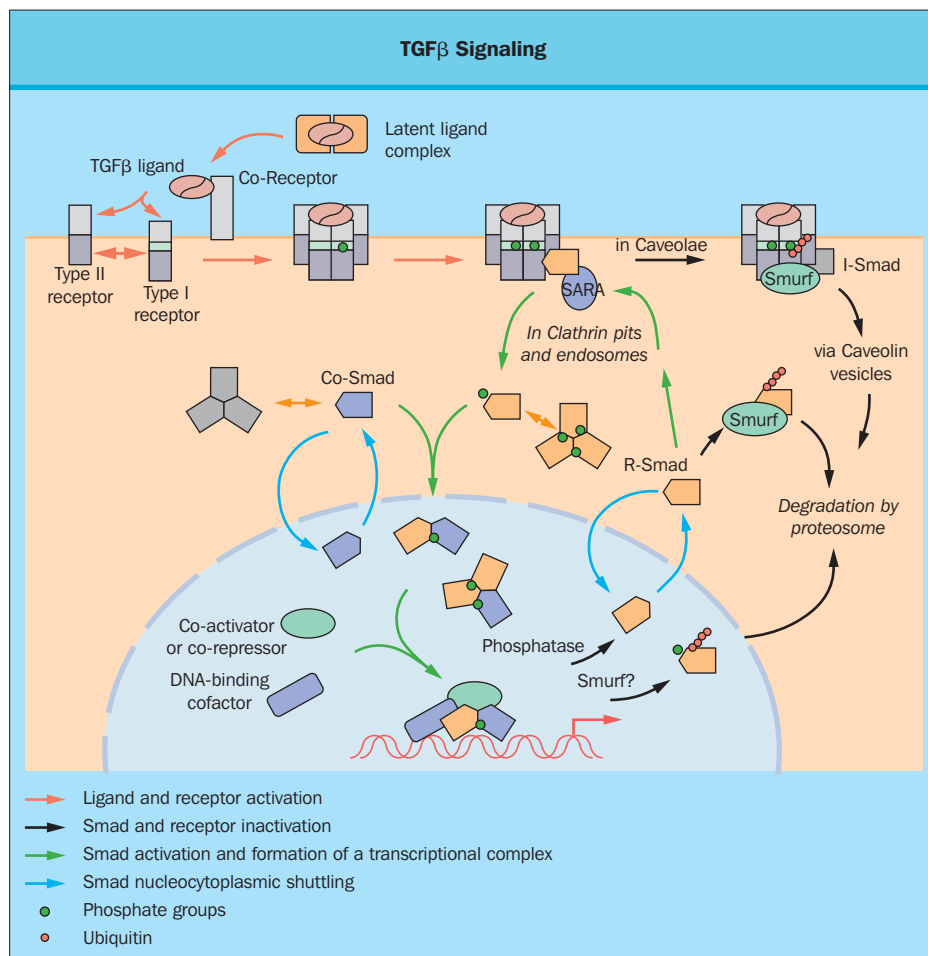
Cell surface receptors in normal liver		
Ligand	Binding sites/cell	Cell type
ASGP	150–250,000	Hepatocyte
LDL	20–50,000	Hepatocyte
Transferrin	31,000	Hepatocyte
Insulin	16,000	Hepatocyte
EGF	300,000	Hepatocyte
M6P (CI)	19,000	Hepatocyte
IgA Fucose	200,000	Hepatocyte Kupffer cell Endothelial cell
GlcNAc (Avian)	33,000	Hepatocyte
Mannose	50–150,000	Kupffer cell Endothelial cell

**Table 2.6 Cell surface receptors in normal liver.** CI, cation-independent; ASGP, asialoglycoprotein; LDL, low-density lipoprotein; EGF, epidermal growth factor; GlcNAc, N-acetylglucosamine; M6P, mannose 6-phosphate.

ponsive to many different ligands (**Fig. 2.15**). In general, there are three main families of cell-surface receptors, each of which transduces extracellular signals in a different way. Ion-channel-linked receptors open or close briefly in response to their respective stimuli. G-protein-linked receptors indirectly activate or inactivate plasma-membrane-bound enzymes or ion channels via trimeric GTP-binding proteins (G-proteins). Enzyme-linked receptors act either directly as or indirectly through enzymes that are usually protein kinases. In general, kinases regulate the phosphorylation state of specific proteins in the target cell. Through cascades of highly regulated protein phosphorylations, elaborate sets of interacting proteins relay most signals from the



**Figure 2.15 Topology of different receptor structures in the membrane.** E, effector protein; G, G protein; C, C-terminus; N, N-terminus; EGF, epidermal growth factor; v-ros, viral ros receptor; LDL, low-density lipoproteins. Reproduced from Hesch (91) by permission of Springer and author.

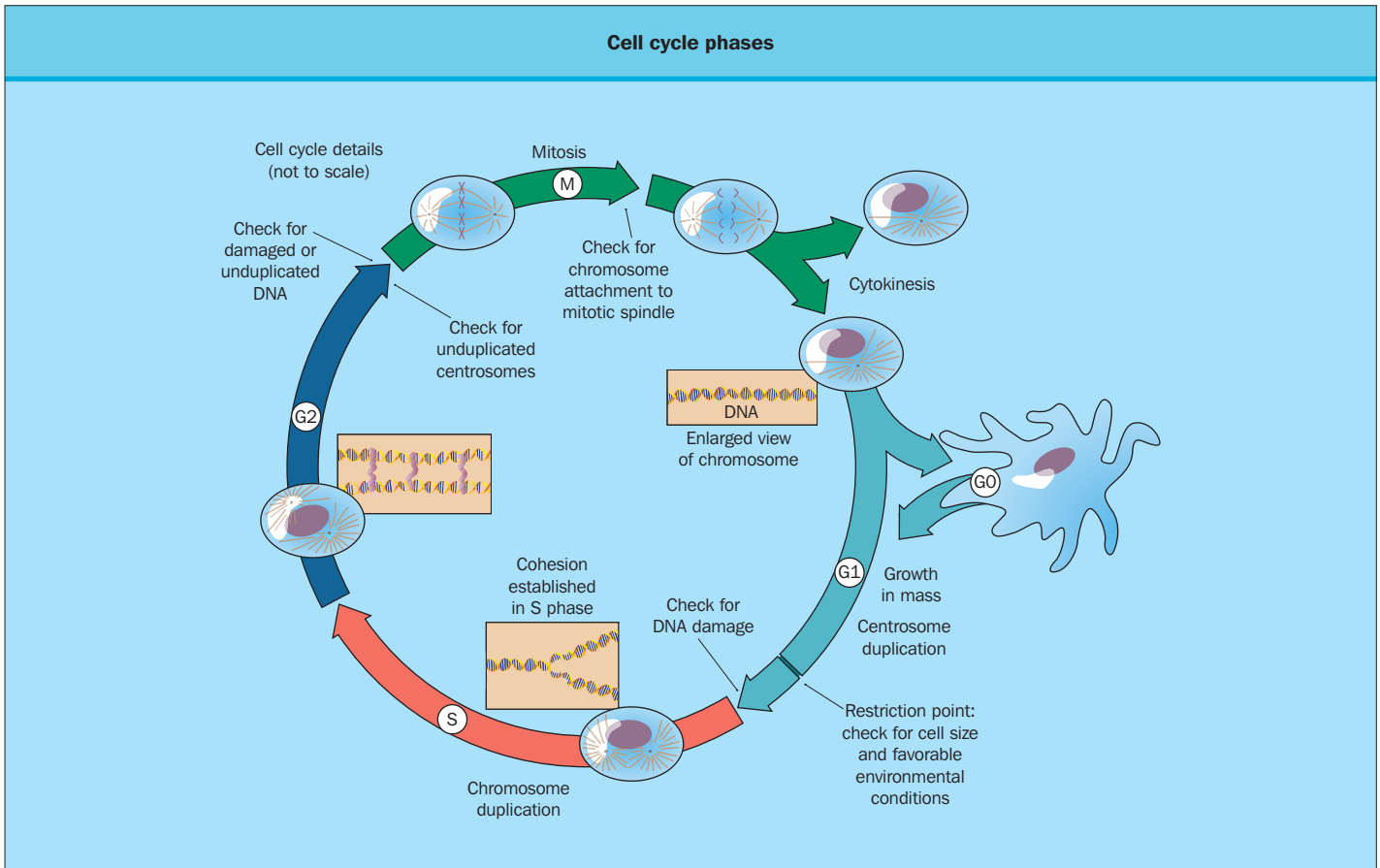


**Figure 2.16 Schematic diagram of TGF- $\beta$  signaling from cell membrane to the nucleus.** The arrows indicate signal flow and are color coded: orange for ligand and receptor activation, gray for Smad and receptor inactivation, green for Smad activation and formation of a transcriptional complex, and blue for Smad nucleocytoplasmic shuttling. Phosphate groups and ubiquitin are represented by green and red circles, respectively. Accessory receptors, such as homo-oligomeric TGF- $\beta$  receptor (TBR) III, may present TGF- $\beta$  to homo-oligomeric TBR II, which is a constitutively active serine/threonine kinase. The TGF- $\beta$ -TBR-II complex recruits TBR-I into the complex, after which TBR-I is phosphorylated by TBR-II and activated. In such a state, TBR-I propagates signals to downstream components. Reproduced from Shi (03).

cell surface to the nucleus, thereby altering the cell's pattern of gene expression and, as a consequence, its behavior.

Transforming growth factor (TGF)- $\beta$  signaling is one of the better understood examples in this class of signal transduction pathways (Fig. 2.16). The isoforms of TGF- $\beta$  exist as homodimers that are excreted from hepatocytes and liver nonparenchymal

cells as latent molecules (pro-TGF- $\beta$ s). The pro-TGF- $\beta$  undergoes plasmin-mediated activation when bound to the mannose 6-phosphate receptor. There are three types of TGF- $\beta$  receptor. Type I and II receptors form heterodimers that transmit the TGF- $\beta$  signal into the hepatocyte. The type III receptor does not appear to transduce an intracellular signal, but serves as a



**Figure 2.17 Cell cycle phases.** Diagram of cellular morphology and chromosome structure across the cell cycle. Reproduced from Pollard & Earnshaw (02) by permission of Elsevier.

repository for TGF- $\beta$  on the plasma membrane. The type II receptor binds ligand with high affinity, resulting in the recruitment of a type I receptor to form a receptor-ligand complex. Upon dimerization, the type II receptor phosphorylates the type I receptor in a cluster of glycine and serine residues near the membrane, known as the GS domain. Phosphorylation of the GS domain stimulates intracellular responses to the bound ligand. Thus, the GS region serves as an important regulatory domain in receptor activation for TGF- $\beta$  signaling.

### Regulation of the cell cycle

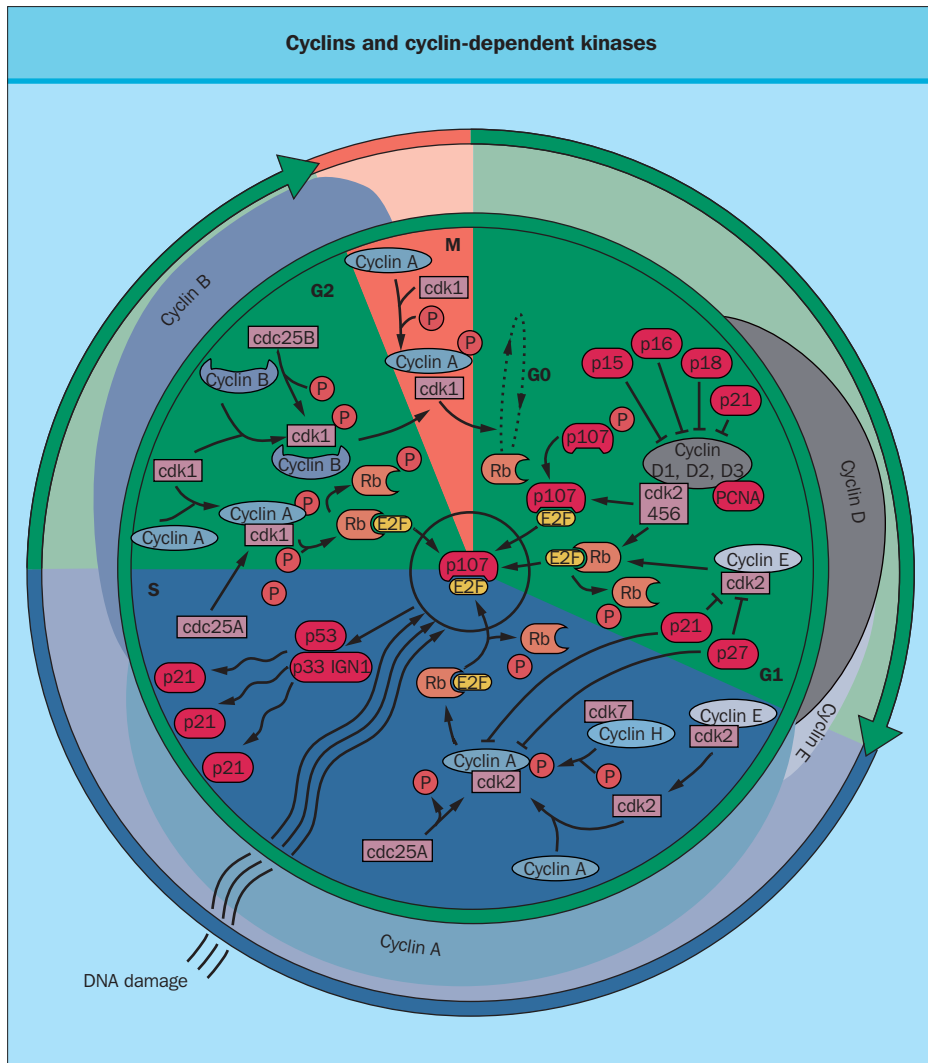
Cells reproduce by duplicating their contents and then dividing. This *cell division cycle* is the fundamental means by which all living things are propagated. Within the liver, the normal state of hepatocytes is a quiescent or non-proliferative one, but they exhibit a tremendous capacity to replicate. For example, after loss of hepatic tissue, hepatocytes, which may have been quiescent until the liver mass is completely restored. Then, just as acutely, they become quiescent again. A typical cell division cycle in somatic cells consists of four major phases (Fig. 2.17). The *G1 phase* is the interval between the completion of mitosis and the beginning of DNA synthesis; *S phase* (synthesis phase) is the period of replication of the nuclear DNA; the *G2 phase* is the interval between the end of DNA synthesis and the beginning

of mitosis; and finally, the *M phase* is the period of mitosis in which nuclear division takes place.

In mitosis, the nuclear envelope breaks down, the contents of the nucleus condense into visible chromosomes, and the cell's microtubules reorganize to form the *mitotic spindle* that will eventually separate the chromosomes. As mitosis proceeds, the cell seems to pause briefly in a state called *metaphase*, in which the chromosomes, already duplicated, are aligned on the mitotic spindle, poised for segregation. The separation of the duplicated chromosomes marks the beginning of *anaphase*, during which the chromosomes move to the poles of the spindle, where they decondense and reform intact nuclei. The cell is then pinched in two by a process called *cytokinesis*, which marks the end of M phase. In most cells the M phase takes only about an hour, which represents only a small fraction of the total cell cycle division time. The much longer period that elapses between one M phase and the next is known as *interphase*, and represents the time required to grow before the next division. Cells which have not yet committed themselves to DNA replication can pause in their progress. They enter a specialized resting state, called *G0*, where they can remain for days, weeks, or years before resuming proliferation.

There are four checkpoints that control transitions between cell cycle stages. They modulate progression of cells through the cycle in response to external or internal signals. The restriction





**Figure 2.18 Cyclins and cyclin-dependent kinases (cdks).** Modulation of cyclins A, B1, D and E provides a mechanism to regulate cdk activity throughout the cell cycle. Cdk activity is further regulated by activating or inhibitory phosphorylation, and by a variety of small proteins, which bind to cyclins, cdks, or their complexes and inhibit cdk activity. The active cyclin-cdk complexes drive cells through the various cell cycle phases, or checkpoints, by phosphorylation of the retinoblastoma family of proteins. Reproduced by permission of BD-Biosciences-PharMingen. Applied Reagents and Technologies (brochure, p.2).]

point is at late G1 and sensitive to the size and physiologic state of the cell and to its interactions with the surrounding extracellular matrix. Cells that do not receive appropriate growth stimuli from their environment arrest at this point and may commit suicide by apoptosis. The restriction point is also called a molecular 'gate' to cell cycle progression that regulates the expression of genes required for cell cycle progression. The gate is based upon the retinoblastoma protein (pRb) and E2F, a family of essential transcription factors, regulated by phosphorylation or dephosphorylation. Two DNA damage checkpoints monitor the integrity of DNA in late G1 or G2 phase. The cells with damaged or partially replicated DNA arrest their progression through the cycle. The damage can either be repaired or undergo programmed cell death (apoptosis). The metaphase checkpoint exists in mitosis and delays the onset of chromosome segregation until all chromosomes have attached properly to the mitotic spindle.

The sequence of the cell cycle events is governed by a control system that delicately balances the expression and activity of a set of interacting proteins. These include the cyclins, cyclin-dependent kinases, the tumor-suppressor protein retinoblastoma and p53, transcription factor E2F and p21. These proteins can induce and coordinate the essential downstream processes that

duplicate and divide the cell's contents. The cell cycle control system is primarily based on the regulation of certain protein kinases. Cyclins and cyclin-dependent kinases (cdks) are essential for cell-cycle regulation in eukaryotes (Fig. 2.18). The cyclins (regulatory subunits) bind to cdks (catalytic subunits) to form active cyclin-cdk complexes in different cell cycle phase. Cdk subunits by themselves are inactive and binding to a cyclin is required for their activity. A number have been identified in eukaryotic cells and participate in diverse processes ranging from transcriptional regulation, cell differentiation and cell cycle regulation. Up to date, more than 16 different cyclins have been identified in humans. Cdk activity is further regulated by a number of modulating pathways, and at least four different mechanisms regulate Cdk activity.

When hepatocytes are stimulated to replicate, an early response is the transcription and translation of G1 cyclins. These G1 cyclins bind to appropriate kinases to form the activated cyclin-cdk complexes that subsequently phosphorylate a set of specific downstream targets. The potential downstream targets of the G1 specific cyclin-cdk complexes include p53, retinoblastoma and p107 (a retinoblastoma-family protein). Retinoblastoma is a nuclear tumor-suppressor phosphoprotein that is hypophosphorylated throughout most of G1 and becomes prog-

ressively phosphorylated in the late G1, S, G2 and M phases of the cell cycle. It regulates through modification of gene expression, achieved by sequestration, active repression or stimulation of a series of transcription factors by the hypophosphorylated form of retinoblastoma that is present in the G1 phase.

In general, a common feature of the retinoblastoma-family proteins, p53 and p33ING1 is their ability to inhibit cell proliferation. For example, in hepatocytes, transforming growth factor- $\beta$  (TGF- $\beta$ ) inhibits cellular proliferation largely through its ability to downregulate the activity of the cyclin-dependent kinases cdk2 and cdk4; TGF- $\beta$  decreases the transcription of cdk4 and downregulates cdk2 activity by inactivating cyclin E-cdk2 complexes. In addition, the proto-oncogene c-Myc is a positive regulator of cyclin-cdk complexes. c-Myc can enhance cdk activity via functional inactivation of the cdk inhibitors as well as by inducing the cdk-activating phosphatase cdc25. All these particular proteins exert control through their kinase activities, which are abruptly switched on or off at particular points in the cycle.

## REGENERATION AND APOPTOSIS

### Liver regeneration

Regeneration is the capability of an organ to replace tissue mass after partial removal or injury. The normal adult liver is a quiescent organ exhibiting minimal replicative activity and, in fact, mitosis is observed only in approximately 1 in every 20 000 hepatocytes. After partial hepatectomy (PH) the remaining hepatocytes can proliferate to restore the mass of the organ within days to weeks. In the rat, peak DNA synthesis occurs 24 h after PH, when approximately 35% of hepatocytes are actively involved in cell replication. Mitosis follows DNA synthesis 6–8 h later, and during the course of regrowth most hepatocytes will have replicated at least once or twice. The replication of non-hepatocyte cells generally is delayed by approximately 24 h, but exhibits a similar synchronous pattern of DNA synthesis and mitosis as observed in hepatocytes. Once the original size of the liver is attained, hepatocytes revert to their non-replicative, quiescent state. Liver regeneration is actually a process of compensatory growth of the remnant liver and is not regeneration of the removed tissue. During the regenerative process, there is an increase in mass resulting from cell proliferation of the remaining hepatocytes. In general, progenitor, or so-called stem cells do not participate in liver regeneration after PH, but are activated and differentiate into hepatocytes only after certain types of toxic injury. In addition, the proliferative response of hepatocytes to PH begins almost immediately. In fact, the immediate-early genes that are rapidly induced as liver cells exit from their normally quiescent state include many of the proto-oncogenes implicated in cancer growth. Their expression is independent of protein synthesis and appears to result from mitogenic stimuli. In contrast, delayed-early genes are expressed during the G0–G1 phase transition and are dependent on protein synthesis. The entire regenerative process is a cascade of events that moves cells from G0 through G1 and DNA synthesis, through G2 to M phase and cell division, and is tightly regulated by both extrahepatic and intrahepatic signals. When the optimal ratio between functional hepatic mass and body mass is reached, the cells return to their resting G0 state.

### Programmed cell death

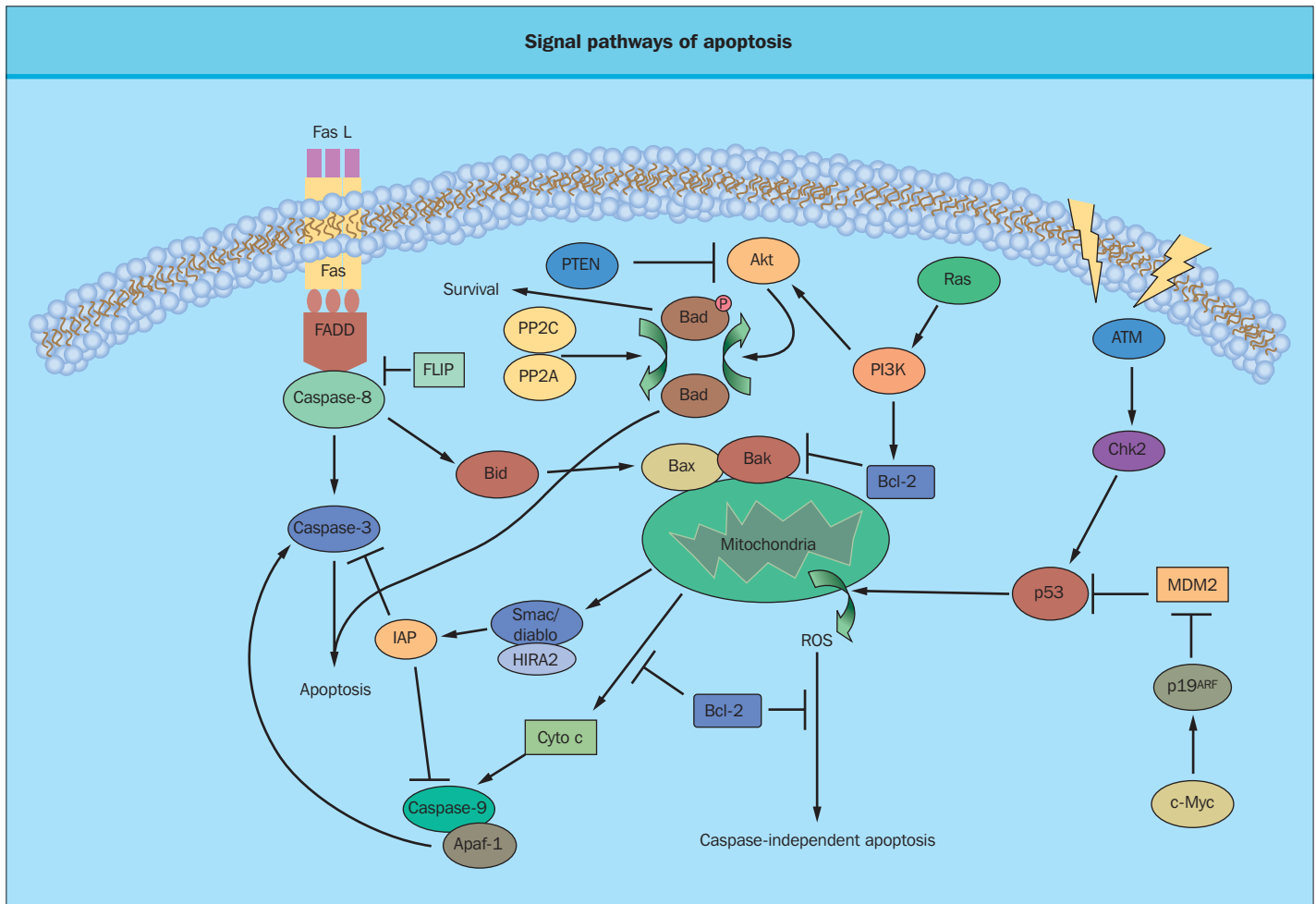
Liver regeneration after PH also involves a remodeling process in which apoptosis, or programmed cell death, plays an important role in reconstruction of the infrastructure of hepatic tissue. In a sense, it fine tunes the regenerative process. In general, apoptosis is a form of cell death that permits the removal of damaged, senescent, or unneeded cells in multicellular organisms, without damage to the cellular microenvironment. Alterations in this normal process can result in the disruption of the delicate balance between cell proliferation and cell death and can lead to a variety of diseases. For example, in many forms of cancer, key proapoptotic proteins are mutated or antiapoptotic proteins are upregulated, leading to the accumulation of cells and the inability to respond to harmful mutations, DNA damage or chemotherapeutic agents. Since effective chemotherapy depends on the induction of programmed cell death, cancers with defects in the cell death signaling pathways are particularly difficult to treat.

Cells undergo programmed death in response to both internal surveillance mechanisms and signals sent (or not sent) by other cells. Thus, some cells effectively ‘volunteer’ to die, whereas other cells are ‘nominated’ for death by others. Cells that ‘volunteer’ to die are due to sensing DNA damage and inappropriate signals to proliferate. Cells that are ‘nominated’ for death occur in at least three ways. First, cells are recognized as foreigners or harboring foreign pathogens. Second, the nurturing signals sent by other cells or the extracellular matrix are withheld. Third, some cells respond to certain growth factors such as TGF- $\beta_1$ .

Programmed cell death is an active cellular process that culminates in cell death. This may occur in response to developmental or environmental cues or as a response to physiologic damage detected by the cell’s internal surveillance network. In recent years, the term programmed cell death and apoptosis have come to be viewed as synonymous. However, apoptosis is only one type of programmed cell death and is characterized by a particular pattern of morphologic changes. In fact, some cells that undergo programmed cell death may exhibit no dramatic structure changes. Apoptotic death occurs in two phases. During the latent phase, the cell looks morphologically normal but is actively making preparations for death. The execution phase is characterized by a series of dramatic structural and biochemical changes that culminate in the fragmentation of the cell into membrane-enclosed apoptotic bodies.

The process of programmed cell death occurs in several stages. In the first step, the apoptotic pathway is triggered, which can be accomplished by a wide variety of stimuli, including DNA damage, growth factor withdrawal, toxins and radiation. Once activated, the signal is transduced by a series of protein–protein interactions that involve a conserved set of signaling modules. In the next stage, cell death is executed by the activation of specific proteases called caspases that cleave multiple substrates, leading to changes characteristic of apoptotic cells such as DNA fragmentation, chromatin condensation, cell shrinkage and membrane blebbing.

There are two major pathways leading to programmed cell death (Fig. 2.19). First is the death receptor pathway. The cells express at least six different cell surface molecules that can function as death receptors. One well-characterized death receptor is called Fas (also known as Apo1 or CD95), a member of the tumor necrosis factor (TNF) receptor family. The Fas ligand interacts with the Fas receptor,



**Figure 2.19 Signaling pathways of apoptosis.** Several mechanisms have been identified in mammalian cells for the induction of apoptosis. Fas is a member of the tumor necrosis factor (TNF) receptor superfamily. Fas ligand (Fas L) transmits signals to Fas on a target cell by inducing trimerization of Fas. Activation of Fas causes the recruitment of Fas-associated protein with death domain (FADD) via interactions between the death domain of Fas and FADD and is followed by pro-caspase-8 binding to FADD via interactions between the death effector domains (DED) of FADD and pro-caspase-8 leading to the activation of caspase-8, Bid and other caspases that initiate a cascade that ultimately results in apoptosis. Fas-induced apoptosis can be effectively blocked at several stages by either FLICE-inhibitory protein (FLIP), by Bcl-2, as well as a growing list of other factors. In addition, activation of caspase-3 by caspase-9 can be blocked by inhibitor of apoptosis proteins (IAPs). Moreover, the protein kinase, Akt, can be activated by various growth factors and its activity can be blocked by PTEN. Akt functions to promote cell survival through two distinct pathways. Akt inhibits apoptosis by phosphorylating the Bcl-2 family member Bad. Alternatively, Akt activates IKK- $\alpha$  that ultimately leads to NF-kappa B activation and cell survival. Proapoptotic Bcl-2 family members, such as Bax and Bak can promote mitochondrial permeability, while Bcl-2 can inhibit their effects. Upon mitochondrial permeability, apoptogenic factors are released from the mitochondrial inter-membrane space and leak into the cytosol. One factor is cytochrome c, which induces the liberation of protease activators (caspases) that ultimately lead to apoptosis through nuclear damage (DNA fragmentation, DNA mutations). In addition, Smac/Diablo is released and can block IAP inhibition of caspase activity. Mitochondrial permeability is also related to the increased generation of reactive oxygen species (ROS), which plays a role in the degradation phase of apoptosis (i.e. plasma membrane alterations). Reproduced with permission from Sigma.

which leads to an interaction between the death domain of the cytoplasmic region of the Fas receptor and the death domain of the adaptor protein, FADD. FADD recruits and activates procaspase-8 through interactions between the death effector domains of these two proteins. Once activated, caspase-8 activates downstream caspases such as caspase-3.

The second major pathway involves mitochondria, which are key players in this alternative pathway to cell death. A variety of toxic insults can trigger this pathway. This process requires either Bax or Bak to open a channel and release the electron transport protein cytochrome c and other proteins from the intermembranous space into the cytoplasm. Cytochrome c binds the scaffolding protein Apaf-1, a mammalian homolog of *Caenorhabditis elegans* CED-4 protein. This complex molecule

interacts with caspases through an N-terminal caspase recruitment domain. Adjacent to this is an ATPase domain. The C-terminal portion of Apaf1 appears to act as a negative regulator. Binding of cytochrome c and deoxyadenosine triphosphate (dATP) removes the negative regulatory influence of C-terminus of Apaf-1, permitting binding and autoactivation of caspase-9. This complex of Apaf-1, cytochrome c and caspase-9 is also called apoptosome. Activated caspase-9 subsequently activate caspase-3 and -7, initiating the cell death cascade. In addition, occurrence of a mitochondrial outer membrane permeabilization plays an important role during apoptosis. The function of the electron transport chain, which is essential for most mitochondrial function including ATP generation, is lost following permeabilization, and this loss is greatly facilitated by caspase activation.



Recent studies have shown that genes involved in apoptosis are actively expressed in the regenerating liver. They include the inducing genes *c-fos*, *c-jun*, *c-myc*, *p53*, *Bax*, *Bad*, *Bak*, and *TGF- $\beta$* ; the apoptosis inhibitory genes, *Bcl-2*, *Bcl-X<sub>L</sub>*, *TRPM-2*/clusterin; and the retinoblastoma gene. Ironically, some of these genes are also involved in cell proliferation, through regulation of the cell cycle. To maintain homeostasis, cell death and proliferation must be precisely balanced, and communication between these two distinct signaling pathways is critical. Liver regeneration after PH is a complicated process, and probably involves an orchestrated balance of cell replication, apoptosis and remodeling by a number of molecular players. When the surviving hepatocytes are also injured, the process may be even more complicated and involve additional factors, including stem-cell proliferation.

## NON-PARENCHYMAL CELLS

The hepatic sinusoidal cells play a critical role in the maintenance of liver function and are composed of four cell types: endothelial cells, Kupffer cells, fat-storing (Ito, stellate) cells and pit cells. The sinusoidal cells represent about 6% of the total liver volume but account for 35–40% of the total number of liver cells. The endothelial and Kupffer cells are in close contact with the bloodstream, whereas parenchymal cells are in contact with the plasma only via the space of Disse. The fenestrated endothelial cells form a physical barrier, which enables direct contact of parenchymal cells with most plasma proteins in the space of Disse, but prevents direct interaction of parenchymal cells with blood cells, large chylomicrons, viruses and bacteria. Kupffer cells, which constitute the majority of the fixed tissue macrophages of the reticuloendothelial system, are anchored to the endothelial cells by long cytoplasmic processes. Within the liver acinus, Kupffer cells are located preferentially in the periportal region. They are highly phagocytic cells, which enables them to phagocytose particulate matter such as bacteria. Both endothelial and Kupffer cells contain several specific receptors on their cell membrane which significantly increases the endocytic capacity of the liver. The fat-storing cells serve as specialized pericytes extraluminally. The pit cells are immunoreactive cells attached to the luminal surface of the sinusoid.

### Kupffer cells

Kupffer cells constitute 80–90% of the total population of fixed macrophages in the body. These cells are components of the wall of hepatic sinusoids and play a significant role in the removal of particulates and cells as well as toxins, infectious agents and foreign substances from the portal blood, particularly those of intestinal origin. Kupffer cells also are the source of a variety of beneficial vasoactive substances, which are thought to be involved in host defense mechanisms as well as in some disease processes in the liver. Kupffer cells appear to derive from monocytes or stem cells in the bone marrow and possess a typical morphology. The cellular morphology includes microvilli, lamellipodia, and occasional filopodia. The cell coat consists of a 50–70 nm thick fuzzy outer layer and a thin layer close to the plasma membrane. Kupffer cells have several specific structures related to pinocytosis, including bristle-coated micropinocytic vesicles, fuzzy-coated vacuoles, and worm-like structures. In

addition, the abundance of lysosomes and their menu of enzymes reflect the prominent role these cells play in the degradation of particles and substances taken up from the bloodstream. Both the number and activity of Kupffer cells may vary due to the immunologic and pathophysiologic condition of the liver. The mean turn over rate of the total Kupffer cell population varies from 21 days to 14 months.

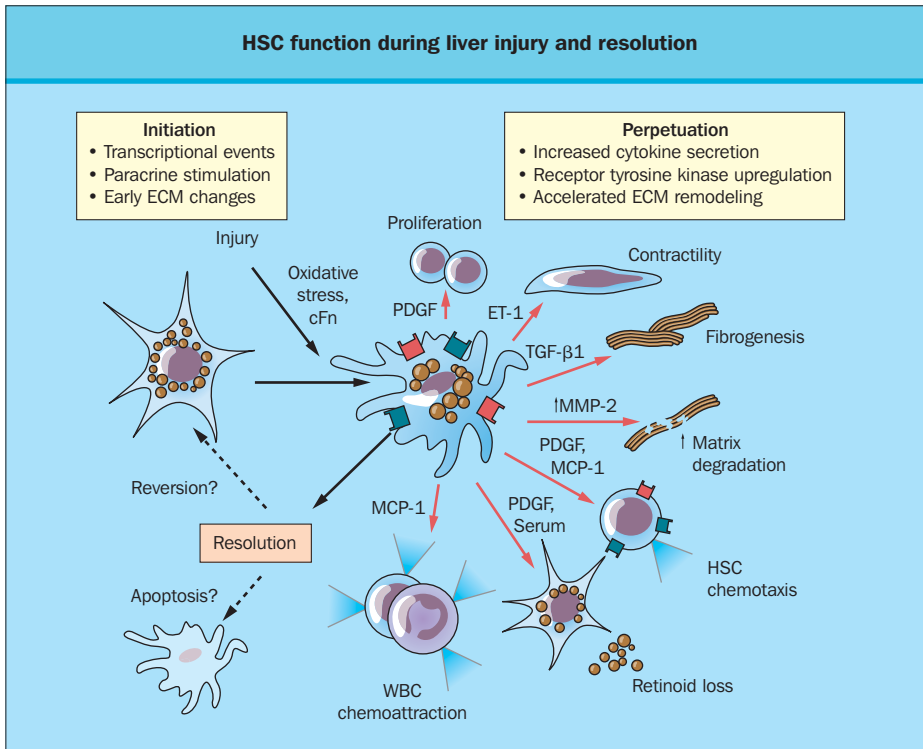
### Endothelial cells

The endothelial cells account for 20% of total liver cells, but for only 3.3% of the protein content of the liver. They possess slender extended processes containing pores (fenestrae) that are arranged in so-called sieve plates. These fenestrae constitute only 6–8% of the surface area of the endothelial lining, so that the endothelium is thought to act as a selective barrier between the blood and the parenchyma. The size of fenestrae is dynamic and can be modified by both physical and chemical forces, which are actively controlled by the actin-containing components of the cytoskeleton, particularly microtubules. The surface of the sinusoidal endothelial cells are relatively smooth compared with those of Kupffer cells, and generally are lacking in filopodia or lamellipodia. They possess numerous bristle-coated membrane invaginations and vesicles and many other lysosome-like vacuoles, which correlate with the high degree of endocytotic activity present in these cells. Unlike cells of the vascular endothelium, liver endothelial cells lack an underlying basement membrane so that solutes and small particles have access to the perisinusoidal space. However, scattered connective tissue elements and extracellular matrix material are associated with the abluminal surfaces of the endothelial cells. The endothelial cells can secrete a wide variety of proteins, such as interleukins, interferon, and *TNF- $\alpha$* . Thus, along with Kupffer cells, the endothelium participates in host defense mechanisms in the liver.

### Hepatic stellate cells (HSCs)

HSCs (vitamin A-storing cells, lipocytes, interstitial cells, fat-storing cells, Ito cells) are located in the space of Disse, under the endothelial lining, in close contact with liver parenchymal cells (Fig. 2.20). HSCs are often surrounded by abundant interstitial collagen bundles. Stellate cells derive from mesenchymal cells in the subendothelial space of the embryonic liver. In normal liver, these cells represent 5–8% of all liver cells. They are phenotypically long-lived cells with a low level of proliferative activity. The nucleated portion of the cell is often located in a recess between two parenchymal cells, and their cytoplasmic processes run in the space of Disse parallel to the endothelial lining. These extrusions cover several sinusoids, not only allowing their contact with a significant portion of the liver blood supply, but also allowing an intimate cell–cell contact with numerous other liver cells. These cytoplasmic extensions show numerous filaments and microtubules. The cytoplasm contains characteristic vitamin A-rich lipid droplets whose number and diameters seem to vary between species and under different conditions.

In physiologic conditions, these cells play pivotal roles in the regulation of retinoid homeostasis; they express specific receptors for retinol-binding protein (RBP), a binding protein specific for retinol, on their cell surface, and take up the complex of retinol and RBP by receptor-mediated endocytosis. In pathologic



**Figure 2.20 HSC function during liver injury and resolution.** Following liver injury, hepatic stellate cells undergo ‘activation’ which connotes a transition 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 chemoattraction. Key mediators underlying these effects are shown. The fate of activated stellate cells during resolution of liver injury is uncertain, but may include reversion to a quiescent phenotype and/or selective clearance by apoptosis. Reproduced from Friedman (00) by permission of The American Society for Biochemistry and Molecular Biology.

conditions such as liver fibrosis, HSCs lose retinoids, and synthesize a large amount of extracellular matrix (ECM) including collagen, proteoglycan and adhesive glycoproteins. Morphology of these cells also changes from the star-shaped stellate cells to that of fibroblasts or myofibroblasts. HSCs have a neural crest origin from their gene expression pattern similar to neural cell type and/or smooth muscle cells and myofibroblasts. The three-dimensional structure of ECM components regulates reversibly the morphology, proliferation, and functions of the hepatic stellate cells. Molecular mechanisms in the reversible regulation of the stellate cells by ECM imply cell-surface integrin binding to ECM components, followed by signal transduction and then cytoskeleton assembly.

HSCs exhibit two major phenotypes, either a ‘quiescent’ or ‘activated’ transdifferentiated state. Cytoplasmic lipid droplets containing retinyl esters and long cytoplasmic processes with fine branching are characteristics of quiescent HSCs, as seen in normal liver tissue. In the activated state, HSCs lack both lipid droplets and long processes, and display proliferative and a fibrogenic myofibroblast-like phenotype. Stellate cell activation is the central event in hepatic fibrosis. Activation consists of two major phases appropriately referred to as (1) initiation and (2) perpetuation. *Initiation* refers to early paracrine-mediated changes in gene expression and phenotype that render the cells responsive to cytokines and other stimuli. The paracrine stimulation process is carried out by all neighboring cell types, including sinusoidal endothelium, Kupffer cells, hepatocytes, platelets and leukocytes. Endothelial cells are also likely to participate in activation, both by production of cellular fibronectin and via conversion of TGF-β from the latent to an active, profibrogenic form. *Perpetuation* results from the effects of these stimuli to maintain the activated phenotype and generate fibrosis. It involves several discrete changes in cell behavior

including proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation, retinoid loss, white blood cell chemoattractant and cytokine release.

HSCs are important sources of paracrine, autocrine, juxtacrine and chemoattractant factors that maintain homeostasis in the microenvironment of the hepatic sinusoid. They display diverse phenotypes with respect to morphology, function and gene expression in hepatic parenchymal, and invaluable roles in vitamin A-storage, extracellular matrix (ECM) remodeling, and liver development and regeneration. Gene expression in HSCs is regulated by cell–cell and cell–ECM interaction or cytokines and growth factors. The number of activated HSCs is modulated by apoptosis, in which apoptosis is influenced by environmental factors in the extracellular milieu including soluble growth factors and the matrix. During fibrogenesis, the overall number of stellate cells reflects the balance of simultaneous proliferation and apoptosis. Stellate cells also exist in extrahepatic organs including the pancreas, lung, kidney and intestine.

In summary, HSCs exert the following functions:

- uptake, storage, and release of retinoids;
- control ECM turnover by synthesis and secretion of ECM molecules (collagens I, III, IV, V, and VI; laminin; tenascin; undulin; hyaluronic acid; and proteoglycans), and by releasing matrix metalloproteinases and their inhibitors;
- control of blood flow through the sinusoidal capillaries by contraction and dilation of the sinusoidal lumen in response to endothelin, angiotensin, thromboxane, prostaglandins;
- production of growth factors and cytokines.

### Pit cells

In normal liver, most pit cells are located within the sinusoidal lumen and are adherent to the sinusoidal wall or anchored with villous extensions (pseudopods). Under physiologic conditions,

pit cells are short-lived and continuously replaced by cells originating from extrahepatic sources. They were originally called large granular lymphocytes. In human liver they are present at very low numbers in contrast to rat liver. The human pit cells have pronounced polarity, abundant cytoplasm containing dense granules, a conspicuous cytocenter, and locomotory shape with hyaloplasmic pseudopods and a uropod. The number of typical granules is significantly smaller than in the rat hepatocyte,

however, and rod-cored vesicles are extremely rare. Another characteristic for human pit cells is the 'parallel tubular arrays', in which the tubular structures frequently associate with dense granules. They are thought to function in the liver:

- against cancer because of their strong cytotoxic activity against various tumor cell lines in vitro;
- in antiviral activity similar to that of natural killer cells; and
- in control of growth and differentiation of liver cells.

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## SECTION I: NORMAL STRUCTURE AND FUNCTION

### CHAPTER

# 3

## Functions of the Liver

Bruce A. Luxon

### OVERVIEW

The liver is a unique organ anatomically located to serve its dual role as a metabolic and biochemical transformation factory. The liver receives blood containing substances absorbed or secreted by the gastrointestinal organs including the pancreas, intestine, stomach and spleen. The liver uses these substances as raw materials and modifies them or synthesizes completely new chemicals. These are then returned to the bloodstream or to bile for excretion.

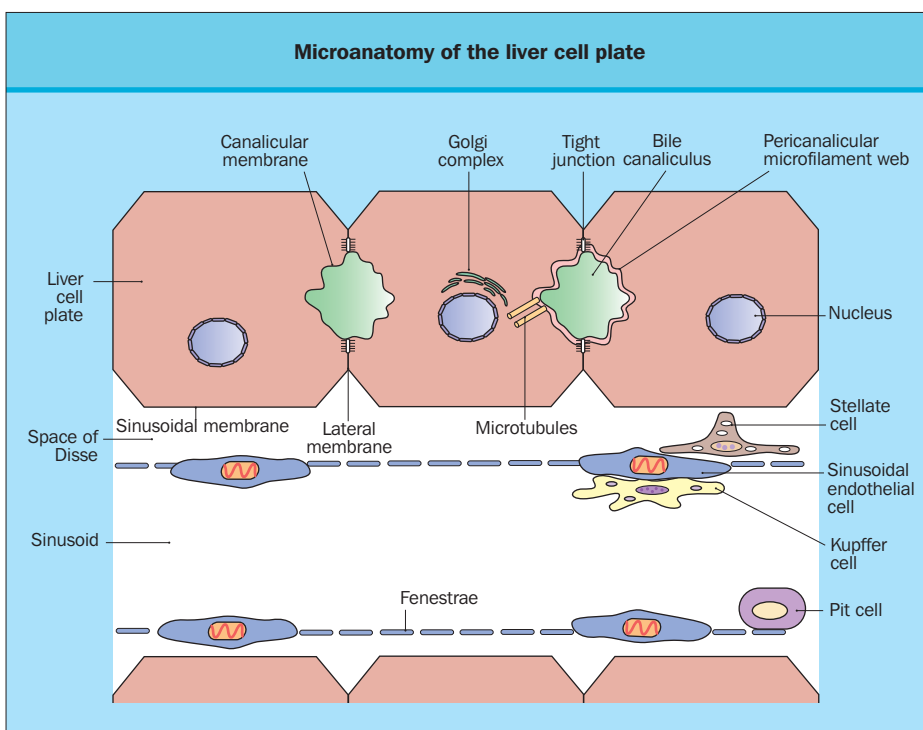
On a cellular level, the liver allows maximal interaction between the blood and liver cells. In its unique design, liver cells are organized into cell plates, each plate being formed by one-cell-thick cords, which are surrounded by blood vessels (sinusoids). The perfusion of these sinusoids is unidirectional, proceeding from the portal tract to the hepatic venule. The specialized capillaries found in the sinusoids have large fenestrations, which allow free exchange between plasma and the extracellular space. Because blood flow proceeds sequentially down the length of the sinusoid, hepatocytes located in different positions along these cell plates contain differing amounts of enzymes and thus have distinct functional capabilities. As blood traverses the liver from its entry point at the portal inlet to its

exit at the hepatic vein, the composition of sinusoidal blood is modified by removal and addition of compounds.

In this chapter, we describe the basic elements of the organization of the liver and emphasize how these elements determine its unique physiology. The structure of the liver lobule is then first described and emphasis is placed on the concept of the liver cell plate. The remaining sections deal with the functional organization of the cell plate, with emphasis on the uptake of compounds from plasma into hepatocytes, their metabolism, excretion into bile and biotransformation.

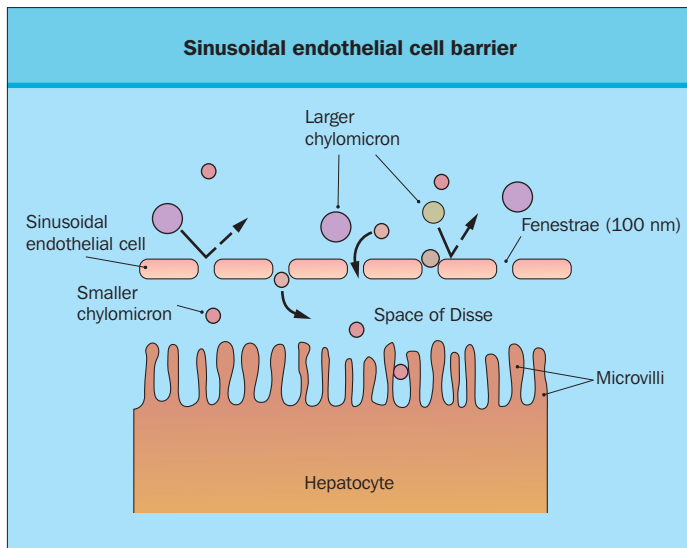
### MORPHOLOGIC CONSIDERATIONS

Hepatocytes are arranged in single-cell thick plates extending from the portal tract to the terminal hepatic venule (**Fig. 3.1**). Adjacent plates of hepatocytes are separated by the hepatic sinusoids, lined by endothelial cells with several characteristic ultrastructural features. Slender processes extending from the cell body contain pores (fenestrae), which are arranged in sieve plates. The fenestrae are dynamic structures under the regulation of an actin cytoskeleton. Unlike other endothelia, sinusoidal endothelial cells lack an underlying basement membrane, allowing direct exchange between plasma and the



**Figure 3.1 Microanatomy of the liver cell plate.**

The relationship of the four non-parenchymal cells to the hepatocytes is shown on the right. Kupffer cells and pit cells lie within the sinusoidal lumen. Endothelial cells separate the sinusoidal lumen from the space of Disse. Stellate cells (lipocytes) lie within the space of Disse. Intracellular organelles and features that play a role in bile secretion are shown within the hepatocytes.



**Figure 3.2 Sinusoidal endothelial cell barrier.** Sinusoidal endothelial cells provide an important barrier to larger molecules such as chylomicrons. Fenestrae have a diameter of approximately 100 nm and exclude large chylomicrons. Smaller molecules permeate into the space of Disse and reach the microvilli of hepatocytes. Modified from Roll et al in Kaplowitz (96) by permission of Lippincott, Williams & Wilkins.

perisinusoidal space of Disse. This feature facilitates the transfer of protein-bound solutes, such as drugs, bilirubin and bile acids, from sinusoidal blood to the hepatocyte and promotes the excretion of, for example, lipoproteins and other proteins from the hepatocyte to the sinusoid (Fig. 3.2). In cirrhosis, a continuous basement membrane accumulates between hepatocytes and endothelial cells, obliterating the fenestrae and impairing solute transfer in both directions. Defenestration also occurs early in liver cancer and in chronic alcohol abuse, contributing to the hyperlipoproteinemia associated with alcoholism.

### Hepatocyte membranes

Neighboring hepatocytes are joined by junctional complexes that serve to demarcate the canalicular space from the basolateral, or sinusoidal, domain. Differences in the structure and function of the sinusoidal and canalicular membrane define the hepatocyte as a polarized cell. A lipid composition of a higher cholesterol–phospholipid and a lower phospholipid–sphingomyelin molar ratio in the canalicular than in the sinusoidal domain confers a relative resistance to the detergent actions of bile acids on the canalicular membrane. Morphologically similar to other transporting epithelia, the surface area of the sinusoidal and canalicular membranes is increased by microvilli. Major alterations in canalicular microvilli occur in intrahepatic and extrahepatic forms of cholestasis, including a reduction in the number of microvilli and the development of giant microvilli secondary to edema and bleb formation, which occlude the canalicular lumen to some degree. These findings suggest that canalicular microvilli, like their morphologic counterparts in the enterocyte, play a role in solute transport.

The sinusoidal membrane is primarily involved in the bidirectional exchange of solutes and has uptake mechanisms for amino acids, glucose and organic anions such as bile acids, fatty

acids and bilirubin; receptor-mediated endocytotic processes;  $\text{Na}^+, \text{K}^+$ -ATPase and glucagon-stimulatable adenylate cyclase activity; and export processes for albumin, lipoproteins and clotting factors. The predominant function at the canalicular membrane surface is secretion, although limited reabsorptive capacity has been demonstrated. Certain membrane enzymes are selectively localized to the canalicular domain, including alkaline phosphatase, leucine aminopeptidase and glutamyltranspeptidase.

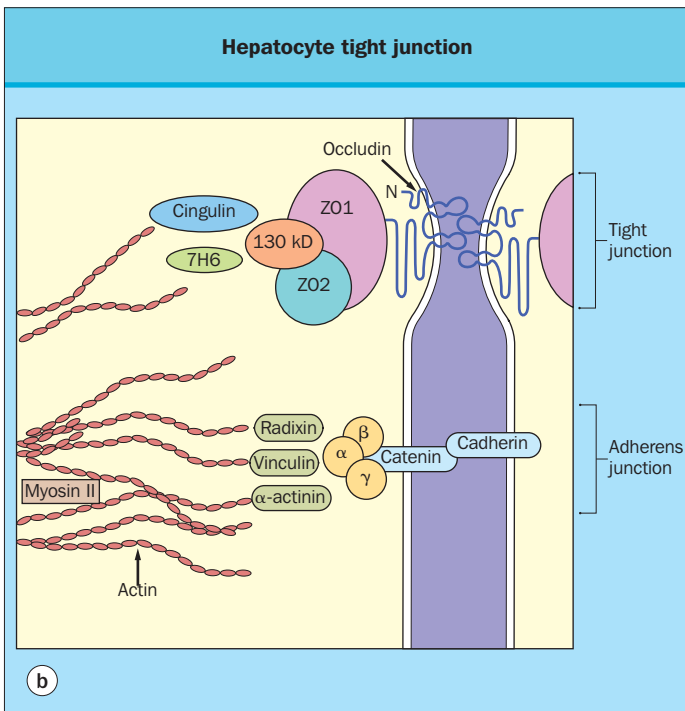
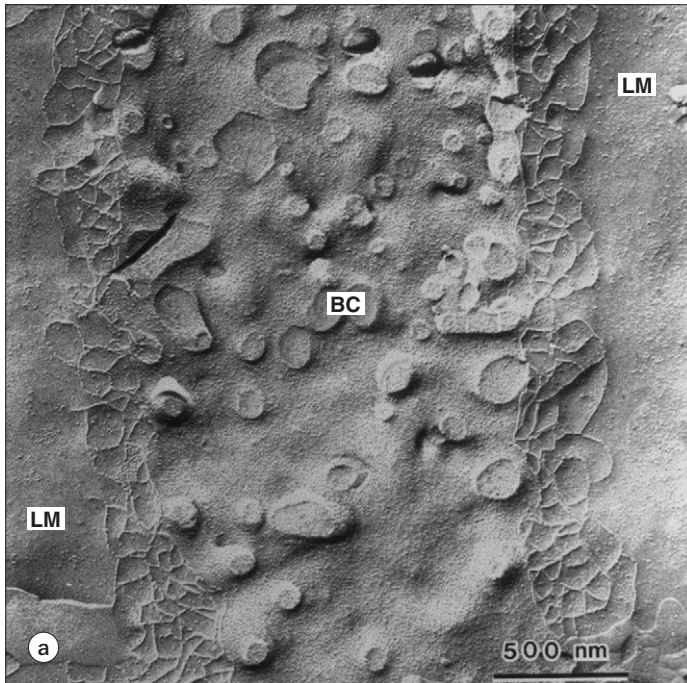
From adjacent canaliculi, bile enters small terminal bile ductules, the canals of Hering, which consist of fusiform cells in close association with neighboring hepatocytes. These short channels traverse the limiting plate to form successively larger ductules and intralobular bile ducts, composed of cuboidal epithelial cells. Interlobular bile ducts, ranging in size from 30 to 40  $\mu\text{m}$ , convey bile to the extrahepatic bile duct, the gallbladder and the duodenum. Cholangiocytes along the intrahepatic biliary tree are morphologically and functionally heterogeneous; regulated transport of water and electrolytes primarily occurs in the medium and large interlobular bile ducts.

### Hepatocyte membrane junctions

The junctional complexes that join adjacent hepatocytes consist of several discrete structures. The tight junctions (i.e. zonulae occludens) function as a barrier to unrestricted movement of solutes from the space of Disse to the canalicular lumen. The tight junction, seen with freeze-fracture electron microscopy, is a network of anastomosing intramembrane strands or fibrils in the outwardly facing cytoplasmic leaflet (Fig. 3.3a). This barrier for entry into bile acts as if it is associated with a net negative charge, since the paracellular movement of negatively charged solutes from blood to bile is impaired. Likewise, negatively charged species, such as conjugated bile acids, secreted across the canalicular membrane may be retained in the canalicular lumen by this selective permeability barrier. Tight junction permeability, altered in intrahepatic and extrahepatic cholestasis, is determined by at least three tight junction-specific proteins. Rows of the transmembrane protein, occludin, act as the intercellular seal. Occludin is linked on its cytoplasmic surface zonula occludens to ZO1 and ZO2 (Fig. 3.3b). Adjacent to the tight junction is the adherens junction or zonula adherens (Fig. 3.3b). The adhesion molecules, the cadherins, form zipper-like connections between hepatocytes. The cytoplasmic domains of cadherins interact with catenins, which are cytoplasmic proteins linked to the actin cytoskeleton.

Located distal to the tight junction is the nexus or gap junction. One of the proposed functions for gap junctions is mediation of intercellular communication under physiologic conditions. Individual gap junction proteins, termed connexins, form hexameric channels that allow the exchange of ions and small molecules, such as calcium, cyclic AMP and inositol trisphosphate, between adjacent hepatocytes.

Spot desmosomes, or macula adherens, are also found in the junctional complex, where they are also involved in cell–cell adhesion. Composed of desmogleins, these structures serve a bridging function, maintaining contact between hepatocytes during pathologic situations that interfere with the function of gap junctions. Actin-containing microfilaments are numerous in the pericanalicular cytoplasm, where they insert into the canalicular microvilli and into the junctional complex to form a pericanalicular web. Coordinated and periodic contractions of



**Figure 3.3 Hepatocyte tight junction.** (a) Freeze-fracture replica of hepatocyte tight junction. The grid-like tight junction elements act as a barrier between the bile canaliculus (BC) and the intracellular space lined by the lateral membrane (LM). Reproduced from Boyer (83) by permission of Lippincott, Williams & Wilkins. (b) Schematic diagram of hepatocyte tight junction. An intercellular seal is formed by contact between pairs of the transmembrane protein, occludin, which are bound on the cytoplasmic surface to ZO1 and ZO2. Junctional proteins without known functions include cingulin and 7H6. The adherens junction is formed by the association of cadherins, which interact with  $\alpha$ -,  $\beta$ - and  $\gamma$ -catenin, cytoplasmic proteins that are linked to the actin cytoskeleton. Adapted from Anderson et al (95) by permission of The American Physiological Society.

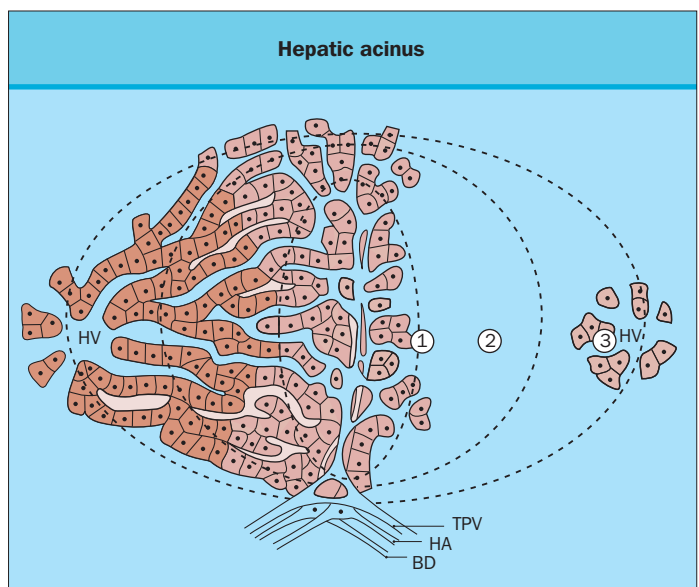
canaliculi, responsive to intracellular calcium and bile acids, have been observed in vitro and in vivo.

### Hepatocyte intracellular organelles

A variety of vesicular transport processes in cells is dependent on the integrity of microtubules. Vesicular transport is an energy-dependent process mediated partially by the microtubule-associated motor ATPases, dynamin, kinesin and dynein, which are all present in abundance in hepatocytes. The role of microtubules in normal bile formation, in particular, appears to be primarily that of delivery of transport proteins to the canalicular membrane from intracellular compartments more proximal than subapical endosomes. The Golgi apparatus is preferentially located at the canalicular pole of the hepatocyte. Besides its role in protein synthesis, intact Golgi function is critical in the intracellular processes involved in bile formation. Dynamins, a family of 100 kDa GTPases that associate with the Golgi apparatus to facilitate the formation of endocytic vesicles and promote intracellular vesicular traffic, also play a role in bile secretion. Bile acids interact with another intracellular organelle, the smooth endoplasmic reticulum (ER). Permeabilization of this intracellular compartment may play a role in the inhibition of bile secretion seen with the hydrophobic bile acids.

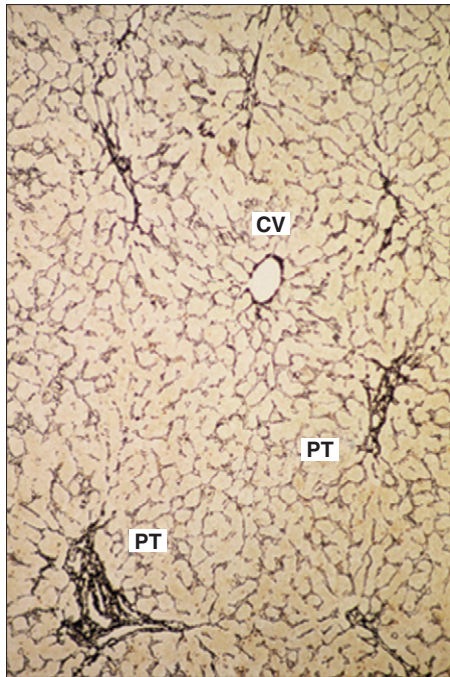
### Hepatic acinus – a functional unit

Histologic units within the liver parenchyma have been appreciated since the late 1600s. Most modern textbooks of histology provide detailed descriptions of the organization of the liver lobule. In the pioneering work of Rappaport (1973), the organizational unit of the human liver was defined as an acinus. In this model, three concentric zones surround the portal triad (Fig. 3.4 and Fig. 3.5). Zone 1 cells are defined as those closest to the afferent blood vessels and are, therefore, the first in the



**Figure 3.4 Hepatic acinus.** The acinar axis is formed by the terminal branch of the portal vein (TPV), hepatic artery (HA) and bile ductule (BD). Blood enters the sinusoids in zone 1 and flows sequentially through zone 2 and zone 3, where it exits the acinus through the terminal branch of the hepatic vein (HV). Reproduced from Traber et al (88) by permission of The American Gastroenterological Association.

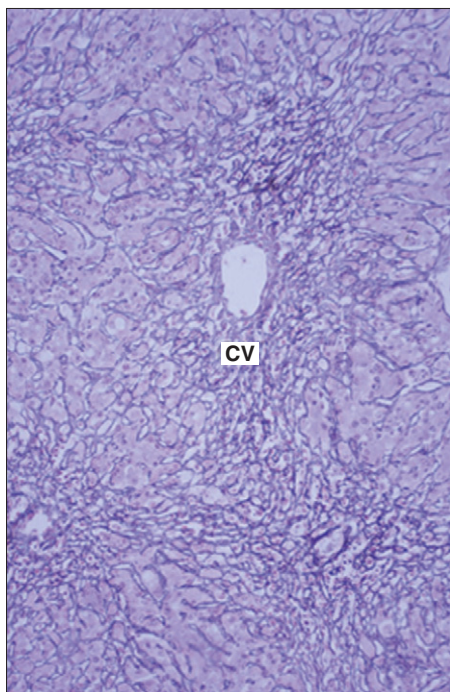




**Figure 3.5**  
**Photomicrograph of a normal human liver biopsy.** The first 5 to 10 hepatocytes comprise the 'periportal' region. Periportal sinusoids are interconnected and relatively narrow. The 'perivenular' portion of the liver cell plate is the distal two-thirds. Sinusoids in this region (zone 3) are wide and relatively straight as they drain into the hepatic vein (CV, central vein; PT, portal triad). (Photograph courtesy of E. Brunt.)

sinusoid to receive blood. This blood contains the highest concentration of oxygenated solutes. Cells in zones 2 and 3 are located at progressively greater distances from the afferent blood vessels and therefore these cells receive relatively oxygen-poor blood. These cells are the ones that are most likely to be damaged by ischemia (Fig. 3.6). In addition, zone 3 hepatocytes actively participate in drug metabolism and disposition. Consequently, most drugs in clinical use, if hepatotoxic, induce zone 3 necrosis.

The key anatomic feature of the acinus is the liver cell plate that consists of a group of 15 to 30 hepatocytes organized as a



**Figure 3.6**  
**Photomicrograph of human liver.** The photomicrograph demonstrates zone 3 hepatocyte damage due to acetaminophen (CV, central vein). (Photograph courtesy of E. Brunt.)

plate that is one cell thick. At one end of the plate, the afferent blood vessels are located in a portal tract. The other end consists of the central vein. Blood enters the liver sinusoid through the portal tract via the portal vein and hepatic arteriole. Blood from these vessels empties into the hepatic sinusoids and flows unidirectionally along the liver cell plate. As it exits the cell plate, it empties into the central vein. Bile flows in the opposite direction to the blood, thus forming a countercurrent system similar to that in the kidney.

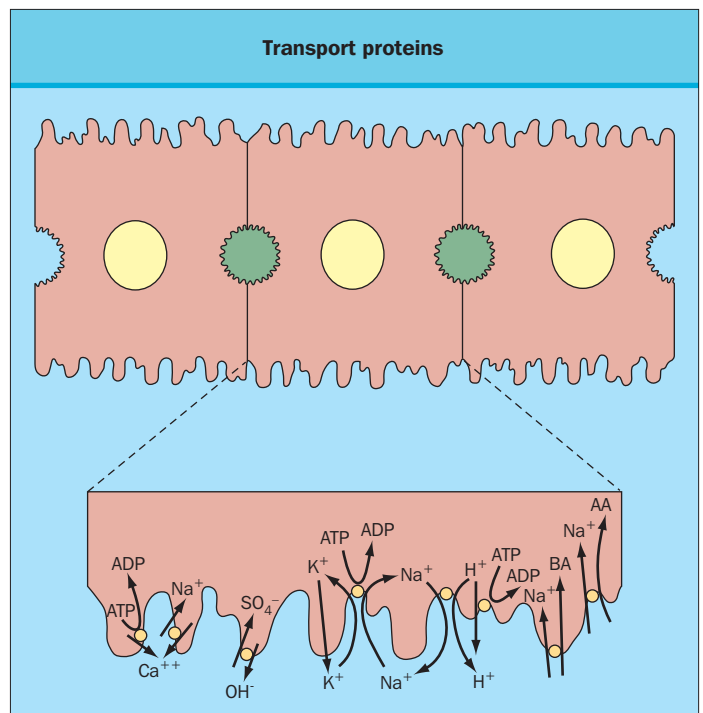
## BILE FORMATION

### Overview

One of the principal functions of the liver is bile formation. There is a great deal of information regarding the mechanism by which individual hepatocytes extract electrolytes and solutes from the plasma, transport them across the plasma membrane and excrete them into bile. Numerous transport mechanisms are associated with the sinusoidal plasma membrane. Specific transporters have been identified to facilitate the uptake of hydrophilic bile acids, amino acids and organic anions such as sulfobromophthalein. All of these transporters rely on the potential difference maintained across the hepatocyte membrane ( $-35$  mV) and the inward sodium concentration gradient ( $12$  mM) (Fig. 3.7).

### Electrolyte and solute transport

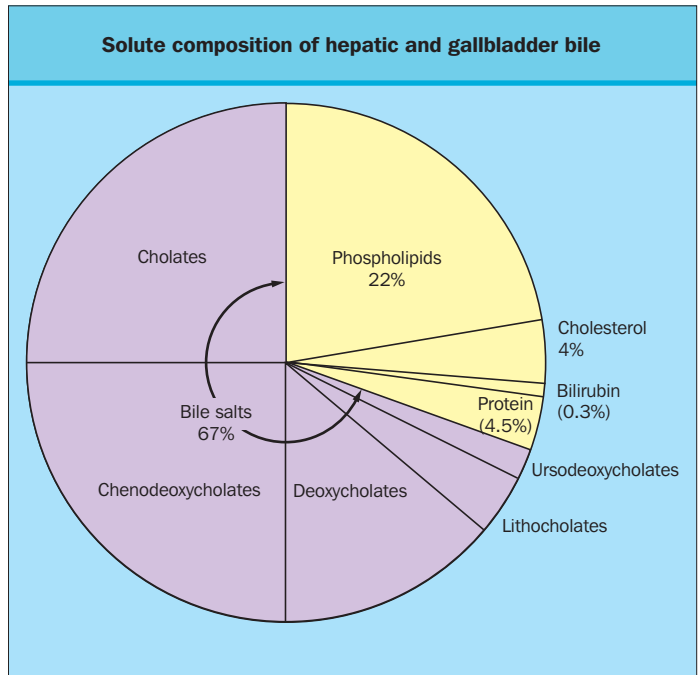
Bile is composed primarily of water, inorganic electrolytes and organic solutes such as bile acids, phospholipids, cholesterol and



**Figure 3.7 Transport proteins.** Multiple transport proteins have been identified in the sinusoidal plasma membrane. Each of these transporters relies on the sodium gradient and provides uptake of bile acids, amino acids and organic anions. Adapted from Scharschmidt in Zakim (90) by permission of Elsevier.

bile pigments (**Table 3.1**). The volume of hepatic bile excreted is between 500 and 600 mL/day. The relative proportions of the major organic solutes of bile are illustrated in **Figure 3.8**. Bile acids are the major organic solutes in bile and are derived from two sources. Primary bile acids (i.e. cholic and chenodeoxycholic acid in humans) are synthesized from cholesterol in the liver. Microsomal 7- $\alpha$ -hydroxylase is the rate-limiting enzyme in bile acid synthesis. The secondary bile acids (i.e. deoxycholic, lithocholic and ursodeoxycholic acid in humans) are produced from primary bile acids by bacterial enzymes in the intestine (**Fig. 3.9**). Bile acids consist of two components that determine their physiologic and physicochemical properties: a steroid nucleus with its hydroxyl substituents and an aliphatic side chain.

The terminal carboxylic acid group of the side chain is modified after the synthesis of the primary bile acids and during the hepatic phase of the enterohepatic cycling of the secondary bile acids by enzymatic conjugation to taurine or glycine. Conjugation decreases the ability of bile acids to traverse cell membranes by passive diffusion. Glycine and taurine conjugates of bile acids also demonstrate selective resistance to hydrolysis by pancreatic enzymes during small intestinal transit. The net



**Figure 3.8** Typical solute composition in percentage by weight of hepatic and gallbladder bile in healthy humans.

Composition of hepatic bile	
Component	Concentration (mmol/L)
<b>Electrolytes</b>	
Na <sup>+</sup>	141–165
K <sup>+</sup>	2.7–6.7
Cl <sup>−</sup>	77–117
HCO <sub>3</sub> <sup>−</sup>	12–55
Ca <sup>2+</sup>	2.5–6.4
Mg <sup>2+</sup>	1.5–3.0
<b>Organic anions</b>	
Bile acids	3–45
Bilirubin	1–2
<b>Lipids</b>	
Lecithin	140–810 (mg/dL)
Cholesterol	97–320 (mg/dL)
Proteins	2–20 (mg/mL)
	0.02–0.2 (g/L)
<b>Peptides and amino acids</b>	
Glutathione	3–5
Glutamate	0.8–2.5
Aspartate	0.4–1.1
Glycine	0.6–2.6

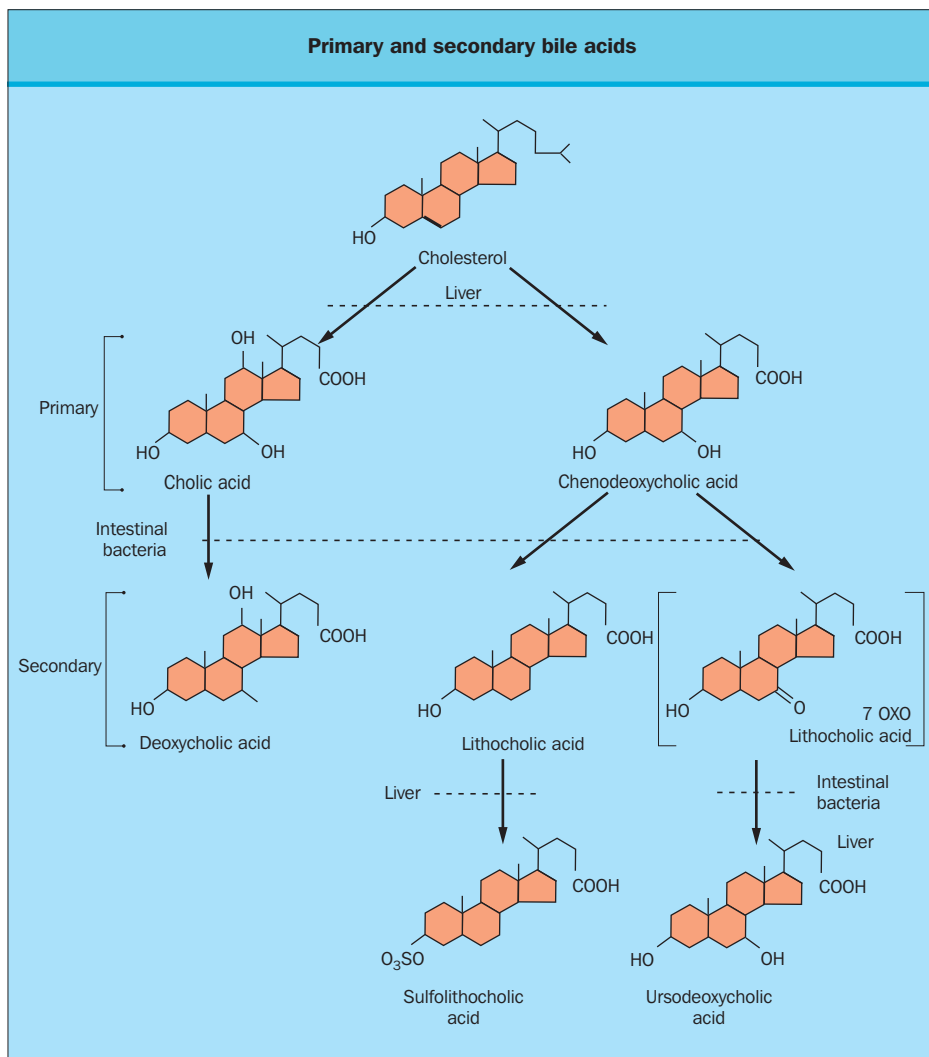
**Table 3.1** Composition of hepatic bile.

effect of conjugation is to permit bile acids to accumulate at an intraluminal concentration in the small intestine high enough to facilitate fat digestion and absorption.

The presence of hydrophilic (i.e. hydroxyl substituents and the amide linkage on the aliphatic side chain) and lipid-soluble or hydrophobic (i.e. the steroid nucleus) regions allows conjugated bile salts to act as amphiphilic molecules that form micelles or polymolecular aggregates above a critical micellar concentration. Bile salt micelles can solubilize other biologically important amphiphilic solutes, such as cholesterol and phospholipids, to form mixed micelles. This detergent-like property of bile acids is important in stabilizing the physical state of bile and in promoting fat digestion and absorption.

The dihydroxy bile acid ursodeoxycholic acid (UDCA) is used in the treatment of chronic cholestatic disorders, such as primary biliary cirrhosis. Under normal conditions, UDCA represents less than 3% of the bile acid pool; it is more hydrophilic than the other major dihydroxy bile acids, chenodeoxycholic acid and deoxycholic acid. The mechanism of action of UDCA is not clear, but it stimulates the biliary secretion and inhibits the intestinal reabsorption of endogenous bile acids, becoming the predominant bile acid in serum and in bile after long-term administration. It may, therefore, protect against bile duct and hepatocyte injury from hydrophobic bile acids, such as chenodeoxycholic acid, deoxycholic acid and lithocholic acid, and other potential hepatotoxins.

As shown in **Table 3.1**, the predominant biliary cation is Na<sup>+</sup> and the concentrations of inorganic electrolytes in bile are similar to their plasma concentrations. The inorganic electrolytes are largely responsible for the osmotic activity of bile. This is because the osmotic activity of most of the organic solutes, such as bile acids, is lost by aggregation into mixed micelles.



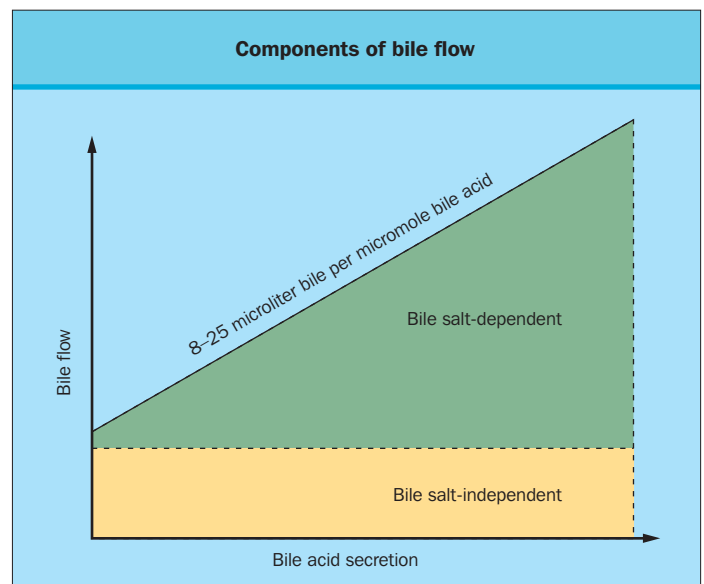
**Figure 3.9** Major primary and secondary bile acids and their sites of synthesis and metabolism.

In contrast to the passive, hydrostatic forces that govern glomerular filtration by the kidney, bile formation by hepatocytes is an osmotic process that involves the active secretion of osmotically active inorganic and organic solutes into the canalicular lumen, followed by passive water movement. In this important respect, hepatic bile secretion can be characterized by the same processes found in more conventional secretory epithelia.

Canalicular bile formation is classically measured using metabolically inert solutes, such as erythritol and mannitol, which are assumed to enter bile passively only at the level of the canaliculus and not to undergo modification by biliary ductular cells. Using these markers, canalicular bile formation has been traditionally divided into two components (**Fig. 3.10**): bile acid-dependent bile flow (BADBF), which is defined as the slope of the line relating canalicular bile flow to bile salt excretion; and bile acid-independent bile flow (BAIBF), which is attributed to the active secretion of inorganic electrolytes and other solutes and defined as the extrapolated y-intercept of this line.

### Bile acid transport

The sinusoidal uptake of conjugated bile acids, such as taurocholate, is primarily mediated by a secondary active transport process, which is driven by the inwardly directed  $Na^+$



**Figure 3.10** Components of bile flow. Bile flow depends on bile acid secretion as well as bile acid independent components. Slope of line indicates that 8–25  $\mu L$  of bile are produced for each  $\mu mol$  of bile salt transported into bile. Adapted from Zakim (03) by permission of Elsevier.



gradient maintained by  $\text{Na}^+, \text{K}^+$ -ATPase activity. The negative electrical potential difference (PD) across the membrane maintained by  $\text{Na}^+, \text{K}^+$ -ATPase activity is also an important driving force, because  $\text{Na}^+$ -taurocholate cotransport is electrogenic (i.e. associated with the net entry of positive charge). This bile salt transporter has been cloned from both rat and human liver and termed the  $\text{Na}^+$ -taurocholate cotransporting polypeptide, *ntcp* and NTCP, respectively. Expression and function of *ntcp* is downregulated in several models of experimental cholestasis, including a model of sepsis-associated cholestasis. Findings in patients with extrahepatic biliary atresia suggest similar regulation of NTCP.

The uptake of unconjugated bile acids at the sinusoidal membrane, in contrast, is a  $\text{Na}^+$ -independent process mediated, in part, by the organic anion transporting polypeptide, *oatp1*. The  $\text{Na}^+$ -independent uptake of bile salts exhibits a broad substrate specificity that includes electroneutral steroids, such as ouabain and progesterone; cyclic oligopeptides, such as phalloidin, somatostatin analogs and cyclosporin; and a wide variety of xenobiotics. Hepatic uptake of amatoxins, the toxic product in toadstools of the genus *Amanita* and the cause of most accidental mushroom poisonings, is also mediated by this multispecific organic anion transport process.

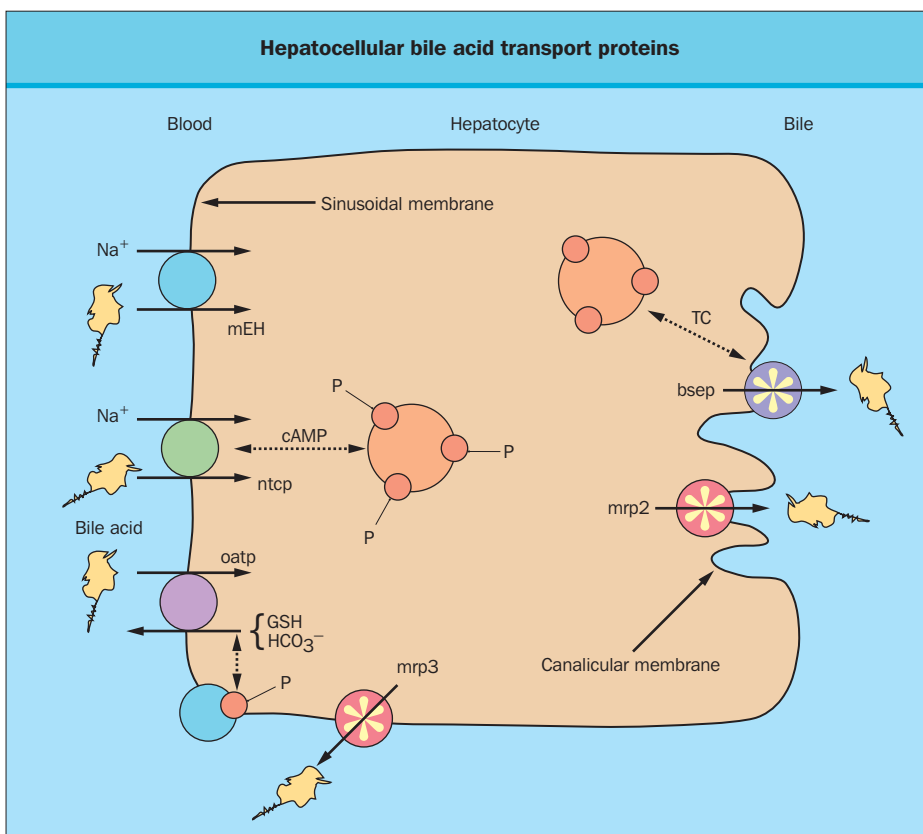
The mechanism for intracellular transport of bile acids from the sinusoidal to the canalicular pole of hepatocytes is not as well understood. Intracellular binding of bile acids has been proposed as a mechanism for hepatic transport and a protective mechanism against potential toxic effects of free bile acids. Two unrelated families of cytosolic proteins with high affinities for bile acids have been identified: ligandins and Y9 proteins. The Y9 bile acid

binders belong to the monomeric reductase gene family that also binds derivatives of polycyclic aromatic hydrocarbon carcinogens. These cytosolic proteins may be involved in the intracellular transport of bile acids and carcinogens to the canalicular membrane or to intracellular organelles by diffusion.

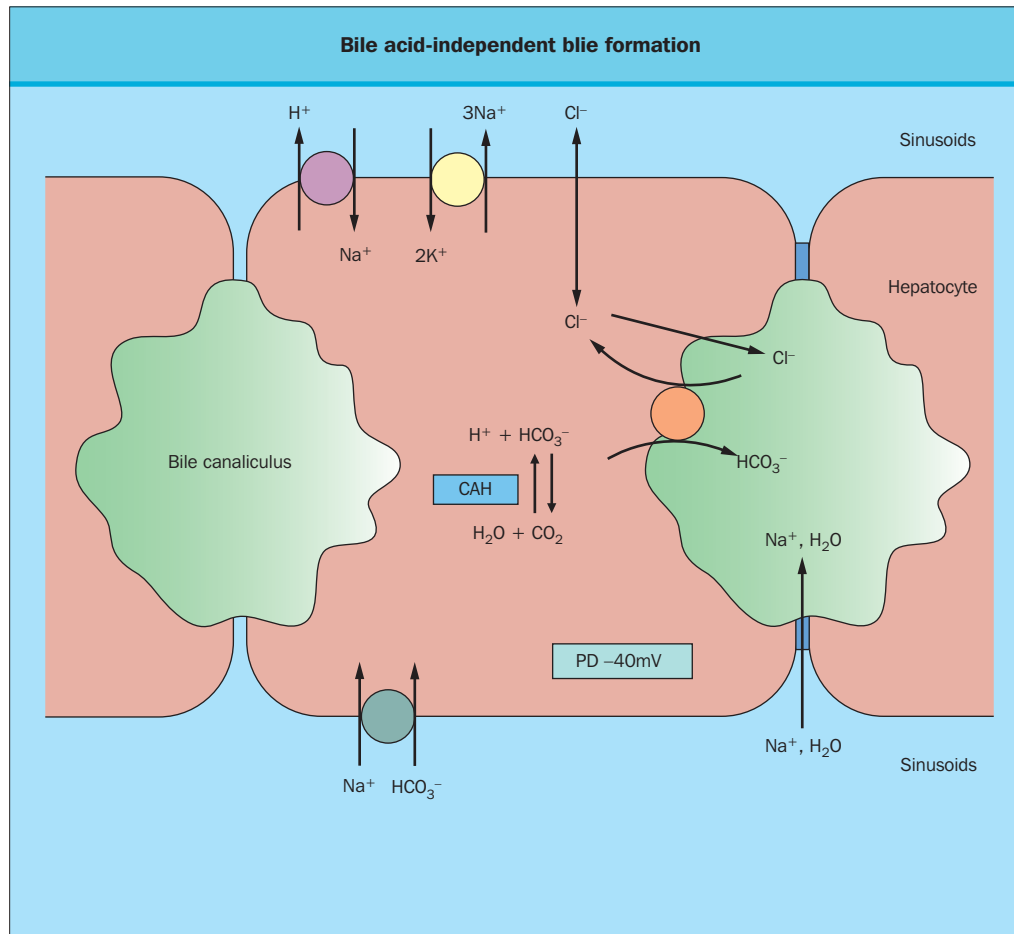
Canalicular excretion represents the rate-limiting step in the hepatic transport of bile acids. Canalicular secretion of anionic bile acids was believed to occur by passive facilitated diffusion down an energetically favorable electrochemical gradient. However, the magnitude of the membrane potential is too small to account for the bile salt concentration gradient that exists across the canalicular membrane, and alternative mechanisms have been identified for carrier-mediated bile acid secretion at this membrane domain. A member of the P-glycoprotein family encoded by the *sggp* gene is an ATP-dependent canalicular bile acid transporter. A defect in this canalicular bile acid transporter is responsible for a form of progressive familial intrahepatic cholestasis, PFIC type 2. The vectorial movement of bile acids from sinusoidal blood to canalicular bile is schematically depicted in Figure. 3.11.

### Electrolyte transport

In contrast to the information regarding BADBF, less is known about the hepatocellular mechanisms that underlie BAIBF. Inhibition of  $\text{Na}^+, \text{K}^+$ -ATPase activity does not appear to have a significant effect on BAIBF and indirect evidence points to a primary role for bicarbonate ( $\text{HCO}_3^-$ ) transport. In other epithelia, such as the pancreas,  $\text{HCO}_3^-$  transport has been attributed to  $\text{Na}^+ - \text{H}^+$  exchange (i.e. antiport) activity. With the identification and characterization of sinusoidal  $\text{Na}^+ - \text{H}^+$



**Figure 3.11 Major known hepatocellular bile acid transport proteins.** Solid arrows indicate transport of the indicated substrate. Dashed arrows denote regulatory pathways of vesicular transport and phosphorylation. \* indicates ATP-dependent transport. bsep, bile salt export pump; GSH, glutathione; mEH, microsomal epoxide hydrolase; mrp2/mrp3, multidrug resistance-associated protein 2/3; ntcp,  $\text{Na}^+$ -taurocholate cotransporting polypeptide; oatp, organic anion transporting protein; TC, taurocholate; P, phosphorylated transporter. Adapted from Wolkoff et al (03) by permission of Elsevier.



**Figure 3.12 Bile acid-independent bile formation.** Sinusoidal  $\text{Na}^+\text{-H}^+$  exchange and canalicular  $\text{Cl}^-\text{-HCO}_3^-$  exchange are functionally coupled by means of cytosolic carbonic anhydrase (CAH) to generate net biliary bicarbonate secretion. A sinusoidal  $\text{Na}^+\text{-HCO}_3^-$  symport in conjunction with canalicular  $\text{Cl}^-\text{-HCO}_3^-$  exchange may also play a role in biliary  $\text{HCO}_3^-$  secretion. Adapted from Moseley et al (86) by permission of The American Physiological Society.

exchange and canalicular  $\text{Cl}^-\text{-HCO}_3^-$  exchange, a model was proposed (Fig. 3.12) in which these two transport processes are functionally coupled by way of cytosolic carbonic anhydrase to generate net biliary bicarbonate secretion. A sinusoidal  $\text{Na}^+\text{-HCO}_3^-$  symport in conjunction with canalicular  $\text{Cl}^-\text{-HCO}_3^-$  exchange may also play a role in biliary bicarbonate secretion. Support for this model comes primarily from studies examining the effects of certain cholestatic and choleretic agents on membrane transport. Ethinyl estradiol, which causes a diminution in BAIBF, inhibits  $\text{Na}^+\text{-H}^+$  exchange activity; UDCA, which results in a  $\text{HCO}_3^-$ -rich choleresis, stimulates  $\text{Na}^+\text{-H}^+$  exchange activity. Amiloride and acetazolamide, inhibitors of  $\text{Na}^+\text{-H}^+$  exchange and carbonic anhydrase activity, respectively, produce a concentration-dependent inhibition of UDCA-stimulated bile flow and biliary  $\text{HCO}_3^-$  output.

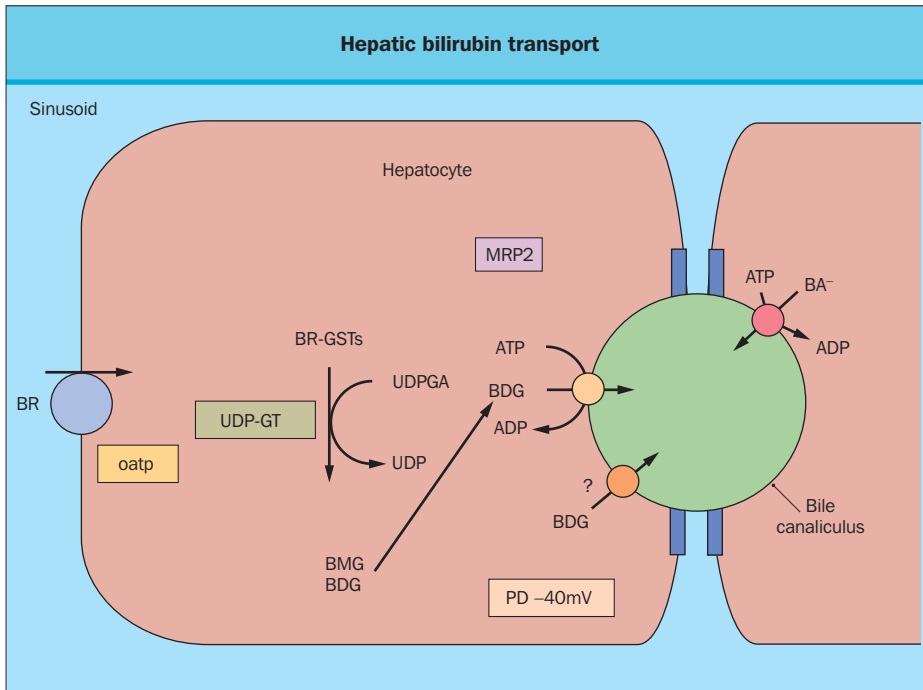
Inorganic electrolytes may not provide a sufficient driving force for BAIBF, however, because their biliary secretion depends primarily on passive diffusion and solvent drag. This has led to the alternative suggestion that non-bile salt organic anions may provide a major driving force for canalicular BAIBF. The tripeptide glutathione ( $\gamma$ -L-glutamyl-L-cysteinylglycine; GSH) may fulfil this role. It is present in bile in high concentrations (see Table 3.1) and, as a result of intrabiliary catabolism of GSH by  $\gamma$ -glutamyltranspeptidase located on the luminal membranes of bile ductule cells and the bile canaliculus membrane, the concentration of this solute in the canaliculus lumen may be substantially higher than that measured in excreted bile. At

concentrations that exceed free (i.e. non-micelle associated) bile acids and bile pigments, GSH may generate a potent osmotic driving force for canalicular bile formation. Because biliary secretion of GSH is a carrier-mediated process, the requirement that the solute providing the driving force for BAIBF is not governed by passive diffusion or solvent drag is met. Additional indirect evidence supports a role for GSH in BAIBF, including the strong correlation of GSH excretion with drug-induced changes in BAIBF and with ontogenic changes in bile formation. However, other unidentified solutes, in addition to GSH, may also contribute to BAIBF.

### Bilirubin metabolism and transport

For a full discussion of bilirubin metabolism and transport, see Chapter 6.

Bilirubin is generated by the breakdown of heme. Approximately 80% of bilirubin is derived from heme released by senescent erythrocytes, while the remainder comes from the heme moieties of other hemoproteins, such as myoglobin and tissue cytochromes. The microsomal enzyme, heme oxygenase, converts heme to biliverdin, which is then converted to bilirubin by biliverdin reductase. The unconjugated bilirubin produced by these enzymatic reactions is transported in the plasma tightly bound to albumin. Competition for albumin binding by certain drugs displaces unconjugated bilirubin and, in the neonate, may result in the diffusion of bilirubin across the blood-brain barrier and bilirubin encephalopathy, or kernicterus.



**Figure 3.13 Hepatic bilirubin transport.**

Unconjugated bilirubin (BR), disassociated from albumin, is taken up at the sinusoidal membrane by a  $\text{Na}^+$ -independent process and mediated by organic anion transporting polypeptide (oatp). Within the hepatocyte, bilirubin and other organic anions bind to cytosolic glutathione S-transferases or ligandins. Conjugation of bilirubin to its monoglucuronides and diglucuronides (BMG and BDG), catalyzed by bilirubin-UDP-glucuronosyl-transferase (UDP-GT), is required for canalicular secretion. Canalicular secretion involves a homolog of the multidrug resistance protein, MRP2.

Bilirubin uptake and secretion is mediated by transport processes distinct from those identified for bile acid transport (Fig. 3.13). Sinusoidal uptake of bilirubin and other non-bile acid organic anions is a  $\text{Na}^+$ -independent process mediated, in large part, by oatp1. Glutathione efflux from the hepatocyte appears to provide the driving force for uptake via oatp1. Within the hepatocyte, bilirubin and other organic anions bind to cytosolic glutathione S-transferases or ligandins. Conjugation of bilirubin to its monoglucuronides and diglucuronides, catalyzed by bilirubin-UDP-glucuronosyl-transferase (UDP-GT), is required for canalicular secretion. Canalicular secretion, in turn, involves a homolog of the multidrug resistance protein, MRP2. A point mutation in MRP2 is the molecular basis for the Dubin-Johnson syndrome, an autosomal-recessive disorder characterized by a conjugated hyperbilirubinemia. Sepsis-associated cholestatic jaundice may be, in part, the result of down regulation of this rate-limiting step in overall hepatic bilirubin transport.

Impaired hepatic uptake at the sinusoidal membrane occurs in Gilbert syndrome and following the administration of certain drugs, such as rifampin (rifampicin). Reduced activity of UDP-GT leads to impaired bilirubin conjugation and is observed in the neonate and in patients with Gilbert and Crigler-Najjar type I and II syndromes. Activity of UDP-GT can be induced by phenobarbital, effectively reducing the jaundice in Crigler-Najjar type II syndrome.

Hepatic bilirubin uptake and conjugating activity are preserved in most forms of liver disease. Accordingly, conjugated hyperbilirubinemias can occur in a wide spectrum of hepatic diseases, including disorders associated with acute and chronic hepatocellular and cholestatic injury and with extrahepatic biliary obstruction.

### Ductular events

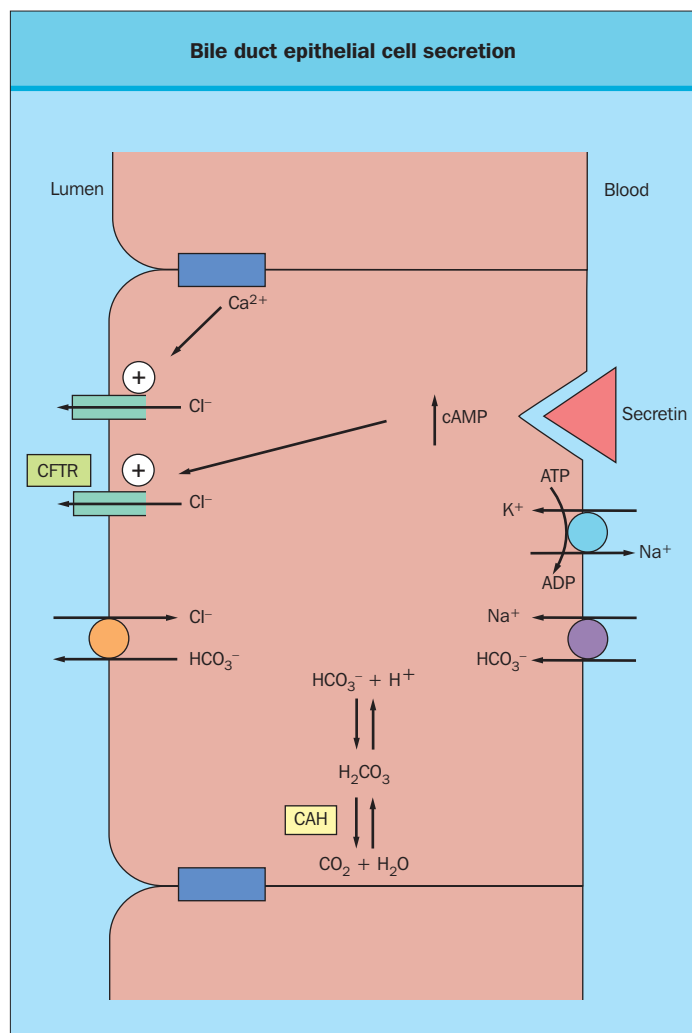
Experimental approaches, such as the isolation of intact segments from small interlobular bile ducts, the site of injury in

the vanishing bile duct syndromes, have led to a better understanding of the biology and pathology of the biliary epithelium; the biliary tract is no longer considered merely a conduit for bile. The mechanism for the  $\text{HCO}_3^-$  rich fluid secretion by bile duct epithelial cells has been examined at both a cellular and molecular level. As shown in Figure 3.14, the binding of the hormone secretin, and possibly of other agonists, to receptors localized to the basolateral domain of the bile duct epithelial cell leads to an increased intracellular level of the second messenger cAMP. Chloride exits from the apical membrane through cAMP-responsive cystic fibrosis transmembrane conductance regulator (CFTR)-associated  $\text{Cl}^-$  channels. The resulting cell depolarization facilitates the uptake of  $\text{HCO}_3^-$  at the basolateral membrane, mediated by a  $\text{Na}^+-\text{HCO}_3^-$  symport (or a  $\text{Na}^+-\text{Cl}^--\text{HCO}_3^-$  cotransporter). Intracellular  $\text{HCO}_3^-$ , increased by either this mechanism or by enhanced carbonic anhydrase activity, then enters the bile duct lumen by means of apical  $\text{Cl}^--\text{HCO}_3^-$  exchange. A clinical correlation of the secretory activity of the biliary epithelium can be found in the increased response to secretin and increased bile flow observed in patients with chronic liver diseases associated with ductular proliferation.

### Gallbladder function

The physiologic functions of the gallbladder include concentration and storage of bile during interdigestive periods, evacuation by smooth muscle contraction in response to cholecystokinin, moderation of hydrostatic pressure within the biliary tract, bile acidification, and absorption of organic components of bile. Although not essential for bile secretion, the gallbladder concentrates bile as much as 10-fold. This process is largely the result of electroneutral  $\text{Na}^+$ -coupled  $\text{Cl}^-$  transport and passive water movement (Fig. 3.15). The exact mechanism is unclear; experimental evidence supports either coupled  $\text{NaCl}$  entry or a  $\text{Na}^+-\text{H}^+$  and  $\text{Cl}^--\text{HCO}_3^-$  exchange operating in parallel. The net result of this concentrative process





**Figure 3.14 Bile duct epithelial cell secretion.** See text for details. CFTR, cystic fibrosis transmembrane conductance regulator; CAH, carbonic anhydrase; cAMP, adenosine 3',5'-cyclic monophosphate.

is the formation of gallbladder bile, isotonic to plasma and composed of higher concentrations of  $\text{Na}^+$ , bile salts,  $\text{K}^+$  and  $\text{Ca}^{2+}$  and lower concentrations of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  than hepatic bile.

Despite the considerable concentration gradient for bile salts and bile pigments, the absorption of highly ionized organic solutes such as taurocholate, sulfobromophthalein and iodipamide is minimal. In acute cholecystitis, increased permeability to water and to highly ionized solutes has been demonstrated and enhanced absorption of iodipamide may account for the non-visualization of the gallbladder that occurs in this setting.

Mucus is released by the exocytosis of secretory granules in the apical portion of gallbladder epithelial cells. Gallbladder mucin synthesis and release are markedly accelerated in animal models of cholesterol cholelithiasis before crystal and stone formation. Formation of an insoluble mucin-bilirubin complex may provide a nidus for cholesterol monohydrate nucleation.

### Enterohepatic circulation

It is best to consider bile acid secretion as a cyclic flow of molecules anatomically limited to the hepatocyte, biliary tree, small intestine, enterocyte and portal blood, known as the

*enterohepatic circulation* (Fig. 3.16). Intestinal conservation of bile acids is approximately 90% efficient, reflecting the additive effects of both passive and active reabsorptive processes. The bile acid pool cycles 5–15 times daily through this pathway.

Passive absorption of bile acids occurs throughout the small intestine and depends on intestinal pH and bile acid structure. The most hydrophobic bile acids (i.e. glycine-conjugated dihydroxy bile acids) are passively absorbed in the more acidic environment of the duodenum, where the fraction in protonated (uncharged) form is greatest. Bile acids are transported across the ileal brush border membrane by a  $\text{Na}^+$ -dependent transporter, referred to as the apical sodium-dependent bile acid transporter (ASBT). Trihydroxy (i.e. cholic acid) bile acids are favored over dihydroxy (i.e. chenodeoxycholic acid) bile acids and conjugated over unconjugated species. Similar to NTCP, ASBT is not fully operative at birth and the inability to conserve bile salts may contribute to the diminished bile salt pool size and 'physiologic steatorrhea' of the immediate postnatal period. Mutations in ASBT have been associated with idiopathic bile acid diarrhea, a chronic diarrheal illness characterized by bile acid malabsorption, lack of association with other forms of ileal dysfunction, and a response to cholestyramine.

Intracellular transport of bile acids in enterocytes is mediated by several cytosolic and microsomal proteins. The best characterized of these proteins is the ileal lipid-binding protein, a member of the fatty acid-binding protein family. At the basolateral membrane of the ileum, bile acids leave the enterocyte by a  $\text{Na}^+$ -independent anion exchange process, recently attributed to a member of the organic anion transporting polypeptide family, oatp3.

The small fraction of bile acids that escapes active or passive absorption in the small intestine undergoes bacterial modification in the colon. The secondary bile acids formed are also reabsorbed to various degrees, depending on their physicochemical properties, their interaction with luminal constituents, and the permeability characteristics of the colon. Lithocholate and deoxycholate, formed from the 7-dehydroxylation of chenodeoxycholate and cholate, respectively, are the major fecal bile acids in humans.

### Bile acid synthesis

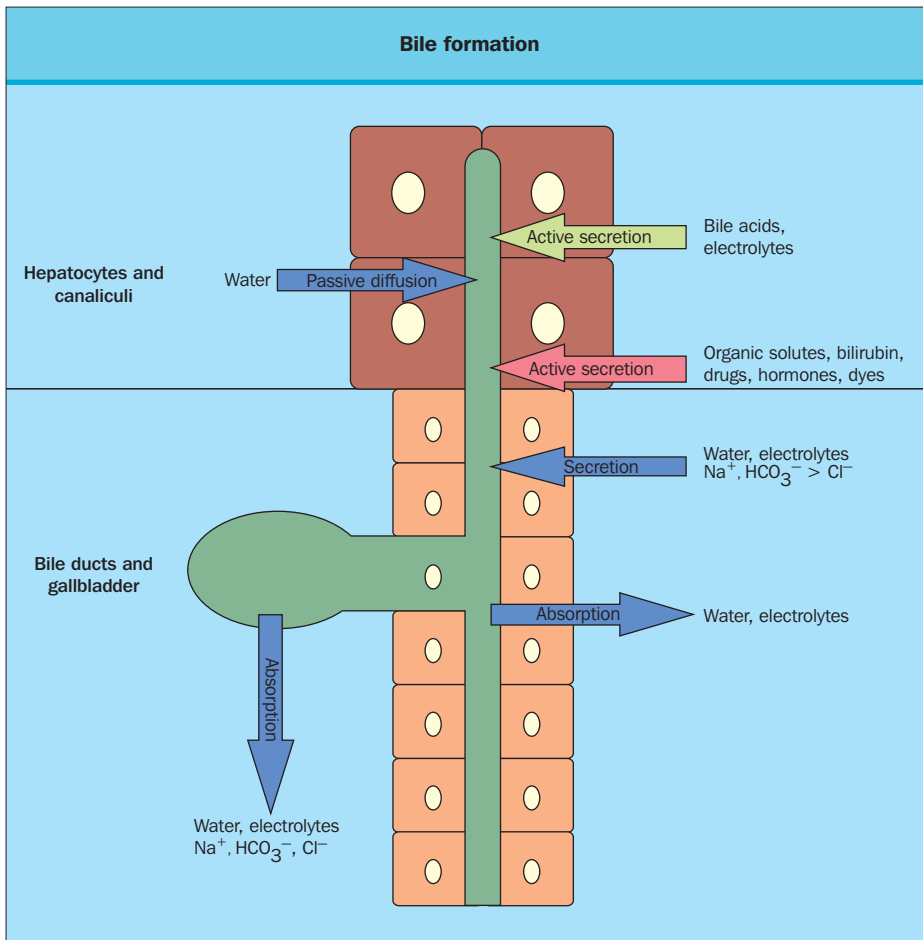
Continuous bile acid synthesis from cholesterol is required to maintain the bile acid pool in the enterohepatic circulation. The maximal rate of synthesis is on the order of 4–6 g/day. The importance of bile acid synthesis in health is evident if the effects of a cessation in synthesis are considered. Fecal loss would not be replenished, cholesterol would not be excreted, bile acid-dependent bile formation would stop, and fat-soluble substances would not be absorbed.

## CENTRAL METABOLIC MECHANISMS

Apart from its unique role in biliary metabolism, the liver is central to the metabolism of lipids, carbohydrates and proteins, as well as being closely involved in drug metabolism. Some of these metabolic pathways are summarized in Figure 3.17.

### Cholesterol and lipoprotein metabolism

The liver plays a pivotal role in normal cholesterol and lipoprotein metabolism. Cholesterol homeostasis is maintained



**Figure 3.15 Bile formation.** Bile formation involves the active transport of electrolytes, bile acids and organic solutes such as bilirubin. Water follows passively into the canaliculi (top). Bile is modified in the bile ducts and gallbladder by secretion and subsequent absorption of electrolytes and water. Modified from Scharschmidt in Zamkin et al (90) by permission of Elsevier.

by pathways that either increase or decrease hepatic cholesterol. Pathways that increase hepatic cholesterol include uptake from lipoproteins [chylomicrons and low-density lipoprotein (LDL)] and de novo synthesis of cholesterol, regulated by the enzyme, 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase. Cholesterol present in the liver exists either as cholesterol esters, a storage form, or free cholesterol. Concentrations of these two forms of cholesterol are governed by acyl-CoA:cholesterol acyltransferase which esterifies free cholesterol, and cholesterol ester hydrolase which hydrolyzes cholesterol esters. The two major pathways that decrease hepatic cholesterol are synthesis of bile acids from free cholesterol and biliary excretion of free cholesterol.

The pathways responsible for cholesterol homeostasis are tightly regulated. Cholesterol inhibits HMG-CoA reductase and bile acids decrease bile acid biosynthesis from cholesterol by inhibition of cholesterol 7 $\alpha$ -hydroxylase.

The major lipoprotein secreted by the liver is very-low-density lipoprotein (VLDL). The amounts of free fatty acids taken up by the liver and diet are the principal regulatory factors in the rate of production, composition and rate of secretion of VLDL.

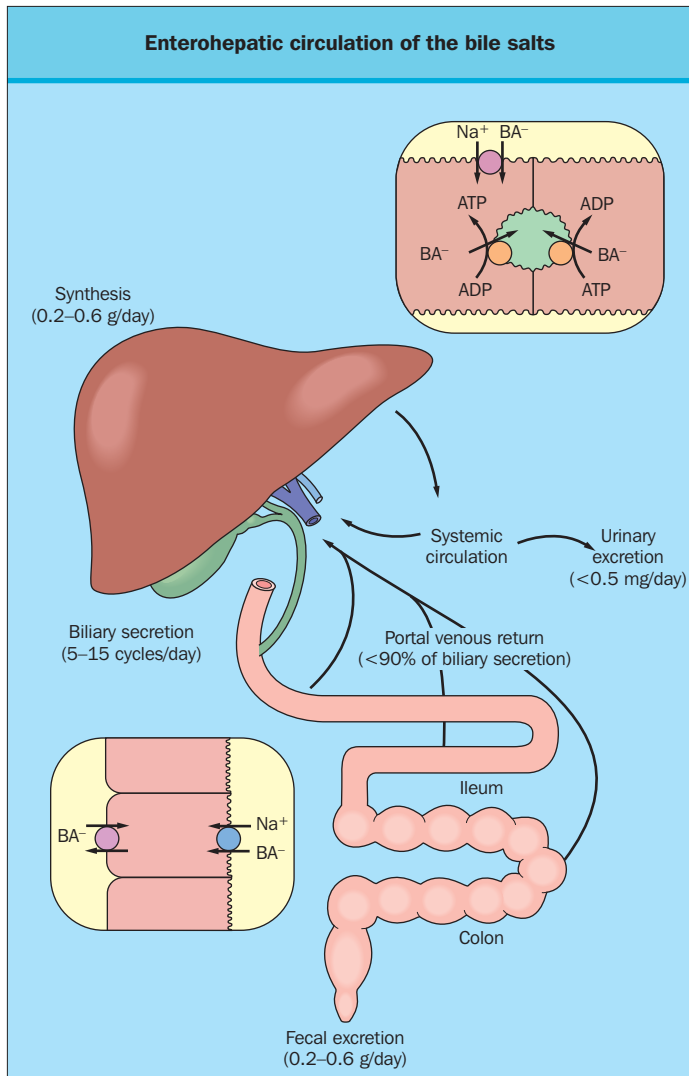
Biliary bile acids promote endogenous lipid secretion and stabilize the physicochemical state of bile. Biliary output of non-esterified cholesterol and phosphatidylcholine (PC) (i.e. lecithin), the two major lipids in bile, is curvilinearly related to bile acid output. Lecithin secretion exceeds cholesterol secretion.

Bile salts appear to promote biliary secretion of phospholipids following their secretion into bile by preferential partitioning into microdomains of the outer leaflet of the canalicular membrane that are also enriched with biliary-type PCs. These bile salts in the canalicular lumen induce vesiculation of the canalicular membrane. The inner or cytoplasmic leaflet of the canalicular membrane is resupplied with biliary-type PCs derived from smooth ER membranes by PC transfer protein and sterol carrier protein 2. Translocation of phospholipid from the inner to the outer leaflet of the canalicular membrane bilayer is mediated by the product of the *mdr2* gene, the mouse homolog of human *MDR3*.

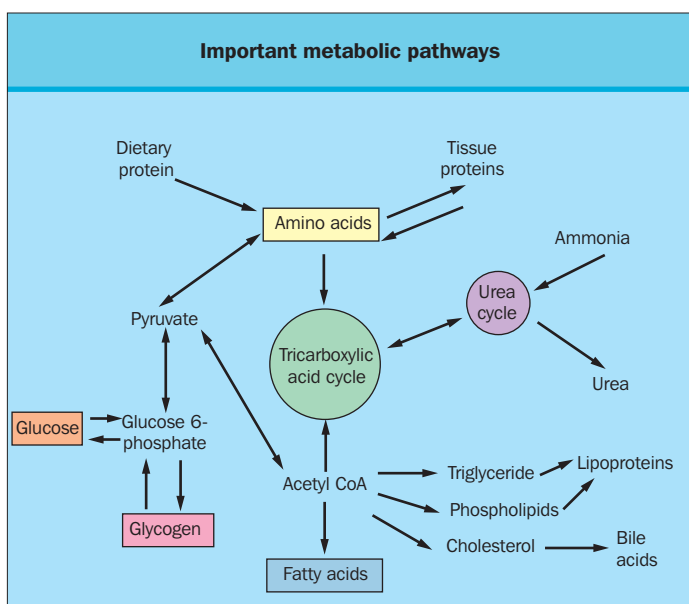
The pathway for phospholipid secretion proposed in **Figure 3.18** appears to be specific for PCs; although the mechanism is unclear, a largely separate secretory pathway exists for cholesterol secretion. However, the initiating event in the pathogenesis of cholesterol cholelithiasis may involve disruption of the coupling of biliary cholesterol excretion to simultaneous secretion of phospholipid and bile acids.

### Drug metabolism

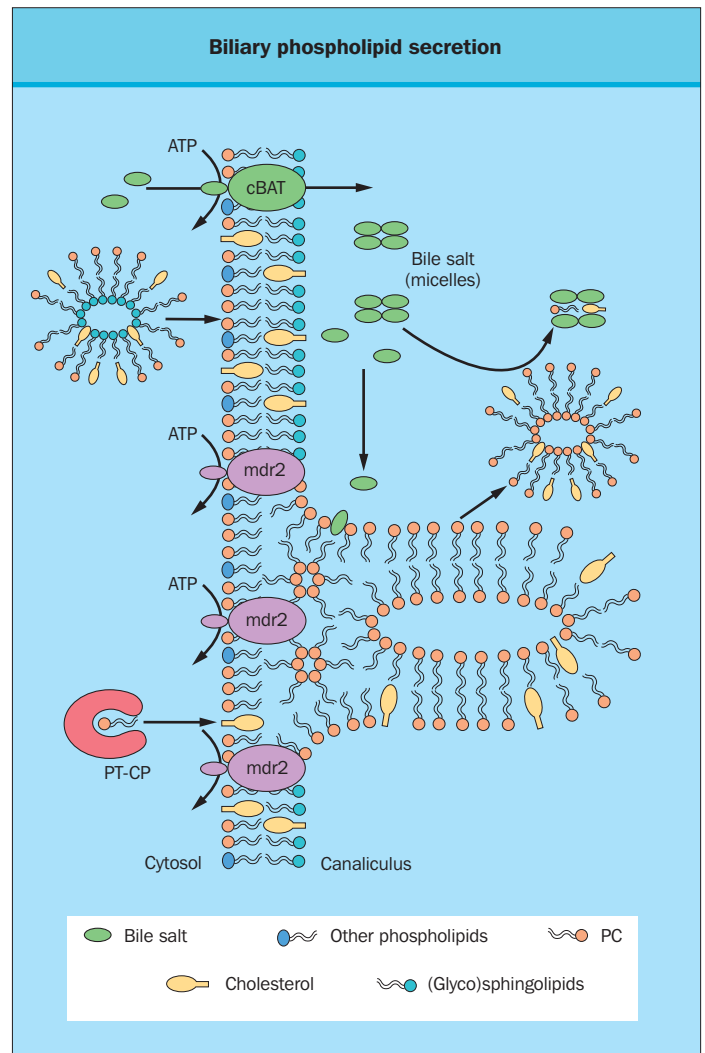
The liver plays a major role in drug metabolism or biotransformation. Products of hepatic biotransformation destined for urinary elimination are transported back into sinusoidal blood. Organic lipophilic drugs undergo biliary excretion, because these substances are poorly filtered at the glomerulus and minimally secreted by the renal tubule. Hepatic uptake of



**Figure 3.16 Enterohepatic circulation of bile salts and typical kinetic values for healthy humans.** Insets depict bile acid transport at the level of the hepatocyte and the enterocyte. BA, bile acid.



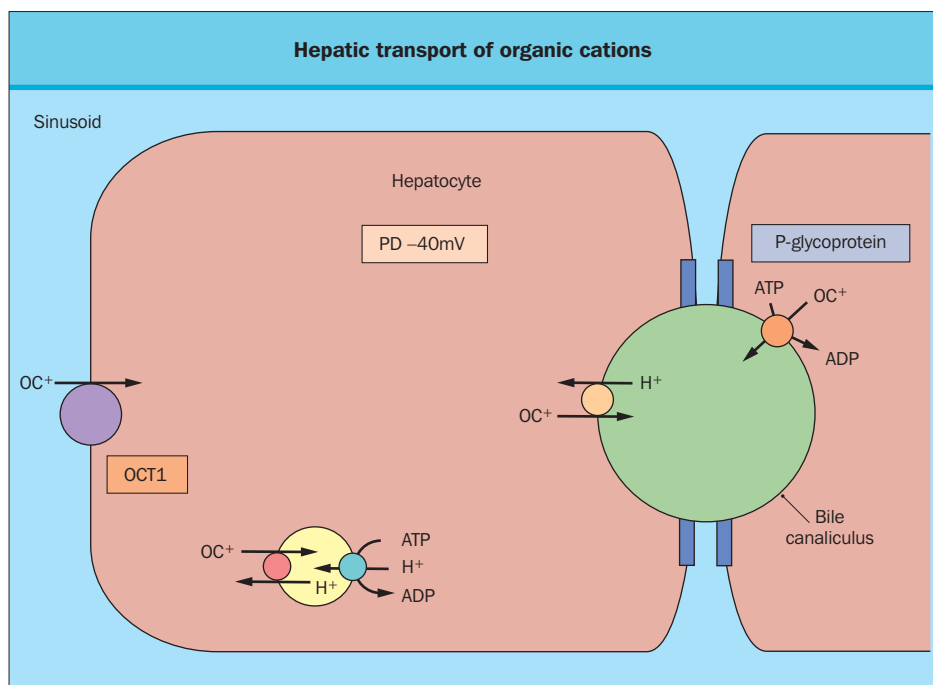
**Figure 3.17 Important metabolic pathways of proteins, carbohydrates and lipids in the liver.**



**Figure 3.18 Biliary phospholipid secretion.** Phosphatidylcholine (PC) is delivered to the cytoplasmic leaflet of the canalicular membrane by phosphatidylcholine transfer protein (PC-TP). PC is then translocated to the outer leaflet by mdr2, resulting in relative phospholipid excess in microdomains of the canalicular membrane. In the presence of bile salts, secreted into the lumen by the canalicular bile acid transporter (cBAT), SPGP, these microdomains are destabilized and vesicular structures develop that pinch off to yield biliary lipid vesicles.

many drugs is mediated by the same transport processes that are involved in hepatic bile formation discussed above. In addition, the hepatic uptake of organic cations, a class of substances that accounts for about 70% of drugs used in clinical practice, is mediated by a membrane potential-dependent carrier on the sinusoidal membrane, termed OCT1 (Fig. 3.19). Once the liver takes up drugs, they are processed or metabolized by several families of enzymes located in the endoplasmic reticulum, the cytosol, and, to a lesser extent, other organelles. The P450 cytochromes are a family of hemoproteins situated predominantly in the endoplasmic reticulum of the hepatocyte in a membrane-bound form. Drug metabolism by the P450 system, although typically detoxifying, occasionally produces toxic intermediates such as electrophiles and free radicals that, if not further metabolized, can lead to covalent binding to cellular proteins and membrane lipid peroxidation, respectively. Conjugation





**Figure 3.19 Hepatic transport of organic cations.** The uptake of organic cations ( $\text{OC}^+$ ) is mediated by a membrane potential-dependent carrier on the sinusoidal membrane, termed OCT1. Organic cations may then be sequestered into acidified intracellular organelles by an  $\text{H}^+$ -ATPase-dependent organic cation- $\text{H}^+$  exchanger. In addition to P-glycoprotein-mediated organic cation efflux, biliary secretion of small-molecular-weight organic cationic drugs may be mediated by an organic cation- $\text{H}^+$  exchanger.

with glutathione is mediated by cytosolic enzymes, the glutathione S-transferases, and plays an important role in the detoxification of electrophiles produced by the P450 system. However, glucuronidation and sulfation are more frequently employed in hepatic biotransformation and are mediated by microsomal UDP-glucuronyl transferases and cytosolic sulfotransferases, respectively.

Hepatic biotransformation can thus be classified into two phases. Phase I reactions involve oxidation or reduction of the parent compound, often generating a carboxyl, hydroxyl, or epoxide group that can subsequently be conjugated in a phase II reaction with, for example, glucuronic acid, glutathione, sulfate, or acetate. However, many drugs do not undergo sequential phase I and II reactions. The effect of phase I reactions is increased polarity, or water solubility, and, therefore, enhanced excretory potential. Phase II reactions further increase the water solubility of a compound. Although the products of phase II reactions tend to be less toxic and less active than the parent compound, phase I reactions may produce toxic intermediates. Exposure to specific substrates of a hepatic drug-metabolizing enzyme may induce the activity of that enzyme. Such induction may be clinically relevant if the enzyme induced produces a toxic intermediate. One of the best examples of such a phenomenon is acetaminophen (paracetamol) hepatotoxicity. Although metabolized largely by sulfation and glucuronidation (phase II reactions), a small percentage of the drug is oxidized by cytochrome P450 to a reactive metabolite. Chronic alcohol exposure induces this form of cytochrome P450, providing an explanation for the enhanced susceptibility of chronic alcoholics to acetaminophen hepatotoxicity.

A specific example of a biliary excretory mechanism for drugs is the multidrug transport protein, known as P-glycoprotein or 9170, encoded by the *MDR1* gene. Originally described in tumor-derived tissue culture systems resistant to cytotoxic hydrophobic agents, such as vinblastine, vincristine and

daunomycin, this ATP-dependent drug efflux system is located on the canalicular membrane and the apical surface of biliary epithelial cells lining small biliary ductules. This selective localization suggests that this membrane transport protein may serve as a pathway for the detoxification of physiologic metabolites and chemotherapeutic agents. In addition to P-glycoprotein-mediated drug efflux, biliary secretion of small-molecular-weight organic cationic drugs may be mediated by an organic cation- $\text{H}^+$  exchanger, a process that shares similarities with renal drug transport (see Fig. 3.19).

### Carbohydrate metabolism

Carbohydrate metabolism by the liver appears to be compartmentalized. In the fed state, the liver is involved in glycogen synthesis from glucose and glycolysis, metabolic processes that are preferentially located in perivenular hepatocytes. In the postabsorptive or fasted state, there is a shift from glucose uptake to glucose production mediated by glycogenolysis and gluconeogenesis predominantly in periportal hepatocytes.

Glucose is not the only ingested carbohydrate that is metabolized by the liver. Fructose is metabolized by the liver via a unique pathway and the metabolism of galactose depends on a conversion to glucose that occurs almost exclusively in the liver. Portosystemic shunting in cirrhosis results in impaired galactose elimination capacity, a finding that may have prognostic importance.

### Fatty acid metabolism

Fatty acids continuously cycle between the liver and adipose tissue. During the fed state, hepatocytes synthesize fatty acids that are then incorporated into lipoproteins to be delivered to adipocytes. During fasting, fatty acids derived from triglycerides stored in adipocytes are delivered to the liver where they undergo oxidation to ketone bodies in mitochondria and, in the case of very long chain fatty acids, in peroxisomes. In cirrhosis, fatty

acids are preferentially used as an energy source, even in the non-fasted state. Mitochondrial oxidation of fatty acids depends on the availability of the amino acid carnitine. Carnitine deficiency may play a role in valproate-induced hepatotoxicity and defects in mitochondrial fatty acid oxidation underlie disorders such as acute fatty liver of pregnancy, Reye syndrome and Jamaica vomiting sickness.

### Ammonia metabolism

Urea synthesis in the liver, through the Krebs–Henseleit cycle, is required for the disposal of the toxic product of nitrogen metabolism, ammonia. Glutamine synthesis plays a minor role in overall ammonia metabolism. Removal of ammonia from the circulation is compartmentalized in the liver. Carbamoylphosphate synthetase, the key regulatory enzyme in urea synthesis from ammonia, is expressed in all but the last one or two hepatocytes surrounding the terminal hepatic venule. The last one or two perivenous hepatocytes, in contrast, express glutamine synthetase and avidly scavenge any remaining ammonia from the circulation to form glutamine, which is subsequently released into the terminal hepatic venule and the systemic circulation. Elevated serum ammonia levels are often observed in both acute and chronic forms of liver disease. The striking elevations seen in fulminant hepatic failure are the result of impaired conversion of ammonia to urea in the setting of severe hepatocellular necrosis, whereas the hyperammonemia present in patients with cirrhosis and portal hypertension reflects loss of glutamine synthetic capacity by the perivenous scavenger cells in the liver as well as portosystemic shunting of ammonia derived from colonic bacteria. Additional factors that influence the level of serum ammonia in patients with cirrhosis include intestinal production of ammonia by bacterial deamination of blood or dietary protein, renal production of ammonia by glutaminase in response to metabolic alkalosis or hypokalemia, intestinal production of ammonia from urea by urease-forming bacteria in the setting of diminished renal function, and hepatic production of ammonia from amino acids in response to increased glucagon secretion.

### Protein synthesis

Albumin is the single most abundant serum protein and the liver synthesizes and exports up to 12 g of albumin/day. Besides serving as a vehicle for the transport of many drugs, serum albumin is a critical factor in the maintenance of plasma oncotic pressure. Albumin synthesis is regulated by changes in nutritional status, osmotic pressure, systemic inflammation and corticosteroids.

The liver also plays an important role in normal blood coagulation. Normal serum activities of the vitamin K-dependent coagulation-factor proenzymes (factors II, VII, IX and X), as assessed by the one-stage prothrombin time, depend on intact hepatic synthesis and adequate intestinal absorption of lipid-soluble vitamin K. Vitamin K is required for the post-translational formation of  $\gamma$ -carboxyglutamyl residues that are essential for physiologic activation of the factors. Prolonged prothrombin times can be observed in both hepatocellular disorders that impair hepatic synthetic function, such as hepatitis and cirrhosis, and in cholestatic syndromes that interfere with lipid absorption. Hepatocellular injury can be differentiated from cholestatic causes of prothrombin time prolongation by the

parenteral administration of vitamin K; intact hepatic function is established by an improvement in prothrombin time greater than 30% within 24 h of administration.

The liver is also a major site for the synthesis of antithrombin III, protein C and protein S. Despite reductions in these plasma inhibitors of hemostasis in chronic liver disease, thrombosis is a rare complication, presumably because of a disproportionate deficiency in procoagulants in most patients.

## HEPATOCTYE HETEROGENEITY ALONG THE LIVER CELL PLATE

Along the liver cell plate, hepatocytes in general appear histologically uniform. However, when examined on a morphologic or histochemical basis, hepatocytes exhibit marked heterogeneity. This heterogeneity (also called zonation) is manifested by cells located in the periportal zone that differ from those downstream in the perivenous zone. Hepatocytes demonstrate zonation with respect to key enzymes, cell receptors, subcellular structures and cell matrix interaction (Fig. 3.20). The heterogeneity is expressed by activation of the cellular genome in response to various inputs, including concentration gradients in hormones, oxygen, metabolic substrates and neural input.

In the 1960s and 1970s, it became clear that there was a zonal organization for specific groups of enzymes and the concept of 'metabolic zonation' became popular. In the original model of metabolic zonation, only two zones of equal size were defined. Later, as the concept of zonation became more prevalent, the periportal and perivenous compartments were subdivided into a proximal and distal part creating four zones.

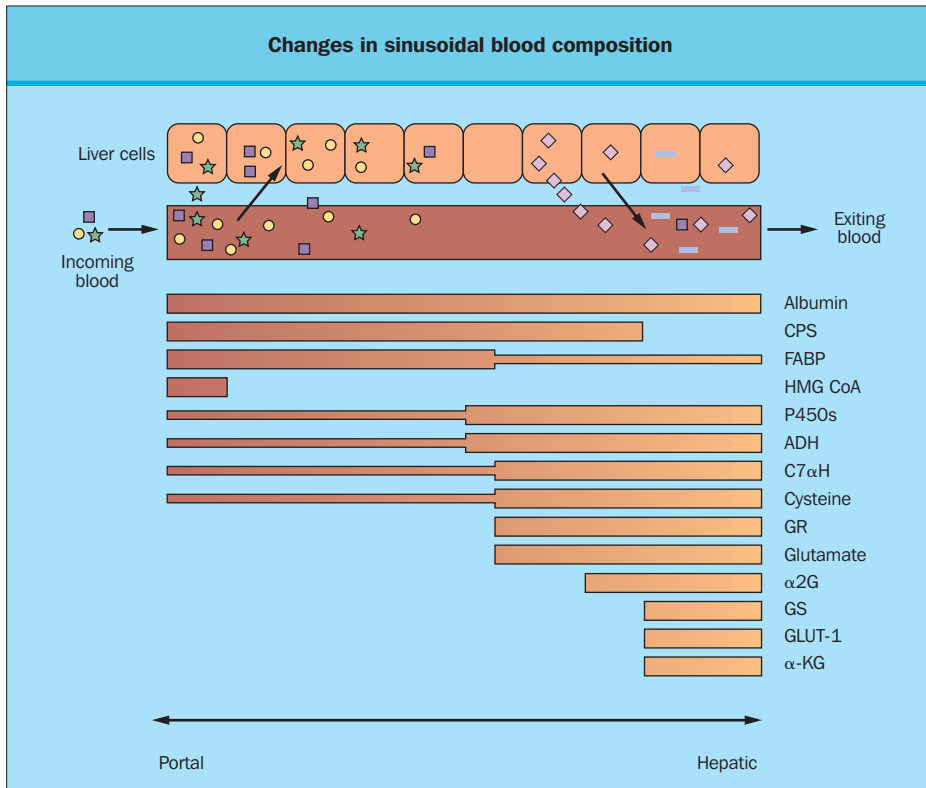
### Zonation of specific metabolic pathways

Numerous experimental approaches using perfused livers, isolated hepatocytes and hepatocyte cultures have been used to study differences in the metabolic specialization of hepatocytes. There are clear zonal differences in the metabolism of carbohydrates, amino acids and ammonia, and fatty acids. Bile formation as well as the metabolism of xenobiotics demonstrate zonation. In the following sections, there is a brief overview of the current understanding of the zonation of each of these pathways (Table 3.2).

### Carbohydrate metabolism

Zonation of carbohydrate metabolism is divided into two phases: absorptive and postabsorptive phase. During the absorptive phase, the perivenous cells primarily take up glucose. In these cells, it is used to synthesize glycogen. Following replacement of glycogen stores, glucose is converted to lactate and released into the hepatic veins. Lactic acid is then taken up by the periportal cells, where it is used as a substrate for gluconeogenesis. This concept is consistent with the observation that glycogen stores are refilled first in the perivenous hepatocytes whereas glycogen degradation starts periportal.

In the postabsorptive phase, glycogen is degraded to glucose in the periportal hepatocytes. As the glucose proceeds down the sinusoid, it is taken up by perivenous cells and degraded to lactate. Lactate is again released into the circulation, and, if unused by the peripheral circulation, it is used as a substrate for gluconeogenesis by periportal cells.



**Figure 3.20 Changes in sinusoidal blood composition.** Sinusoidal blood composition changes as incoming solutes are removed by liver cells (top). As solutes are taken up by hepatocytes, their concentration decreases. 'Perivenular' plasma contains increased concentrations of material that have been secreted by zone 3 hepatocytes. Hepatic gene expression along the liver cell plate (bottom). The 20 to 30 hepatocytes lining the liver sinusoid contain varying amounts of key enzymes. (CPS, carbamoyl phosphate synthetase; FBP, fatty acid-binding protein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; P-450s, cytochromes of the P-450 family; ADH, alcohol dehydrogenase; C7 $\alpha$ H, cholesterol 7  $\alpha$ -hydroxylase; Cysteine, cysteine transporter; GR, glucagon receptor; Glutamate, glutamate transporter;  $\alpha$ 2G,  $\alpha$ 2-globulin; GS, glutamine synthetase; GLUT-1, glucose transporter;  $\alpha$ -KG,  $\alpha$ -ketoglutarate transporter.) Modified from Gumucio et al in Kaplowitz (96) by permission of Lippincott, Williams & Wilkins.

Heterogeneity of hepatocyte metabolism and biotransformation					
	Periportal (zone 1) cells			Pericentral (zone 3) cells	
Physiologic function	Metabolic function	Enzyme involved	Physiologic function	Metabolic function	Enzyme involved
Oxidation		Succinate dehydrogenase			
Glucose formation	1. Gluconeogenesis 2. Glucose from glycogen 3. Glycogen from pyruvate	Glucose-6 phosphatase Phosphoenolpyruvate carboxykinase	Glucose uptake	1. Glycolysis 2. Glycogen from glucose 3. Glycogen to pyruvate	Glucokinase
Urea formation	Urea from amino acid nitrogen and from NH <sub>3</sub>	Carbamoyl phosphate synthetase	Glutamine formation		
Protective metabolism	1. Glutathione peroxidation 2. Glutathione conjugation	Glutathione peroxidase Glutathione level	Xenobiotic metabolism	1. Mono-oxygenation 2. Glucuronidation	Cytochrome P450 UDP-glucuronosyl-transferase
Plasma protein synthesis		Albumin	Plasma protein synthesis		α-Fetoprotein
		α <sub>2</sub> -Macroglobulin Fibrinogen			Angiotensinogen α <sub>1</sub> -Antitrypsin
Cholesterol synthesis	Hydroxymethyl glutaryl-coenzyme A reductase				
Bile formation	Taurocholate uptake carrier Bile acid export carrier				

**Table 3.2 Heterogeneity of hepatocyte metabolism and biotransformation.**



**Fatty acid metabolism**

The zonation of fatty acid metabolism is less pronounced than that described for carbohydrate metabolism. Perivenous cells appear to synthesize preferentially VLDL, whereas periportal cells perform  $\beta$ -oxidation and ketogenesis. Fatty acid-binding protein, the chief intracellular transport protein for long chain fatty acids, is located predominantly in periportal cells. It is unclear whether the presence of this intracellular carrier promotes the preferential  $\beta$ -oxidation and ketogenesis seen in periportal cells. HMG-CoA reductase is located almost exclusively in periportal cells.

**Amino acid and ammonia metabolism**

Nutrient proteins in the form of amino acids are supplied to hepatocytes via the portal vein. Periportal cells preferentially convert the ammonia to urea. If the quantity of ammonia presented to the periportal region is sufficient to escape ureagenesis, perivenous hepatocytes take up the ammonia and convert it to glutamine. Glutamine is then released into the systemic circulation and returned to periportal cells, where again the ammonia is preferentially formed into urea. The periportal and perivenous systems have different kinetics with the periportal system being a high-capacity, low-affinity pathway and the perivenous pathway having a high affinity but a low capacity. Work using perfused livers has demonstrated that the removal of ammonia in periportal cells (urea synthesis) is restricted to incoming ammonia concentrations higher than 50  $\mu$ M and that glutamine synthesis in the perivenous cells occurs at concentrations of ammonia below 50  $\mu$ M.

**Zonation of xenobiotic metabolism**

The cytochrome P-450 system is responsible for the metabolism of many xenobiotics. This process often involves mono-oxygenation followed by conjugation with either glucuronic or sulfuric acid. Sulfate formation predominates in periportal cells, whereas glucuronide formation is the major conjugation reaction in perivenous cells. The mono-oxygenation also occurs preferentially in the perivenous zone, leading to the formation of potentially toxic electrophiles. The perivenous zone, therefore, is predisposed to the toxicity of reactive oxygen intermediates. The combination of this predilection as well as the decreased ability of the cell to detoxify oxygen intermediates by glutathione leads to the perivenous necrosis caused by some hepatotoxins.

**Regulation of hepatocyte heterogeneity**

It is generally assumed that hepatocyte zonation develops as a consequence of the heterogeneity in the microenvironment of individual cells. This is controlled by various signals such as substrate concentration (including oxygen), hormones, neuro-

mediators, nerves and cell-to-biomatrix interactions. The differential expression patterns seen in the zonation may be attributed to different rates of mRNA transcription, mRNA degradation, mRNA translation, or end-protein degradation.

The expression of the genes for the enzymes of gluconeogenesis is regulated at the pretranslational level. Similarly, the amino acid metabolizing enzymes are regulated at the pretranslational level. Each of these alterations in gene expression occurs during a normal feeding rhythm. In contrast, glycolytic enzymes appear to be regulated mainly at the post-translational level during a normal feeding pattern. Each of these generalities regarding the regulation of zonal gene expression may change depending on the nutritional conditions.

Ammonia detoxification also appears to be regulated at the pretranslational level. Both the mRNA and the proteins of the key ureagenic enzymes are located exclusively in the periportal cells. In contrast, the mRNA and the protein for glutamine synthesis are located exclusively in parenchymal cells in the distal perivenous area. Neither these mRNA nor the enzyme levels themselves seem to vary with the nutritional state, suggesting that the organism needs to maintain tight control over ammonia despite external influences.

**Physiologic significance of hepatocyte heterogeneity**

The pattern of organization described earlier indicates the complexity of the liver's ability to respond to a large variety of input and output solute concentrations and the overall metabolic demands of the organism. Two control elements seem to dictate the degree of zonal responses: the sequential delivery of substrates and the zonal patterns of gene expression. Because of the unidirectional perfusion of the liver acinus, periportal cells are exposed to a high concentration of incoming solutes. Solutes using transport systems that have a great capacity for uptake (ammonia and bile acids) are preferentially taken up by the first hepatocytes that they encounter. Refluxed material or substances escaping uptake by periportal cells are subsequently presented to 'downstream' cells. The removal of ammonia, either as urea or by glutamine synthesis, is an example of this complexly controlled system. Each of these systems allows minute adjustments in the outflow concentration of substances of a splanchnic origin as well as new products formed by the synthetic machinery of the liver.

In summary, the zonation of hepatocytes allows the liver acinus to accomplish the regulation of substrate production and the metabolism of proteins and hormones in a dynamic fashion. Zonation appears to depend on the unidirectional perfusion of substrates as well as on basic gene expression by hepatocytes. Each of these control mechanisms is dynamic, responding to the overall needs of the organism.

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## SECTION II: EVALUATION OF PATIENTS WITH LIVER DISEASE

### CHAPTER

# 4

## History and Physical Examination

Peter D. Howdle

### INTRODUCTION

Patients with liver disease present in a variety of ways and have a multiplicity of symptoms, and signs, of varying severity. At one end of the spectrum is the patient who is found incidentally to have an abnormal liver test but no clinical features of liver disease; at the other end is the patient who has obvious symptoms and signs of acute or chronic hepatocellular failure. Some of these symptoms and signs are quite specific for liver disease; others are suggestive but may also reflect disease or dysfunction in another system. Thus the physician has to maintain a high standard of clinical expertise and a low diagnostic threshold for liver disease in order to evaluate patients properly.

### THE HISTORY

Although good clinical practice always entails obtaining a comprehensive history from the patient, this is especially true for patients with liver disease. Some vague or non-specific symptoms may be of considerable significance e.g. lethargy, itch, and their importance may not be apparent to the patient. Important clues regarding the etiology of liver disease may also be gleaned from social behavior many years previously (e.g. needle-sharing when using IV drugs up to 30 years previously), travel experiences, current and previous exposure to therapeutic medications, and family history (e.g. history of emphysema in an older generation might be a clue that  $\alpha_1$ -antitrypsin deficiency is the cause of liver disease). Some patients may be reticent about disclosure of relevant issues, especially with regard to sexual activity, drug abuse and alcohol consumption. These issues need to be handled sensitively in order to obtain the most accurate possible history.

The quality of the history available in patients with liver disease may be suboptimal because of impairment of consciousness or higher mental functions. Usually this is due to the presence of encephalopathy or cognitive dysfunction (see below). Subclinical encephalopathy can be difficult to detect in the absence of formal psychometric evaluation. Accompanying histories from family or friends may provide valuable insight in these situations.

### SYMPTOMS OF LIVER DISEASE

#### General ill health

Weakness, increased fatigability and general malaise are commonly encountered, but obviously non-specific symptoms of both acute and chronic liver disease have been estimated to occur in up to 60% of patients. Lethargy is particularly prominent in primary biliary cirrhosis (PBC) and chronic viral

hepatitis and is often disproportionately severe given the stage of the underlying liver disease. It is not uncommon, however, for such symptoms to persist for a considerable period of time following acute hepatitis. These symptoms may improve rapidly when specific treatment is given for the liver disease (particularly following successful liver transplantation). However, in many cases there is no effective treatment for the weakness and malaise, the pathogenesis of which is poorly understood. Altered manganese homeostasis in the central nervous system has been postulated as a contributory factor.

#### Gastrointestinal symptoms

Gastrointestinal symptoms are common in liver disease, although they are very non-specific. Anorexia, particularly in jaundiced patients, frequently occurs in acute hepatitis of whatever cause. Long-term anorexia inevitably leads to weight loss; in patients who have end-stage chronic liver disease the muscle wasting and loss of adipose tissue is often striking. There is often a remarkable contrast between the thin, wasted chest and the distended abdomen in end-stage patients who have developed ascites.

Nausea and vomiting are common with gallstones and with many forms of liver disease. Nausea is noteworthy in the prodromal phase of acute hepatitis, while vomiting is particularly associated with obstructive biliary disease. Severe nausea and vomiting occur in the first 24–48 h after acetaminophen overdose but tend to settle before the expression of severe liver injury. It should be remembered that excess alcohol also causes these symptoms, particularly in the morning.

Hematemesis is frequent in patients with liver disease. In patients with portal hypertension it should be assumed that the site of bleeding is esophageal or gastric varices until proven by investigation (see Ch. 9). Alternative explanations for bleeding are found in a substantial cohort of patients. In acute liver failure, patients often develop erosive gastritis (see Ch. 33).

Abdominal pain is also common in hepatobiliary disease. It can be present anywhere across the upper abdomen, but is frequently localized to the right upper quadrant. Many chronic liver diseases are associated with a dull ache which has been attributed to stretching of the capsule of the liver. The pain may be more severe if there is rapid or gross enlargement of the liver. Tenderness over the liver is observed in acute hepatitis, hepatic abscess or hepatic malignancy. Peritoneal pain is present if the capsule and/or parietal peritoneum is involved. Severe pain, especially if associated with jaundice or fever, suggests biliary disease and/or cholangitis. On occasion, an enlarged spleen is painful, especially if splenic infarcts develop.

Abdominal discomfort, as opposed to pain, may also be described by patients who have ascites. Patients who have gross





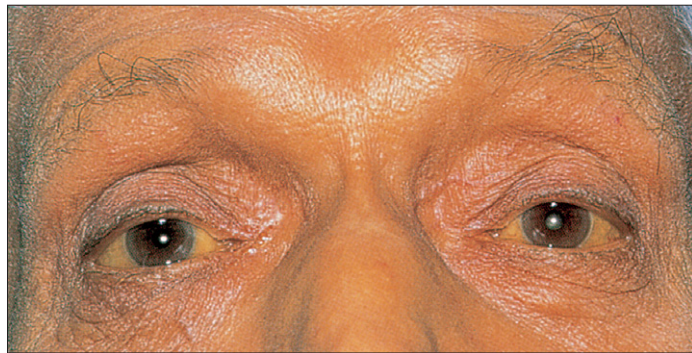
**Figure 4.1 Ascites in a patient with chronic liver disease.** There are dilated abdominal wall veins, striae and an everted umbilicus.

ascites often complain of the development of an umbilical hernia, difficulty in mobilization and dyspnea (Fig. 4.1). There is an increased risk of spontaneous bacterial peritonitis and this should always be borne in mind, particularly when a patient with ascites suddenly deteriorates. The sudden development of ascites raises the possibility of Budd-Chiari syndrome.

There is commonly a change in bowel function or stool appearance in liver disease. Mild diarrhea with soft stools is frequent. Stools suggestive of steatorrhea are less common, but if present suggest biliary obstruction or cholestasis; pancreatic insufficiency, often present in chronic alcoholism, should not be forgotten. Severe diarrhea should prompt the search for a cause other than liver disease, although associated conditions such as ulcerative colitis or celiac disease should be borne in mind. Drinkers of excess alcohol also develop diarrhea. Melena is obviously an important symptom and in patients with portal hypertension should trigger the same care plan described for hematemesis. Melena and constipation, although not a direct consequence of liver disease, are also relevant as common precipitants of hepatic encephalopathy (see Ch. 11).

### Jaundice and associated symptoms

Jaundice is the most striking symptom of hepatobiliary disease and is well recognized as such by lay people. It may initially be brought to the attention of the patient by friends, relatives or a primary care physician. Jaundice is overt when the serum bilirubin level exceeds approximately  $45 \mu\text{mol/L}$  ( $2.6 \text{ mg/dL}$ ) and is most easily discernible in the sclerae. The serum bilirubin may be much higher in a range of hepatobiliary diseases whether due to a major hepatocellular pathology or to extrahepatic cholestasis. The pattern of associated symptoms may help to differentiate between these two major categories of disease leading to jaundice. Prodromal symptoms of fever, nausea, anorexia and malaise suggest acute hepatitis. The urine may be dark with either hepatocellular or cholestatic disease, but the presence of pale stools strongly suggests cholestatic disorders. Symptoms of infection or severe pain also suggest biliary problems such as cholangitis or cholelithiasis. In hepatocellular damage there are often associated generalized symptoms and the patient feels unwell, whereas with a slowly evolving cholestatic disorder



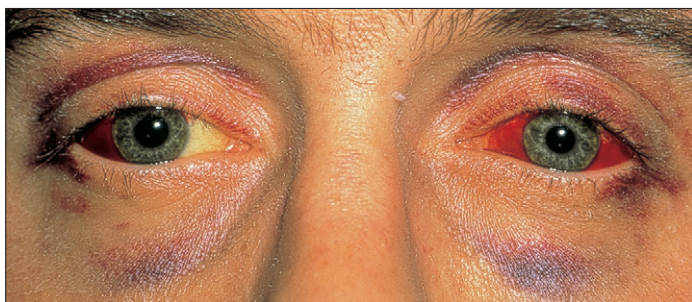
**Figure 4.2 Jaundice of the skin and sclerae in a patient with malignant nodes at the porta hepatis.**

the patient often feels surprisingly well despite being significantly jaundiced (Fig. 4.2). Progressive pruritus (prior to jaundice) suggests emerging cholestatic liver disease. Pruritus can be one of the most distressing symptoms of liver disease and when severe is fully deserving of professional sympathy and attention. It can, when incessant, become quite disabling and seriously interfere with sleep patterns. The latter is in part due to the fact that the pruritus is usually more intense at night.

### Circulatory, cardiorespiratory and hematologic symptoms

The fluid retention that is characteristic of advanced cirrhosis often results in manifestations other than ascites. Many patients will have dependent edema. Some will have a pleural effusion, usually right-sided, and on occasion this can be severe and represent the dominant manifestation of the fluid retention. Dyspnea may be due to splinting of the diaphragms by severe ascites, pleural effusions, pulmonary hypertension or hepatopulmonary syndrome. Oliguria and nocturia occur as fluid retention develops and liver disease increases in severity.

Spontaneous (or easily induced) bleeding is commonly seen in patients who have liver disease and are developing either acute or chronic liver failure. Petechiae and ecchymoses occur in chronic liver disease. Subconjunctival hemorrhages are often seen in acetaminophen-induced acute liver failure. These symptoms are attributable to the defective hepatic synthesis of coagulation factors combined with the thrombocytopenia that occurs in both acute and chronic liver failure. In severe acute liver failure, as seen in acetaminophen overdose, patients may develop an almost uncontrollable bleeding tendency (Fig. 4.3).



**Figure 4.3 Subconjunctival hemorrhage, periorbital bruising and jaundice in a patient who has acute liver failure.**

### Nervous system

The most striking neurologic symptoms occurring in liver disease are those associated with encephalopathy (see Ch. 11). Encephalopathy occurs to some degree in up to 80% of patients who have chronic liver disease; in such cases, it is chronic and variable in extent. In severe acute liver failure, encephalopathy develops rapidly with the increasing severity of the liver failure (see Ch. 33). Encephalopathy is a complex neuropsychiatric syndrome, but patients develop such symptoms as sleep disturbance, altered personality, deterioration of higher mental functions, increasing drowsiness and confusion and eventually coma. Feter hepaticus and a coarse flapping tremor are characteristic of encephalopathy.

More specific neurologic symptoms may occur if particular areas of the central nervous system are affected by pathologic change, for example symmetric demyelination of the cord leading to a paraplegic gait. Specific diseases can also lead to characteristic neurologic symptoms, for example the extrapyramidal features seen in Wilson disease, cerebellar dysfunction in alcoholic liver disease or the night-blindness characteristic of vitamin A deficiency associated with chronic cholestatic disease.

### Endocrine system

Sexual dysfunction and infertility are common in liver disease. Men develop decreased libido, impotence and sterility. Males may have painful or embarrassing gynecomastia (Fig. 4.4), or other features of feminization or testicular atrophy. Women are vulnerable to masculinization, reduced libido, oligomenorrhea and infertility. These symptoms are more severe in patients with alcoholic liver disease. Bilateral parotid enlargement is frequently seen in patients with alcoholic liver disease. Sexual function returns in many men within days of successful liver transplantation while menstruation and fertility can return within 3 months in premenopausal women.

### Past medical history

This is of great importance for a wide range of reasons, and examples include:

- a previous episode of jaundice might suggest chronic viral hepatitis, especially hepatitis B,

- transfusion of blood products could lead to acquisition of hepatitis B or C,
- a history of ulcerative colitis often predates the diagnosis of primary sclerosing cholangitis by considerable periods,
- gallstone related disease can be episodic and may recur after cholecystectomy, and
- previous biliary surgery may be the cause of a biliary stricture.

A history of neonatal jaundice or umbilical sepsis could also have relevance to patients presenting with liver disease or portal hypertension later in life. These examples illustrate the necessity for a comprehensive lifelong evaluation of the medical history in each patient.

### Family history

A positive family history may help to establish the cause of liver disease in some patients. An index case of a genetic liver disease may already have been established e.g. genetic hemochromatosis or Wilson disease. Patients with autoimmune liver disease often have relatives with the same or other autoimmune liver diseases, or non-hepatic autoimmune diseases like thyroid disease or rheumatoid arthritis. A family history of diabetes could be relevant to the diagnosis of genetic hemochromatosis, non-alcoholic steatohepatitis (NASH) or autoimmune hepatitis. A family history of hepatitis B may also be relevant, especially if the mother is involved (see Ch. 15). Alcoholism tends to have a familial tendency whether or not liver disease has been diagnosed.

### Drug history

Many drugs can cause hepatic dysfunction and disease, thus a careful drug history is important, even from the distant past. For example, hepatic dysfunction can occur after patients have been taking amiodarone or methotrexate for some months. Routine monitoring of liver function is recommended and full assessment may require liver biopsy. Drug-induced liver disease is discussed in detail in Ch. 32.

### Social history

Intravenous drug abuse, alcohol consumption, sexual orientation and practice, foreign travel and type of work (now and previously), including exposure to industrial chemicals and toxins, must all be enquired about when seeking a cause of liver disease in a newly presenting patient. The amount of alcohol consumed can be notoriously difficult to assess. Patients with hepatitis A often have a transient aversion to cigarette smoking.

## THE PHYSICAL EXAMINATION OF THE PATIENT WITH LIVER DISEASE

A comprehensive history should be complemented by a thorough physical examination with careful documentation of both the presence and absence of pertinent physical signs. The physical signs of liver disease vary in their significance. Some can be specific for a particular condition (e.g. Kayser-Fleischer rings in Wilson disease) or for a complication of liver disease (e.g. liver flap in hepatic encephalopathy). Others are quite non-specific but in the appropriate clinical setting do suggest the presence of liver disease. Thus, an excessive number of spider nevi is strongly indicative of cirrhosis in a male who abuses alcohol, but would be of no clinical relevance in a woman in the later stages of pregnancy.



**Figure 4.4**  
**Gynecomastia in a patient with chronic liver disease.** Striae are seen on the abdominal wall.



In order to elucidate properly and reliably the physical signs in liver disease, the young physician must practice regularly; some of the signs are genuinely difficult to elicit, and there is considerable inter- and intra-observer variation.

## SIGNS OF LIVER DISEASE

The physical signs found in liver disease are summarized in Figure 4.5.

### General examination

It is important to note the general body build of the patient. Muscle wasting, loss of fat deposits and thinning of the skin are all signs of malnutrition, which occur as chronic liver disease progresses. The onset of fluid retention can mask some of these signs. There is often a remarkable contrast between a thin wasted chest and a distended abdomen due to ascites (Fig. 4.6).

Patients who have acute liver disease usually look well-nourished. Obesity is strongly correlated with abnormal liver tests and other manifestations of NASH.

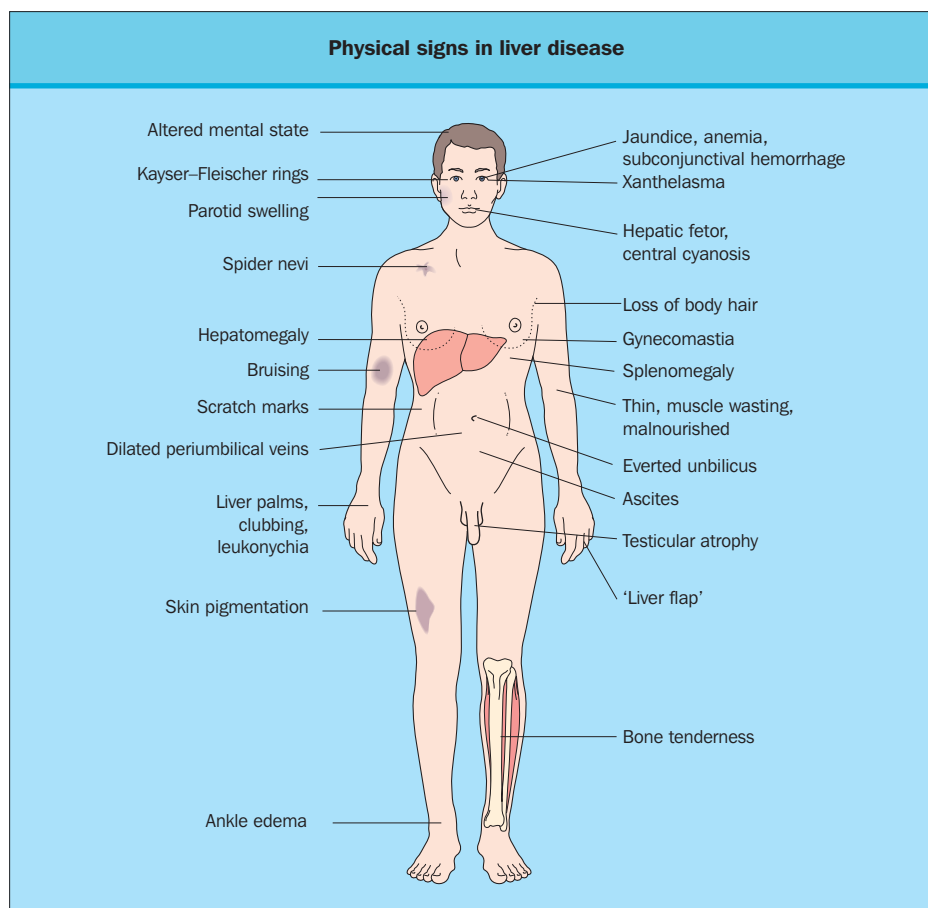
### Fever and infection

Patients who have liver disease have an increased risk of infection. Up to 50% of patients present with, or acquire in hospital, a bacterial infection. Urinary tract infections are common, as is pneumonia, particularly in alcoholics. Septicemia can quickly develop, leading to shock. Sepsis should always be suspected in



**Figure 4.6 Gross ascites in a patient who has chronic liver disease.** There is an umbilical hernia, dilated abdominal wall veins and pigmented skin. Marked muscle wasting is evident.

a patient with cirrhosis who deteriorates for no apparent reason. There is a poor correlation between body temperature and the presence or absence of infection. Approximately 30% of patients who have decompensated cirrhosis have a continuous low-grade fever, as do patients with alcoholic hepatitis. In 50% of these the fever persists for more than 2 weeks. Significant infection can be associated with normal or reduced body temperature. In



**Figure 4.5 Physical signs in liver disease.**



patients with severe chronic liver disease, there is a high mortality from infections (38%). In acute liver failure, fungal infections are an important complicating problem (see Ch. 33). Blood, urine, sputum and ascites should be cultured routinely and a chest X-ray should be obtained.

### Facial signs

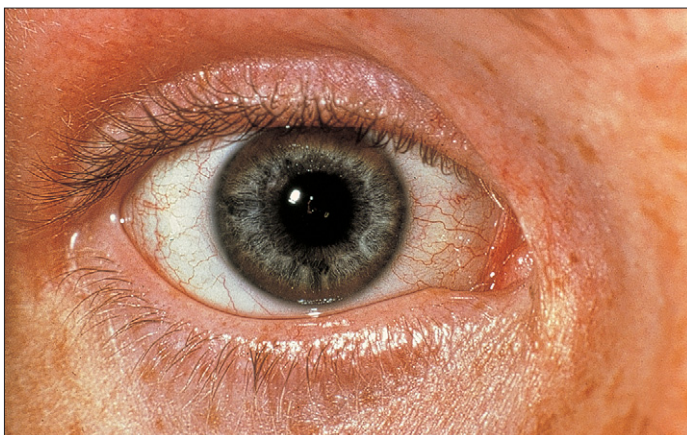
There are no particular facial characteristics denoting liver disease, but there are rare instances of specific features (e.g. Alagille syndrome – widely set eyes, prominent forehead, flat nose and small chin associated with congenital hepatic duct hypoplasia). Other facial signs include:

- telangiectasia as one of the common cutaneous stigmata of chronic liver disease,
- pseudo-Cushingoid appearance and parotid swelling in alcoholic liver disease,
- subconjunctival hemorrhage (see Fig. 4.3) which is commonly seen in severe acute liver failure,
- xanthelasma around the eyes suggesting chronic cholestatic liver disease (e.g. PBC) and,
- conjunctival pallor, suggesting anemia which is common in liver disease.

A specific sign in the eye is the Kayser-Fleischer (K-F) ring (Fig. 4.7). This is a ring due to the deposition of copper-containing pigment in the Descemet's membrane at the periphery of the posterior surface of the cornea. The K-F ring is usually greenish-brown in color; although visible to the naked eye, it is best seen by an ophthalmologist using a slit-lamp. It is present in more than 90% of all patients who have Wilson disease with neurologic signs, and in the majority of those with hepatic involvement (see Ch. 24). It may be absent (or very difficult to visualize) in approximately 50% of adolescents with acute presentations of Wilson disease. A similar ring may rarely be found in cases of chronic cholestasis or cirrhosis.

### Fetor

Hepatic fetor or fetor hepaticus complicates severe hepatocellular disease with associated encephalopathy. It is described as a sweet, slightly fecal smell of the breath which is presumed to be of intestinal origin, being particularly prevalent in patients with an extensive portal collateral circulation.



**Figure 4.7** A Kayser-Fleischer ring in a patient with Wilson disease.



**Figure 4.8** Jaundice in a patient with alcoholic liver disease. There is also a spider nevus and paper money skin.

### Skin signs

Jaundice is the physical sign most often regarded as synonymous with hepatobiliary disease (Figs 4.2 and 4.8). It is detectable by the physician when the serum bilirubin level exceeds approximately  $45 \mu\text{mol/L}$  ( $2.6 \text{ mg/dL}$ ). Patients are referred to as being 'jaundiced' or 'icteric'. The earliest sign is a yellow discoloration of the sclerae but as the serum bilirubin rises the jaundice is obvious in the skin and, if prolonged, the skin can develop a greenish tinge. Bilirubin binds preferentially to elastic tissues, but body fluids such as ascites, sweat or urine can also become yellow. There are several mechanisms affecting the depth of jaundice, although in general hepatobiliary diseases cause deeper jaundice than does hemolysis. The severity of the jaundice does not necessarily correlate with the prognosis of the underlying pathology.

Very rarely a yellow pigmentation of the skin may be due to excessive consumption of foods containing carotene or lycopene (e.g. tomatoes and carrots). Some drugs may also cause discoloration (e.g. mepacrine). However, in these circumstances, the sclerae remain white.

There are other signs in the skin that are strongly associated with liver disease. Hyperpigmentation is common in chronic liver disease, and is due to the increased deposition of melanin. It is seen particularly in primary biliary cirrhosis and hemochromatosis (bronze diabetes). Local areas of hyperpigmentation occur at sites of irritation, trauma or in more exposed areas. In addition, patients with marked hyperbilirubinemia can have general hyperpigmentation. Vitiligo is much more obvious in pigmented patients, and it is associated with autoimmune disease (e.g. PBC and chronic hepatitis). Pruritus is a frequent symptom of cholestatic conditions and associated scratch marks on the skin are commonly observed. These are in accessible areas and so the center of the back is usually spared. The nails in such patients are highly polished; this may be a striking phenomenon on examination.

Lichen planus is associated with PBC and the increased plasma lipids in chronic cholestatic conditions lead to xanthelasma around the eyes and xanthomata over pressure areas such as buttocks, knees and elbows (Fig. 4.9). Porphyria cutanea tarda is associated with hepatitis C, with up to 80% of these patients being hepatitis C virus (HCV) positive.

Excessive bruising and purpura occur in patients where the coagulation factors and platelet count are disturbed. Often sites of venipuncture show excessive extravasation of blood. Needle



**Figure 4.9** Extensive xanthoma in the neck of a pigmented patient with primary biliary cirrhosis.



**Figure 4.11** A large spider nevus on the cheek.

marks from intravenous drug abuse should be noted, since they may suggest the cause of viral hepatitis. Tattoos similarly should be noted. Leukocytoclastic vasculitis, a necrotic rash with transient erythema multiforme, may be an early manifestation of both hepatitis B and C infection.

Vascular 'spiders' are commonly seen in association with chronic liver disease. There are several synonyms: spider telangiectasia, spider nevi, arterial spiders, spider angiomas. The lesion consists of a central arteriole with numerous small radiating vessels resembling a spider's legs, hence the various names for this lesion (**Fig. 4.10**). Pressure on the central prominence causes blanching of the whole lesion. Occasionally such lesions can be seen or felt to pulsate. The spiders can vary in size from 1 mm to 1–2 cm in diameter (**Fig. 4.11**). They are found in the drainage area of the superior vena cava and are commonly found in the neck, face, arms and hands. Approximately 2–3% of normal individuals have two or three spiders, but any more than that, especially if they are developing or enlarging, suggests parenchymal liver disease. Spiders can occur briefly in acute hepatitis, but usually reflect cirrhosis. They can develop during pregnancy, but fade after delivery. There are two other lesions

associated with spiders: white spots on the arms and buttocks on exposure to cold; these indicate the position of a spider's central arteriole. Secondly, in the area of the spiders, there may be numerous, random small vessels. These resemble the pattern on the paper used for US dollar bills, hence the skin is called 'paper money skin' (see **Fig. 4.8**).

The skin may occasionally reveal several relatively specific signs. The CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, telangiectasia) suggests primary biliary cirrhosis; hereditary hemorrhagic telangiectasia may cause cirrhosis and hepatic vascular ectasia; striae and a facial rash occur with autoimmune hepatitis.

### Examination of the limbs

Examination of the limbs in liver disease patients may reveal proximal myopathy associated with alcohol abuse or vitamin D deficiency, bone tenderness related to osteopenia of chronic liver disease or the arthritides associated with primary biliary cirrhosis or hemochromatosis.

Pitting edema, particularly in the legs, is common, especially in patients with chronic liver disease who have already developed ascites (**Fig. 4.12**).



**Figure 4.10** Spider nevi on the neck.



**Figure 4.12** Peripheral edema in a patient with chronic liver disease.



### Hand and nail signs

Examination of the hands can reveal several signs in patients with liver disease. None of these signs, however, are specific to liver disease. The hands may be warm and the palms bright red. This palmar erythema is a mottled red discoloration affecting the thenar and hypothenar eminences, the pulps and bases of the fingers (**Fig. 4.13**). The feet may be similarly affected. This appearance is associated with acute and chronic liver disease but can also occur in rheumatoid arthritis, thyrotoxicosis, fever and pregnancy.

Dupuytren's contracture has been traditionally associated with alcoholic cirrhosis. In this condition there is a thickening and shortening of the palmar fascia causing flexion deformities of the fingers. The abnormality is now known to have a multifactorial origin but to be strongly associated with cigarette smoking and alcohol consumption rather than primary liver disease. It affects approximately 10% of men over 65 years of age and consequently will be commonly seen in older males who drink regularly, whether or not liver disease is present.

Reference has already been made to the highly polished nails associated with pruritus. The nails can also appear white (leukonychia). This is due to opacity of the nail bed and occurs in up to 80% of patients with chronic liver disease. The distal border may retain a 1–2 mm pink edge, but the lunulae are often obscured by the white nail bed (**Fig. 4.14**). An alternative appearance is one of transverse white lines, parallel to the lunula, band leukonychia (Muehrcke lines) (**Fig. 4.15**). Both these forms of leukonychia are said to be related to a low serum albumin, such as occurs in chronic liver disease. Other conditions in which leukonychia is found include nephrotic syndrome, diabetes mellitus, pulmonary tuberculosis, rheumatoid arthritis and multiple sclerosis. Blue discoloration of the lunulae has been described in Wilson disease and argyria.

Finger clubbing occurs in chronic liver disease and even regresses after hepatic transplantation. It is associated, however, with many other conditions. It may be associated with hypoxia



**Figure 4.13** Palmar erythema in a patient who has chronic liver disease.

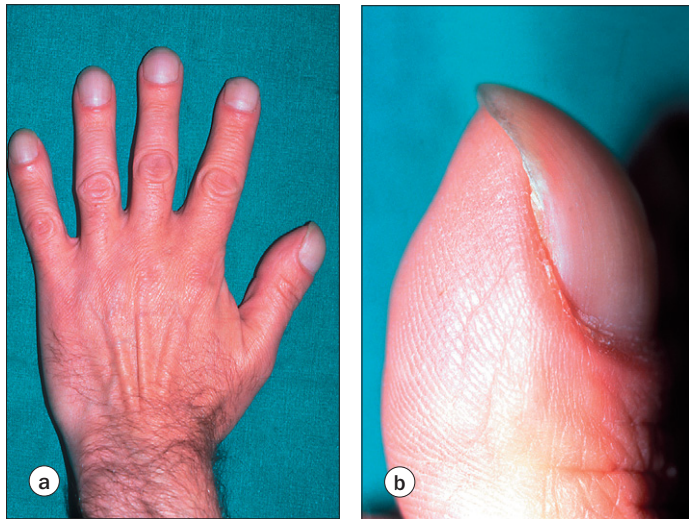


**Figure 4.14** Leukonychia of the proximal nail bed in a patient who has alcoholic liver disease.



**Figure 4.15** (a) Band leukonychia in a patient who has hemochromatosis. (b) Close up.





**Figure 4.16** (a) Clubbing of the fingers in a patient who has chronic alcoholic liver disease. (b) Close up.

and pulmonary hypertension and, in these conditions, hypertrophic osteoarthropathy with periostitis occurs. This is seen radiologically in up to 40% of patients with chronic liver disease (Fig. 4.16).

### Cardiovascular examination

Patients in the early stages of liver disease have a normal cardiovascular system on clinical examination. Alcohol has an effect on blood pressure and this may contribute towards hypertension in some cases. Patients who have advanced hemochromatosis and alcohol abuse can develop cardiomyopathies with the consequent heart failure.

Cardiovascular changes develop as patients with chronic liver disease slowly deteriorate. Similar hemodynamic effects occur rapidly if the patient has acute liver failure. In these conditions a hyperdynamic circulation develops and the patient manifests warm peripheries, a bounding pulse, tachycardia at rest and systemic hypotension. There is an evident precordial impulse and an ejection flow murmur. As the disease progresses the blood pressure falls further and this contributes to the progressive compromise of renal, hepatic and cerebral blood flow and ultimately to terminal multisystem failure. The deterioration in the hemodynamic parameters may be reversible in acute-on-chronic liver failure, i.e. when an acute deterioration is triggered by a potentially reversible or treatable complication like sepsis or gastrointestinal hemorrhage. These circulatory changes are the result of increased cardiac output and reduced peripheral vascular resistance. The cause is thought to be the release of a variety of vasodilators, often acting via the induction of nitric oxide synthase.

Pulmonary hypertension rarely produces symptoms. However, significant asymptomatic pulmonary hypertension is found in 2–10% of patients with portal hypertension. The incidence depends on the pressure threshold used to define pulmonary hypertension. It can contribute to the signs of right heart failure and low cardiac output which may be found in severely affected patients. When severe, it represents a contraindication to liver transplantation.

### Respiratory examination

There is a variety of pulmonary pathologies that can affect patients who have liver disease and these produce abnormal physical signs. Cyanosis occurs in up to 30% of cases of chronic liver disease. Reduced chest expansion, evidence of pleural effusions, dyspnea, orthopnea and signs of intrapulmonary fluid, infection or pulmonary fibrosis can all be found in patients with acute or chronic liver disease. The hepatopulmonary syndrome is another important cause of dyspnea (see Ch. 13). In this condition there is evidence of intrapulmonary arteriovenous (A-V) shunts, vasodilatation leading to ventilation–perfusion mismatching, and capillary wall thickening leading to diffusion defects. The impairment of oxygenation is classically worsened by rising from the lying to the standing position (orthodeoxia).

Pleural effusions occur in approximately 6% of cirrhotic patients but are more common in decompensated cirrhosis, usually occurring on the right and in association with ascites. However, they can occur bilaterally and in the absence of ascites, or can still be present when ascites has responded to diuretic treatment. A right-sided effusion is the norm in the post-operative phase following liver transplantation.

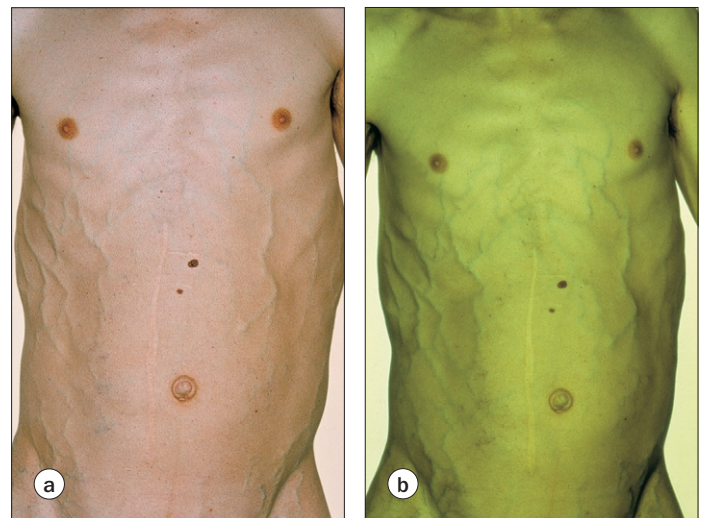
More specific associations between the lung and liver are seen in autoimmune conditions such as fibrosing alveolitis and Sjögren syndrome. In  $\alpha_1$ -antitrypsin deficiency emphysema may be present and in cystic fibrosis chronic suppurative lung disease is usual.

### Abdominal examination

#### Observation

Observation of the abdomen may reveal wasting due to severe malnutrition or cachexia due to malignancy. Alternatively, distention, especially in the flanks, may be present, suggestive of ascites. Normal abdominal wall movement may be restricted by intra-abdominal pathology causing tenderness and pain. The abdominal skin may show petechiae, scratch marks or striae, all of which are related to possible liver disease (Figs 4.1 and 4.4).

Dilated abdominal wall veins are a notable finding in liver disease (see Figs 4.1, 4.6 and 4.17). Prominent superficial veins



**Figure 4.17** Abdominal wall veins in a patient with chronic liver disease. (a) Routine photograph. (b) Infrared photograph.

occur around the umbilicus if the portal venous blood flow is obstructed beyond the origin of the umbilical veins from the left branch of the portal vein, as occurs in intrahepatic pathology. Such portal hypertension allows the umbilical and paraumbilical veins to open up and produce dilated veins on the abdomen and the umbilicus. Blood in these veins flows away from the umbilicus. The appearance is classically called ‘caput medusae’ since the serpiginous veins are supposed to resemble snakes on the head of the mythological gorgon Medusa. However, this classical appearance is rarely observed in practice, and the term ‘caput medusae’ has come to be used to describe the more common finding of dilated veins that flow away from the umbilicus in patients with cirrhosis. Dilated veins due to an obstructed inferior vena cava are usually contrasted with paraumbilical veins. In inferior vena caval obstruction the distended veins are more commonly seen in the sides of the abdomen and the flow is upwards from legs to chest.

### Palpation

Palpation of the abdomen may reveal general features such as abnormal masses or an area of tenderness. These may be associated with hepatobiliary problems, for example intra-abdominal malignancy or biliary infection. More specifically, the clinician needs to palpate the liver and spleen. The shape and size, the contour and the consistency of the liver are all important features. Normally the liver may just be palpable. A Riedel’s lobe (anatomical variant of the right lobe) or downward displacement by pulmonary disease may inappropriately suggest the liver is enlarged. True hepatomegaly is usually indicative of liver disease, unless it is a manifestation of right ventricular failure or related conditions (Table 4.1).

The development of cirrhosis often leads to shrinkage of a previously enlarged liver, perhaps with compensatory hypertrophy of the left lobe. The cirrhotic liver becomes irregular and firm. An enlarged, hard, irregular liver suggests malignancy.

Splenomegaly is an important physical sign in liver disease (Table 4.2). The spleen is normally palpable although, in one study, it was felt in 3% of 2200 healthy college students. It enlarges due mainly to portal hypertension and hyperplasia of the reticuloendothelial component and can thus be felt in chronic or acute liver disease. In the presence of chronic liver disease,

Common causes of hepatomegaly	
Causes	Examples
Chronic liver disease	Multiple causes often leading to cirrhosis
Inflammation	Viral, bacterial (including abscess), parasitic
Venous congestion	Cardiac, venous occlusion (e.g. hepatic vein)
Tumors	Primary hepatobiliary, secondary carcinoma, hematologic malignancies
Biliary disease	Extrahepatic obstruction, congenital cystic disease
Metabolic	Fatty liver, amyloidosis, storage diseases

**Table 4.1 Common causes of hepatomegaly.** The differential diagnosis of hepatomegaly

Common causes of splenomegaly	
Causes	Examples
Venous congestion	Liver disease, hepatic/portal vein occlusion
Infection	Viral, bacterial, parasitic
Inflammation	Rheumatoid arthritis, sarcoidosis, systemic lupus erythematosis
Hematologic disturbances	Chronic hemolysis, hemoglobinopathies, myeloproliferative disease, leukemia, lymphoma
Miscellaneous	Storage diseases, amyloidosis

**Table 4.2 Common causes of splenomegaly.** The various etiologies of splenomegaly

splenomegaly is the most important sign of portal hypertension. When splenomegaly is present, the peripheral blood may show features of hypersplenism. The splenic size is most easily assessed by ultrasound examination.

Palpation of the kidneys is usually unhelpful in liver disease, although 70% of cases with adult renal polycystic disease may develop liver cysts.

### Percussion

Percussion of the abdomen is useful in assessing the size of the liver, especially when it is small, and in detecting the presence of ascites (see Figs 4.1 and 4.6). The cardinal sign of ascites is ‘shifting dullness’ present on both sides of the abdomen. At least 2 L of ascites must be present before it can be thus detected. A fluid thrill requires marked ascites before it is detected. An everted umbilicus is frequently associated with gross ascites (see Figs 4.1 and 4.6).

### Auscultation

Auscultation of the abdomen in relation to liver disease may reveal three types of sounds:

- *Arterial bruits* heard over the liver suggest the presence of a vascular hepatocellular carcinoma or other malignancy, an A-V malformation, very rarely tortuous arteries in a cirrhotic liver, or severe alcoholic hepatitis.
- *Venous hums* are occasionally heard in portal hypertension and reflect turbulent flow in collateral veins. A venous hum at the umbilicus in association with dilated abdominal wall veins and occasionally a venous thrill is termed the Cruveilhier-Baumgarten syndrome. This rare and unusual association may be due to a congenitally patent umbilical vein or portal hypertension in well-compensated cirrhosis.
- *Friction rubs* over the liver suggest inflammation or infiltration. They may be caused by tumor or abscess invading the peritoneum or by hepatic infarction. A transient rub is often heard over the site of a liver biopsy. In the Fitz-Hugh-Curtis syndrome a rub is heard as a result of perihepatitis often caused by chlamydial infection.

### Examination for endocrinologic abnormalities

Endocrine changes may occur in the presence of liver disease and are common when there is an alcoholic cause. In patients with

cirrhosis, hypogonadism is common, which in men produces feminization leading to testicular atrophy, a female body habitus and distribution of body hair, and gynecomastia (see Fig. 4.4). The latter particularly occurs in alcoholics, but is also seen in patients taking spironolactone. This combination of factors may be frequently present in patients with liver disease. Gonadal atrophy in women produces very few clinically detectable physical signs, although the premenopausal patient loses female characteristics such as breast and pelvic fatty tissue.

It is important to recognize that alcohol abuse can produce features of pseudo-Cushing’s syndrome and that some of the above features in men with hemochromatosis can occur before evidence of chronic liver disease.

Neurologic examination

Neurologic abnormalities due to underlying liver disease are rare, apart from hepatic encephalopathy. The neurologic examination is usually, therefore, unremarkable. Specific associations should be borne in mind, for example the neurologic features of Wilson disease, the effects of vitamin A and E deficiency, and the neurologic consequences of viral hepatitis or alcohol abuse.

The clinical features of encephalopathy vary from subtle signs with mild cognitive impairment (minimal hepatic encephalopathy) to coma. A clinical grading system from 1 to 4 is useful (Table 4.3), particularly when frequent observations need to be made to follow the development of the condition or the effect of treatment. Early changes may be monitored by serial use of a number connection test where the time taken by a patient to join together sequential but randomly spaced numbers is recorded. The time taken increases progressively as the severity of the encephalopathy increases.

One of the most characteristic signs of encephalopathy is that of a flapping tremor (‘asterixis’ or ‘liver flap’). This is demonstrated best with the arms outstretched, the wrists dorsiflexed and the fingers separated. This position may have to be maintained for 3–5 min to elicit a flapping tremor. The flapping tremor consists of brief, rapid flexion and extension movements of the fingers or wrists. If it is severe, a similar tremor may be seen in the arms, head and feet. The tremor is seen best on sustained posture; it is absent at rest and disappears if coma develops. The ‘liver flap’ is not specific for liver disease and can occur in renal failure, respiratory failure, metabolic disturbances and drug intoxication.

When neurologic deterioration of this nature occurs, the clinician should evaluate the patient carefully because intracranial lesions such as infection, hemorrhage or tumor can

Hepatic encephalopathy: precipitating factors in chronic liver disease	
Causes	Examples
Gut factors	Constipation, high protein intake, upper gastrointestinal bleeding
Electrolyte/water imbalances	Vomiting, diarrhea, diuretic treatment, renal failure, paracentesis
Infection	Chest, urinary, spontaneous bacterial peritonitis
Drugs	Hypnotic/sedative drugs, alcohol
Severe factors affecting major metabolic functions	Hypoxia, hypotension, hypoglycemia

Table 4.4 Hepatic encephalopathy: precipitating factors in chronic liver disease. A number of clinical conditions not involving the liver can worsen hepatic encephalopathy

produce a similar clinical picture, as can metabolic and drug-induced pathology. In patients who have chronic liver disease and consequent hemostatic disturbances, even minor head injury can lead to significant intracranial bleeding. Hepatic encephalopathy can progress rapidly to coma in acute liver failure and is a severe prognostic sign. In cases of chronic liver disease, it is often precipitated by a number of factors, particularly if there is significant portal-systemic shunting (Table 4.4).

More focal neurologic signs may rarely be present in chronic hepatic encephalopathy as a result of irreversible neurologic degeneration in the cerebral cortex, the basal ganglia, the cerebellum or the spinal cord, such as occurs in the acquired hepato-cerebral degeneration syndrome or in hepatic myelopathy.

DIAGNOSIS AND PROGNOSIS

The evaluation of the patient with liver disease needs careful clinical assessment and the use of various investigations in order to arrive at a definitive diagnosis and management plan. Analysis of the information obtained also allows an assessment of the severity of the disease and the prognosis for the patient. Grading the overall severity of liver disease is now more important than it used to be because of the routine use of liver transplantation in the management of patients. An estimate of prognosis is essential if the timing of transplantation is to be optimal.

Clinical grading of hepatic encephalopathy		
Clinical grade	Clinical signs	Flapping tremor
Grade 1 (prodrome)	Alert, euphoric, occasionally depression Poor concentration, slow mentation and affect, reversed sleep rhythm	Infrequent at this stage
Grade 2 (impending coma)	Drowsiness, lethargic, inappropriate behavior, disorientation	Easily elicited
Grade 3 (early coma)	Stuporous but easily rousable, marked confusion, incoherent speech	Usually present
Grade 4 (deep coma)	Coma, unresponsive but may respond to painful stimulus	Usually absent

Table 4.3 Clinical grading of hepatic encephalopathy. The spectrum of encephalopathy is divided into four clinical grades



Child's grading of disease severity in chronic liver disease with Pugh's 1973 modifications			
Criteria assessed	Points scored for increasing abnormality		
	1	2	3
Encephalopathy (grade)	None	1–2	3–4
Ascites	Absent	Slight	Moderate
Serum bilirubin [ $\mu\text{mol/L}$ (mg/dL)]	<35 (<2)	35–50 (2–3)	>50 (>3)
In primary biliary cirrhosis	<70 (<4)	70–170 (4–10)	>170 (>10)
Serum albumin [g/L (g/dL)]	>35 (3.5)	35–28 (3.5–2.8)	<28 (<2.8)
Prothrombin time [prolongation (s)]	1–4	4–10	>10
Total score	5–6	7–9	10–15
Child's grade equivalent	A	B	C
Overall mortality in Pugh's series (%)	29	38	88
Modified from Pugh et al (73).			

**Table 4.5 Child's grading of disease severity in chronic liver disease with Pugh's 1973 modifications.** The Child-Pugh score is universally used to quantify the severity of liver disease

Various classifications have been used to assess severity of disease. In patients with chronic liver disease Child's grading with Pugh's modification is frequently used. This system uses a combination of clinical and laboratory parameters. Each feature is scored from one to three points and the total score equated with a Child's grade (from A to C) (Table 4.5). The Child grade has been equated with survival (Table 4.6), C being the most severe with an overall mortality of 88% (Table 4.5). MELD is a score of disease severity that is based exclusively on laboratory parameters, and this is increasingly replacing the Child's classification in some clinical situations.

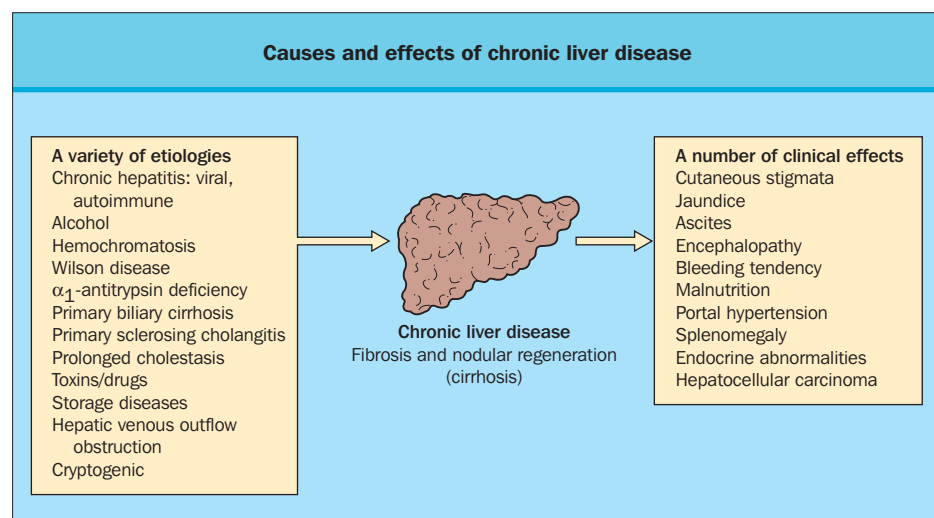
There are some important clinical points that have a bearing on survival in individual patients who have chronic liver disease when they are assessed. Serious factors include:

- persistent jaundice,
- intractable ascites,
- spontaneous bacterial peritonitis,
- progressive encephalopathy,
- persistent hypotension,
- low serum albumin,
- persistent hyponatremia, and
- prolonged prothrombin time.

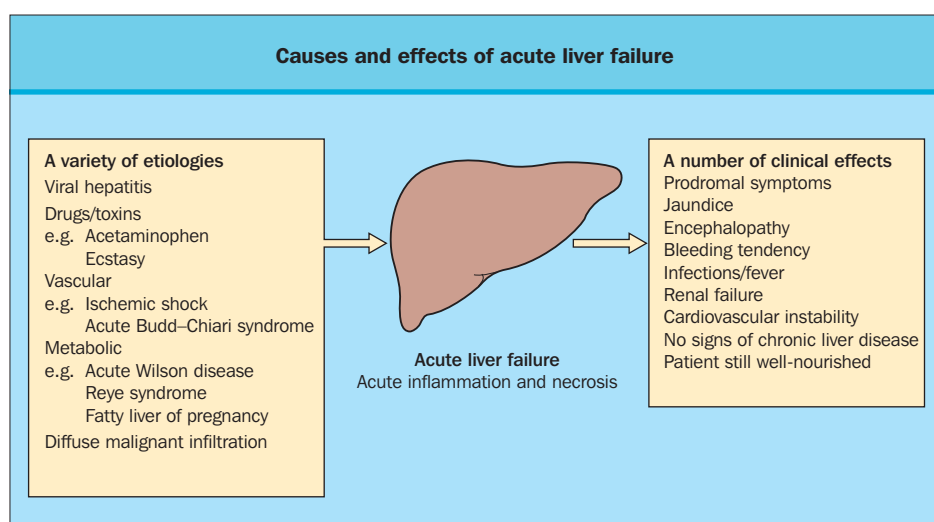
Survival in chronic liver disease			
Percentage survival in chronic liver disease			
Child's grade	At 1 year	At 5 years	At 10 years
A	84	44	27
B	62	20	10
C	42	21	0
Modified from Christensen et al (84).			

**Table 4.6 Survival in chronic liver disease.** The risk of death in patients with chronic liver disease correlates well with the Child's grade

In clinical practice, the experienced physician uses his or her skills to elicit many of the features described in this chapter. Clinical diagnoses are dependent upon a process of pattern recognition and the astute physician integrates all the information obtained in evaluating the patient. It is perhaps useful to remember that the liver is affected by a variety of etiologies but often reacts in a similar, diffuse pathologic fashion. As a result, a number of clinical effects can be reliably predicted (Figs 4.18 and 4.19).



**Figure 4.18 Causes and effects of chronic liver disease.** This figure shows the common clinical manifestations of chronic liver disease together with the common etiologies of cirrhosis.



**Figure 4.19 Causes and effects of acute liver failure.** This figure shows the common clinical manifestations of acute liver failure together with the common etiologies of acute liver injury.

## FURTHER READING

### Historical interest

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### Specific complications

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## SECTION II: EVALUATION OF PATIENTS WITH LIVER DISEASE

### CHAPTER

# 5

## Laboratory Tests

Joanne C. Imperial and Emmet B. Keeffe

A broad array of biochemical tests is used to provide indirect evidence of hepatobiliary disease. The term *liver function tests* (LFTs) is firmly entrenched in routine medical language, although this terminology has been criticized because the tests most commonly employed in the evaluation of liver disease (i.e. serum aminotransferase and alkaline phosphatase) assess hepatocyte integrity rather than synthetic function. Biochemical tests on routine automated chemistry panels that may indicate liver disease can be divided into those that truly evaluate liver function, such as serum albumin or prothrombin time, and those that are simply markers of hepatobiliary disease, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and  $\gamma$ -glutamyltransferase (GGT). The clinical approach to the diagnosis of conditions that are associated with mild elevation of liver enzymes that are markers of hepatocellular necrosis, i.e. AST and ALT and enzymes indicating cholestasis, i.e. ALP and GGT, is the focus of this review.

Screening biochemical testing of healthy, asymptomatic populations has revealed that up to 6% have abnormal serum aminotransferases. However, the prevalence of liver disease in

the general population, excluding simple fatty liver, is lower (i.e. 2–4%). The relatively low prevalence of liver disease, as well as the fact that abnormal liver test results are often minor, and that the screened patient is often asymptomatic, may explain the frequent failure of physicians to perform confirmatory testing or additional evaluations. Despite this tendency to dismiss minor abnormalities of liver tests results, it is important that all such abnormalities be investigated and that, at least, a plausible explanation for the abnormality be determined. Elevated liver enzyme levels may be explained by significant liver disease, which may be treatable, as well as by insignificant liver disease, especially fatty liver, or occasionally diseases of other organs, such as muscle or bone. Additionally, even abnormal markers of hepatic synthetic function (albumin and prothrombin) can be attributable to non-hepatic causes (Table 5.1). On rare occasions, minor elevation of liver enzyme levels is explained by a laboratory error, artifact or macroenzyme formation. Taking into account these considerations, the clinical challenge is to determine the etiology of an abnormal liver test result in an asymptomatic individual using a stepwise and cost-effective approach.

Non-hepatic causes of abnormal liver tests		
Test	Non-hepatic causes	Discriminating tests
↓Albumin	Protein-losing enteropathy Nephrotic syndrome Malnutrition Congestive heart failure	Serum globulins, $\alpha_1$ -antitrypsin clearance Urinalysis, 24 h urinary protein Clinical setting Clinical setting
↑Alkaline phosphatase	Bone disease Pregnancy Malignancy Myocardial infarction Muscle disorders	GGT, SLAP, 5'-NT GGT, 5'-NT Alkaline phosphatase electrophoresis MB-CPK Creatine kinase
↑Bilirubin	Hemolysis Sepsis Ineffective erythropoiesis  Shunt hyperbilirubinemia	Reticulocyte count, peripheral smear, urine bilirubin Clinical setting, cultures Peripheral smear, urine bilirubin, hemoglobin electrophoresis, bone marrow examination Clinical setting
↑Prothrombin time	Antibiotic and anticoagulant use, steatorrhea, dietary deficiency of vitamin K (rare)	Response to vitamin K, clinical setting
GGT, $\gamma$ -glutamyltranspeptidase; SLAP, serum leucine aminopeptidase; 5'-NT, 5'-nucleotidase. Modified from Moseley (96).		

**Table 5.1 Non-hepatic causes of abnormal liver tests.**



TESTS THAT REFLECT HEPATOBILIARY INJURY

Aminotransferases

The aminotransferases (previously called aminotransferases) are the most frequently utilized and specific indicators of hepatocellular integrity. These enzymes – AST (formerly serum glutamic oxaloacetic transaminase, or SGOT) and ALT (formerly serum glutamic pyruvic transaminase, or SGPT) – catalyze the transfer of the α-amino groups of aspartate and alanine, respectively, to the α-keto group of ketoglutaric acid. ALT is localized primarily in the liver, but AST is present in a wide variety of tissues, including the heart, skeletal muscle, kidney, brain and liver. Whereas AST is present in both the mitochondria and cytosol of hepatocytes, ALT is localized to the cytosol. The evaluation of an asymptomatic individual with an isolated elevation of ALT is outlined in **Figure 5.1**.

Although no uniform definition of ‘mild’, ‘moderate’ or ‘severe’ elevation of liver enzymes exists, a working definition has been suggested (**Table 5.2**). Diagnostic clues can often be obtained by assessing the degree of elevation. Mild elevations are typically found in non-alcoholic fatty liver disease (NAFLD), including simple fatty liver and non-alcoholic steatohepatitis (NASH), as well as in chronic viral hepatitis. Moderate elevations may be seen in chronic viral hepatitis, alcoholic hepatitis and a number of miscellaneous chronic liver diseases with or without cirrhosis, including extrahepatic biliary obstruction. Marked elevation of aminotransferases are typical of severe acute viral hepatitis, toxic or drug-induced hepatic injury, and shock or ischemia to the liver. The finding of extremely high aminotransferase levels, i.e. greater than 2000–3000 U/L, should always raise concern

Characteristics of elevated concentrations of liver enzymes

Test	Normal*	Mild†	Moderate†	Marked†
AST	11–32	<2–3	2–3 to 20	>20
ALT	3–30	<2–3	2–3 to 20	>20
ALP	35–105	<1.5–2	1.5–2 to 5	>5
GGT	2–65	<2–3	2–3 to 10	>10

\*Units for normal are U/L; normal ranges vary with the assay used and should be obtained from the laboratory performing the test.  
†Numbers in the table refer to multiples of the upper limits of normal.  
ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γglutamyltranspeptidase.  
Reproduced from Keeffe (94).

Table 5.2 Characteristics of elevated concentrations of liver enzymes.

for acetaminophen overdose, use of excessive therapeutic doses of acetaminophen by the alcoholic patient or shock and/or ischemia to the liver. In the great majority of liver diseases, however, AST and ALT elevations are mild or moderate. The height of the elevation of aminotransferase levels does not appear to correlate with the extent of necrosis on liver biopsy specimens and therefore has no prognostic value. In fact, rapidly declining aminotransferase levels may be a reflection of decreased numbers of viable hepatocytes and indicate a poor prognosis in

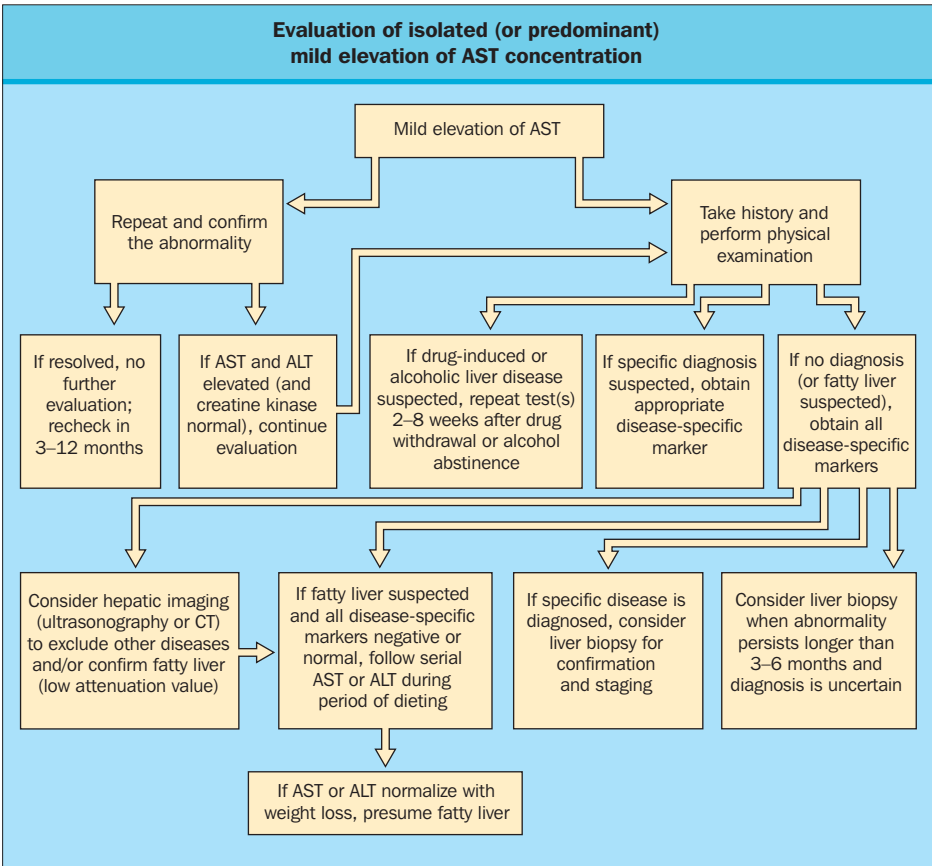


Figure 5.1 Evaluation of isolated (or predominant) mild elevation of AST concentration. AST, aspartate aminotransferase. Reproduced from Keeffe (94) by permission of The American Gastroenterological Association.

fulminant hepatic failure. In patients with extrahepatic biliary tract obstruction, serum levels of AST and ALT may increase to greater than 300 U/L but usually decline rapidly after peaking within the first 24–48 h after obstruction. Although elevations in aminotransferase levels may be the first clue to liver disease and screening has proved useful for detecting subclinical liver disease in asymptomatic persons, patients with normal levels may also have significant liver damage. For instance, recent studies have demonstrated that asymptomatic carriers of hepatitis C virus (HCV) may have evidence of chronic hepatitis and even cirrhosis on liver biopsy despite having repeatedly normal aminotransferase levels. Another characteristic feature of chronic hepatitis C is the episodic, fluctuating pattern of serum ALT levels: periods of elevated enzyme activity alternating with periods of normal or near-normal ALT levels.

The ratio of AST to ALT may be helpful diagnostically, particularly in patients with alcoholic liver disease who characteristically have a ratio of greater than 2.0 (Fig. 5.2). The reason for the elevation of the AST level out of proportion to the ALT level appears to be a differential reduction in hepatic ALT caused by a deficiency of the cofactor, pyridoxine-5-phosphate. In fact, oral administration of this cofactor to patients with alcoholic liver disease has been shown to result in an increase in serum ALT levels. Although an AST:ALT ratio of greater than 2.0 strongly suggests the diagnosis of alcoholic liver disease, it does not preclude other diagnoses. Other biochemical clues to the presence of alcoholic liver disease include elevations of

GGT, erythrocyte mean corpuscular volume and desialylated transferrin. Conversely, NAFLD is typically associated with an ALT level greater than the AST level. In viral hepatitis, the ratio of AST to ALT is also usually less than 1.0 but may rise to values greater than 1.0 as cirrhosis develops.

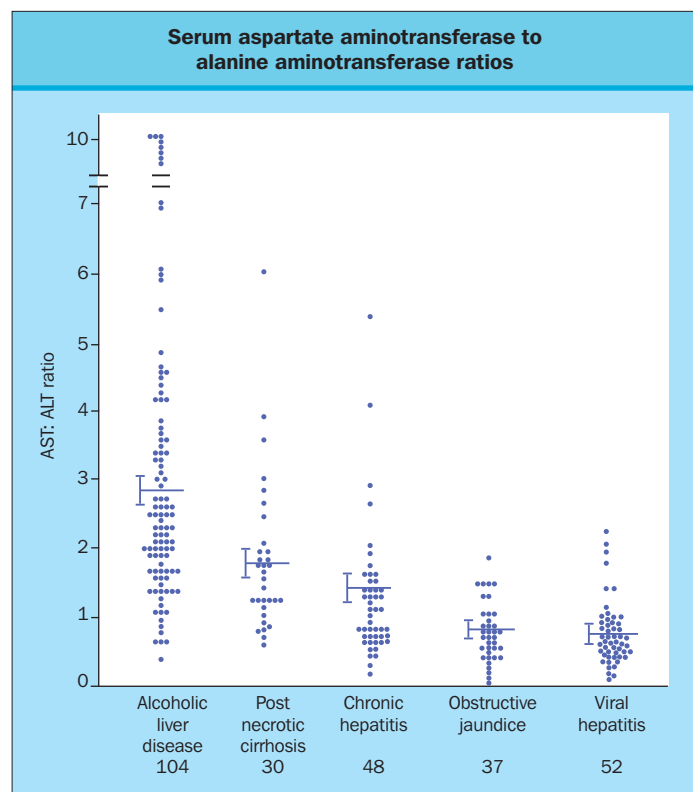
It is important to note that certain clinical situations are associated with relatively low levels of aminotransferases. Yauda and associates have shown that values higher than 20 U/L are definitely abnormal and possibly indicative of liver disease in patients undergoing hemodialysis, which suggests that the upper normal limits of AST and ALT in these patients should be reduced considerably. There is considerable evidence that hepatitis C viremia without biochemical evidence of hepatic dysfunction is common in patients with renal failure. **Figure 5.3** outlines the evaluation of elevated liver enzymes in hemodialysis patients. The reduced sensitivity of antibody to HCV (anti-HCV) in this population warrants direct measurement of serum HCV RNA when clinical suspicion of HCV infection is high.

Clinical studies of patients with chronic, asymptomatic and mild-to-moderate elevation of AST or ALT is limited, particularly studies that include liver biopsy as part of the evaluation process. One of the earliest, most comprehensive studies of the significance of mild-to-moderate elevation of AST is a Scandinavian investigation by Hultcrantz et al (1986) of 149 asymptomatic patients, all of whom had elevation of liver enzymes for >6 months and underwent liver biopsy. These patients were not only asymptomatic, but also had normal physical examinations; the remainder of the standard liver profile was normal except for a minority of patients with less than twofold elevation of ALP. The histologic diagnoses on liver biopsy included:

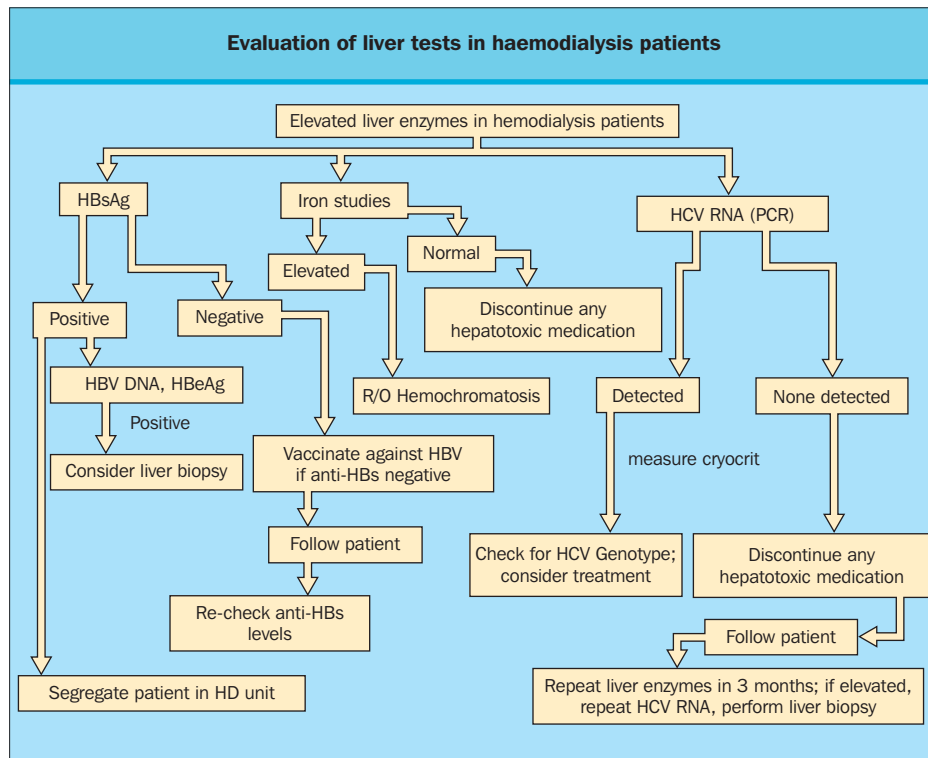
- fatty liver in 63%;
- CAH (chronic active hepatitis) or CPH (chronic persistent hepatitis) presumably viral, in 20%; and
- miscellaneous liver disease in 17%.

Fatty liver was associated with high body weight, alcohol intake, hyperlipidemia and diabetes mellitus. These patients had all undergone evaluation with hepatic imaging and a number of immunoserologic tests. The authors found that hepatitis B serology, serum ferritin and  $\alpha_1$ -antitrypsin levels were the only useful specific tests. It is important to note that this study was performed prior to the availability of serologic testing for HCV infection with anti-HCV.

In a study of the cause of an elevated ALT in 100 blood donors, which represents a different population from patients referred to a tertiary care center, as in the above investigation, presumptive fatty liver was once again the most common diagnosis based on exclusion of viral, autoimmune and genetic diseases and on the association with daily alcohol use and/or obesity. This study did not include liver biopsies as part of the evaluation, but relied on clinical diagnoses. A practical finding of this study was that ALT was elevated on only one occasion in one-third of the subjects, i.e. at the time of blood donation and elevated intermittently (36%) or persistently (28%) in the other two-thirds of the subjects. These same authors later applied testing for anti-HCV by the second generation enzyme-linked immunosorbent assay-2 (ELISA-2) to this group, and anti-HCV was detected in 17 of the 100 blood donors. The anti-HCV positive donors had a higher mean ALT, a persistent rather than transient or intermittent ALT elevation, more often admitted use of injection drugs, were lean rather than obese and were less likely to be regular alcohol



**Figure 5.2 Serum aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratios in patients with biopsy proven liver disease.** Of patients with alcoholic liver disease, 70% had ratios greater than 2.0 compared with 26% of patients with postnecrotic cirrhosis, 8% with chronic active hepatitis, 4% with viral hepatitis, and none with obstructive jaundice. Reproduced from Cohen et al (79) by permission of Springer Science and Business Media.



**Figure 5.3 Algorithm for evaluation of liver tests in a hemodialysis (HD) patient.** HBsAg, hepatitis B surface antigen; anti-HBs, antibody to hepatitis B surface antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction. Modified from Rosen et al (96).

drinkers. Thus, chronic hepatitis C is more often the explanation of asymptomatic elevation of ALT in persons who are not obese or use alcohol, while anti-HCV is likely to be negative in those subjects who are obese or drink alcohol regularly. These latter patients are more likely to have fatty liver.

The two studies cited above document that fatty liver, now referred to as NAFLD is frequently the cause of mildly elevated levels of AST or ALT (as well as ALP and GGT). This observation appears to be especially true in the healthy blood donor population, where obesity has been proposed as the major cause of persistent elevation of ALT. NAFLD is often associated with obesity, hyperlipidemia, or diabetes mellitus and can be predicted by reduced attenuation values on computed tomography (CT) of the liver. A study by Palmer & Schaffner (1990) of overweight adults with abnormal aminotransferases and no primary liver disease by specific immunoserologic testing demonstrated that weight reduction of >10% corrected abnormal LFTs and decreased hepatosplenomegaly. These authors recommend that, after other causes of liver disease are eliminated by clinical and biochemical parameters, weight reduction should be tried before proceeding with more expensive and invasive tests, such as liver biopsy.

The prevalence and clinicopathologic features of NAFLD, with or without NASH, are now recognized as a major cause of liver disease in the USA. NASH has traditionally been thought to most commonly affect obese, diabetic, especially insulin-resistant, middle-aged women, but recent experience documents that men are also affected and obesity and diabetes may be absent. In some patients, it may progress to clinically significant liver disease, including decompensated cirrhosis requiring liver transplantation and even hepatocellular carcinoma. AST and ALT levels are mildly to moderately elevated, while bilirubin, albumin and prothrombin time are usually normal. The absence

of elevated AST and ALT levels does not rule out the possibility of histologically significant liver disease, however. This entity is pertinent to this review, since 48–100% of patients have no symptoms of liver disease. Thus, liver biopsy is essential to establish a diagnosis and/or stage the disease in this population of patients with a negative immunoserologic work-up. Pathologic features of NASH include acinar zone 3 (central vein) injury with hepatocellular fat accumulation, Mallory bodies and perivenular and pericellular fibrosis. This lesion may be histologically indistinguishable from that of alcoholic liver disease. Fibrosis or cirrhosis occurs in approximately 15–50% of patients.

In a large prospective study of French blood donors from 1987 to 1989, non-A, non-B hepatitis was assessed with third generation tests for anti-HCV and serum HCV RNA by polymerase chain reaction (PCR) and fatty liver was once again found to be common. In this study, 184 subjects (1.7%) had elevated ALT $\times$ 2 over 6 months and 88 agreed to follow-up. Of these, 45 subjects had persistently elevated ALT over 6 months and underwent liver biopsy. Findings included:

- fatty liver in 29/45 (65%), with 23 cases explained by alcohol, obesity or hyperlipidemia; ALT normalized in 22 patients with dietary management; and
- chronic hepatitis in 16/45 (35%); 12 of 16 patients had documented chronic hepatitis C, but 4 patients remained unexplained, suggesting the possibility of another virus (non-A, non-B, non-C virus).

A unique condition that can present with persistently elevated aminotransferase levels and/or fatty liver is heterozygous familial hypobetalipoproteinemia. This entity is related to apolipoprotein B (ApoB) deficiency, which can be suspected when hypocholesterolemia is found on a routine chemistry panel. These patients are generally otherwise healthy.



Finally, elevated serum ALT levels may be unexplained or associated with muscle injury from rigorous exercise in individuals undergoing military basic training. In a study of US Air Force recruits, 99 of 19 877 individuals (0.5%) had an ALT elevation greater than 55 U/L. In the majority (88%) of subjects, the cause of the elevation was not apparent after clinical evaluation and screening serologic and immunologic laboratory tests. The elevated ALT was presumably related to individual variation from a non-Gaussian distribution, non-hepatic (muscular) disease, or undetected liver disease.

The development and current wide availability of testing for viral, autoimmune and genetic liver diseases now generally results in a specific etiologic diagnosis of liver disease prior to biopsy. For example, serologic markers for hepatitis A, B, C and D are commercially available and facilitate the specific diagnosis of acute and chronic viral hepatitis (Table 5.3). Seroprevalence studies show that 1.4% of the US population is reactive to anti-HCV, with 70–85% being viremic, and approximately 0.3% of the US population is HBsAg carriers. Hemochromatosis is a relatively common disease that occurs in 1 in 250 individuals in the general population and can be diagnosed by the finding of an elevated serum ferritin and a high percentage iron saturation, or the detection of the *HFE* gene. A subsequent liver biopsy with measurement of quantitative iron content and calculation of the hepatic iron index provides confirmation of disease. Wilson disease is rare, occurring in approximately 1 in 30 000 population, but can be specifically diagnosed by the finding of low serum ceruloplasmin and high urinary copper excretion. Liver biopsy with determination of quantitative copper content provides a confirmatory diagnosis.  $\alpha_1$ -antitrypsin deficiency can be diagnosed by the finding of a reduced  $\alpha_1$ -antitrypsin level, typically to 20% of normal, and confirmed by phenotyping or by liver biopsy showing the characteristic periodic acid-Schiff, diastase-resistant inclusions.

Most common liver diseases	
Common	Uncommon
Non-alcoholic fatty liver disease	Drug-induced liver disease
Chronic hepatitis C	Autoimmune hepatitis
Chronic hepatitis B	$\alpha_1$ -antitrypsin deficiency
Alcoholic liver disease	Wilson disease
Hemochromatosis	Miscellaneous conditions

Table 5.4 Most common liver diseases.

A reasonable diagnostic approach to the evaluation of a patient with a mild isolated (or predominant) elevation of AST (and/or ALT) is shown in Figure 5.1. In the application of the above algorithm, the most common hepatic conditions should be kept in mind, i.e. fatty liver, chronic hepatitis C, chronic hepatitis B, alcoholic liver disease, and hemochromatosis, as should those that are less common, i.e. drug-induced liver disease, autoimmune hepatitis,  $\alpha_1$ -antitrypsin deficiency and Wilson disease (Table 5.4). Depending on logistic and cost issues, an asymptomatic patient with mildly elevated ALT might be evaluated sequentially for the above two groups of liver conditions rather than in a shotgun fashion.

## TESTS THAT REFLECT CHOLESTATIC LIVER INJURY

### Alkaline phosphatase and $\gamma$ -glutamyltranspeptidase

The usual markers to identify cholestasis are ALP and GGT, with 5' nucleotidase and leucine aminopeptidase used less

Types of hepatitis viruses						
	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E	Hepatitis G
Virus	HAV	HBV	HCV	HDV	HEV	HGV
Size	27 nm	42 nm	30–60 nm	35 nm	37 nm	30–60 nm
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA	ssRNA
Antigens	HAAg*	HBsAg HBeAg HBcAg*	HCAg*	HDAg*	HEAg*	None available
Antibodies	IgM anti-HAV Anti-HAV	IgM anti-HBc Anti-HBc Anti-HBs Anti-HBe	Anti-HCV	IgM anti-HDV Anti-HDV	anti-HEV	None available
Viral markers	HAV RNA*	HBV DNA DNA polymerase	HCV RNA†	HDV RNA	none	HGV RNA
*Research test. †See the text for a discussion of HCV genotyping. ss, Single stranded. Modified from Lindsay et al in Kaplowitz (96).						

Table 5.3 Types of hepatitis viruses.

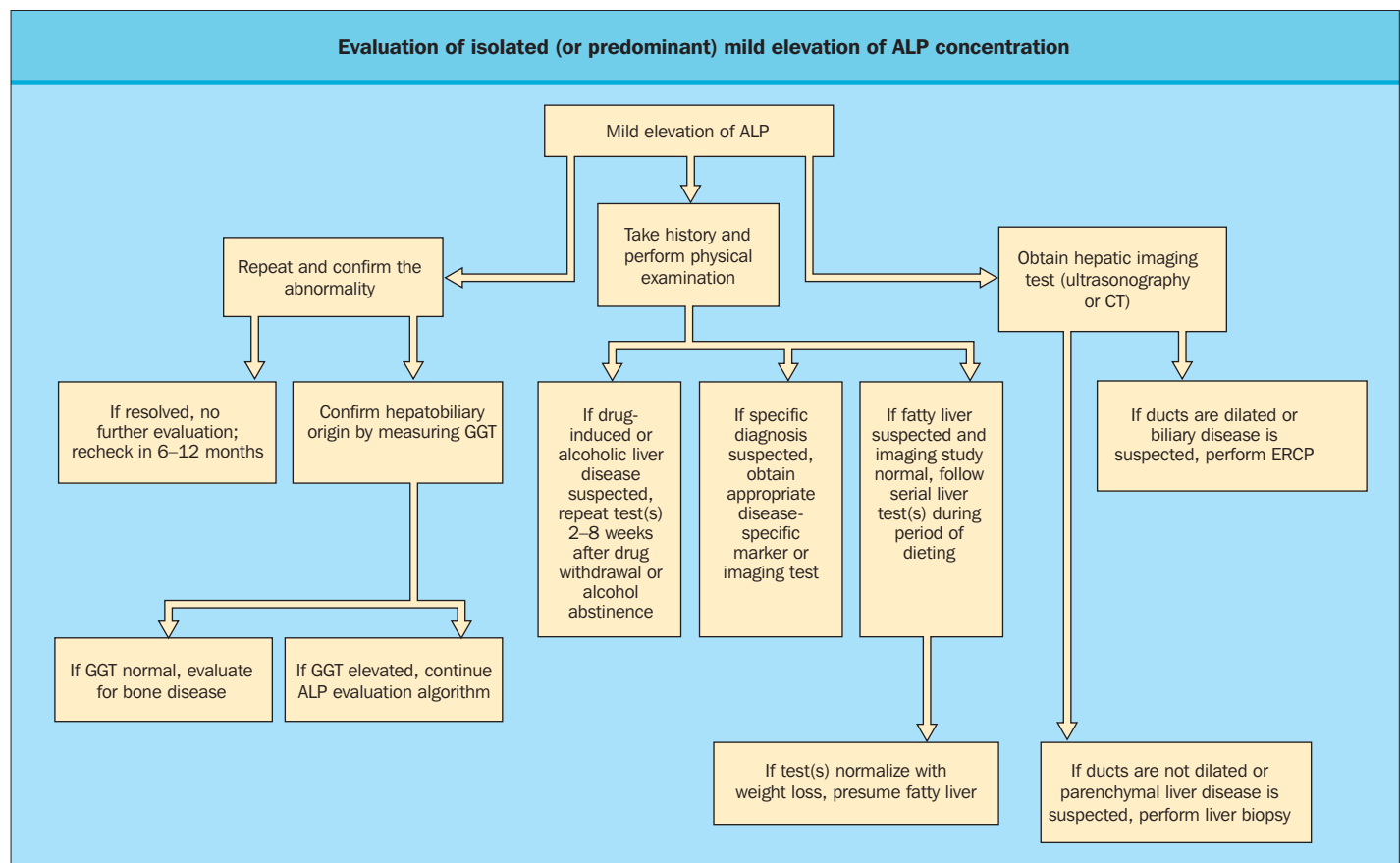
commonly. Serum ALP is the name applied to a group of enzymes that catalyze the hydrolysis of phosphate esters at an alkaline pH. The enzymes are widely distributed and may originate from bone, liver, intestine, or placenta. In children and adolescents, in whom bone growth is active, the serum ALP may increase up to threefold. In patients with hepatobiliary disorders, elevated ALP levels result from increased hepatic production with leakage into the serum, rather than failure to clear or excrete circulating ALP. Markedly elevated levels of ALP suggest the possibility of disorders such as extrahepatic biliary obstruction, infiltrative diseases of the liver, including primary and metastatic tumors, primary biliary cirrhosis (PBC), primary sclerosing cholangitis, or drug-induced cholestasis. The degree of elevation does not differentiate extra- and intrahepatic etiologies of cholestasis. In partial extrahepatic biliary obstruction, ALP may be elevated in the absence of hyperbilirubinemia or pruritus. ALP may also be mildly elevated in parenchymal liver disease, particularly alcoholic liver disease.

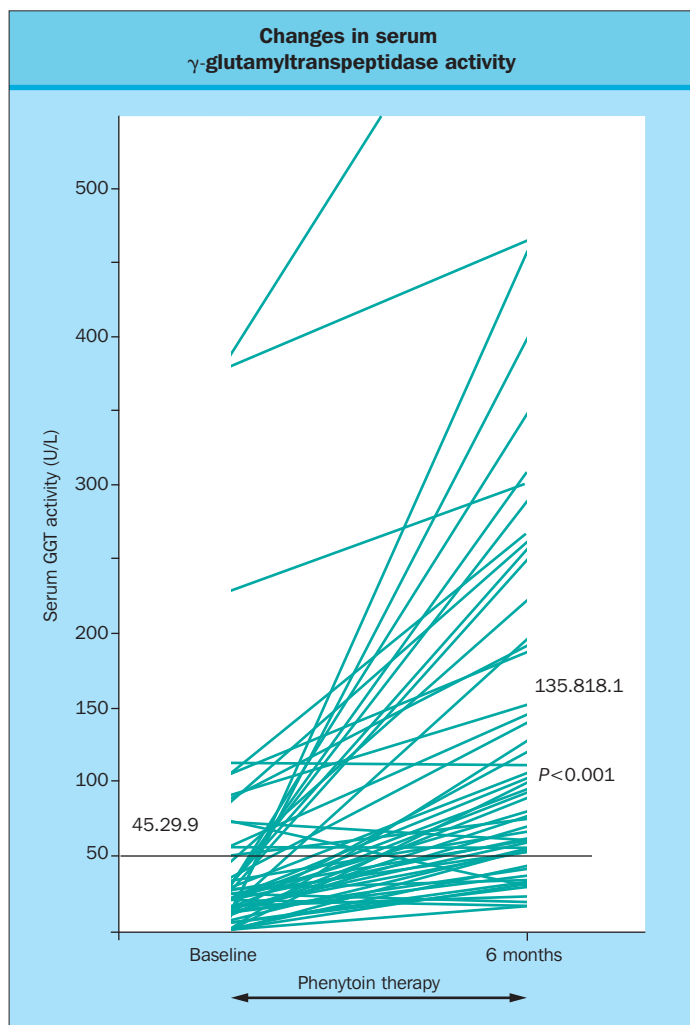
Mild to moderate ALP abnormalities are seen in infiltrative processes such as amyloidosis, granulomatous disease and some neoplasms. Patients with Hodgkin disease and renal cell carcinoma may have elevated ALP without any direct involvement of bone or the hepatobiliary system. Mild elevation of ALP is also common in physiologic situations, such as pregnancy and physiologic bone growth and is seen not uncommonly in subjects >50 years of age. In most cases, the ALP elevation is evident on the basis of history and physical examination and hepatic imaging tests.

Low serum levels of ALP have been associated with congenital hypophosphatasia, hypothyroidism, pernicious anemia and zinc deficiency. Moreover, patients with fulminant Wilson disease complicated by hemolysis can present with undetectable levels of serum ALP. Whether this is due to replacement of the cofactor, zinc, by copper and subsequent inactivation of ALP remains unknown.

The significance of isolated mild elevation of ALP ( $<1.5\text{--}2 \times$  normal) is problematic and has undergone only limited investigation. Rubenstein et al (1986) found that most ambulatory patients with unexplained ALP elevation detected during multiphasic screening had no overt disease during a 2-year follow-up period. Lieberman & Phillips (1990) evaluated the clinical outcome of hospitalized patients with isolated ALP elevation. A total of 87 patients with isolated ALP were identified and 45 of these patients (52%) had normalization of ALP, usually within 1–3 months. For 12 patients (14%) there was no apparent explanation for the transient rise in ALP, while miscellaneous diagnoses explained the transient rise in the remainder of patients. In the 42 patients with persistent ALP elevation, 14 had terminal malignancies and all but 7 of the remaining patients had obvious diagnoses.

Finally, elevation of ALP  $>1.5\text{--}2$ -fold, particularly when associated with abnormalities of other liver tests, points to the presence of an underlying hepatobiliary disease. A reasonable diagnostic approach to the evaluation of a patient with a mild isolated or predominant elevation of ALP is shown in Figure 5.4.





**Figure 5.5 Changes in serum  $\gamma$ -glutamyltranspeptidase (GGT) activity.** Changes monitored from baseline to 6 months of phenytoin therapy in 58 patients. Of the total group, 52 patients demonstrated a rise and six patients a fall in GGT activity. Reproduced from Keeffe et al (86) by permission of Springer Science and Business Media.

Serum GGT is useful to confirm whether an elevated ALP is secondary to hepatobiliary disease or is of extrahepatic origin. Some automated chemistry batteries include GGT, which may lead to the clinical problem of whether or not to evaluate an isolated elevation. Unfortunately, because the enzyme is ubiquitous, an elevated GGT has poor specificity and may be due to induction by alcohol, phenytoin (Fig. 5.5), or other drugs. Although the diagnostic yield of an isolated elevation of GGT has not been studied, the consensus is that extensive evaluation is usually not appropriate beyond a routine history, physical examination and general chemistry panel. 5'-Nucleotidase, on the other hand, is specific for liver disease and its measurement is particularly helpful in diagnosing liver disease in children and pregnancy, those clinical settings in which ALP is elevated physiologically.

## TESTS THAT REFLECT HEPATIC SYNTHETIC FUNCTION

### Bilirubin

Bilirubin is an endogenous organic anion derived primarily from the degradation of hemoglobin released from red blood cells.

Hyperbilirubinemia is classified as either unconjugated or conjugated. (Ch. 6). In patients with hyperbilirubinemia due to hepatocellular dysfunction or cholestasis, the serum bilirubin is predominantly conjugated and hence water soluble, allowing easy renal excretion. Extreme hyperbilirubinemia (i.e.  $>25$  mg/dL) usually signifies severe liver disease in association with another cause of unconjugated hyperbilirubinemia (i.e. hemolysis) or renal dysfunction.

The level of serum bilirubin has been utilized to predict the natural history of specific liver diseases. Dickson and others have reported that serum bilirubin is the most important variable for predicting survival in patients with PBC. The level of hyperbilirubinemia also has prognostic significance in patients with fulminant hepatic failure and acute alcoholic hepatitis, comprising the majority of the discriminant function scores derived by Maddrey and associates. Moreover, recent studies of candidates for liver transplantation have shown that serum bilirubin is an important component of the Model for End-stage Liver Disease (MELD) scale and a reliable measure of disease severity and mortality risk in patients with end-stage liver disease awaiting liver transplantation. The MELD scale is a tool originating from the Mayo Clinic, originally used to determine prognosis after a TIPS (transjugular intrahepatic shunt), and now applied to liver transplantation for assessment of clinical status while waiting for transplantation. MELD has replaced CPT score as a predictor of pre and post transplant survival and disease severity for patients awaiting liver transplantation.

## SERUM ALBUMIN

Albumin is quantitatively the most important circulating protein synthesized by the liver, accounting for three-quarters of the plasma colloid oncotic pressure. The liver is the only site of synthesis with approximately 12–15 g produced daily in the normal adult. Plasma albumin concentration is decreased in severe acute and chronic liver disease and is one of the criteria for the CTP (Child-Turcotte-Pugh) classification widely used to grade severity of cirrhosis. In addition to liver disease, hypoalbuminemia may also result from gastrointestinal and renal losses, hormonal changes, increased catabolism and hypergammaglobulinemia, which may lead to feedback inhibition of albumin synthesis. Prealbumin (or thyroid-binding prealbumin) appears to be a more sensitive index of hepatic protein synthesis than albumin, particularly in acute liver failure because its half-life is shorter (1.9 days).

## PROTHROMBIN TIME AND SERUM COAGULATION FACTOR LEVELS

With the exception of factor VIII, all the clotting factors are synthesized within hepatocytes. The clotting factors that determine prothrombin time (PT) are short-lived (hours), making the test suited for evaluating acute liver injury; indeed, PT is one of the key elements in prognostic scores for fulminant hepatic failure and acute alcoholic hepatitis. In general, a prolongation of  $\geq 2$  s more than control is considered abnormal. Unfortunately, the PT is not a sensitive index of chronic liver disease, because it may be normal even in patients with cirrhosis; moreover, its specificity is low in the presence of malabsorption, because factors II, VII and X are dependent on vitamin K. It is now



generally accepted that the international normalized ratio should replace the PT, because the latter is based on different types of thromboplastin that have considerable variation in different countries.

## OTHER QUANTITATIVE TESTS OF LIVER FUNCTION

Patients with essentially normal conventional serum liver tests may have significantly diminished hepatic function; conversely, hepatic function may be well preserved in some patients with abnormal serum liver tests. Although quantitation of functional reserve is not performed routinely in clinical practice, quantitative serum liver tests may provide useful data regarding the prognosis and effects of therapeutic interventions. Some examples of these tests are described below.

Indocyanine green (ICG) is a dye removed from the circulation by the liver after intravenous injection. Because it is taken up exclusively by hepatocytes, the clearance of low doses of ICG is used to measure liver blood flow. With administration of higher doses of ICG, the uptake process becomes saturated; the maximal removal of ICG can be calculated and this reflects functional hepatic mass rather than blood flow in the liver.

The metabolism of aminopyrine is sensitive to alterations in the redox state, thyroid disease, infection, amino acid deficiency and glutathione deficiency as well as folate and vitamin B<sub>12</sub> deficiency. The aminopyrine breath test has been used to predict short-term prognosis and mortality in patients with alcoholic hepatitis, and in one study was a better predictor of outcome than standard serum liver tests. The caffeine breath test is comparable to the aminopyrine test, except without the need for radioisotope usage. Caffeine clearance appears to be impaired in

advanced liver disease and has limited utility for screening mild or early liver disease.

The <sup>14</sup>C-galactose breath test is a simple test that may provide good prognostic information in fulminant hepatic failure where the galactose elimination capacity (GEC) is reduced. In addition, serial measurements of the GEC were more accurate in predicting death in patients with PBC than the Mayo score. However, in multivariate analysis of survival in other patients with cirrhosis, determination of GEC appeared to provide little additional information compared with the Child-Pugh score. Unlike the ICG, the GEC is not suitable for assessment of hepatic reserve before hepatic resection for malignancy because some tumors may metabolize galactose.

## SUMMARY

The differential diagnosis of abnormal serum liver tests includes hepatic and extrahepatic conditions and is greatly narrowed by a comprehensive history and physical examination and by the recognition of classic patterns of liver injury. The limitations of these liver tests, including problems with specificity and sensitivity, must always be taken into account. Patients with essentially normal conventional serum liver tests may have significantly impaired hepatic function; conversely, hepatic function may be well preserved in some patients with abnormal serum liver tests. Furthermore, other indices of hepatic function, such as bilirubin and PT, are important prognostic indicators of the natural history of specific conditions such as PBC, alcoholic hepatitis and end-stage liver disease.

Serologic tests are invaluable tools in diagnosing the many etiologies of abnormal liver enzymes and may obviate the need for liver biopsy in many cases. All abnormalities of liver enzymes should be investigated.

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## SECTION II: EVALUATION OF PATIENTS WITH LIVER DISEASE

### CHAPTER

# 6

## Jaundice

Steven D. Lidofsky

### INTRODUCTION

Jaundice, which gives rise to a yellow appearance of the skin, occurs as a consequence of bilirubin deposition into subcutaneous tissues. In darker skinned individuals (or early in the course of jaundice), this yellow discoloration may be more readily apparent in the sclerae and mucous membranes. Yellow pigmentation of the skin can also result from excessive ingestion of vegetables that are abundant in the content of carotene or other carotenoids. Distinction of these conditions from true jaundice is generally straightforward, however, as scleral icterus and hyperbilirubinemia are not seen with ingestion of vegetable products. Jaundice is classically associated with liver and biliary tract disease, but it is important to emphasize that there are extrahepatic causes of jaundice as well.

The cause of jaundice can be identified in the vast majority of cases. Indeed, with a simple history, physical examination and routine biochemical screening tests, the presence versus absence of biliary tract obstruction can be correctly distinguished 75% of the time. Additional tests are often necessary to make a precise diagnosis, however. The diversity of currently available laboratory and imaging techniques requires judicious selection in order to minimize cost and patient discomfort.

This chapter covers five principal areas. It begins with a discussion of bilirubin formation and metabolism, especially as it relates to the pathophysiology of jaundice. It then presents a rational clinical classification of the causes of jaundice. Some of the causes described are delineated with brevity, as they are discussed in greater detail in other chapters in this book. This section is followed by clinical clues to various types of jaundice. With this as a background, the chapter moves toward a presentation of the strengths and weaknesses of different modalities for the diagnosis of jaundice. The chapter concludes with a general approach, built upon the conceptual framework already established, to the management of the jaundiced patient.

### PATHOPHYSIOLOGY

#### Overview

Jaundice is a manifestation of an elevated serum bilirubin concentration. Serum bilirubin levels are generally less than 1–1.5 mg/dL (17–25  $\mu$ mol/L) and jaundice is not usually detectable until bilirubin concentration exceeds 3 mg/dL (50  $\mu$ mol/L). The level of bilirubin elevation is a critical determinant of overall prognosis in several hepatobiliary disorders. These include acute liver failure, alcoholic hepatitis, primary biliary cirrhosis and sclerosing cholangitis. An understanding of the origins of an elevated bilirubin concentration is particularly germane to the management of these clinical entities, as is

elucidating the cause of jaundice in patients who do not have a defined illness.

#### Bilirubin formation

Bilirubin is a potentially toxic compound that is an end-product of heme degradation. It has the chemical structure of a tetrapyrrole, which renders it highly water-insoluble due to internal hydrogen bonding. As such, the elimination of bilirubin from the circulation requires chemical conversion in the liver to water soluble conjugates that are normally excreted into bile. This multistep process is schematically illustrated in **Figure 6.1**. An abnormality at any of these steps can lead to hyperbilirubinemia and jaundice.

Daily bilirubin production is considerable. Most (70–80%) is derived from hemoglobin degradation from senescent erythrocytes and a small proportion comes from premature destruction of developing erythrocytes in the bone marrow (ineffective erythropoiesis). The liver represents the dominant source of the remaining 20–30% of bilirubin formed. The principal intracellular sources for this are heme-containing enzymes such as catalase and cytochrome oxidases. Other heme-containing proteins (e.g. myoglobin) are present in extrahepatic tissues. However, the overall contribution of such proteins to bilirubin production is minimal due to their low mass or slow degradation rate.

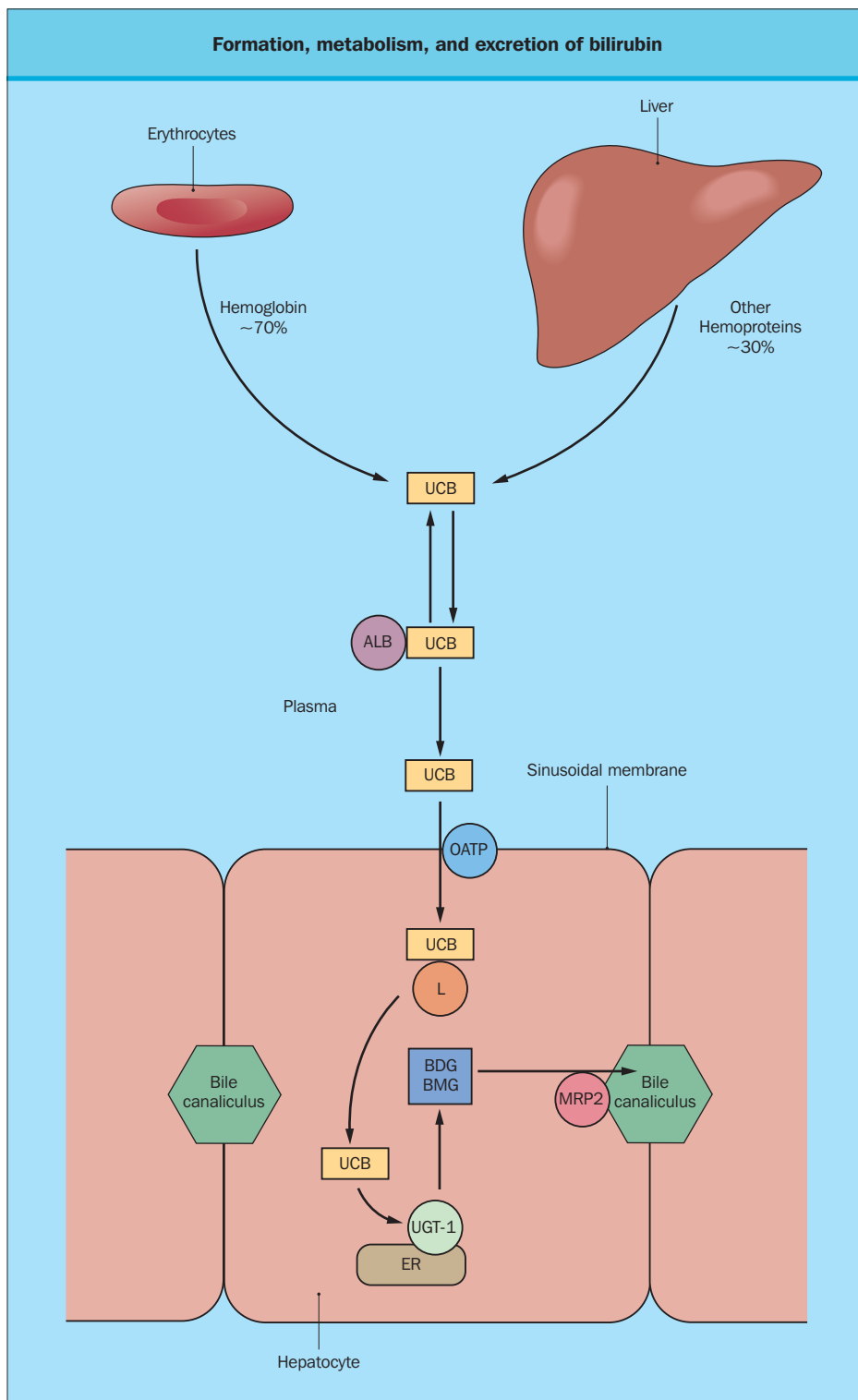
Heme is converted to bilirubin via a two-step process (**Fig. 6.2**). First, heme is converted to biliverdin by the microsomal enzyme heme oxygenase. Second, biliverdin is converted to bilirubin by the cytosolic enzyme biliverdin reductase. Degradation of heme derived from erythrocyte hemoglobin primarily takes place in macrophages in the spleen, liver and bone marrow. By contrast, hepatocytes produce bilirubin from heme derived from free hemoglobin, haptoglobin-bound hemoglobin and methemalbumin.

#### Bilirubin metabolism

Bilirubin metabolism is a complex process in which several inherited disorders and associated gene products have been identified. This is illustrated in **Figure 6.1**. Bilirubin circulates in plasma tightly bound to albumin. Extraction of bilirubin from plasma occurs in the liver, where bilirubin is metabolized and transported into bile through the actions of hepatocytes.

Bilirubin uptake occurs at the sinusoidal (basolateral) membrane of hepatocytes. The mechanism appears to be carrier-mediated. This process is competitively inhibited by certain organic anions such as bromsulfophthalein (BSP) and indocyanine green. In addition, the drug rifampin (rifampicin) may lead to hyperbilirubinemia by a similar process. A candidate gene product, the organic anion transporting polypeptide OATP2 (SLC21A6),





**Figure 6.1 Formation, metabolism and excretion of bilirubin.** Unconjugated bilirubin (UCB), formed from breakdown of hemoglobin from erythrocytes and heme-containing proteins from other tissues (principally liver), circulates in plasma bound to albumin (ALB). It is taken up by the hepatocyte (possibly via a member of the OATP family of transport proteins), diffuses through the cytosol bound to ligands (L) and undergoes conjugation in the endoplasmic reticulum (ER) by bilirubin glucuronyltransferase (UGT-1). Mono- and diglucuronidated bilirubins (BMG, BDG) are exported into the bile canaliculus via the transport protein MRP2.

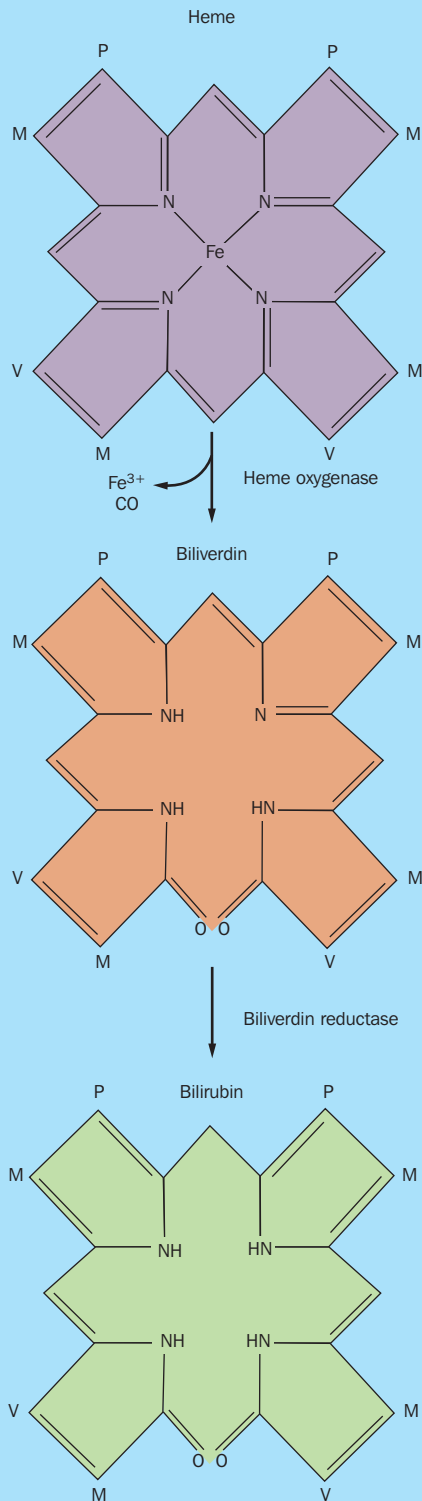
has been proposed to mediate sinusoidal bilirubin uptake, but its role remains controversial.

Once sinusoidal uptake has occurred, bilirubin is directed to the endoplasmic reticulum by several cytosolic binding proteins, which serve to facilitate intracellular diffusion of this hydrophobic molecule. These cytosolic proteins include the ligandins and fatty acid binding protein. Bilirubin conjugation occurs in the endoplasmic reticulum. There it is principally converted to

water-soluble monoglucuronides and diglucuronides via covalent linkage with uridine diphosphate (UDP)-glucuronic acid. This is accomplished by the enzyme bilirubin UDP-glucuronosyl transferase (bilirubin UGT-1), the *HUG-Br1* gene product. Conjugation serves to convert hydrophobic bilirubin into a water-soluble form that can be readily excreted into bile.

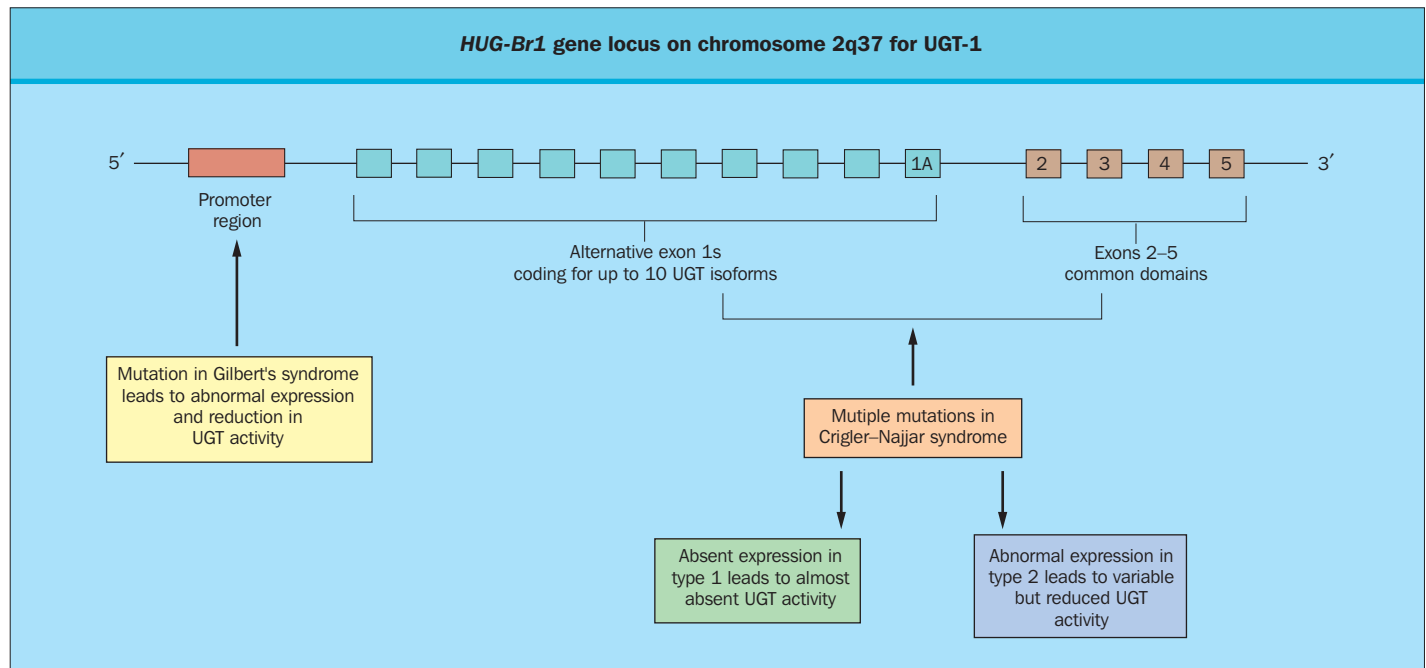
Three inherited disorders of bilirubin metabolism are associated with defects in bilirubin UGT-1 activity (Fig. 6.3). In

### Formation of bilirubin from heme



**Figure 6.2 Formation of bilirubin from heme.**

Shown here are the chemical reactions that are catalyzed by heme oxygenase and biliverdin reductase, in which heme is sequentially converted to biliverdin and then to bilirubin. In the chemical structure shown, side groups are abbreviated as follows: methyl (M), propionyl (P) and vinyl (V).



**Figure 6.3** *HUG-Br1* gene locus on chromosome 2q37 for UGT-1.

Gilbert syndrome, bilirubin UGT-1 activity is decreased in response to an apparent decrease in *HUG-Br1* gene transcription. Mutations in the promoter region of the *HUG-Br1* gene have been reported in patients with Gilbert syndrome and have been thought to contribute to this decrease in transcription. In the Crigler-Najjar syndromes, bilirubin UGT-1 activity is either undetectable due to absence of the *HUG-Br1* gene product (type I Crigler-Najjar syndrome), or enzyme activity is reduced due to missense mutations in the coding region of the *HUG-Br1* gene (type II Crigler-Najjar syndrome). Developmental delay of *HUG-Br1* expression appears to be the cause of physiologic jaundice of the newborn, a condition that resolves spontaneously. An acquired reduction in bilirubin UGT-1 activity occurs during use of the HIV protease inhibitor, indinavir and unconjugated hyperbilirubinemia is detected in up to 25% of individuals taking this drug.

Once conjugated, bilirubin is directed toward the canalicular (apical) membrane of hepatocytes, where it is transported into the bile canaliculus by an ATP-dependent export pump. The responsible protein has been identified as the multidrug resistance-associated protein MRP2 (ABCC2), a member of the ATP-binding cassette protein superfamily. MRP2 appears to function as a multispecific transporter of a variety of organic anions (including glutathione and BSP, but not hydrophilic bile acids). Of interest, MRP deficiency has been identified as the cause of Dubin-Johnson syndrome, another inherited hyperbilirubinemic disorder.

### Bilirubin excretion

Virtually all bilirubin in human bile is conjugated. Approximately 80% is in the form of bilirubin diglucuronides. Nearly all the rest is in the form of bilirubin monoglucuronides and only trace amounts are unconjugated. Bilirubin is propelled with biliary contents into the duodenum, where it is ultimately

eliminated in the stool. Clearly, obstruction of the biliary tree at any level, from its origins at the canals of Hering to its exit at the ampulla of Vater, can lead to jaundice (Fig. 6.4).

Resorption of conjugated bilirubin by the gallbladder and gut is minimal. However, bilirubin breakdown products can be reabsorbed by the gut. In particular, conjugated bilirubin can be hydrolyzed by bacterial  $\beta$ -glucuronidase in the terminal ileum and colon. The resulting unconjugated bilirubin is converted in the intestinal lumen to tetrapyrroles called urobilinogens. Up to 20% of these colorless compounds are resorbed by the gut and are ultimately excreted in bile and urine.

## CAUSES

### Overview

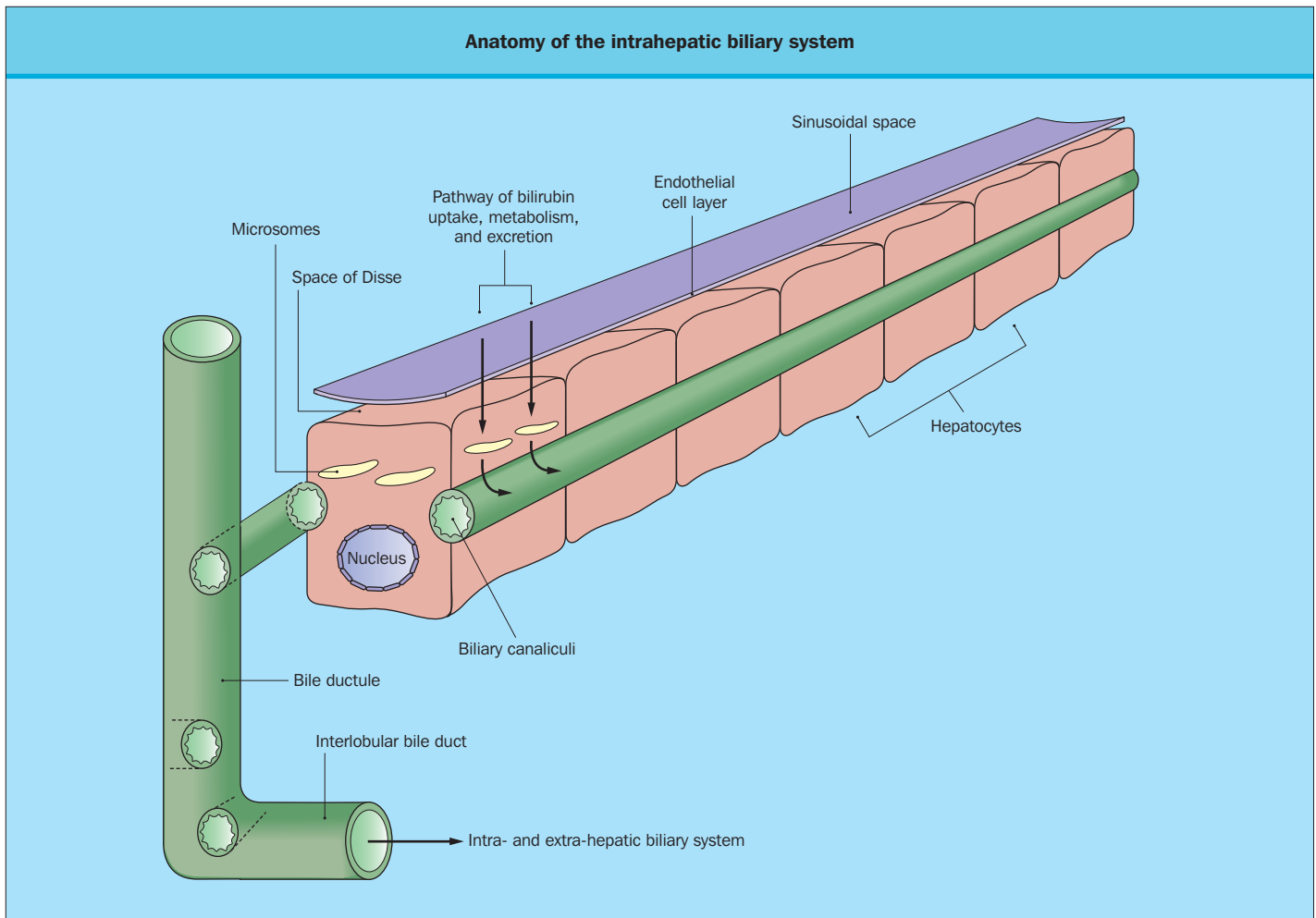
Jaundice can result from either an increase in bilirubin production or a decrease in hepatobiliary elimination of bilirubin (Table 6.1). In practice, the latter entity can be subdivided into three broad categories:

- isolated disorders of bilirubin metabolism;
- intrinsic liver disease; and
- obstruction of the bile ducts.

### Disorders associated with increased bilirubin production

Several processes can lead to increased bilirubin production. These include hemolysis, ineffective erythropoiesis, or resorption of a hematoma. For these reasons, jaundice may complicate the clinical course of patients with hemolytic anemias, either hereditary or acquired. Megaloblastic anemia from either folate or vitamin B<sub>12</sub> deficiency, iron deficiency anemia, sideroblastic anemia and polycythemia vera may also increase bilirubin production. With these disorders, bilirubin concentration does not generally exceed 4–5 mg/dL (68–85  $\mu$ mol/L).





**Figure 6.4 Anatomy of the intrahepatic biliary system.**

Massive transfusion can lead to jaundice because the shortened life span of transfused erythrocytes leads to excessive bilirubin production. This, in conjunction with resorption of hematomas, is a significant factor in the development of hyperbilirubinemia that follows major trauma. Each of these disorders involves excessive delivery of unconjugated bilirubin to the liver without intrinsic hepatobiliary dysfunction. Characteristically, these disorders produce an unconjugated hyperbilirubinemia.

#### Isolated disorders of bilirubin metabolism

Each of the following inherited conditions is characterized by a disorder at a specific step of bilirubin metabolism (see Fig. 6.1) without generalized hepatocellular dysfunction. In some of these disorders, the mutated gene has been identified. These disorders can be divided into unconjugated hyperbilirubinemias and conjugated hyperbilirubinemias (Table 6.2; also see Fig. 6.1 and Table 6.1).

#### Gilbert syndrome

The most common of the inherited hyperbilirubinemias is Gilbert syndrome. Gilbert syndrome is present in up to 10% of Caucasian individuals. As discussed above, Gilbert syndrome is thought to be attributable to decreased bilirubin glucuronidation. Thus, it is characterized by unconjugated hyperbilirubinemia.

Gilbert syndrome is entirely benign and uncommonly produces clinical jaundice. Because fasting is associated with down-regulation of bilirubin UGT-1 activity, serum bilirubin may rise twofold to threefold with fasting in Gilbert syndrome. This is the basis of the diagnostic 'caloric test' for Gilbert syndrome, which involves a prolonged fast. The test, however, has low sensitivity. In general, bilirubin remains below 4 mg/dL ( $68 \mu\text{mol/L}$ ) in affected individuals. Patients with Gilbert syndrome are commonly identified during or after adolescence, when isolated hyperbilirubinemia is detected incidentally during routine multiphasic biochemical screening, or during an intercurrent illness in which symptoms of nausea and vomiting or fasting may thus provoke an unconjugated hyperbilirubinemia.

#### Crigler-Najjar syndromes

More profound defects in bilirubin conjugation are encountered in the Crigler-Najjar syndromes. In type I Crigler-Najjar syndrome, unconjugated bilirubin levels often exceed 20 mg/dL ( $340 \mu\text{mol/L}$ ) and the majority of afflicted individuals die of kernicterus in the neonatal period. Liver transplantation can be life-saving. Patients with type II Crigler-Najjar syndrome have serum bilirubin levels between those of Gilbert syndrome and those of type I Crigler-Najjar syndrome (see Table 6.2). In contrast to type I Crigler-Najjar patients, patients with type II

Differential diagnosis of jaundice
<b>Isolated disorders of bilirubin production or metabolism</b> Unconjugated hyperbilirubinemia Increased bilirubin production e.g. hemolysis, ineffective erythropoiesis, massive blood transfusion, resorption of hematomas Decreased hepatocellular uptake of bilirubin e.g. rifampin (rifampicin) Decreased bilirubin conjugation e.g. Gilbert syndrome, Crigler-Najjar syndrome types I and II, physiologic jaundice of the newborn
Conjugated hyperbilirubinemia Impaired canalicular excretion of bilirubin e.g. Dubin-Johnson syndrome, Rotor syndrome
<b>Liver disease</b> Hepatocellular injury Acute or subacute hepatocellular injury e.g. viral hepatitis, hepatotoxins (e.g. ethanol), drugs [e.g. acetaminophen (paracetamol), many others], ischemia, metabolic disorders (e.g. Wilson disease, Reye syndrome), pregnancy-related (e.g. acute fatty liver of pregnancy, pre-eclampsia), autoimmune hepatitis Chronic hepatocellular injury e.g. viral hepatitis, hepatotoxins (e.g. ethanol), non-alcoholic steatohepatitis, autoimmune hepatitis, metabolic (Wilson disease, hemochromatosis, $\alpha_1$ -antitrypsin deficiency)
Intrahepatic cholestasis Diffuse infiltrative disorders e.g. granulomatous disease, amyloidosis, malignancy Inflammation of intrahepatic bile ductules and/or portal tracts e.g. primary biliary cirrhosis, graft versus host disease, drugs Miscellaneous conditions e.g. benign recurrent intrahepatic cholestasis, intrahepatic cholestasis of pregnancy, drugs (many, e.g. estrogens, anabolic steroids), total parenteral nutrition, sepsis, atypical presentations of viral or alcoholic hepatitis, postoperative cholestasis
<b>Obstruction of the bile ducts</b> Choledocholithiasis Cholesterol gallstones Pigment (black or brown) gallstones Diseases of the bile ducts Inflammatory/infection e.g. primary sclerosing cholangitis, AIDS cholangiopathy, hepatic arterial chemotherapy or trauma, postsurgical strictures Neoplasms (e.g. cholangiocarcinoma)
Extrinsic compression of the biliary tree Neoplasms e.g. pancreatic carcinoma, hepatocellular carcinoma, metastatic lymphadenopathy Pancreatitis Vascular enlargement (e.g. aneurysm, cavernous transformation of portal vein)

Table 6.1 Differential diagnosis of jaundice.

Crigler-Najjar syndrome experience a fall in serum bilirubin concentration to levels of 2–5 mg/dL (34–85  $\mu$ mol/L) with phenobarbital, which increases bilirubin UGT-1 activity. With appropriate phototherapy, these individuals generally survive to adulthood without neurologic impairment.

#### Dubin-Johnson and Rotor syndromes

Two inherited disorders are associated with conjugated hyperbilirubinemia. Each appears to result from a selective decrease in bilirubin secretion into the bile canaliculus. Individuals with Dubin-Johnson syndrome, which has been linked to mutations in the MRP2 gene (see discussion above), also exhibit impaired

canalicular transport of other organic anions such as BSP, glutathione and conjugated bile acids. The molecular basis of Rotor syndrome is currently unknown. Both Dubin-Johnson syndrome and Rotor syndrome are not associated with impaired hepatic function.

#### Liver disease

Jaundice is a classic feature of hepatic dysfunction. In distinction to isolated disorders of bilirubin metabolism, icteric liver disease is characterized by an increase in serum bilirubin concentration in association with abnormalities in other standard biochemical liver tests. The extensive differential diagnosis of

Inherited disorders of hepatic bilirubin metabolism and transport					
	<i>Gilbert syndrome</i>	<i>Type I Crigler-Najjar syndrome</i>	<i>Type II Crigler-Najjar syndrome</i>	<i>Dubin-Johnson syndrome</i>	<i>Rotor syndrome</i>
Incidence	<10% of population	Extremely rare	Uncommon	Uncommon	Rare
Genetic defect	Mutations in promoter region of <i>HUG-Br1</i>	Absent <i>HUG-Br1</i> expression	Mutations in coding region of <i>HUG-Br1</i>	Absent expression of MRP2	Unknown
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive
Mechanism	Decreased bilirubin conjugation	Absent bilirubin conjugation	Markedly decreased bilirubin conjugation	Impaired canalicular excretion of conjugated bilirubin via multispecific anion transporter	Unknown
Serum bilirubin concentration (mg/dL) (μmol/L)	<3–4 (51–68), virtually all unconjugated	Usually >20 (340), unconjugated	Usually <20 (340), virtually all unconjugated	Usually <7 (120), about 50% conjugated	Usually <7 (120), about 50% conjugated
Other laboratory abnormalities	Bilirubin decreases with phenobarbital	No change in bilirubin with phenobarbital	Bilirubin decreases with phenobarbital	Normal total urinary coproporphyrin (>80% type I)	Elevated total urinary coproporphyrin (<80% type I)
Liver histology	Normal	Normal	Normal	Dark pigment, predominantly centrilobular	Normal
Prognosis	Good	Poor, death in infancy from kernicterus unless specific therapy employed	Good	Good	Good
Treatment	None required	Phototherapy initially, liver transplantation necessary for long-term survival	Phenobarbital for marked hyperbilirubinemia	None available; avoid estrogens (worsens hyperbilirubinemia)	None

Table 6.2 Inherited disorders of hepatic bilirubin metabolism and transport.

icteric liver disease is briefly outlined here. Two broad categories of liver disease can be defined: that in which hepatocellular injury is the predominant mechanism; and that in which reduced bile formation (cholestasis) predominates. The latter types of diseases are the most problematic from a diagnostic standpoint, because they are often difficult to distinguish clinically from obstruction of the bile ducts. The following discussion is arbitrarily organized according to the above classification. Clearly, however, there are instances in which hepatocellular injury and cholestasis contribute similarly to the development of jaundice.

### Acute hepatocellular injury

A variety of disorders can produce acute or subacute hepatocellular injury, including viral hepatitis, exposure to hepatotoxins, hepatic ischemia and certain metabolic derangements. In these situations there is usually evidence of severe hepatocyte injury with widespread necrosis and/or apoptosis and often associated inflammatory cell infiltration. This severe damage leads to metabolic dysfunction and destruction of excretory mechanisms, resulting in jaundice.

Five major hepatotropic viruses have been isolated. Hepatitis A and E viruses are transmitted enterally. Each typically pro-

duces a self-limited illness that does not progress to chronic liver disease. By contrast, hepatitis B, C and D viruses are transmitted parenterally and illness produced by these agents may be prolonged and progress to chronic disease. The diagnosis of each these disorders is aided by serologic testing (see Chs 14, 15 and 16).

Toxins and drugs can produce hepatocellular injury, either in a dose-dependent fashion, or idiosyncratically. The most common agent to produce acute dose-dependent hepatocellular injury is acetaminophen (paracetamol). A wide variety of drugs produce idiosyncratic hepatocellular injury and jaundice. These include isoniazid, HMG-CoA reductase inhibitors and minocycline (see Ch. 32). In a patient with ethanol dependency, alcoholic hepatitis should always be a diagnostic consideration when there is jaundice and biochemical evidence of hepatocellular injury. Of note, alcoholic hepatitis can also have an atypical cholestatic presentation that can create diagnostic confusion (see Ch. 21).

Several forms of hepatic ischemia produce hepatocellular injury and jaundice. These include hepatic ischemia caused by systemic hypotension, and/or hypoxia, or occlusive vascular disorders. Thrombosis of the hepatic veins (Budd-Chiari syndrome)



should be suspected in patients with hypercoagulable states and, in particular, myeloproliferative disorders. Hepatic veno-occlusive disease should be suspected in a patient receiving cytotoxic agents in the setting of bone marrow transplantation (see Ch. 30).

Jaundice is a recognized complication of Wilson disease, an inherited disorder of copper excretion into bile with copper overload and hepatocellular injury. Patients with Wilson disease can also develop a Coombs negative hemolytic anemia, which contributes to the disproportionate hyperbilirubinemia that is often present (see Ch. 24). Several mitochondriopathies are associated with impaired fatty acid metabolism and hepatocellular injury from fatty acid overload. These include Reye syndrome, which classically follows a viral illness in children, valproic acid toxicity and fatty liver of pregnancy.

### Chronic hepatocellular injury

Jaundice is a well-recognized indicator of significant hepatic dysfunction in the setting of liver diseases produced by chronic hepatocellular injury. In such situations, there are a variety of pathogenetic mechanisms that cause liver damage. In general, there is hepatocyte necrosis and/or apoptosis, as well as inflammation of varying severity, and progressive hepatic fibrosis that ultimately leads to cirrhosis. The hepatocytes are therefore severely affected at a functional level, and biliary excretion is also substantially affected at both the canalicular and more distal levels due to the grossly deranged architecture. As a rule, jaundice is unusual in chronic hepatocellular injury unless cirrhosis has developed. Although the broad categories of chronic hepatocellular injury are similar to those of acute injury, there are differences in the specific details. For example, causes of chronic viral hepatitis are largely limited to hepatitis B, C and D viruses. The major hepatotoxin-associated chronic hepatocellular disease is alcoholic cirrhosis, whereas drug toxicity is a much less common cause of chronic as opposed to acute hepatocellular injury. Non-alcoholic fatty liver disease (which is histologically indistinguishable from alcoholic liver disease) has emerged as a leading cause of chronic hepatocellular injury in industrialized nations; it is most prevalent in overweight individuals, in particular those with type 2 diabetes mellitus. The possibility of industrial exposure to toxic compounds such as vinyl chloride should be a diagnostic consideration in jaundiced patients with an appropriate background; carbon tetrachloride, a classic industrial hepatotoxin, is no longer used in the USA.

Two metal storage diseases merit consideration. Wilson disease clearly produces chronic hepatocellular injury (see Ch. 24). However, the most common metal storage disease worldwide is the iron overload disorder genetic hemochromatosis, with an estimated prevalence of 0.5% in Caucasian populations. Hepatocytes are a major target of iron-mediated injury in hemochromatosis, although cholangiocytes can be affected when iron overload is significant (see Ch. 23). Miscellaneous causes of chronic hepatocellular injury include autoimmune hepatitis and  $\alpha_1$ -antitrypsin deficiency.

### Cholestasis

Cholestasis is a term referring to a reduction in bile flow or bile formation. It can therefore occur anywhere from the sinusoidal membrane of the hepatocyte to the exit of the common bile duct into the duodenum. Thus, cholestasis can be classified as

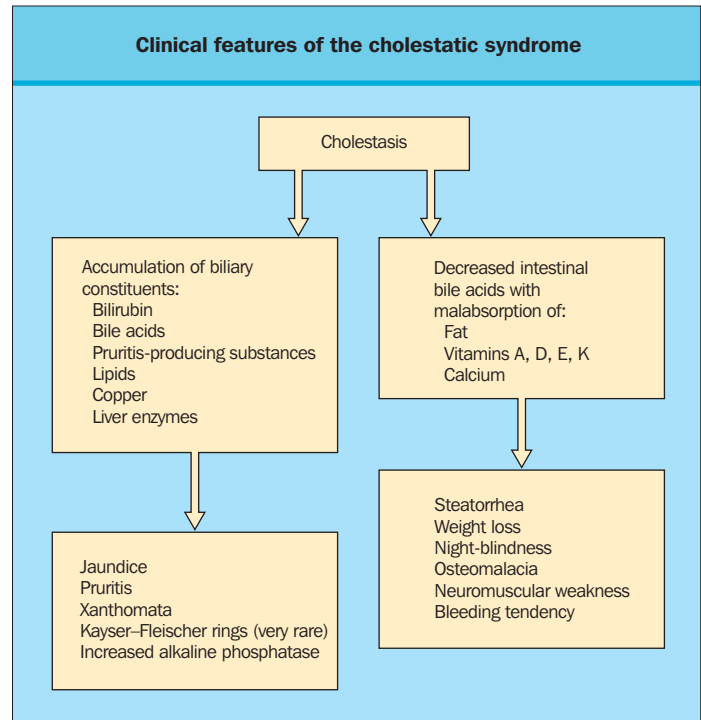


Figure 6.5 Clinical features of the cholestatic syndrome.

intra- or extrahepatic, and there are many causes of each. This classification is adhered to here with a discussion of hepatic disorders that cause intrahepatic cholestasis and of obstruction of the bile ducts that causes extrahepatic cholestasis.

Functionally, if bile flow is interrupted, at whatever level, there will be decreased hepatic secretion of water and organic anions (including bile acids and bilirubin). Normal bile flow is obviously necessary for bilirubin excretion, and thus, many of the pathologic processes affecting overall bile production also affect bilirubin clearance (see Figs 6.1 and 6.4). Morphologically, bile will accumulate in the liver and biliary system and evidence of this can be seen on liver biopsy.

Clinically, there will be features resulting from the decreased secretion and retention of bile, such as jaundice, pruritus and evidence of malabsorption (Fig. 6.5).

As indicated, in the diagnosis of jaundice, one of the most difficult differentials is between intra- and extrahepatic cholestasis.

### Hepatic disorders with prominent cholestasis (intrahepatic cholestasis)

#### Overview

The following diseases are characterized by biochemical abnormalities that mimic obstruction of the bile ducts. These disorders have the greatest potential to generate diagnostic confusion. Such disorders can be categorized histologically according to those that infiltrate the liver, those that mainly involve injury to intrahepatic bile ductules or portal triads, and those in which major histologic changes are not evident.

#### Infiltrative diseases

Many forms of infiltrative liver diseases are often associated with striking cholestasis and jaundice. Infiltrative diseases of the

liver can be conveniently divided into granulomatous diseases, amyloidosis and neoplastic replacement of hepatic parenchyma. The latter should be suspected if there is a known underlying malignancy. These disorders are likely to produce cholestasis by compression or obliteration of small bile ductules. Granulomatous diseases of the liver include:

- infections, such as tuberculosis, *Mycobacterium avium* complex (particularly in the immunocompromised host), leprosy, brucellosis, Q fever, syphilis, fungal diseases, parasitic diseases and mononucleosis;
- toxins, such as beryllium, quinidine, allopurinol and sulfonamides; and
- systemic disorders, including sarcoidosis, lymphoma (in particular, Hodgkin disease) and Wegener granulomatosis.

Tuberculosis and sarcoidosis are the most common forms of granulomatous liver diseases to produce jaundice. Although amyloidosis is in the differential diagnosis of infiltrative liver diseases, jaundice is extremely uncommon in that setting.

### Disorders involving bile ductules

Inflammation and loss of small intrahepatic bile ductules are characteristic of primary biliary cirrhosis. When jaundice occurs, the disease is generally advanced. In particular, the presence of a serum bilirubin concentration of greater than 10 mg/dL (170  $\mu$ mol/L) carries an extremely poor prognosis without liver transplantation. A distinct form of bile ductular injury occurs with hepatic involvement in graft versus host disease, encountered primarily after bone marrow transplantation but occasionally in organ transplant recipients as well. Certain drugs can produce inflammation of the portal tracts with resultant cholestasis. These include chlorpromazine, erythromycin (particularly the estolate salt), chlorpropamide and methimazole. Cholestasis generally resolves within several months after drug discontinuation.

### Cholestasis with minimal histologic abnormalities

Jaundice may accompany conditions characterized by minimal hepatocellular injury or histologic abnormalities. The mechanism of cholestasis in these conditions is not well understood at present and may be multifactorial. In benign recurrent cholestasis, an autosomal recessive disorder that has been mapped to the same gene (*PFIC1*) that is mutant in progressive familial intrahepatic cholestasis type I (Byler disease), there is a defect of canalicular secretion of several classes of organic anions, including bile acids, into bile.

Sex hormones can produce a histologically bland intrahepatic cholestasis. Estrogens reduce bile formation through several mechanisms. These include:

- inhibition of the hepatocellular plasma membrane sodium pump, an important modulator of solute transport from blood to bile;
- impaired acidification of intracellular organelles, which may disrupt the targeting of organic anion transporters to their proper membrane domain;
- inhibition of the activity of the bile salt export pump (BSEP) (ABCB11), and;
- decreased membrane fluidity, which may perturb the function of such transporters.

Each of these mechanisms may contribute to jaundice resulting from the use of oral contraceptives. However, jaundice is

rare with the low doses of estrogen in modern contraceptives. Anabolic steroids can produce a syndrome that is clinically indistinguishable from that of estrogen-induced cholestasis. Of interest, the anabolic steroids methyltestosterone and norethandrolone may impair the integrity of hepatocellular microfilaments and thus increase back diffusion of biliary solutes through tight junctions into serum.

The features of cholestasis associated with total parenteral nutrition (TPN) can resemble those of cholestasis associated with estrogens and anabolic steroids. However steatohepatitis can also be seen with TPN. It has been proposed that TPN-induced cholestasis is also related to an altered enterohepatic circulation as well as diminished neuroendocrine stimulation of bile flow.

Cholestasis and jaundice may also develop during bacterial infections. Several mechanisms have been proposed, each related to cytokines, including tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$  released during infection. Tumor necrosis factor- $\alpha$  reduces expression of MRP2, interleukin-1 $\beta$  reduces expression of BSEP, and each reduces expression of the basolateral bile acid uptake protein NTCP (SLC10A1), all of which result in decreased bile flow. Sepsis-related cholestasis in the critically ill patient may be extremely difficult to distinguish from obstruction of the bile ducts. Helpful diagnostic clues are discussed below.

### Atypical presentations of cholestasis

Viral hepatitis may on occasion produce profound cholestasis with marked pruritus. Unless there are risk factors for viral hepatitis, no features reliably distinguish this disorder from those of other cholestatic syndromes or biliary tract obstruction. A high level of suspicion and appropriate serologies will aid in establishing the diagnosis. The course can be quite prolonged. Similarly, alcoholic hepatitis can uncommonly have a cholestatic presentation. This is one setting in which urgent liver biopsy may be required to make the diagnosis.

### Jaundice in pregnancy

Several cholestatic disorders associated with pregnancy merit discussion. Each of these characteristically occurs in the third trimester. Intrahepatic cholestasis in pregnancy typically presents with pruritus. Infrequently, it is associated with jaundice. It generally resolves within 2 weeks of delivery and tends to recur with subsequent pregnancies. Two far more serious syndromes are acute fatty liver of pregnancy and pre-eclampsia with HELLP syndrome (*hemolysis, elevated liver function and low platelets*). The former histologically resembles Reye syndrome with microvesicular steatosis in hepatocytes. The latter is a microvascular disorder of the third trimester that is characterized by hemolysis, coagulopathy and low platelet counts. Each requires prompt delivery.

### Jaundice in the postoperative patient

Postoperative jaundice is often multifactorial and includes both cholestatic disorders and those that produce hepatocellular injury. Potential contributors include inhalational anesthetic agents, other potentially hepatotoxic drugs, hepatic ischemia, blood transfusions, TPN and sepsis. These can be distinguished from benign postoperative cholestasis, a self-limited (less than 1–2 weeks) syndrome characterized by transient conjugated

hyperbilirubinemia without biochemical evidence of hepatocellular injury.

The liver transplant recipient represents a special case, in which the differential diagnosis of jaundice may not only include the disorders common to all postoperative patients, but those that relate to transplantation in particular. Specific diagnostic considerations include graft dysfunction due to ischemia-reperfusion injury or vascular occlusion, graft rejection, obstruction of the bile ducts, bile leak, viral hepatitis (e.g. cytomegalovirus, recurrent hepatitis B or C), immunosuppressive drug toxicity (e.g. azathioprine) and lymphoproliferative disorders.

### Obstruction of the bile ducts

Obstructive disorders of the biliary tree include occlusion of the bile duct lumen by gallstones, intrinsic disorders of the bile ducts and extrinsic compression.

#### Cholelithiasis

The most common cause of biliary obstruction is cholelithiasis. Two types of gallstones are associated with this problem: cholesterol gallstones and pigment gallstones. Cholesterol gallstones typically originate in the gallbladder, migrate into the common bile duct and either impact at the ampulla of Vater or produce partial obstruction in a ball-valve fashion. Calcium bilirubinate (black pigment) gallstones characteristically develop in patients with unconjugated hyperbilirubinemia. Black pigment stones, like cholesterol stones, can form in the gallbladder, but they may also form in situ at any level of the biliary tree including the common bile duct. A distinct type of bilirubinate stone, so-called brown pigment gallstones, also forms in situ within the biliary tree. Obstruction of the bile ducts by these stones leads to repeated bouts of cholangitis (recurrent pyogenic cholangitis) in patients from certain regions in Asia, in association with biliary parasitic infestations, and in patients with prior biliary tract surgery.

#### Diseases of the bile ducts

Intrinsic disorders of the bile ducts may be inflammatory, infectious, or neoplastic. Primary sclerosing cholangitis, an inflammatory disorder of the bile ducts, characterized by focal and segmental strictures, is extensively discussed elsewhere in this book (see Ch. 20). A similar picture of focal narrowing and localized obstruction of the bile ducts is seen in patients infected with human immunodeficiency virus (so-called AIDS cholangiopathy), but jaundice is distinctly unusual in this setting. Biliary strictures may also follow hepatic arterial infusion of certain chemotherapeutic agents or result from surgical injury to the bile duct or hepatic artery. Neoplasms of the biliary tree, including cholangiocarcinoma, are discussed elsewhere in this book (see Chs. 28 and 29).

#### Extrinsic compression of the bile ducts

Extrinsic compression of the biliary tree may result from neoplastic involvement or inflammation of surrounding viscera. Rarely, marked enlargement of surrounding vessels (e.g. arterial aneurysms, cavernous transformation of the portal vein) can compress the bile ducts as well.

Painless jaundice is a classic sign of carcinoma of the head of the pancreas. Occasionally, hepatocellular carcinoma or periportal lymph nodes enlarged by any metastatic tumor or

lymphoma obstruct the extrahepatic bile ducts. An unusual complication of cholecystitis, Mirizzi syndrome, occurs when a large gallstone becomes impacted in the cystic duct leading to compression of the common bile duct. Pancreatitis may also produce extrinsic biliary compression as a result of edema, pseudocyst formation, or fibrosis.

## CLINICAL FEATURES

### Overview

In a jaundiced individual, associated clinical features can be very helpful in distinguishing isolated disorders of hyperbilirubinemia from intrinsic liver disease or from biliary tract obstruction. Important clues can be obtained from the history and physical examination. These, in conjunction with standard biochemical tests, will substantially improve the efficiency of subsequent diagnostic studies.

It should be remembered that jaundice is only one of the features of cholestasis, but that other effects of cholestasis are encountered in such patients. Some of these features can be present without jaundice (see Fig. 6.5).

### History

The absence of any symptoms other than jaundice is consistent with hemolysis or an isolated disorder of bilirubin metabolism. However, it does not exclude intrinsic liver disease or biliary tract obstruction. Clues to the presence of liver disease versus biliary tract obstruction are featured in **Table 6.3**.

Symptoms compatible with a viral prodrome, such as anorexia, malaise and myalgias, make viral hepatitis a strong possibility, as does a history of known infectious exposure, injection drug use, or receipt of blood products. The presence of arthralgias and rash, and other autoimmune disorders, heightens the suspicion for autoimmune hepatitis. Symptoms of pruritus are encountered with several disorders of intrahepatic cholestasis, such as primary biliary cirrhosis. Important clues to chronic liver disease include a history of fluid retention or confusion. A history of rapid fluid retention and jaundice raises the concern of Budd-Chiari syndrome or veno-occlusive disease. A careful history may suggest that environmental hepatotoxins, ethanol, or medications underlie hepatic dysfunction that is responsible for jaundice. Finally, a family history of liver disease suggests the possibility of a hereditary disorder, such as hemochromatosis, Wilson disease, or  $\alpha_1$ -antitrypsin deficiency.

There are several historic clues to the presence of biliary tract obstruction. A history of fever, especially when accompanied by rigors, or abdominal pain, particularly in the right upper quadrant, is suggestive of cholangitis due to obstructive diseases (particularly cholelithiasis). A history of prior biliary surgery also increases the likelihood that biliary tract obstruction is present. Although weight loss is often a non-specific symptom, it is suggestive of malignancy in an older individual with jaundice. Obstructive jaundice from gallstone disease is also more common in the elderly.

### Physical examination

Like the history, physical examination can offer important clues that distinguish intrinsic liver disease from biliary tract obstruction. Several signs suggest that liver disease is the cause of jaundice. These include evidence of portal hypertension (i.e.



Features that differentiate biliary tract obstruction from cholestatic liver disease in the diagnosis of jaundice		
	<i>Favors biliary obstruction</i>	<i>Favors liver disease</i>
History	Abdominal pain Fever, rigors Poor biliary surgery Older age	Malaise, myalgias, arthralgias, suggestive of viral syndrome Known infectious exposure Receipt of blood products, intravenous or nasal use of illicit drugs Exposure to known hepatotoxin Family history of liver disease
Physical examination	Fever Abdominal tenderness Palpable abdominal mass Surgical scar	Ascites Signs of chronic liver disease (e.g. prominent abdominal veins, gynecomastia, spider angiomas, asterixis, encephalopathy) Ascites Signs of specific liver disease (e.g. Kayser-Fleischer rings, xanthelasma)
Routine laboratory studies	Predominant elevation of alkaline phosphatase Prothrombin time that is normal or normalizes with vitamin K administration Elevated serum lipase	Predominant elevation of serum aminotransferases Prolonged prothrombin time that does not correct with vitamin K administration Decreased albumin concentration

**Table 6.3** Features that differentiate biliary tract obstruction from cholestatic liver disease in the diagnosis of jaundice.



**Figure 6.6** Xanthomata (fatty deposits) in the skin creases and palm of a patient with primary biliary cirrhosis.

ascites, splenomegaly, or prominent abdominal veins) or other characteristic features, such as spider angiomas, gynecomastia and asterixis. Certain physical findings may suggest particular liver diseases. Examples of such classic signs include hyperpigmentation in hemochromatosis, xanthomas in primary biliary cirrhosis (see **Fig. 6.6**), and Kayser-Fleischer rings in Wilson disease. By contrast, certain findings suggest biliary tract obstruction. For example, high fever or abdominal tenderness (particularly right upper quadrant) suggests cholangitis. A palpable abdominal mass or palpable gallbladder suggests a neoplastic cause of obstructive jaundice. Finally, an abdominal scar in the midline or right upper quadrant may be the only clinical clue to prior biliary surgery.

### A caveat

All clues in the history and physical must be interpreted with caution, because fever and abdominal pain accompany diseases other than biliary obstruction, and patients with prior biliary surgery may develop viral hepatitis. Conversely, anorexia and malaise are not exclusively symptoms of viral hepatitis, and patients with parenchymal liver disease can certainly develop gallstones. Nonetheless, when these clues are evaluated with physical findings and routine laboratory tests, jaundice is correctly characterized as obstructive or non-obstructive in most cases.

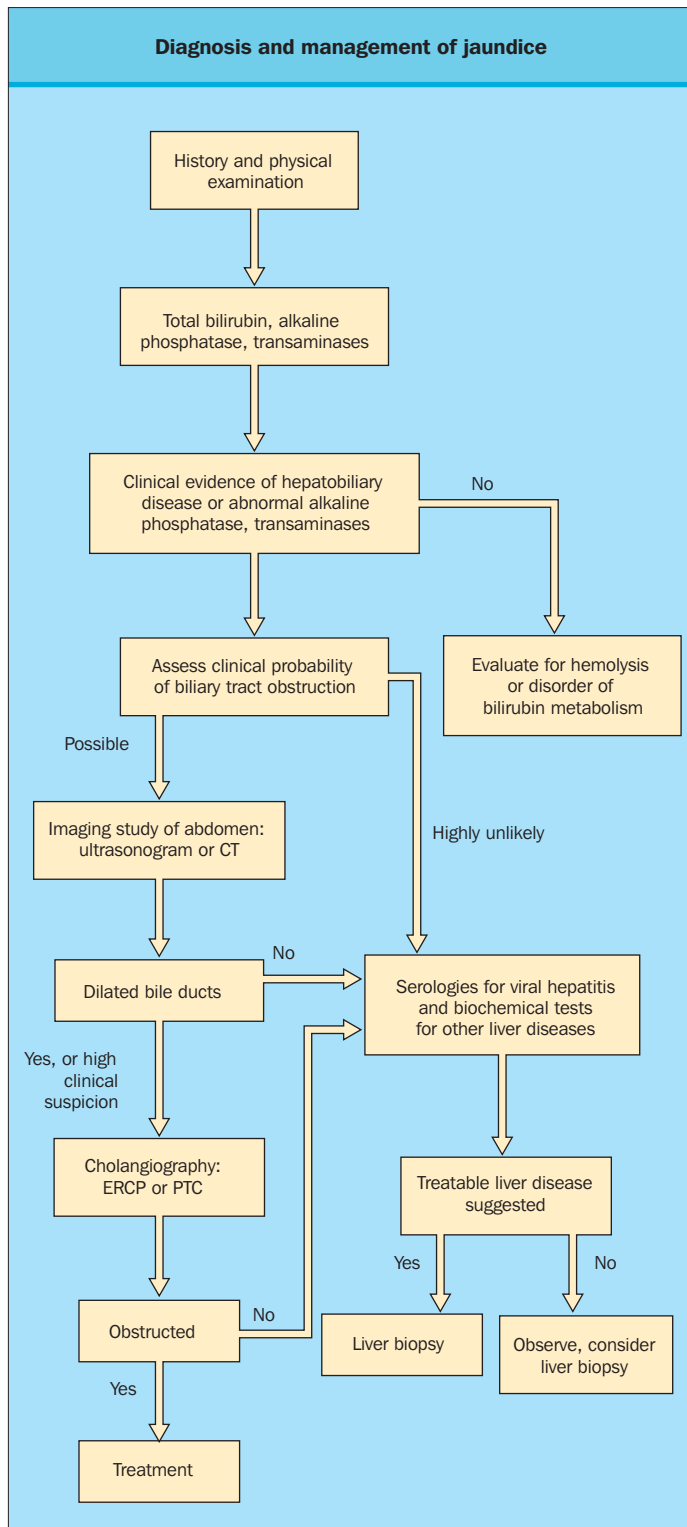
## DIAGNOSIS AND EVALUATION

### Overview

A general algorithm for evaluating the patient with jaundice is presented in **Figure 6.7**. The sequential approach involves: a careful patient history, physical examination and screening laboratory studies; formulation of a working differential diagnosis; selection of further specialized tests to narrow the diagnostic possibilities; and development of a strategy for treatment or further testing if unexpected diagnostic possibilities are suggested.

### Screening laboratory studies

Essential laboratory studies include serum total bilirubin, alkaline phosphatase, aminotransferases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], albumin, and prothrombin time. Serum alkaline phosphatase activity reflects the presence of a number of related enzymes of different tissue origins. In liver, alkaline phosphatase is a membrane-associated protein that is present on the apical domain of the plasma membrane of hepatocytes and cholangiocytes. Under physiologic conditions, this protein is



**Figure 6.7** Diagnosis and management of the jaundiced patient.

enzymatically cleaved and released into bile, but small amounts are released into serum as well. Biliary obstruction or intrahepatic cholestasis increases serum alkaline phosphatase activity by increasing its synthesis, targeting to the basolateral domain of the plasma membrane and release into serum. However,

increased serum alkaline phosphatase activity may reflect release of alkaline phosphatase isoenzymes that are released from extrahepatic tissues. Hence, other more specific markers, such as the serum activities of the canalicular enzymes  $\gamma$ -glutamyltranspeptidase, leucine aminopeptidase, or 5'-nucleotidase are useful to confirm the hepatobiliary origin of alkaline phosphatase. In a jaundiced patient, a predominant increase in (hepatic) alkaline phosphatase activity relative to those of serum aminotransferases suggests the possibility of biliary tract obstruction. It should be noted that intrahepatic cholestatic disorders can produce an identical biochemical picture. By contrast, in hemolysis or an isolated disorder of bilirubin metabolism, alkaline phosphatase and serum aminotransferases are normal.

Measurements of aminotransferases, in particular, AST and ALT, are useful in the evaluation of jaundice. Serum AST reflects a mixture of isozymes released from both the cytosol and mitochondria of hepatocytes as well as AST released from other tissues. ALT is a cytosolic enzyme found predominantly in hepatocytes. Both AST and ALT ordinarily circulate in low concentrations in serum. However, hepatocyte damage, due to ischemia, viral infection, or toxins, significantly increases serum aminotransferase activity. Thus, predominant elevation of serum aminotransferase activity in comparison with alkaline phosphatase activity suggests that jaundice is due to intrinsic hepatocellular disease. By contrast, in hemolysis or an isolated disorder of bilirubin metabolism, serum aminotransferases are normal.

Of note, a serum activity of AST that exceeds that of ALT by at least a factor of two, but is less than 10 times the upper limit of normal, represents a clue to the diagnosis of alcoholic liver disease. However, acute Wilson disease may present with similar biochemical abnormalities. There are exceptions to the above generalizations. For example, transient biliary obstruction from choledocholithiasis is occasionally associated with a brief but dramatic elevation (exceeding 10 to 20 times normal) of serum aminotransferase activity.

Serum albumin is a useful marker of hepatic synthetic function, as it is decreased in the presence of significant liver disease. However, other conditions such as malabsorption, nephrotic syndrome and systemic inflammation can depress serum albumin levels. Thus test results are best interpreted in conjunction with the prothrombin time. The prothrombin time is a measure of the plasma activities of the coagulation factors I, II, V, VII and X, each of which is synthesized in the liver. Prolongation of the prothrombin time can result from impaired hepatic synthesis of these proteins but may also reflect deficiency of vitamin K, which is essential for post-translational  $\gamma$ -carboxylation of lysine residues of factors II, VII, IX and X. Vitamin K absorption requires an intact enterohepatic circulation (hence an unobstructed biliary tree) of bile acids. Thus, parenteral administration of vitamin K will generally normalize a prolonged prothrombin time in patients with obstructive jaundice but not in patients with liver disease. In hemolysis or an isolated disorder of bilirubin metabolism, serum albumin and prothrombin time are (generally) normal.

In most cases, the measurement of the total bilirubin concentration is sufficient to aid in the diagnosis of jaundice, especially when it is interpreted in conjunction with the alkaline phosphatase and aminotransferases. In patients with liver disease

or biliary tract obstruction, there are either signs or symptoms of hepatobiliary disease, or more than one of the above laboratory tests is abnormal. By contrast, patients with hemolysis or isolated disorders of bilirubin metabolism have no signs or symptoms of hepatobiliary disease, and the only abnormality in screening laboratory studies is hyperbilirubinemia.

Although some laboratories provide colorimetric estimates of conjugated and unconjugated bilirubin, these are not usually necessary in practice (with the exception of neonatal jaundice, in which unconjugated hyperbilirubinemia has therapeutic implications). In general, such tests may have greatest utility in distinguishing unconjugated hyperbilirubinemias (e.g. hemolysis, Gilbert and Crigler-Najjar syndromes), from isolated conjugated hyperbilirubinemias (e.g. Rotor and Dubin-Johnson syndromes). These are uncommon issues. Moreover, accurate distinction between these conditions usually requires more sophisticated chromatographic tools that are available only in specialized centers. Thus, reliance on the relative concentrations of unconjugated versus conjugated bilirubin is not helpful in most cases.

### Decision analysis

In aggregate, history, physical examination and screening laboratory studies provide a good estimate of the likelihood that obstructive jaundice is present or absent. For example, an asymptomatic hyperbilirubinemic patient who has an unremarkable physical examination and normal serum alkaline phosphatase, ALT, AST, albumin and prothrombin is unlikely to have liver disease or biliary obstruction. Further testing for specific disorders, such as hemolysis or isolated defects in bilirubin metabolism, is warranted (see Fig. 6.9).

If classic features of liver disease are present on the basis of history, physical examination and screening laboratory studies, and biliary tract obstruction is not suspected, then a further evaluation for causes of liver disease should ensue. On the other hand, if the history, physical examination and laboratory studies suggest that biliary tract obstruction is possible, an imaging study is appropriate to confirm either the presence or absence of biliary tract obstruction. Selection of an imaging study depends on the likelihood of obstruction, diagnostic accuracy, cost, complication rate and availability, especially if simultaneous therapeutic intervention is anticipated.

### Imaging studies (see Ch. 8 also)

#### Abdominal ultrasonography

Abdominal ultrasonography is often the initial imaging study in the evaluation of hepatobiliary disease, because it determines the caliber of the extrahepatic biliary tree, evaluates the liver parenchyma and it reveals intra- or extrahepatic mass lesions. The sensitivity of abdominal ultrasonography for the detection of biliary obstruction in jaundiced patients ranges from 55 to 91%, and the specificity from 82 to 95% (Table 6.4). Ultrasonography can also demonstrate cholelithiasis (although common duct stones may not be well seen) and space occupying lesions in the liver greater than 1 cm in diameter. Ultrasonography has three major advantages. First, it is non-invasive. Second, it is portable (this may be invaluable in the evaluation of the critically ill patient). Third, it is relatively inexpensive. However, there are several potential disadvantages with ultrasound. For example, studies may be difficult to interpret in

obese patients or patients in whom overlying bowel gas obscures the biliary tree. Also, dilatation of the common bile duct, which usually indicates biliary tract obstruction, is common in patients who have undergone previous cholecystectomy. A final caveat is that in patients with cirrhosis and other conditions associated with poorly compliant hepatic parenchyma such as primary sclerosing cholangitis, intrahepatic ducts may not dilate with obstruction.

#### Computed tomography of the abdomen

Computed tomography (CT) of the abdomen with intravenous contrast is an excellent alternative to ultrasound in evaluating the possibility of biliary tract obstruction in jaundiced patients. Abdominal CT has a diagnostic accuracy comparable to that of ultrasound in the diagnosis of biliary obstruction, with a sensitivity and specificity of 63–96% and 93–100%, respectively (see Table 6.4). Abdominal CT has several advantages over ultrasound. First, it detects space occupying lesions as small as 5 mm. Second, it is not operator-dependent as in ultrasonography. Third, it provides technically superior images in obese individuals and in patients in whom the biliary tree is obscured by bowel gas. Additional caveats that apply to the accuracy of ultrasonography for the diagnosis of biliary obstruction also apply to abdominal CT. Other considerations in the utilization of abdominal CT in patients with jaundice are its lack of portability, requirement for use of intravenous contrast and expense.

#### Endoscopic retrograde cholangiopancreatography

Endoscopic retrograde cholangiopancreatography (ERCP) permits direct visualization of the biliary tree as well as the pancreatic ducts. In contrast to abdominal ultrasonography and CT, ERCP is more invasive. The procedure involves passage of an endoscope into the duodenum, introduction of a catheter into the ampulla of Vater and injection of contrast medium into the common bile duct and/or pancreatic duct. Importantly, conscious sedation and even in some cases general anesthesia is necessary.

The technique of ERCP is highly accurate in the diagnosis of biliary obstruction, with a sensitivity of 89–98% and specificity of 89–100% (see Table 6.4). It offers the possible implementation of other diagnostic maneuvers in addition to simple visualization and radiography. For example, it permits biopsies and brushings for cytology of periampullary lesions. Moreover, if a focal cause for biliary obstruction is identified (e.g. choledocholithiasis, biliary stricture), maneuvers to relieve obstruction (e.g. sphincterotomy, stone extraction, dilatation, stent placement) can be performed during the same session. It should be noted that acquisition of biopsy material and therapeutic intervention via ERCP are largely limited to lesions that are distal to the bifurcation of the right and left hepatic bile ducts.

The technical success rate of ERCP is higher than 90%; the rate limiting step is cannulation of the ampulla of Vater. This may be a particularly important consideration in patients with prior abdominal surgery and altered anatomy (e.g. cholecystojejunostomy, gastrojejunostomy). As an invasive procedure, ERCP should be employed thoughtfully. The morbidity and mortality associated with ERCP from untoward events such as respiratory depression, aspiration, bleeding, perforation, cholangitis and pancreatitis, are 3 and 0.2%, respectively. These



Imaging studies for the evaluation of jaundice					
Test	Sensitivity (%)	Specificity (%)	Morbidity (%)	Mortality (%)	Comments
Abdominal ultrasonogram	55–91	82–95	–	–	Advantages: Non-invasive, portable, less expensive Disadvantages: Bowel gas may obscure common bile duct; interpretation difficult in obese individuals and in those with ileus
Abdominal CT	63–96	93–100	–	–	Advantages: Non-invasive, not operator-dependent, interpretation less affected by obesity or ileus Disadvantages: Not portable, intravenous contrast required (potential for nephrotoxicity)
ERCP	89–98	89–100	3	0.2	Advantages: Provides direct imaging of bile ducts, permits direct visualization of perampullary mucosa, potential for simultaneous tissue acquisition or therapeutic intervention, especially useful for lesions distal to bifurcation or hepatic ducts Disadvantages: Cannot be performed if altered anatomy precludes endoscopic access to ampulla (e.g. gastro- or choledochojunostomy)
PTC	98–100	89–100	3	0.2	Advantages: Provides direct imaging of bile ducts, potential for simultaneous tissue acquisition or therapeutic intervention, especially useful for lesions proximal to common hepatic duct Disadvantages: More difficult with non-dilated intrahepatic bile ducts
CT, computed tomography; ERCP, endocrine retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiography					

Table 6.4 Imaging studies for the evaluation of jaundice.

rates are increased when interventional procedures are concomitantly employed. A final consideration is cost, as ERCP is more expensive than non-invasive imaging procedures.

#### **Percutaneous transhepatic cholangiography**

Percutaneous transhepatic cholangiography (PTC) is a procedure that complements ERCP. It requires the passage of a needle through the skin and subcutaneous tissues into the hepatic parenchyma and advancement into a peripheral bile duct. When bile is aspirated, a catheter is introduced through the needle and radio-opaque contrast medium is injected. It has accuracy comparable to that of ERCP in the diagnosis of biliary tract obstruction in the setting of jaundice, with a sensitivity and specificity of approximately 98–100% and 89–100%, respectively (see Table 6.4). As in ERCP, interventional procedures, such as balloon dilatation and stent placement to relieve amenable focal obstructions of the biliary tree, can be performed at the time of PTC.

Percutaneous transhepatic cholangiography is potentially technically advantageous under conditions in which the level of biliary obstruction is proximal to the common hepatic duct or in which altered anatomy precludes ERCP (see above). However, when the intrahepatic ducts are not dilated, multiple passes are frequently required and PTC may be technically unsuccessful in up to 25% of attempts. The morbidity and mortality of PTC from bleeding, perforation and cholangitis are 3 and 0.2%, respectively. Like ERCP, it is more expensive than abdominal ultrasonography or CT.

#### **Other imaging studies**

Magnetic resonance cholangiography (MRC) is a technical refinement of standard magnetic resonance imaging that permits rapid clear-cut delineation of the biliary tree without the requirement for intravenous contrast. Emerging evidence suggests that its accuracy for detecting biliary tract obstruction approaches that of ERCP. Endoscopic ultrasonography (EUS) appears to

have performance characteristics that are comparable to those of MRC in the diagnosis of biliary stones or strictures. Although MRC and EUS are promising imaging modalities, they are expensive, making it uncertain whether they will supplant abdominal ultrasonography or CT as an initial imaging test. Moreover, at the time of this writing, it is not clear whether MRC or EUS should be performed before ERCP or PTC if ultrasonography or CT is inconclusive.

Nuclear scintigraphy of the biliary tree, while helpful in the diagnosis of cholecystitis, is not sufficiently sensitive to justify its use in the diagnostic evaluation of jaundice. Furthermore, hepatic uptake of radiolabeled derivatives of iminodiacetic acid [e.g. 2,6-dimethyl iminodiacetic acid (HIDA)] is quite limited when the serum bilirubin exceeds 7–10 mg/dL (119–170  $\mu$ mol/L).

### Suggested strategies for imaging

The order of imaging studies will depend largely on the clinical likelihood of obstructive jaundice (see Fig. 6.9). Clinical decision analysis has compared several diagnostic strategies by mathematical modeling. For example, if the probability of biliary obstruction is 20%, the positive and negative predictive values of a strategy that employs ultrasonography as the initial test would be 96 and 98%, respectively. This compares quite favorably with a strategy that employs ERCP as the initial test. On the other hand, if the probability of biliary obstruction is only 60%, a strategy that employs ultrasonography as the first test would yield a positive predictive value of 99%. However, the negative predictive value would fall to 89%. By contrast, when ERCP is the initial diagnostic procedure, the predictive value of a positive test would be 99% and that of a negative test would be 95%.

Stated differently, among patients in whom the probability of biliary obstruction is felt to be low (but not zero), information regarding the hepatic parenchyma may be just as important as excluding the possibility of biliary obstruction. Therefore, in such patients an abdominal ultrasonogram or CT is appropriate as initial imaging studies. If no evidence of biliary obstruction is found, the patient can be followed and should undergo further evaluation for hepatic parenchymal disease as appropriate (see Fig. 6.9). On the other hand, if dilated bile ducts are visualized, then direct imaging of the biliary tree should be performed.

If biliary obstruction is felt to be very likely on clinical and biochemical grounds, ERCP (or PTC) could be considered as the initial study, since the absence of dilated ducts on abdominal ultrasound or CT would not rule out biliary obstruction. If ERCP or PTC fails to show biliary obstruction, then abdominal ultrasonography or abdominal CT should be performed (if this has not been done) to image the hepatic parenchyma, and further studies, as outlined below, should be obtained.

The decision to employ ERCP versus PTC will be influenced by a variety of factors (see above and Table 6.4). These include the availability of each procedure at a particular facility, the presence or absence of dilated bile ducts, the suspected level of biliary obstruction, and the importance of accurately localizing the obstructing lesion (proximal with PTC, or distal with ERCP) in order to facilitate a plan of therapy. Under most circumstances, ERCP should be the procedure of choice, since it is comparable to PTC in availability, accuracy, technical success

rate and frequency of complications, and offers a broader range of interventional options than PTC.

### Further studies

#### Serologic testing

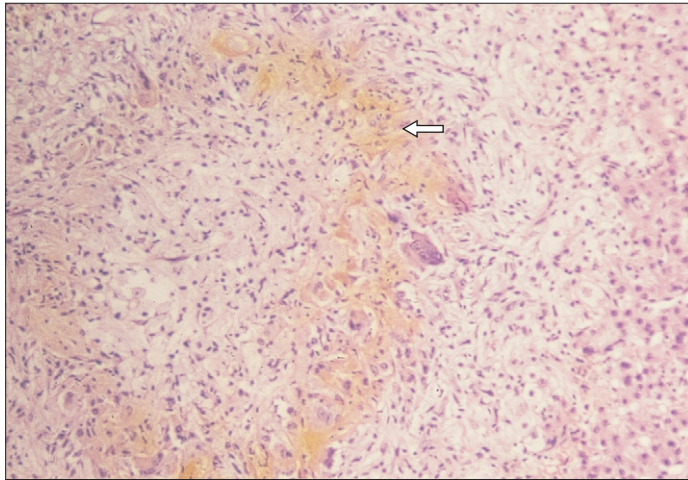
When imaging studies do not suggest biliary obstruction, evaluation for underlying liver disease is indicated in jaundiced patients with biochemical evidence of hepatocellular dysfunction or cholestasis (see Fig. 6.9). If there is evidence of hepatocellular injury, appropriate screening laboratory studies would include viral serologies (including those for hepatitis B and C viruses; if acute then hepatitis A virus as well), serum levels of iron, transferrin and ferritin (for hemochromatosis), ceruloplasmin (for Wilson disease, especially in patients younger than 40 years of age), autoantibodies and serum protein electrophoresis (for autoimmune hepatitis), and  $\alpha_1$ -antitrypsin phenotype analysis (for  $\alpha_1$ -antitrypsin deficiency). If there is biochemical evidence of predominant cholestasis, anti-mitochondrial antibodies (for primary biliary cirrhosis) should be measured. Confirmation of these diagnoses as well as elucidation of diagnoses not revealed by serologic analysis may be made by liver biopsy.

#### Liver biopsy

Liver biopsy provides precise information regarding details of hepatic lobular architecture. It has greatest utility in two types of patients. These are patients in whom a treatable cause of liver disease is suspected by serologic evaluation or patients with persistent jaundice that remains undiagnosed. With special histologic stains and/or quantitation of copper or iron content as appropriate, liver biopsy permits the accurate diagnosis of a number of liver diseases. These include viral hepatitis, steatohepatitis, Wilson disease, hemochromatosis,  $\alpha_1$ -antitrypsin deficiency, primary biliary cirrhosis, granulomatous hepatitis and neoplasms. Occasionally, liver biopsy provides clues to otherwise unsuspected biliary tract obstruction (Table 6.5 and

Abnormalities seen on liver biopsy that are associated with obstructive jaundice
Findings seen with either extra- or intrahepatic cholestasis
Bile plugs in canalicular spaces Bile staining of hepatocytes or Kupffer cells
Findings suggestive of biliary obstruction
Feathery degeneration of hepatocytes Portal tract 'edema' Proliferation of bile ductules Periductular fibrosis
Findings 'diagnostic' of biliary obstruction
Bile infarct Bile lake Neutrophils in the wall or lumen of interlobular bile (cholangitis) Bile plugs in interlobular ducts

**Table 6.5 Abnormalities seen on liver biopsy that are associated with obstructive jaundice**



**Figure 6.8** Histologic section showing biliary cirrhosis and a bile lake (arrow) in a patient with prolonged extrahepatic biliary obstruction.

Fig. 6.8). The absence of these findings does not exclude biliary tract obstruction, however.

Despite its potential advantages, liver biopsy carries a small risk of complications. These include bleeding and perforation, with a morbidity of less than 0.5% and mortality of 0.1%. For these reasons, liver biopsy should be employed only after a careful evaluation of the jaundice has been performed.

## MANAGEMENT

### Biliary obstruction

In the patient with obstruction of the bile ducts, the goal of therapy is mechanical relief of obstruction. There are three alternative approaches: endoscopic, radiologic and surgical. Both interventional endoscopic or radiologic approaches permit sphincterotomy, balloon dilatation of focal strictures and placement of drains or stents. These techniques are less invasive than surgery, but they may not permit definitive treatment under specialized circumstances. The therapeutic strategy chosen will depend, in part, on the location and likely etiology of the obstructing lesion. For example, focal intrahepatic strictures may be best treated by an interventional radiologic approach. By contrast, lesions distal to the bifurcation of the hepatic ducts may be more suitably managed endoscopically. Mass lesions may require surgery.

### Cholestatic liver disease

In cholestatic liver disease, the optimal treatment is directed toward the underlying etiology. Such treatments might include cessation of ethanol, discontinuation of a drug or administration of specific medical therapy, such as antiviral agents, phlebotomy for hemochromatosis, corticosteroids for autoimmune hepatitis, ursodiol (ursodeoxycholic acid) for primary biliary cirrhosis, and trientine for Wilson disease. In irreversible liver disease, liver transplantation is a clear-cut option.

In adults, therapy for hyperbilirubinemia per se is not usually required. In particular, the neurotoxicity of bilirubin is confined to disorders characterized by extreme elevations of unconjugated bilirubin such as type I Crigler-Najjar syndrome. In these special cases, clearance of bilirubin can be achieved by phototherapy. With this technique, exposure to blue or green light produces photoisomerization of bilirubin to more water-soluble enantiomers that can be excreted without the need for conjugation.

## Management of other cholestatic features

### Pruritus

Pruritus is a complication of cholestasis that is worthy of discussion. It should be emphasized that it develops independently of jaundice. Not all jaundiced individuals have pruritus, and not all patients with pruritus and cholestasis are jaundiced. Regardless of the mechanism, pruritus can be devastating to the quality of life of patients with cholestasis.

Although it has been assumed that the pruritogen is excreted in bile and undergoes an enterohepatic circulation, its chemical structure remains unknown. Indirect evidence suggests that it may be chemically related to bile acids, since bile acid-binding resins such as cholestyramine have been of some benefit. Other lines of evidence suggest that endogenous opiate-like compounds may be involved. Opiates are excreted in bile and endogenous opiomimetic activity is elevated in animal models of cholestasis.

The choleretic bile acid ursodiol improves biochemical indices and can slow disease progression in primary biliary cirrhosis, but it has variable efficacy with respect to pruritus. Ursodiol appears to improve biochemical markers in TPN-induced cholestasis as well as pruritus in intrahepatic cholestasis of pregnancy. However, it does not appear to be of benefit in non-alcoholic steatohepatitis. Its role in primary sclerosing cholangitis remains under investigation. Ursodiol has not been extensively studied in other cholestatic disorders. Phenobarbital, an inducer of xenobiotic metabolism, may relieve pruritus in individual patients, but its utility has not been supported in controlled trials. Rifampin (rifampicin), another inducer of xenobiotic metabolism, has been shown in some, but not all studies, to reduce pruritus in primary biliary cirrhosis and pediatric cholestatic disorders. The opiate antagonist naltrexone has shown promise in small clinical trials, but its widespread use may be limited by the development of somatic pain. In addition to these specific therapies, simple measures have been recommended, such as less frequent bathing, wearing of light clothing and frequent cutting of fingernails. The use of skin emollients and mild fragrance-free soaps has been recommended as well. Each of these approaches is summarized in Table 6.6.

### Effects of malabsorption

Malabsorption is an important consequence of chronic cholestasis. If steatorrhea is present, a low fat diet may help the resulting diarrhea and medium chain triglycerides, as a dietary supplement that does not require bile acids for absorption, may improve nutrition. Fat soluble vitamins A, D, E and K should be given, together with calcium supplements.



Medical therapy for cholestasis-associated pruritus			
Drug	Regimen	Efficacy	Adverse effects
Antihistamines, e.g: Diphenhydramine Hydroxyzine Cholestyramine	25–50 mg q6h 25 mg q8h 4–6 g 30 min before meals (may take double dose at breakfast and skip evening dose)	Rarely provides significant relief apart from sedation Beneficial in most patients	Drowsiness Constipation, fat malabsorption, decreased absorption of other medications
Ursodiol (ursodeoxycholic acid)	10–15 mg/kg per day	Beneficial in primary biliary cirrhosis, possibly in intrahepatic cholestasis of pregnancy	No major toxicity reported
Rifampin (rifampicin)	300 mg q12h	Beneficial in most but not all controlled trials to date	Inducer of hepatic enzymes involved in drug metabolism, potential hepatotoxicity, red–orange discoloration of secretions
Naltrexone	50 mg/day	Beneficial in primary biliary cirrhosis, no extensive study in other disorders	Opiate withdrawal symptoms, generally transient

**Table 6.6 Medical therapy for cholestasis-associated pruritus.**

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## SECTION II: EVALUATION OF PATIENTS WITH LIVER DISEASE

### CHAPTER

# 7

## Liver Biopsy: Indications, Technique, Complications and Interpretation

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### INDICATIONS

Liver biopsy evaluation remains the gold standard for diagnosing and staging a variety of liver disorders (Table 7.1). The most common indications for biopsy are to assess fibrosis in chronic hepatitis C and to evaluate elevated liver tests. Liver biopsy evaluation is also indicated to assess response to treatment and/or progression of disease. Finally, biopsy can be useful in evaluation of systemic conditions, including sarcoidosis, amyloidosis and fever of unknown origin.

### TECHNIQUE OF PERCUTANEOUS LIVER BIOPSY

The importance of obtaining adequate tissue cannot be over-emphasized. While 'skinny needles' may be adequate in sampling mass lesions, larger bore needles are preferred for sampling diffuse parenchymal disease. Two types of needles are used for percutaneous liver biopsy (PLB): cutting needles (e.g. Tru-Cut) and aspiration-type needles such as the Menghini, Jamshidi and Klatskin. The use of cutting needles causes less fragmentation of fibrotic tissue and may provide superior specimens in cases of cirrhosis, but has been associated with higher complication rates than aspiration needles in some studies.

The aspiration biopsy is performed while the patient is recumbent with his or her right arm extended. Liver span is identified by percussion. A site several centimeters caudad to the top of the liver in the right mid-axillary line is selected, usually in the 8th or 9th intercostal space. The site is prepped, draped and

infiltrated with local anesthetic extending to the liver capsule; a 2–3 mm skin incision is made so that the needle can pass easily. The needle is attached to a syringe containing 2–3 mL of sterile saline. It is inserted over the superior margin of the rib (in order to avoid the intercostal artery) with its tip directed toward the xiphoid process. Once through the intercostal tissues, 1–2 mL of the saline is expelled to flush the needle. The patient holds his or her breath in expiration. With the syringe plunger retracted to create negative pressure, the needle is passed quickly into the liver and withdrawn. The specimen is transferred to a specimen container. If additional tissue is required, one or two additional passes can be made through the same entry site without significantly increasing the risk of complications. The patient is placed in the right lateral decubitus position for 1 h and kept at bed rest for an additional 2–3 h while vital signs are monitored frequently. Outpatients may be observed for 6–8 h afterward, as virtually all complications become evident within this period.

### CONTRAINDICATIONS AND COMPLICATIONS

Liver biopsy-related mortality occurs in 0.1–0.01% of cases. Hemorrhage is the most common cause of fatalities. Although serious bleeding complicates 0.06–0.35% of cases, clinically insignificant intrahepatic or subcapsular hematomas are found in about a quarter of patients undergoing ultrasound examination a day after biopsy. Less common complications of liver biopsy include perforation of the gallbladder, bile peritonitis, puncture of the intestine or kidney, pneumothorax, hemobilia, hemothorax, arteriovenous fistula and sepsis.

To diminish the risk of bleeding, patients are usually instructed to discontinue medications that interfere with platelet function at least 7 days before the biopsy. These include non-steroidal anti-inflammatory agents, clopidogrel and ticlopidine. Prior to performing the procedure, the adequacy of hemostatic mechanisms is estimated using the platelet count and prothrombin time (PT). Although acceptable levels are somewhat arbitrary, platelets  $<50\,000/\text{mm}^3$  or PT  $>3$  s above control would be regarded as contraindications to PLB in most cases. While blood product transfusion may correct these abnormalities, use of transjugular biopsy (TJB) is probably more prudent. Bleeding times are not checked in our center unless platelet dysfunction is suspected, such as in uremic patients. In these cases, options include DDAVP just prior to biopsy, performing the biopsy immediately after dialysis or TJB; however, data regarding the efficacy of these approaches are lacking. Other situations in which TJB is preferable include massive ascites, morbid obesity, hemophilia and inability to cooperate.

Ultrasound guidance has been advocated as a means of reducing complications from PLB. While ultrasound may be

#### Indications for liver biopsy

Grading and staging of chronic liver disease
Hepatitis C and B
Non-alcoholic fatty liver disease (NAFLD)
Alcoholic liver disease
Autoimmune hepatitis
Evaluation of elevated liver tests of unclear etiology
Aid in diagnosis and evaluation of cholestatic disease
Primary biliary cirrhosis
Primary sclerosing cholangitis
Evaluation of hepatomegaly
Evaluation of liver diseases related to iron overload
Aid in diagnosis of Wilson disease
Evaluation of a liver mass (CT or ultrasound guided biopsy)
Assessing suitability of potential donor livers
Evaluation of engrafted liver post-liver transplantation
Evaluation of fever of unknown origin

Table 7.1 Indications for liver biopsy.

helpful in avoiding inadvertent puncture of adjacent organs or unsuspected liver lesions (e.g. hemangioma), it is less clear that ultrasound alters the risk of hemorrhage. Data suggest that use of ultrasound results in less pain and fewer hospitalizations; however, no differences in transfusion requirement, surgery or mortality have been observed. Indeed, given the relatively low rate of serious complications associated with ‘blind’ PLB, none of the randomized studies to date have adequate statistical power to assess these outcomes. Despite the lack of clear evidence for its cost-effectiveness, ultrasound is commonly used and will likely increase as hand-held devices become more readily available.

PROCESSING, STAINS AND GENERAL MORPHOLOGIC APPROACH

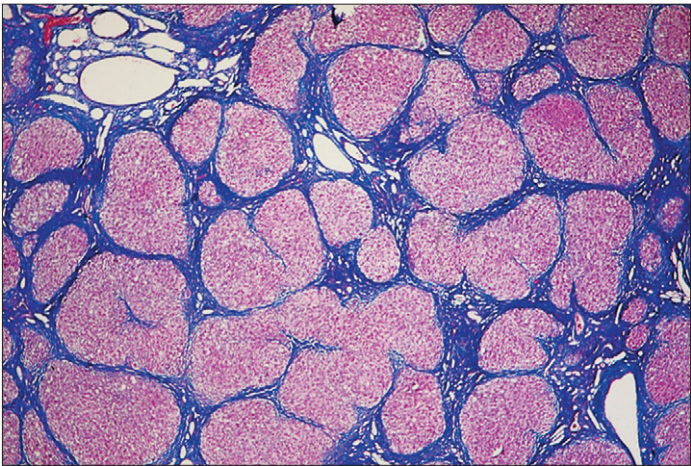
Processing and stains

‘Routine’ handling for histopathologic interpretation of most liver diseases begins with immediate fixation in 10% neutral buffered formalin; it is recommended that the specimen should not be placed on a biopsy sponge due to well-described artifacts. In some clinical settings, special handling of the tissue is necessary for evaluation (Table 7.2). A 1 mm<sup>2</sup> portion of the biopsy may be postfixed in 2.5% glutaraldehyde for further processing if ultrastructural evaluation is necessary. Pathologists’ practice for utilization of histochemical stains may differ; however, in general, ‘routine’ stains are utilized to highlight features in addition to those easily noted on the standard hematoxylin and eosin stain. In our practice, routine stains include: trichrome and reticulin stains for demonstrating fibrosis and cord architecture respectively; iron stain for hemosiderin; and diastase-pretreated periodic acid-Schiff (PAS-d) stain. Each of these stains can serve multiple purposes in evaluation and the findings for these stains will be discussed in the following sections. Table 7.3 lists additional histochemical and immunohistochemical stains, the disease processes for which they are useful, and the expected reactivity. Sophisticated techniques, such as in situ hybridization and polymerase chain reaction are available in limited numbers of research facilities and are variably available for diagnostic testing.

General interpretation

Interpretation of liver biopsy is a multistep process that incorporates architectural and cellular assessment. A standard liver biopsy is estimated to represent only 1/50 000–1/65 000 of the organ; thus, assessment of sample adequacy is the initial step in interpretation. To a large extent, adequacy depends on the nature of the process under consideration, whether a focal, space-occupying lesion, or diffuse parenchymal disease. The former may be able to be diagnosed with a small fragment of representative tissue, while the latter requires larger cores. An adequate sample is also one that is not entirely subcapsular.

Even with adequate sample size, there are recognized difficulties in interpretation due to inherent inhomogeneity of parenchymal involvement in liver disease, including the full spectrum of acute and chronic hepatitis and vascular processes, and the chronic cholestatic diseases. For these reasons, pathologists commonly recommend more than five portal tracts, or a core length of 1.5–2.0 cm for adequacy of evaluation of parenchymal disease. Figure 7.1 illustrates this difficulty.



**Figure 7.1** Explant liver, cirrhosis. The distorted, remodeled hepatic architecture of cirrhosis, characterized by nodules of hepatocytes surrounded by scar tissue, is easily recognized. However, a liver biopsy could sample various areas within this section and ‘miss’ the diagnostic lesions of cirrhosis (Masson trichrome).

Tissue processing for specialized analysis		
Disease process	Diagnostic evaluation	Tissue preparation
Hereditary hemochromatosis	Iron concentration; µg/g dry weight	Fresh tissue or paraffin block*
Wilson disease	Copper concentration; µg/g dry weight	Fresh tissue or paraffin block*
Microvesicular steatosis Fatty liver of pregnancy Reye syndrome	Microvesicular steatosis best seen with Oil Red O or Sudan Black stains	Frozen sections of fresh or formalin-fixed tissue; cannot be done on paraffin-embedded tissue
Porphyria cutanea tarda	Intracytoplasmic needle-shaped crystals	Unstained tissue sections examined under polarized light; ferric ferricyanide reduction test
Inborn errors of metabolism Bacterial, viral, fungal, mycobacterial infections	Enzymatic assays Culture and identification	Fresh tissue obtained at biopsy* Fresh tissue supplied to the microbiology laboratory or PCR analysis on fresh or fixed tissue
*Submission of tissue to a reference laboratory is necessary. PCR, polymerase chain reaction.		

Table 7.2 Tissue processing for specialized analysis.

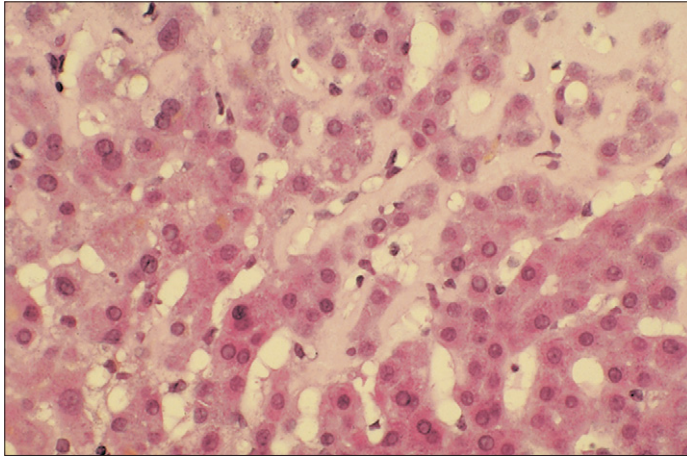


<b>Histochemical and immunohistochemical stains</b>			
<i>Histochemical stains</i>	<i>Use in diagnostic liver pathology</i>	<i>Pathologic processes</i>	<i>Comments</i>
Vierhoff Van Geison	Vessel wall connective tissue	VOD, Budd-Chiari syndrome	Helpful in the identification of obliterated vessels not readily seen by standard stains
Rhodanine	Copper in cytoplasm and lysosomes of hepatocytes	Wilson disease  Chronic cholestasis	Most useful in late stages of Wilson disease; uneven distribution in the liver so that a negative stain does not rule out the possibility of Wilson disease Granular reactivity in zone 1 or periseptal hepatocytes
Orcein	Copper-associated protein  Elastic fibers  Hepatitis B virus (HBV) surface antigen	Wilson disease, chronic cholestasis Recent necrosis, collapse Scar tissue formation Eccentric cytoplasmic reactivity corresponds with the ground-glass change seen by hematoxylin and eosin; indicates the presence of viral components in hepatocytes	Less sensitive than rhodanine for copper Passive septa: no elastic fibers detected Active septa: elastic fibers present Less sensitive than immunohistochemical stain for HBV surface antigen
Oil Red O	Red globules correspond to fat droplets	Diseases in which microvesicular steatosis occurs in hepatocytes	Confirms the presence of microvesicular steatosis
Congo Red Crystal Violet Thioflavin-T	Confirmation of amyloid protein deposition in the space of Disse or hepatic arterioles	Amyloid infiltration	Immunohistochemical stains for protein A or immunoglobulin light chain will distinguish secondary (AA) from primary/plasma cell dyscrasia-related (AL) amyloidosis
<i>Immunostains</i>	<i>Antigen</i>	<i>Reactivity in liver tissue</i>	<i>Diagnostic utility</i>
HBV surface antigen	HBsAg	Cytoplasmic reactivity: diffuse or inclusion; membrane reactivity	Confirmatory of the presence of HBV surface antigen in hepatocytes
HBV core antigen	HBcAg	Nuclear reactivity; may see cytoplasmic reactivity in occasional hepatocytes	Confirmatory of HBV core antigen in hepatocytes; indicates active replication of the virus
A <sub>1</sub> AT	α <sub>1</sub> -Antitrypsin protein	Immunoreactive globules or diffuse cytoplasmic blush	Reactivity with globules may be positive or negative with a positive rim; this is more specific for A <sub>1</sub> AT in the secretory apparatus than diffuse cytoplasmic reactivity
Other viral antigens			
HSV	Herpes simplex, I and II	Immunoreactivity localizes to infected cells	Confirmatory of HSV in intranuclear inclusions
CMV	Cytomegalovirus	As above	Confirmatory of CMV in intranuclear and intracytoplasmic inclusions
Cytokeratins	CK 8, 18  CK 8, 18, 7, 19	Hepatocyte cytokeratin  Biliary epithelium	May be useful in confirming the hepatocellular origin of the tumor Useful in delineating biliary differentiation in tumors; helpful in identifying bile duct epithelium in ductopenic conditions
Note: Immunohistochemical markers for other viral hepatitis markers, hepatitis δ agent, hepatitis C antigen, and hepatitis A antigen are available in research facilities. VOD, veno-occlusive disease; A <sub>1</sub> AT, α <sub>1</sub> -antitrypsin deficiency; HSV, herpes simplex virus; CMV, cytomegalovirus.			

**Table 7.3 Histochemical and immunohistochemical stains.**

Liver biopsy assessment begins with an initial impression of overall pattern of injury (inflammatory or not) or neoplastic growth; in non-neoplastic liver disease, this is broadly categorized as predominantly hepatic, cholestatic, mixed, or vascular. Microscopic regions of greatest involvement, commonly sub-

divided as portal-based or lobular, are useful distinctions. Lobular, or more correctly, acinar, involvement is subdivided into zone 3 (or perivenular) or zone 1 (or periportal). These categorizations reflect differing pathophysiologic mechanisms in liver disease and are useful for diagnostic algorithms. Within acini and portal



**Figure 7.2 Amyloidosis.** Accumulation of amyloid is in the space of Disse, with consequent compression of hepatocytes within the cords (hematoxylin and eosin).

tracts vascular structures are evaluated for location and structural integrity; biliary structures are assessed for inflammation and cellular or architectural alterations. Hepatocytes are noted for inclusions and structural alterations. Sinusoids are evaluated for infiltrates; **Figure 7.2** is an example of amyloidosis infiltrating the sinusoids. The cells of the sinusoids, collectively referred to as sinusoidal lining cells, are assessed. **Table 7.4** lists examples of common histologic features and associated disease processes. As will be noted in the following sections, few microscopic diagnoses can be made based on single histologic features.

## HEPATITIS

Hepatitis is microscopically characterized by chronic inflammation, varying degrees of hepatocellular degeneration, apoptosis and/or necrosis, and concurrent hepatocellular regeneration. Bile stasis, damage to bile ducts and inflammation of vessels may variably be present. This pattern of liver injury is commonly attributed to hepatotropic viruses; other processes include drugs, autoimmune hepatitis (AIH) and some metabolic liver diseases, discussed below. Fibrosis indicates chronicity.

### Acute hepatitis

Histologic changes of acute hepatitis are commonly accentuated in the lobules compared with portal tracts; often, this accentuation is in zone 3, or perivenular areas. Disarray, or 'lobular unrest', is due to concomitant hepatocellular necrosis, dropout and regeneration (**Fig. 7.3**). Mononuclear cell inflammation and sinusoidal lining cell 'activation' are panlobular; ballooning hepatocellular degeneration is most often in perivenular hepatocytes; acidophilic (apoptotic) degeneration of hepatocytes is non-zonal and seen as single eosinophilic fragments. Kupffer cell hypertrophy, PAS-d (+/- iron) pigmentation and foci of reticulin condensation are common.

Confluent necrosis [zone 3 in hepatitis B virus (HBV), AIH, or zone 1 in hepatitis A virus (HAV), pre-eclampsia], bridging necrosis and reticulin collapse (submassive necrosis) or panacinar collapse (massive necrosis) characterize severe injury. Acinar collapse is noted on reticulin stain (**Fig. 7.4**); distinguishing recent and remote collapse requires evaluation by orcein stain

Clues to etiology of acute and chronic hepatitis in liver biopsies	
Histologic features	Etiology
Zone 1 necrosis, predominantly plasma cell infiltrates, cholestasis, and iron-laden Kupffer cells	Hepatitis A
Cytoplasmic ground-glass change (demonstrates HBsAg by orcein or immunostain), sanded nuclei (demonstrate HBcAg by immunostain)	Hepatitis B (chronic)
Portal lymphoid follicles, bile duct injury, mild macrovesicular steatosis, scattered acidophil bodies (immunostains for HCV, PCR utilized in research)	Hepatitis C (chronic)
Severe hepatitis with massive or submassive necrosis, with HBsAg immunostaining	Hepatitis D ( $\delta$ virus)
Predominantly plasma cell infiltrates, hepatocellular rosettes, giant cell transformation of hepatocytes, confluent acinar necrosis, collapse, or scar	Autoimmune hepatitis
Cholestasis, eosinophils, granulomas, steatosis Acute hepatitis Chronic hepatitis Granulomatous hepatitis Submassive or massive necrosis	Drug-induced hepatitis e.g. isoniazid e.g. $\alpha$ -methyl dopa e.g. aspirin, valium e.g. acetaminophen, halothane
Atypical lymphocytes in portal areas and sinusoids	Epstein-Barr virus
Focal granulomatous necrosis; microabscesses, nuclear and cytoplasmic viral inclusions in immunocompromised patients	Cytomegalovirus
Steatosis, glycogen nuclei, anisocytosis, copper demonstrated by rhodanine or orcein stains, Mallory hyaline	Wilson disease
Eosinophilic PAS-d-positive globules in zone 1 hepatocytes PAS, periodic acid-Schiff	$\alpha_1$ -Antitrypsin deficiency

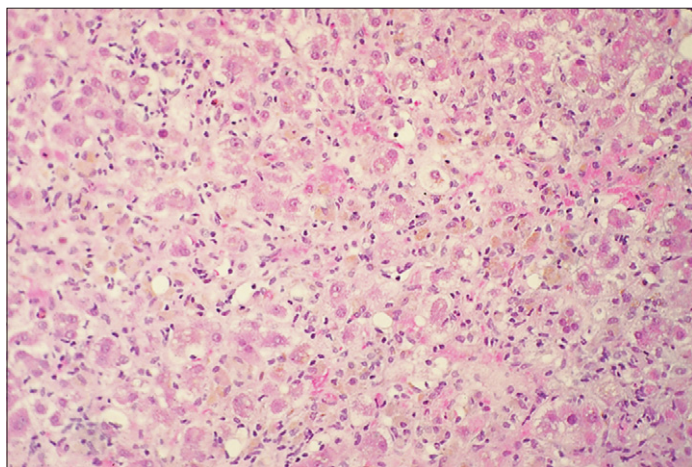
**Table 7.4 Clues to etiology of acute and chronic hepatitis in liver biopsies.**

(Table 7.3). Features of recovery include regenerative hepatocellular changes, increased lipofuscin pigment in zone 3 hepatocytes, and mild portal inflammation. Pigmented Kupffer cells denote phagocytosed necrotic hepatocytes or apoptotic hepatocytes (**Fig. 7.5**).

### Chronic hepatitis

Chronic hepatitis may show lesions described above for acute hepatitis. Interface hepatitis and portal involvement predominant to lobular activity are characteristic. Fibrosis and

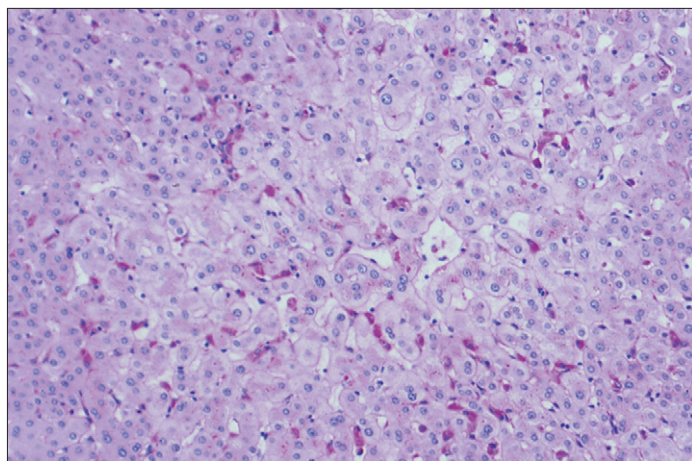




**Figure 7.3 Acute hepatitis, hepatitis C virus.** Hepatocellular swelling with panacinar disarray, sinusoidal inflammation and ceroid-pigmented Kupffer cells are seen (hematoxylin and eosin).



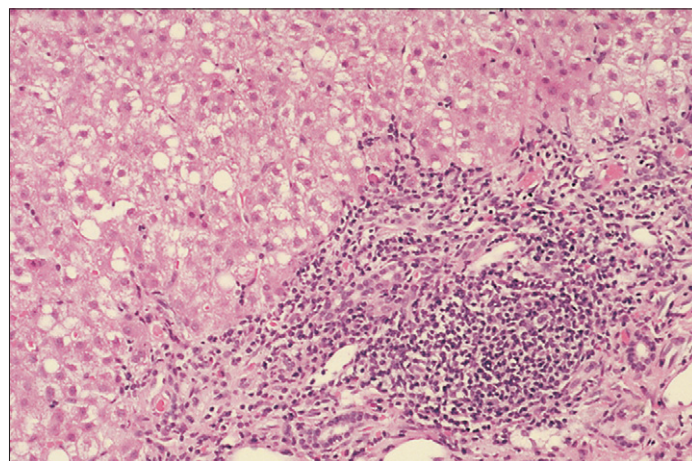
**Figure 7.4 Reticulin collapse.** The reticulin stain highlights zonal collapse seen on either side of the centrally located regenerative hepatic cords (Sweet reticulin).



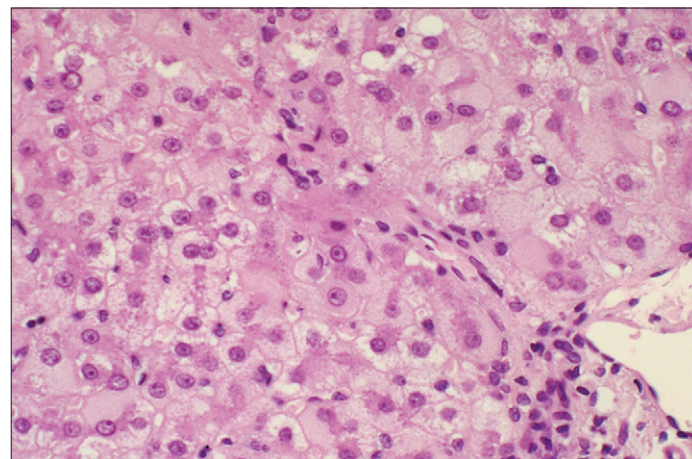
**Figure 7.5 Intracellular pigment.** The diastase-pretreated periodic acid-Schiff (PAS-d) stain highlights Kupffer cells in sinusoids that contain phagocytosed remnants of necrotic hepatocytes. These findings are indicative of a recent or resolving acute hepatitic process (PAS-d).

cirrhosis may be noted. Inflammatory infiltrates and hepatocellular alterations, while not diagnostic, may be suggestive of etiology: portal lymphoid aggregates are common in hepatitis C virus (HCV); plasma cell-rich infiltrates, in combination with hepatic rosette formation, suggest autoimmune hepatitis; notable numbers of eosinophils suggests hypersensitivity reaction associated with drugs; 'ground-glass' hepatocyte inclusions may be detected in HBV. Serologic testing is required for the final diagnosis in chronic hepatitis (Figs 7.6–7.10).

Fibrosis and architectural remodeling may or may not be present; in most forms of chronic hepatitis, fibrosis is portal-based. Current practice for clinical evaluation and therapeutic trials in chronic hepatitis is for use of standardized nomenclature that includes the underlying etiology, written and/or numeric statements of degrees of necroinflammatory 'activity', and descriptors or numeric assignment of fibrosis and architectural change. Of the systems in use today, Knodell, Ishak,

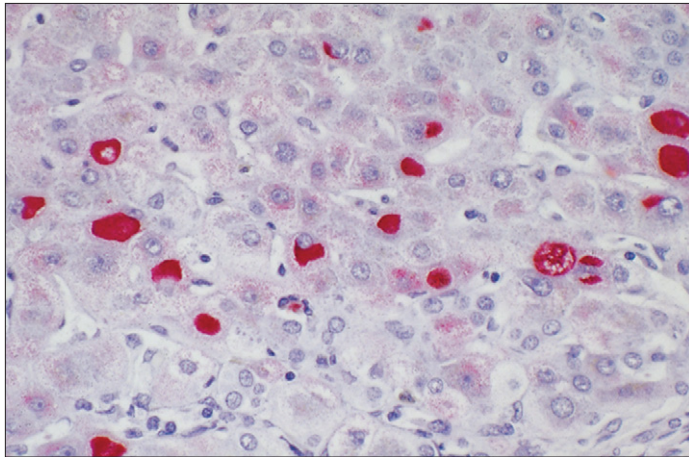


**Figure 7.6 Chronic hepatitis C virus.** Portal tract expansion by a lymphoid aggregate is a characteristic lesion of chronic hepatitis C infection. Interface hepatitis, characterized by loss of demarcation of the limiting plate due to inflammation and single hepatocyte necrosis, is present. Mild bile duct injury and occasional hepatocytes with macrovesicular steatosis are also seen (hematoxylin and eosin).

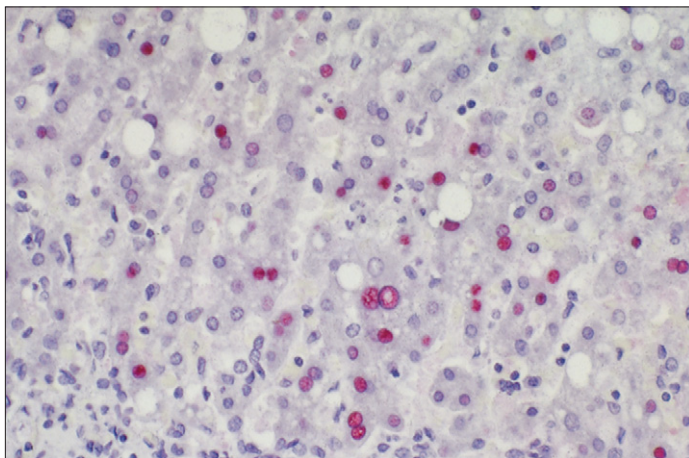


**Figure 7.7 Chronic hepatitis B virus.** One of the hallmarks of chronic hepatitis B infection is the presence of intracytoplasmic ground-glass inclusions in hepatocytes, as shown (hematoxylin and eosin).

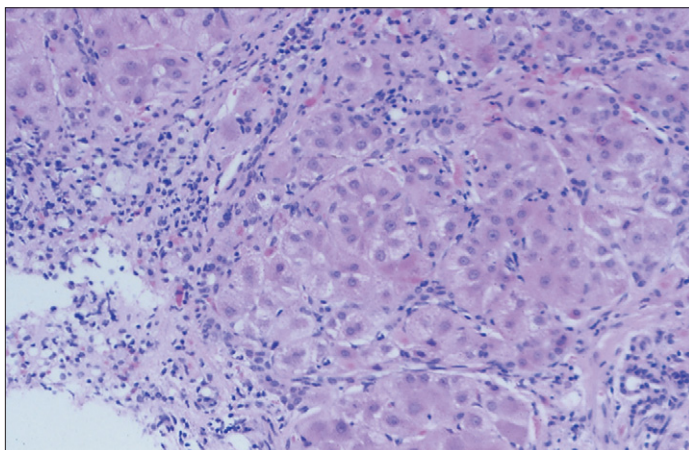




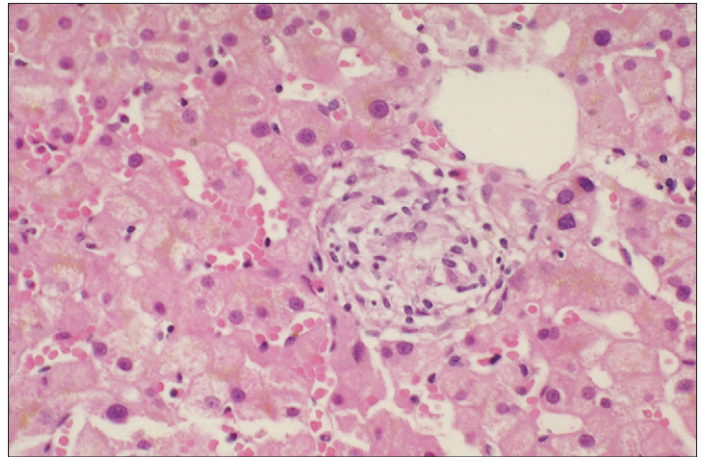
**Figure 7.8 Chronic hepatitis B virus.** This immunoperoxidase stain shows the intracytoplasmic inclusions of hepatitis B surface antigen, which corresponds with the ground-glass change described in the previous photomicrograph (alkaline phosphatase reaction).



**Figure 7.9 Chronic hepatitis B virus.** Intranuclear immunohistochemical reactivity of hepatitis B core antigen is illustrated (alkaline phosphatase reaction).



**Figure 7.10 A low-power view illustration of serologically proven autoimmune hepatitis.** Hepatic rosettes and a prominent component of plasma cells, although not diagnostic of autoimmune hepatitis, strongly correlate with the clinically-suspected disease (hematoxylin and eosin).



**Figure 7.11 The well-formed epithelioid granuloma located near a terminal hepatic venule contains an occasional eosinophil.** This raises the possibility of a drug-related etiology. 13–36% of granulomas remain cryptogenic (hematoxylin and eosin).

Scheuer and METAVIR are most often utilized. All of these derive from the original study of Knodell et al (1981) in which the concepts of histologic activity index were introduced. A recent review summarizes the details of these systems.

### Granulomas

Granulomas are histiocyte and macrophage aggregates; additional features may include mixed chronic inflammation, caseating or inflammatory necrosis, forms of multinucleated histiocytes and fibrosis (Fig. 7.11). While occasional, small granulomas are a common finding in liver biopsies, the term granulomatous hepatitis is reserved for extensive parenchymal and/or portal involvement. Search for organisms by special stains and in-depth clinical correlation are warranted. The latter includes determination of drug exposure, occupational history and serological testing. Etiologies of non-caseating, non-necrotizing epithelioid granulomas include various infections, drugs, sarcoidosis and other systemic granulomatous diseases (including Crohn disease), Hodgkin disease, primary biliary cirrhosis and reaction to foreign material. Other changes that may be clues to a diagnosis are shown in Table 7.5.

### ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Although no specific histologic features are solely attributable to human immunodeficiency virus (HIV) infection of the liver, opportunistic infections, high-grade lymphoma and Kaposi sarcoma as well as granulomas, steatosis, peliosis and Kupffer cell siderosis may be encountered. Common infections include cytomegalovirus, *Mycobacterium avium intracellulare*, *Mycobacterium tuberculosis*, cryptococcosis, histoplasmosis, candidiasis, *Pneumocystis carinii* and many others, in addition to hepatitis B and C. Infection of the large bile ducts may lead to a sclerosing cholangitis-like syndrome (AIDS cholangiopathy), with biopsy changes of mechanical duct obstruction. Biopsies from patients with AIDS should be cultured and routinely evaluated with special histochemical stains for fungal and acid-fast organisms, whether or not granulomas are present.

Histologic clues to etiology of hepatic granulomas	
Histology	Etiology
Necrotizing granulomas	Infections (e.g. tuberculosis, brucellosis, fungi)
Epithelioid granulomas with eosinophils	Drugs, parasitic infestations
Epithelioid granulomas 'segmented' by fibrosis; Schaumann bodies	Sarcoidosis
Fibrin ring granulomas	Q fever, drugs (e.g. allopurinol)
Lipogranulomas	Steatohepatitis, exogenous lipids (e.g. mineral oil in processed foods)
Granulomas with polarizable talc crystals	Injection drug use

**Table 7.5** Histologic clues to etiology of hepatic granulomas.

## CHOLESTATIC LIVER DISEASE

Cholestatic liver disease may result from necroinflammatory lesions, congenital or metabolic processes, or external ductal compression. **Table 7.6** details clinical conditions. Two broad categories reflect the anatomic sites of abnormal bile retention: intrahepatic and extrahepatic. The distinction is important for diagnostic and therapeutic options: extrahepatic obstruction may respond to bile duct decompression procedures, whereas intrahepatic cholestatic processes will not.

The well-known morphologic changes of acute extrahepatic obstruction do not discriminate between the various etiologic

Cholestatic liver disease	
Intrahepatic cholestasis	
Primary liver disease	Systemic conditions
Primary biliary cirrhosis	Cholestasis of pregnancy
Autoimmune cholangiopathies	Sepsis
Primary sclerosing cholangitis	Hodgkin and non-Hodgkin lymphoma
Paucity of intrahepatic bile ducts	Toxic shock syndrome
Idiopathic adulthood ductopenia	Congestive heart failure
	Sarcoidosis
	Cystic fibrosis
Metabolic liver disease	Miscellaneous liver disease
Byler disease	Drug-induced hepatotoxicity
$\alpha_1$ -Antitrypsin deficiency	Cholestatic viral hepatitis A and C
Wilson disease	Alcoholic hepatitis
	Orthotopic liver transplant rejection
	Benign recurrent intrahepatic cholestasis
Extrahepatic cholestasis	
Cholelithiasis	
Benign and malignant strictures of extrahepatic bile ducts	
Carcinoma of the bile ducts	
Biliary atresia	
Extrahepatic bile duct compression by tumors	

**Table 7.6** Cholestatic liver disease.

Histopathologic features of cholestasis		
	Acute cholestasis	Chronic cholestasis
Zone 3 hepatocyte feathery degeneration	+	–
Canalicular bile plugs	+	±
Bile lakes/infarcts	+	–
Portal edema; cholangiolar proliferation; pmn infiltrates	+	–
Cholangiolar proliferation	+	+
Cholestatic liver cell rosettes	±	+
Periductal fibrosis	–	+
Ductopenia or loss of bile ducts	–	+
Xanthomatous transformation of hepatocytes	–	+
Periportal feathery degeneration; periseptal edema with loose fibrosis (cholelate stasis)	–	+
Copper accumulation, periportal or periseptal hepatocytes	–	+
Mallory hyaline, periportal or periseptal hepatocytes	–	+
Biliary fibrosis; cirrhosis	–	+

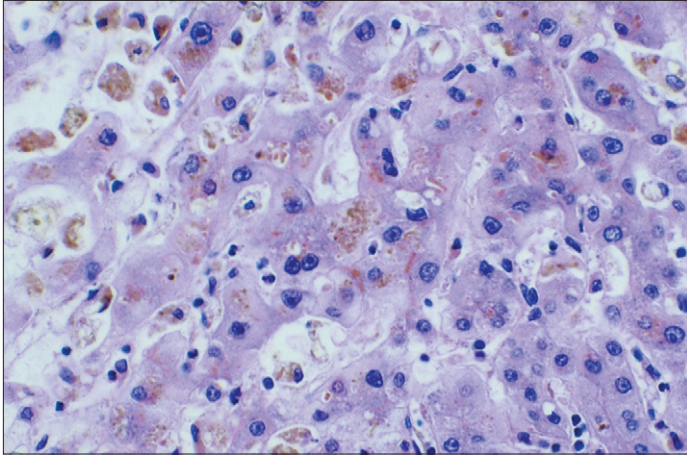
**Table 7.7** Histopathologic features of cholestasis.

possibilities. Histologic features in intrahepatic 'chronic' cholestasis, however, may be characteristic. Histologic patterns are described in **Table 7.7**. Zonal involvement differs in acute and chronic cholestasis: hepatocellular changes of acute cholestasis are present in zone 3 hepatocytes, whereas zone 1, periportal or periseptal hepatocellular changes are characteristic of chronic cholestatic conditions. When present, the location of bile plugs is useful information. Canalicular bile plugs (**Fig. 7.12**) are seen most commonly in acute obstructive or necroinflammatory lesions and may also be seen in end-stage chronic cholestatic syndromes characterized by the loss of intrahepatic bile ducts. Bile infarcts are indicative of obstruction (**Fig. 7.13**). Bile plugs in dilated zone 1 canals of Hering are characteristic of sepsis.

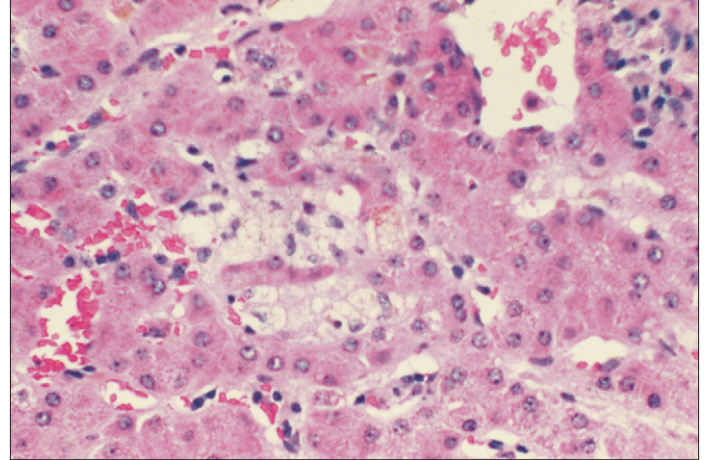
## CHRONIC CHOLESTATIC SYNDROMES

The clinical and histologic features of the major chronic cholestatic syndromes of adults, primary biliary cirrhosis (PBC), autoimmune cholangitis, primary sclerosing cholangitis (PSC) and idiopathic adulthood ductopenia, are clinically distinguished from one another by serologic assays for antimitochondrial antibodies, antinuclear antibodies and cholangiographic findings. These processes share histologic features of chronic cholestasis such as cholelate stasis, copper reactivity, xanthomatous transformation of hepatocytes and irregular 'biliary' fibrosis (**Figs 7.14–7.16**). In addition, in each process, there may be portal chronic

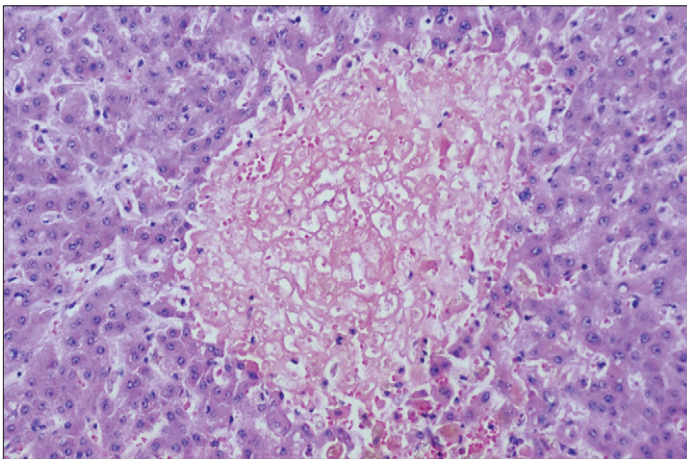




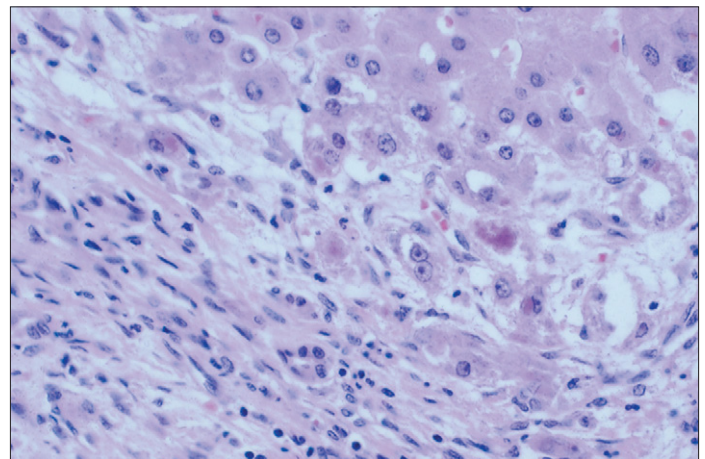
**Figure 7.12 Acute cholestasis.** Golden-brown bile can be seen in the intercellular canaliculi spaces in hepatocytes and intracytoplasmically in enlarged, intrasinusoidal Kupffer cells. These changes, which are seen in acute obstruction, are predominantly in zone 3 (hematoxylin and eosin).



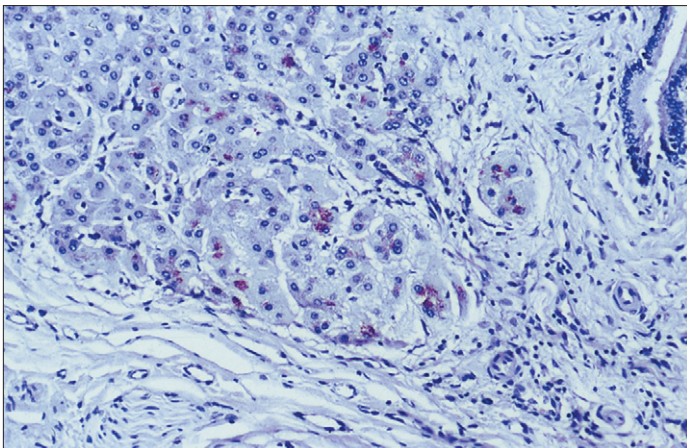
**Figure 7.15 Chronic cholestasis.** Xanthomatous cells are seen in clusters in sinusoids in chronic cholestasis (hematoxylin and eosin).



**Figure 7.13 Bile infarcts** These are usually associated with severe, acute cholestasis due to obstruction (hematoxylin and eosin).



**Figure 7.16 Chronic cholestasis.** Mallory hyaline, dense eosinophilic intracytoplasmic aggregates can be seen in zone 1 or periseptal hepatocytes in chronic cholestasis (hematoxylin and eosin).



**Figure 7.14 Chronic cholestasis.** Chronic cholestasis results in copper accumulation in zone 1 or periseptal hepatocytes. The rhodanine stain reacts as red-brown granules with intracytoplasmic copper (Rhodanine).

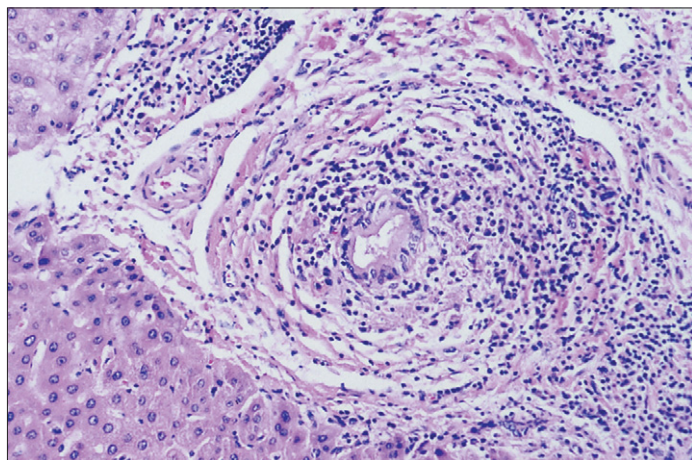
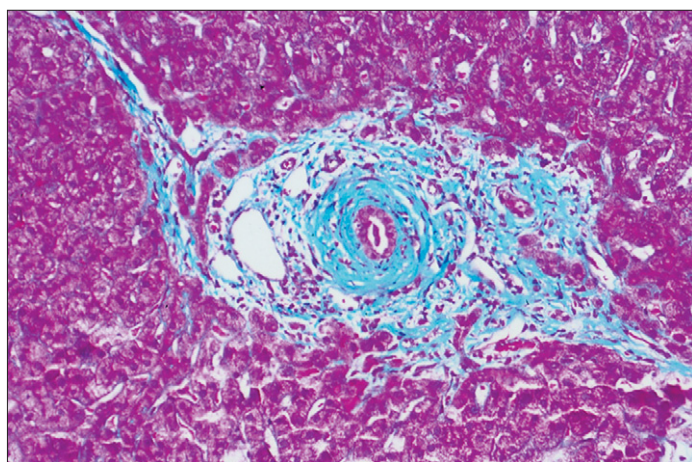
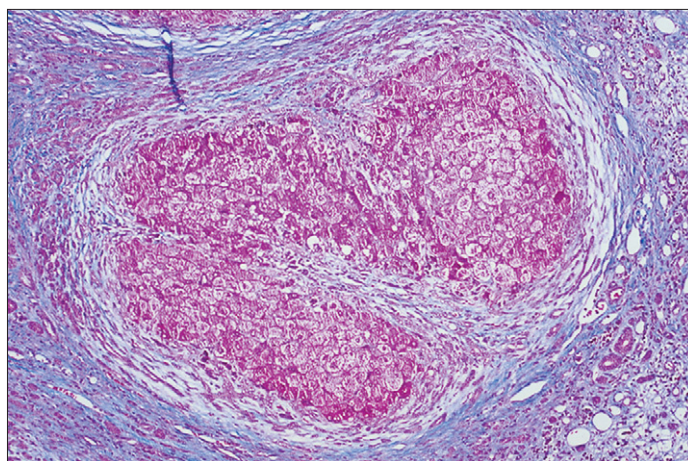
inflammation, interface activity and lobular chronic inflammation. Discriminating features are described in **Table 7.8**. The histologic stages in use for evaluation of PBC and PSC reflect the progression of processes from bile duct-centered lesions to cirrhosis. Typically, these processes inhomogeneously affect the liver and overlap of lesions among the four stages may be seen. Ductopenia is a result of each of these processes (**Figs 7.17–7.19**).

## DRUG-INDUCED LIVER DISEASE

Drug-induced liver disease may manifest as nearly any form of pathologic pattern including forms of hepatitis, cholestatic disease and tumors. The diagnosis of drug-induced liver disease is clinicopathologic and requires a high index of suspicion and careful clinical documentation. In some cases, the changes are suggestive, but not diagnostic of an offending agent; in others,



Chronic cholestatic syndromes			
Morphology	PBC/AIC	PSC	IAD
<b>Portal tracts/bile ducts</b>			
Ductopenia	++	+	+
• With lymphoid aggregates	++	–	–
• With residual fibrosis	–	+	–
Lymphoid aggregates	++	–	–
Granulomatous inflammation	++	–	–
Florid duct lesion	++	–	–
Concentric periductal fibrosis	–	+	–
Interface activity	++	+	±
PBC, primary biliary cirrhosis; AIC, autoimmune cholangiopathy; PSC, primary sclerosing cholangitis; IAD, idiopathic adulthood ductopenia.			

**Table 7.8 Chronic cholestatic syndromes.****Figure 7.17 Primary biliary cirrhosis.** A periductal granuloma is present; the florid duct lesion of primary biliary cirrhosis is characterized by lymphocytic infiltration around and into the interlobular bile duct (hematoxylin and eosin).**Figure 7.18 Primary sclerosing cholangitis.** The interlobular bile duct in the center of the photomicrograph appears to be enlarged when compared with the hepatic artery branch nearby. This is due to the concentric, 'onion-skin' fibrosis investing the duct. This fibro-obliterative lesion is characteristic of primary sclerosing cholangitis, but the diagnosis requires cholangiographically demonstrated ductal changes (Masson trichrome).**Figure 7.19 Primary sclerosing cholangitis.** The cirrhotic nodule in biliary cirrhosis is irregular and surrounded not only by scar tissue but also by the loose, edematous stroma shown in this photomicrograph. This latter change is known as cholate stasis (Masson trichrome).

the association of a particular drug with histologic alterations is well-known. Examples of the latter include zone 3 'bland' necrosis (acetaminophen), submassive necrosis (halothane), bile duct inflammation (amoxicillin-clavulanic acid), hepatic adenoma (oral contraceptives), Budd-Chiari syndrome (oral contraceptives).

## VASCULAR LESIONS

Primary vascular disorders of the liver are uncommon. Vascular changes due to systemic circulatory disease processes are more common; these include congestive heart failure and/or circulatory collapse. In these conditions, liver biopsy for elevated liver tests is uncommon. An exception is the consideration of amiodarone toxicity in patients with refractory arrhythmias.

Disease processes of the hepatic outflow veins are broadly divided into occlusion of large outflow veins, as in severe right-sided cardiac failure and Budd-Chiari syndrome, and damage to the smallest terminal hepatic venules, as noted in **Table 7.9**. The latter has been referred to as 'veno-occlusive' disease in the past, but based on recent work, is currently referred to as 'sinusoidal obstruction syndrome'. **Figure 7.20** illustrates lesions of acute large vein outflow obstruction. Without relief from the obstruction, perisinusoidal fibrosis and bridging fibrosis can be documented. The red cell extravasation lesion can also still be recognized. **Figure 7.21** illustrates the perivenular necrosis and dropout, and terminal hepatic venule intraluminal fibrosis associated with sinusoidal obstruction syndrome.

Decreased afferent blood flow to the liver, such as in systemic shock, may result in ischemic 'hepatitis'. The histologic changes are those of pauci-inflammatory zone 3 coagulative necrosis (**Fig. 7.22**). When severe, there may be zone 3–3 bridging. True infarction of the liver is rare because of the dual blood supply; however, in the non-allograft liver, damage to arterial branches may occur in processes such as polyarteritis nodosa, or as an 'innocent bystander' in primary biliary cirrhosis; resultant parenchymal changes may be subtle and include nodular regenerative hyperplasia. Acute arterial obstruction, such as inadvertent surgical ligation, rarely results in infarction, but may result in biliary strictures. Thrombosis of portal vein branches leads to



	Causes of hepatic venous outflow obstruction
Obstruction of large veins	Budd-Chiari syndrome
	Hematologic abnormalities, hypercoagulable states, myeloproliferative disorders, such as polycythemia vera
	Membranous obstruction, often associated with hepatocellular carcinoma
	Chemotherapy
	Radiation therapy
	Neoplasms, primary and metastatic
	Oral contraceptives
	Trauma
	Pregnancy
	Abscess
Damage to small veins	Hepatic sinusoidal obstruction syndrome (veno-occlusive disease)
	Chemotherapy, including thioguanine and 6-mercaptopurine
	Azathioprine
	Ingestion of toxic pyrrolizidine alkaloids (bush tea)
	Radiation
	After bone marrow transplantation

Table 7.9 Causes of hepatic venous outflow obstruction.

sharply demarcated albeit subtle cord atrophy, known as the ‘infarcts’ of Zahn.

Hepatoportal sclerosis may manifest clinically as non-cirrhotic portal hypertension and is characterized by eccentric scarring, and luminal narrowing of portal tract portal vein branches (Fig. 7.23). Biopsy samples may or may not contain the affected portal vein branches, but may be useful in either confirming or excluding the fibrosis and nodularity of cirrhosis.

Nodular regenerative hyperplasia (NRH) results from altered regional blood flow and encompasses lesions of the smallest acini to complex acini. The lesions of NRH are subtle on liver biopsy and consist of nodules formed by alternating groups of atrophic and hypertrophic cords without surrounding fibrosis. Reticulin stain may be useful for documentation (Fig. 7.24).

METABOLIC LIVER DISEASE

Most metabolic liver diseases are encountered in pediatric patients. Common findings may be chronic hepatitis, fibrosis and storage of abnormal products in hepatocytes or Kupffer cells. Histochemical stains, polarizing light and ultrastructural evaluation are useful techniques in categorizing some metabolic disorders while other metabolic defects may require enzymatic or molecular analyses in fresh tissue specimens in order to provide an accurate diagnosis. Primary metabolic disorders of concern in Western adults are hereditary hemochromatosis and  $\alpha_1$ -antitrypsin deficiency; Wilson disease and the porphyrias are much less common, albeit clinically significant disorders.

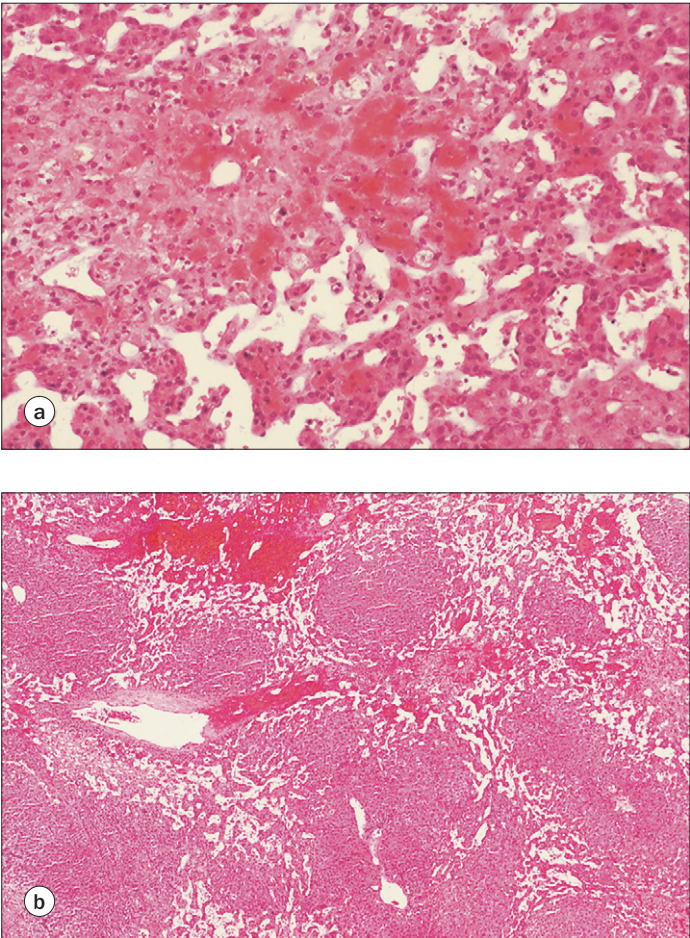


Figure 7.20 Budd-Chiari syndrome. (a) The lesions of acute Budd-Chiari syndrome are characterized by the striking sinusoidal dilatation and red blood cell extravasation into hepatic cords (hematoxylin and eosin). (b) This low-power photomicrograph illustrates ‘reverse lobulation’ with the illusion that outflow veins and portal triads have altered relationships and the latter occupy the ‘center’ of the acinus (hematoxylin and eosin).

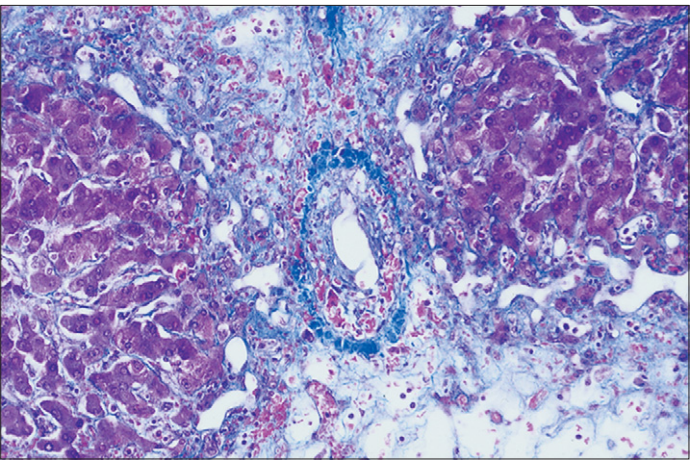
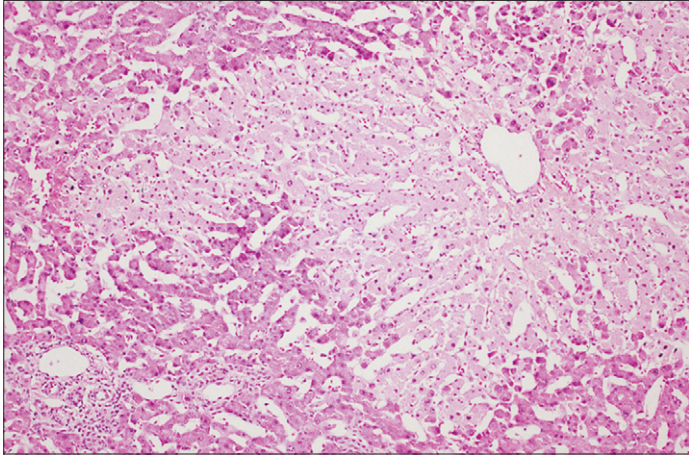
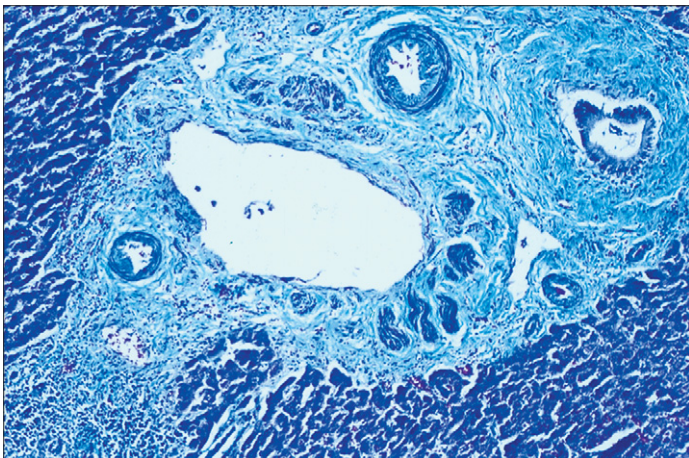


Figure 7.21 Veno-occlusive disease. The obliterative lesion of small, terminal hepatic venules characteristic of veno-occlusive disease is well documented on a connective tissue stain, such as the trichrome stain (Masson trichrome).

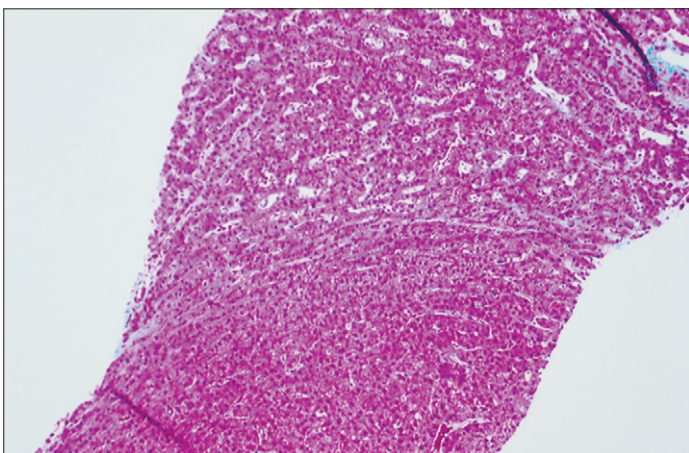




**Figure 7.22 Ischemia.** Zone 3 coagulative necrosis, as illustrated in this photomicrograph, is characteristic of ischemic liver disease and is usually associated with profound hypotension or shock (hematoxylin and eosin).



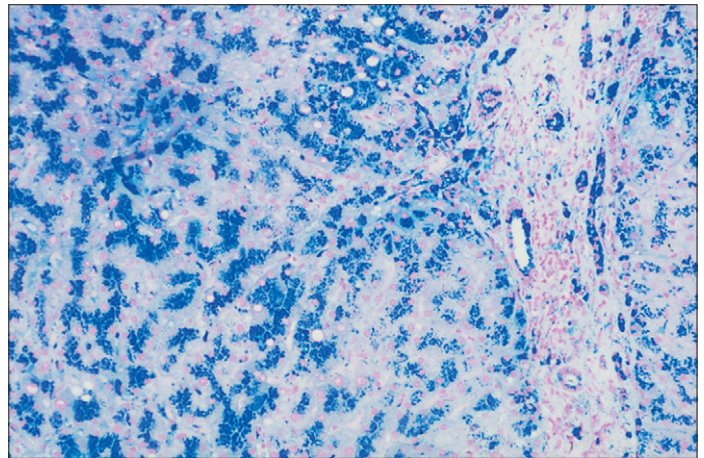
**Figure 7.23 Hepatoportal sclerosis.** The abnormalities of the portal tract and the portal vein branch within it are characteristic; numerous muscle bundles are absent (Masson trichrome).



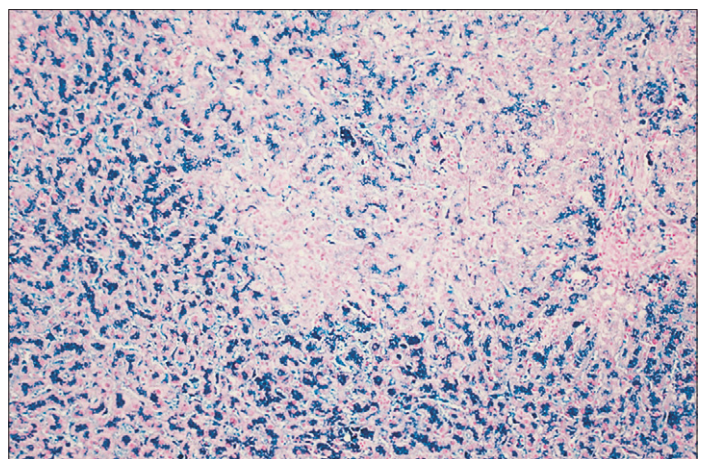
**Figure 7.24 Nodular regenerative hyperplasia.** The architecture of the hepatic parenchyma in this biopsy is very distorted; compressed cords of hepatocytes noted centrally, are separated from the hypertrophic cords above. This case of nodular regenerative hyperplasia was secondary to azathioprine (Masson trichrome).

### Hereditary hemochromatosis (HH)

Currently, indications for liver biopsy are limited to assessment for fibrosis. Iron pigment is visible by routine hematoxylin and eosin stain as a granular, slightly chunky golden-brown refractile pigment within hepatocytes, but is best semiquantitated by iron stain. Initial deposits are in the periportal (zone 1) hepatocytes. The iron stain highlights the distinct pericanalicular location of iron as well as gradient of decreasing deposition from zones 1 to 3; in later stages, iron granules are seen in bile duct epithelia, sinusoidal lining cells (Kupffer cells and endothelial cells) and endothelia of portal and/or terminal hepatic venules (Fig. 7.25). 'Grading' iron depends on the use of an iron stain; published systems vary in complexity, but are relatively reproducible among pathologists and correlate with chemically-determined quantitation. When necessary, exact quantitation can be accomplished utilizing paraffin-embedded tissue by chemical analysis. In the setting of HH, foci of non-iron-reactive hepatocytes, 'iron-free foci' are considered preneoplastic lesions (Fig. 7.26). Secondary

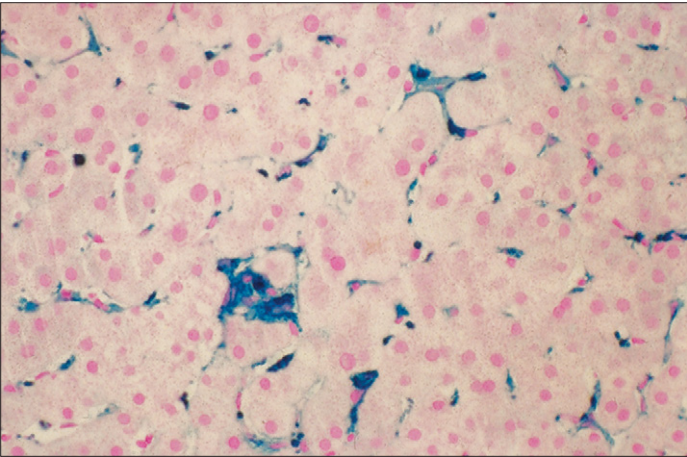


**Figure 7.25 The Prussian blue stain highlights the pericanalicular location in hepatocytes, as well as iron in Kupffer cells and ductular epithelium.** (Prussian blue).



**Figure 7.26 Non-reactive hepatocytes in a cluster in an otherwise iron-loaded liver.** This is an example of an 'iron-free focus'. There was hepatocellular carcinoma elsewhere in the liver (Prussian blue).



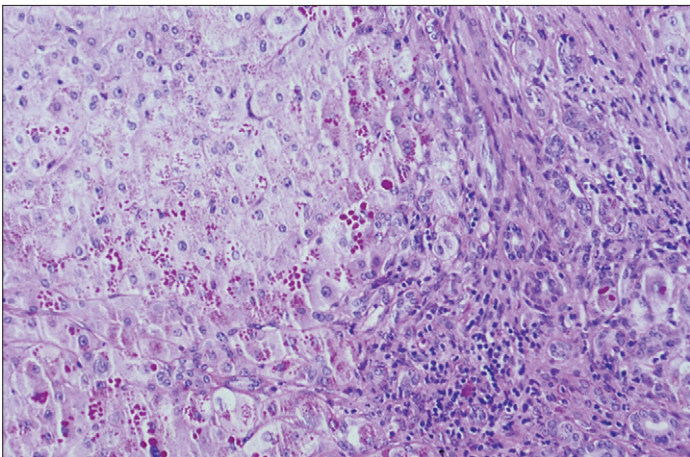


**Figure 7.27 Iron overload, secondary to blood transfusion.** This Prussian blue-stained liver biopsy shows the early lesions of secondary iron overload: Sinusoidal lining cell reactivity is highlighted by the stain. The hepatocytes are non-reactive (Prussian blue).

iron overload is characterized by preferential Kupffer cell iron deposition (Fig. 7.27). Other conditions that result in excess iron accumulation are listed in Table 7.10; some may or may not be histologically distinguishable from HH by light microscopic evaluation. Genetic testing is required for a definitive diagnosis.

**$\alpha_1$ -Antitrypsin deficiency**

This protein storage disorder is histologically apparent by varying-sized round eosinophilic 1–30  $\mu$ m cytoplasmic inclusions within zone 1 or periseptal hepatocytes. The globules may be noted by routine hematoxylin and eosin stains, but the strong PAS-d positivity readily brings them to the pathologist’s attention (Fig. 7.28). Immunohistochemical confirmation is not commonly required, but the halo-like peripheral reactivity is characteristic. Biliary cirrhosis may occur in affected children; in neonates, the globules may not be present prior to 12 weeks of age. Clinical evaluation is required for differentiating the variety of processes that result in neonatal cholestatic liver disease.



**Figure 7.28  $\alpha_1$ -Antitrypsin deficiency.** In the adult  $\alpha_1$ -antitrypsin may show changes similar to chronic hepatitis of other causes including cirrhosis and interface hepatitis. The diastase pretreated periodic acid-Schiff (PAS-d) stain shows the characteristic intracytoplasmic globules of varying sizes located in hepatocytes adjacent to a fibrous septum (PAS-d).

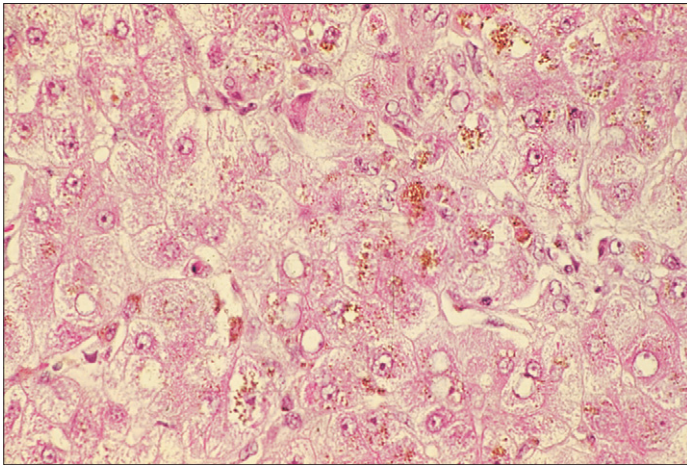
**Wilson disease**

The liver biopsy in Wilson disease typically exhibits various histologic findings, none of which are diagnostic. Copper or copper-binding protein stains may or may not be positive, and diagnosis relies on a high index of clinical and/or pathologic suspicion; ultimately, the diagnosis rests with tissue copper quantitation. Microscopic findings include non-zonal macrovesicular steatosis, glycogenated nuclei, anisonucleosis, abundant and ‘chunky’ lipofuscin deposits; iron deposits and canalicular cholestasis may also be present. Portal and lobular inflammatory lesions of chronic hepatitis may be noted; alternatively, bridging fibrosis and features of submassive necrosis may or may not be present. Mallory hyaline may be present in periportal hepatocytes (Fig. 7.29). Ultrastructural changes include ‘fishmouth’ canaliculi and mitochondrial alterations, but are not sufficient to be considered diagnostic (Fig. 7.30).

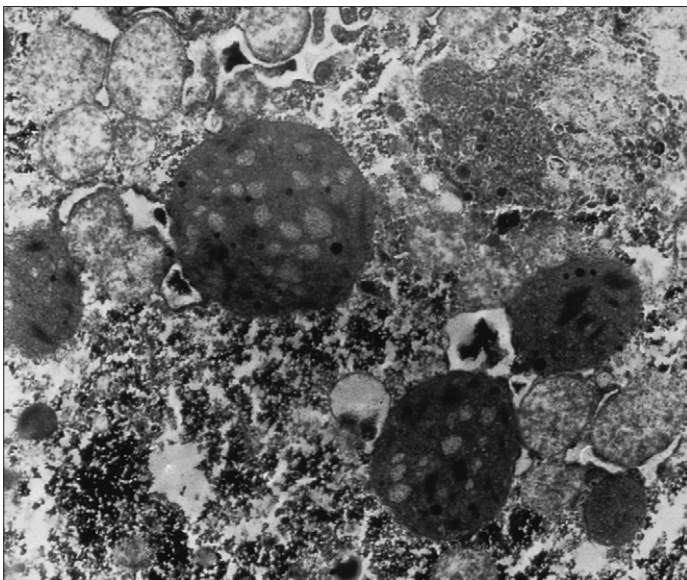
Distribution of hepatic iron* in clinical conditions with iron overload			
Clinical condition	Hepatocellular iron	Kupffer cell iron	Bile duct iron
Hereditary hemochromatosis	1+–4+, zone 1> zone 3	Large aggregates, only with 4+ hepatocellular iron	+
After portacaval shunt	1+–4+, zone 1> zone 3	Only with 4+ hepatocellular iron	+
Alcoholic liver disease	1+–2+, non-zonal	+, panacinar	–
Porphyria cutanea tarda	1+–2+, non-zonal	+, panacinar	–
Transfusions, hemolytic anemias	1+–2+, up to 4+ with massive Kupffer iron	+, panacinar	±
Ineffective erythropoiesis (sideroblastic and chronic disease anemias, myelofibrosis)	1+–2+	+, panacinar	–
*The Prussian blue histochemical stain is necessary when evaluating increased iron deposition in liver parenchyma.			

**Table 7.10 Distribution of hepatic iron in clinical conditions with iron overload.**





**Figure 7.29 Wilson disease.** The hematoxylin and eosin of a liver specimen from a patient with acute liver failure illustrates several of the lesions described in Wilson disease, including hepatocellular anisonucleosis, anisonucleosis, glycogenated nuclei and golden-brown intracellular pigment. By special stains, some of the pigment can be shown to be bile; some is iron; and some is atypical lipofuscin (hematoxylin and eosin).



**Figure 7.30 Electron microscopy in Wilson disease.** Abnormally enlarged mitochondria with intracystic widening and crystalloid inclusions are illustrated (lead citrate and uranyl acetate).

### Porphyria

Of the numerous porphyrias, only porphyria cutanea tarda is encountered with any frequency in adult populations. Biopsies may show macrovesicular steatosis, focal necrosis and mild accumulation of hepatocellular and Kupffer cell iron, or may include features of alcoholic liver disease. Histologic lesions suggestive of chronic hepatitis C or HH should initiate further clinical evaluation for these often-associated conditions. Auto-fluorescence of uroporphyrin in hepatocytes may be detected in fresh or formalin-fixed frozen sections and less commonly in paraffin-embedded, unstained sections with polarized light.

### Diabetes mellitus (DM)

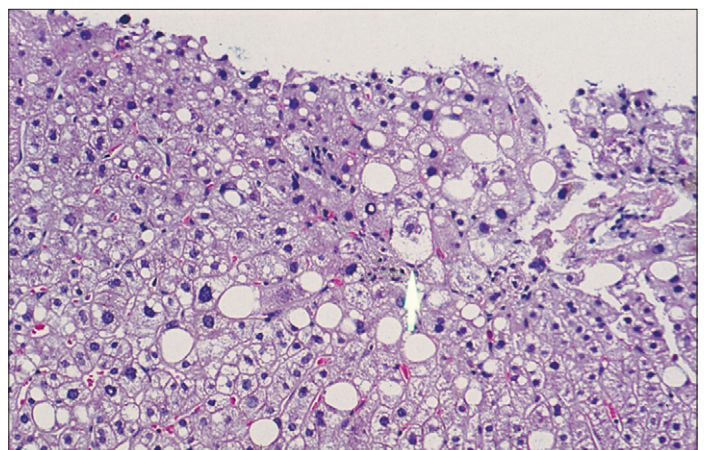
Diabetes is a metabolic disorder seen with increasing frequency. The most common hepatic morphologic findings of obesity-

related type II DM are steatosis and steatohepatitis. Associations with type I DM include hepatomegaly due to hepatocellular glycogen accumulation; recently, non-zonal deposition of dense perisinusoidal fibrosis has been reported.

## FATTY LIVER DISEASES

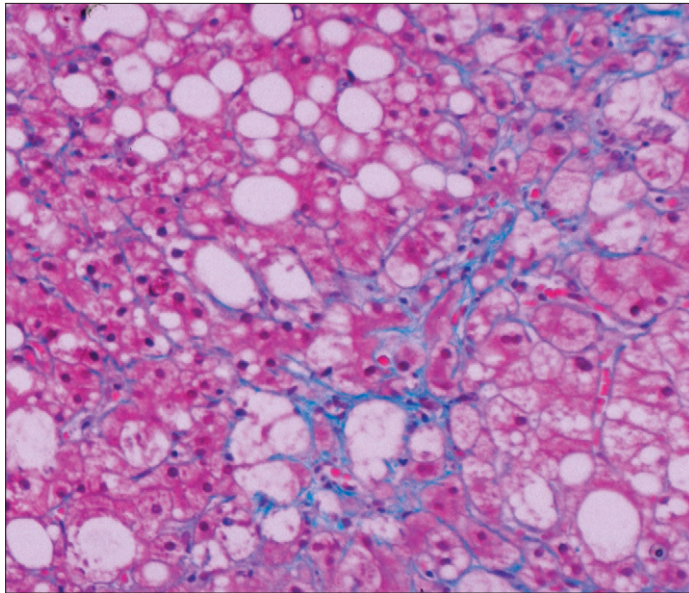
Macrovesicular steatosis is commonly encountered in liver biopsies. The lower limit of steatosis to be considered 'normal' is not established. Steatosis is common in, but not restricted to, alcoholic liver disease, hepatitis C, obesity, and in the increasingly recognized 'metabolic syndrome' that includes increased body mass index, insulin resistance and DM, hyperlipidemia, and hypertension. Steatosis is also associated with malnutrition, various obesity-related luminal bypass procedures, Wilson disease and several drugs, such as corticosteroids. Studies have documented a strong relationship of steatosis and fibrosis.

Steatohepatitis (SH) is comprised a constellation of histopathologic findings; *non-alcoholic* SH remains one of the few remaining diagnoses that requires both clinical and microscopic evaluation as pathologists are not always able to distinguish the milder forms of SH from those related to alcohol abuse. Unlike steatosis, the necroinflammatory lesions and fibrosis of SH are not detectable by clinical testing, radiographic or laboratory evaluation. The constellation of lesions accepted for SH, regardless of etiology, include macrosteatosis, hepatocyte ballooning and mild, mixed lobular inflammation; these lesions are commonly accentuated in zone 3 (Figs 7.31 and 7.32). Portal inflammation may be present but is commonly mild. Marked portal inflammation may be a harbinger of concurrent chronic liver disease. Other lesions may include Mallory hyaline, megamitochondria, glycogenated nuclei, small lobular lipogranulomas and mild iron deposition in sinusoidal lining cells. The fibrosis of SH is initially present as perisinusoidal bands of collagen in zone 3 that appear to separate hepatocytes, a pattern referred to as 'chickenwire' fibrosis. With progression, periportal fibrosis, bridging fibrosis and cirrhosis may occur. A system for



**Figure 7.31 Steatohepatitis.** In this example of non-alcoholic steatohepatitis zone 3 steatosis, ballooning and mild mixed inflammation are noted. A ballooned hepatocyte contains a poorly-formed Mallory hyaline (hematoxylin and eosin).





**Figure 7.32 Steatohepatitis.** The trichrome stain may be necessary to highlight delicate zone 3 perisinusoidal (chickenwire) fibrosis (Masson trichrome).

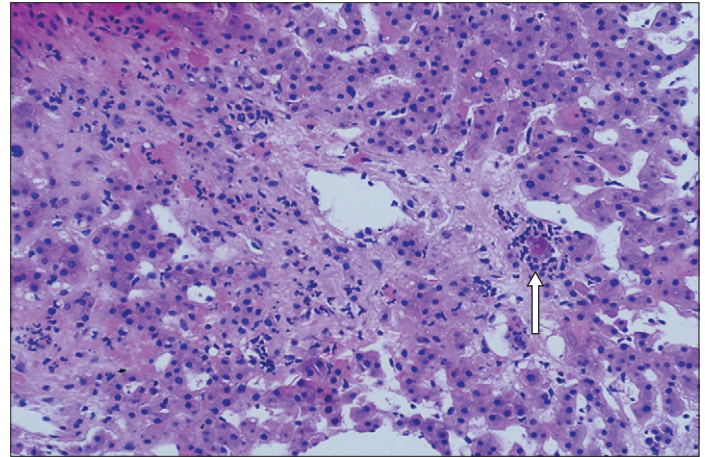
semi-quantitative evaluation of SH followed the paradigm developed for 'grading' and 'staging' in chronic hepatitis and results in separate descriptive and numeric values for the necroinflammatory lesions (grade) and fibrosis (stage).

While microscopic findings do not distinguish alcoholic SH from the various conditions related to non-alcoholic, or metabolic SH, authorities have noted that the presence of numerous Mallory hyaline, marked ductular proliferation and associated periductular polymorphonuclear leukocytes, terminal hepatic venous phlebitis and the lesions collectively referred to as sclerosing hyaline necrosis (SHN) strongly support a consideration of alcoholic liver disease. SHN, a poor prognostic marker, is characterized by obliteration of terminal hepatic venules, extensive perisinusoidal fibrosis, marked ballooning, numerous Mallory hyaline with satellitosis (polymorphonuclear leukocyte aggregates adjacent to hepatocytes with Mallory hyaline) (Figs 7.33 and 7.34). Finally, as experience is gained with this field, it is apparent that pediatric SH may result in different pathologic findings: less zone 3 accentuation of lesions, markedly greater steatosis and portal-based fibrosis.

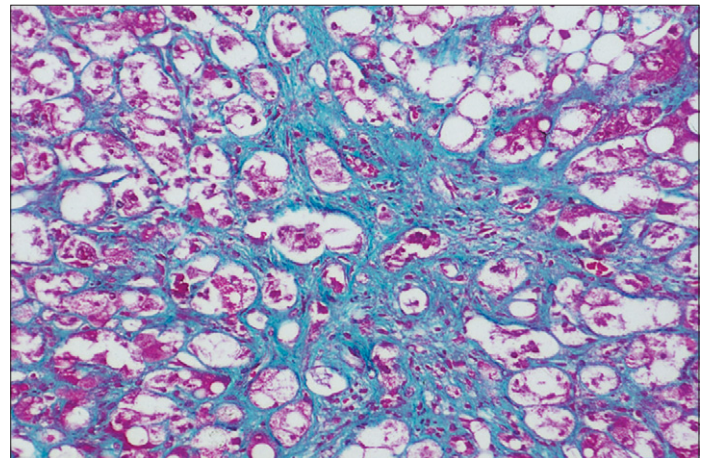
Pure microvesicular steatosis is uncommon and is associated with a limited list of clinical conditions, all of which manifest as severe hepatic dysfunction. Conditions include Reye syndrome, acute fatty liver of pregnancy, valproic acid toxicity, salicylate toxicity, alcoholic foamy degeneration and some pediatric metabolic disorders. The histology is bubbly-appearing cytoplasm with central retention of the nucleus. The change may be seen as delicate septa within hepatocytes and an Oil red O stain on tissue that has not been paraffin-embedded may be necessary for confirmation of steatosis.

## SPACE-OCCUPYING LESIONS

The means of tissue procurement from space-occupying lesions may determine the assessment to be done: as a cytologic



**Figure 7.33 Alcoholic liver disease.** Sclerosing hyaline necrosis is characterized by perivenular necrosis and fibrosis; the arrow points to a Mallory body surrounded by polymorphonuclear leukocytes (satellitosis) (hematoxylin and eosin).



**Figure 7.34 Alcoholic liver disease.** Trichrome stain shows a dense perisinusoidal, pericellular matrix investing ballooned, steatotic hepatocytes. There is near obliteration of the terminal hepatic venule (Masson trichrome).

preparation, or as a surgical pathology specimen. As in any liver biopsy evaluation, clinical history, such as underlying chronic liver disease, clinical cirrhosis, medication use, gender, lesional size and associated symptoms, are helpful in interpreting the biopsy. The differential diagnosis for an incidental nodule found in a young woman during cholecystectomy is quite different from that of a liver mass in a young woman taking oral contraceptives who presents with hemoperitoneum. The concern of sampling adequacy is significant even with radiologic guidance; perilesional 'mass effect' changes in the liver are well known and include obstructive biliary changes, vascular lesions or both.

## Benign lesions

### Hepatocellular adenoma (HA)

This benign tumor of hepatocytes consists of a circumscribed mass that may have hemorrhage or necrosis on the cut section; HA consists of sheets of benign, uniform eosinophilic or clear hepatocytes separated by thin, sinusoidal-like vascular spaces. No portal tracts or biliary structures are present within the



tumor. The lack of biliary epithelium and lack of a central scar distinguish hepatocellular adenoma from focal nodular hyperplasia (Fig. 7.35).

#### **Focal nodular hyperplasia (FNH)**

Typically 2–3 cm in diameter, FNH may be embedded in the liver or pedunculated. Cut section shows a central scar; histologically, there are abnormal vascular structures within the scar. The scar radiates into and separates groups of benign hepatocytes; ductular structures with associated inflammation are seen adjacent to the scar. Bile or copper reactivity may be present. Hepatocyte cytology is similar to the surrounding uninvolved liver (Fig. 7.36).

#### **Dysplastic hepatocellular nodule**

The dysplastic or ‘borderline’ hepatocellular nodules are challenging on liver biopsy. Grossly, the lesions bulge above the

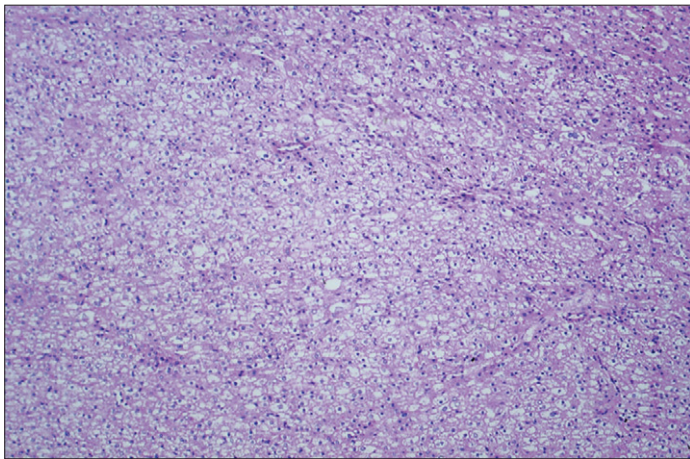
surface of a cirrhotic liver and appear bile stained or pale. Microscopically, dysplastic nodules consist of a group of similar-appearing hepatocytes with atypical nuclear or architectural features that are worrisome but not diagnostic of hepatocellular carcinoma. Cytologic changes may include nuclear crowding (increased N/C ratio), cytoplasmic basophilia, eosinophilia, clear cell change, fatty change, Mallory hyaline, increased bile or iron, or iron-free focus in an iron-loaded liver.

#### **Bile duct adenoma (peribiliary gland hamartoma)**

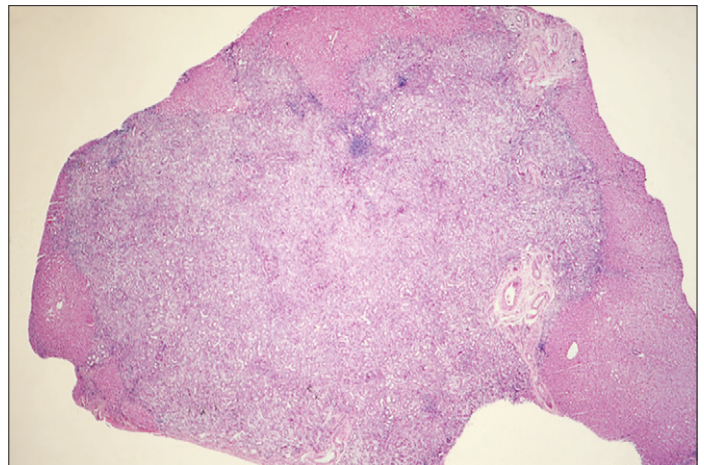
This superficial subcapsular circumscribed lesion is characterized by densely packed benign glandular structures embedded in a fibrous matrix; the differential considerations include metastatic adenocarcinoma and cholangiocarcinoma (Fig. 7.37).

#### **Angiomyolipoma**

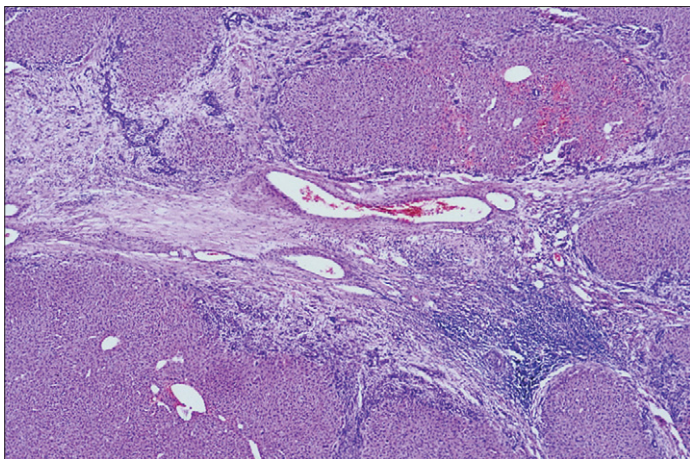
These large tumors are composed of vascular spaces, eosinophilic spindle cells reactive with smooth muscle and HMB-45 immunohistochemical markers, and large droplet fat-containing cells (Fig. 7.38).



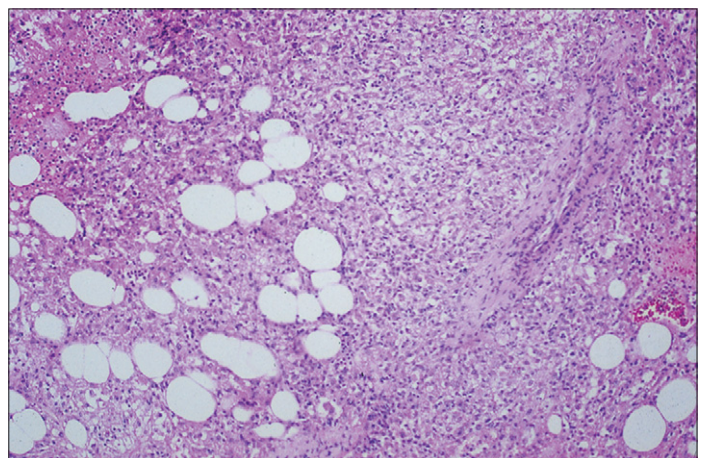
**Figure 7.35 Hepatocellular adenoma.** This benign tumor is characterized by sheets of cytologically bland hepatocytes. Although small vascular structures are present within the tumor, portal tracts are not (hematoxylin and eosin).



**Figure 7.37 Bile duct adenoma.** The subcapsular mass consists of benign ductular structures in a fibrous matrix (hematoxylin and eosin).



**Figure 7.36 Focal nodular hyperplasia.** The central scar appears to radiate into nodules of hepatocytes and is demarcated from the hepatocytes by proliferated ductules and lymphocytic inflammation. Large, abnormal vascular structures are present in the center portions of the scar (hematoxylin and eosin).



**Figure 7.38 Angiomyolipoma.** The three components of angiomyolipoma are seen in this photomicrograph: a large arterial vascular structure, the diffuse collection of eosinophilic cells with abundant cytoplasm, and small nuclei, and the foci of steatosis (hematoxylin and eosin).



### Malignant primary tumors of the liver

#### Hepatocellular carcinoma (HCC)

World Health Organization classifications describe the following architectural patterns: trabecular, pseudoglandular, compact and scirrhous. Cytologic changes of HCC are variable and include an eosinophilic, clear cell change or steatosis and nuclear atypia. Mallory hyaline or eosinophilic PAS-d positive globules may be present. Interstitial bile is diagnostic when present. Nuclear features include increased N/C ratio, hyperchromasia, and less frequently, abnormal mitoses. Vascular invasion is a useful diagnostic finding. Reticulin stain shows loss of reticulin fibers. Immunohistochemical studies for CD 34 demonstrate aberrant sinusoidal reactivity; polyclonal CEA and CD10 show a canalicular staining pattern and tumor cells show cytoplasmic reactivity with antibodies directed against HepPar1 (hepatocyte) (Figs 7.39–7.41).

#### Fibrolamellar hepatocellular carcinoma

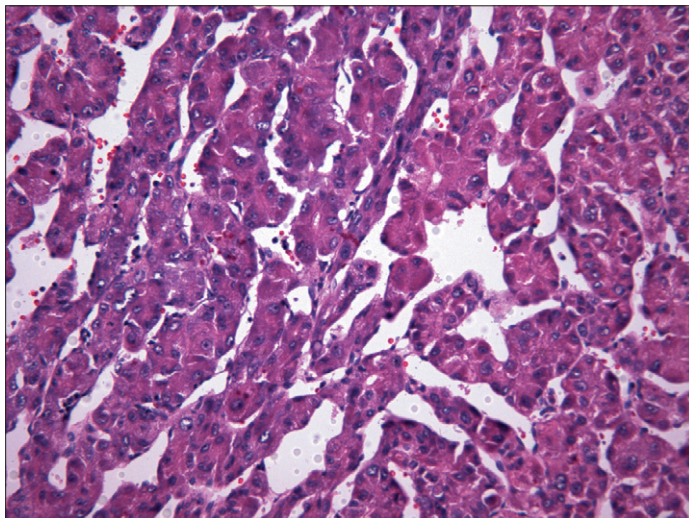
This variant of HCC is characterized by large, eosinophilic granular tumor cells with large nuclei and prominent nucleoli; tumor cells may contain intracytoplasmic pale inclusion bodies. Clusters of tumor cells are separated by characteristic broad bands of lamellar fibrosis (Fig. 7.42).

#### Cholangiocarcinoma (CCA)

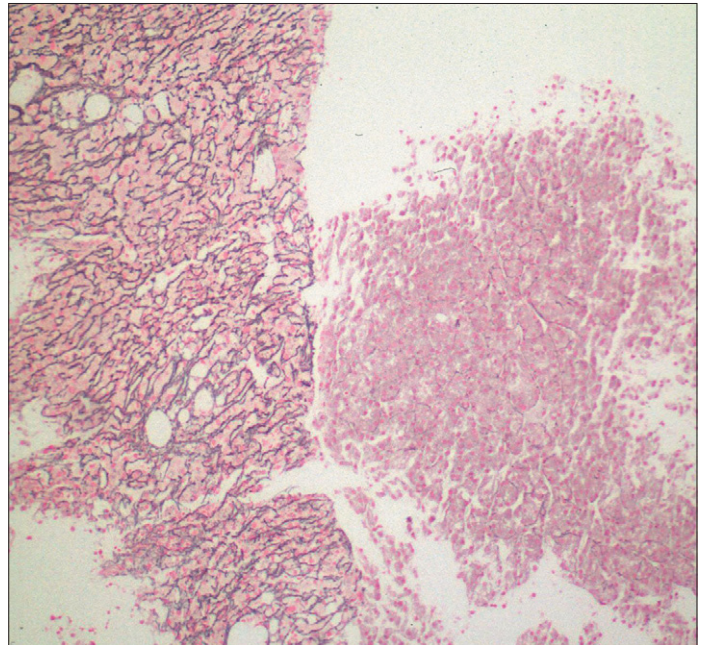
Malignant glandular or ductular structures are embedded in desmoplastic stroma. Carcinoma in situ may be seen in the bile ducts. Tumors may or may not be mucin-positive, but are typically CK 7 positive (Fig. 7.43). In some situations, distinction between CCA and fibrosing HCC may be challenging. Perineural invasion is characteristic of CCA (Fig. 7.44).

#### Combined cholangiohepatocellular carcinoma

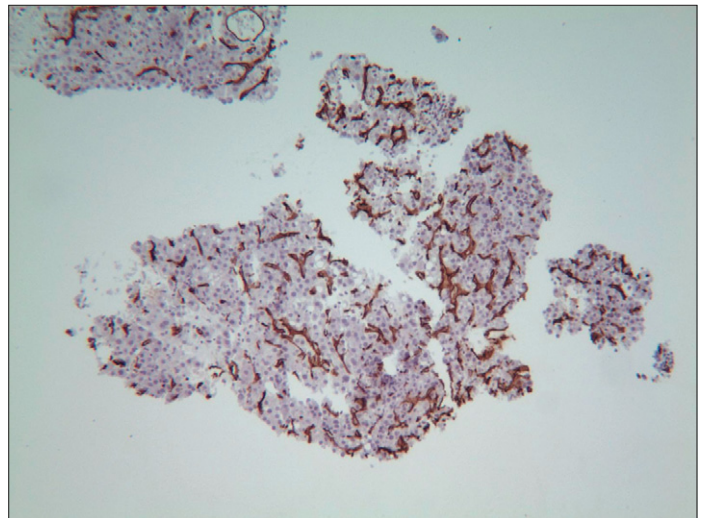
Some cases may represent a 'collision' tumor with distinct areas of HCC and CCA; other cases show a transition between areas of hepatocellular carcinoma and tumor cells with ductular or glandular architecture. Interstitial bile pigment is present in



**Figure 7.39 Hepatocellular carcinoma (HCC).** Markedly thickened trabecula, nuclear hyperchromasia and pleomorphism, and delicate sinusoidal spaces are noted in this HCC (hematoxylin and eosin).



**Figure 7.40 Hepatocellular carcinoma.** The reticulin stain shows normal reactivity in cirrhotic nodule on the left and loss of reticulin fibers in the nodule on the right (Reticulin).



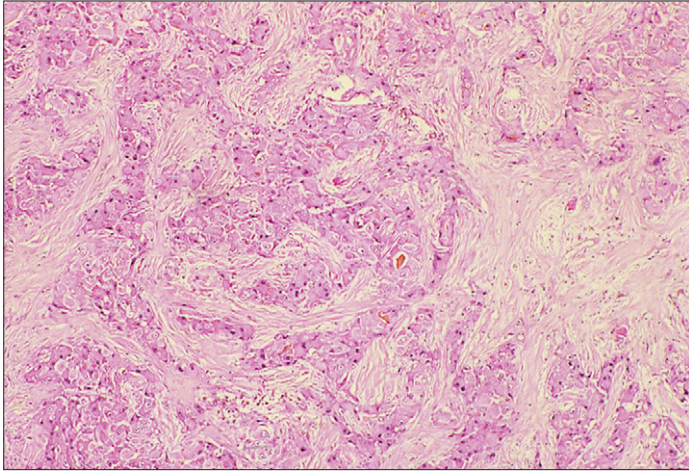
**Figure 7.41 Hepatocellular carcinoma (HCC).** The CD 34 immunostain diffusely decorates the vasculature of this HCC; non-neoplastic hepatic parenchymal sinusoids have very limited reactivity with CD 34 (DAB, counterstain with hematoxylin).

the HCC component; mucin may be present in the CCA component. The foci of CCA will react with biliary-type cytokeratins Moll 7, 19.

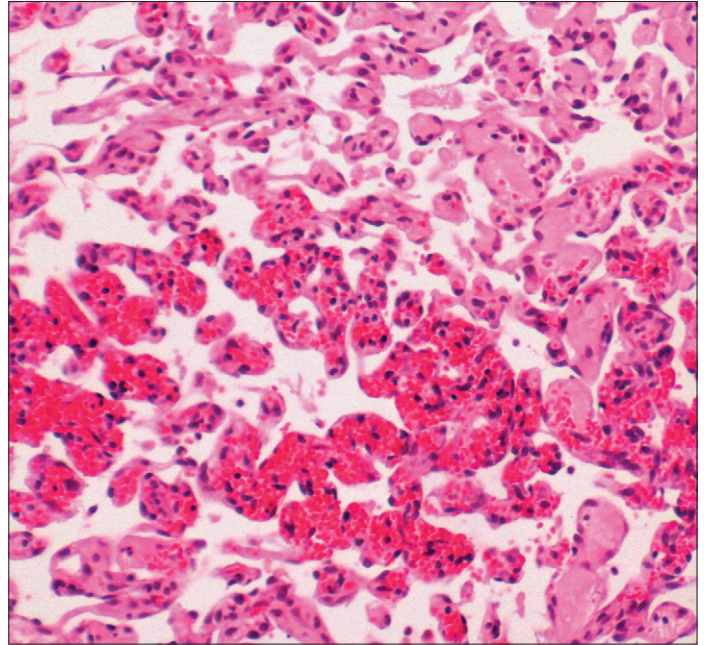
#### Angiosarcoma

This multifocal destructive tumor is characterized by interdigitating vascular channels lined by atypical, large endothelial cells. The tumor may infiltrate along the sinusoids and endothelial surfaces of the portal or central veins. Tumor cells are reactive to antibodies against endothelia, factor VIII, CD 31 and CD 34 (Fig. 7.45).

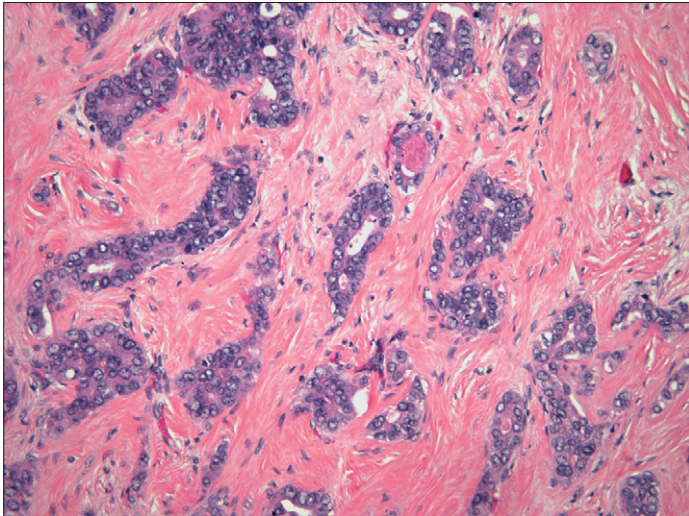




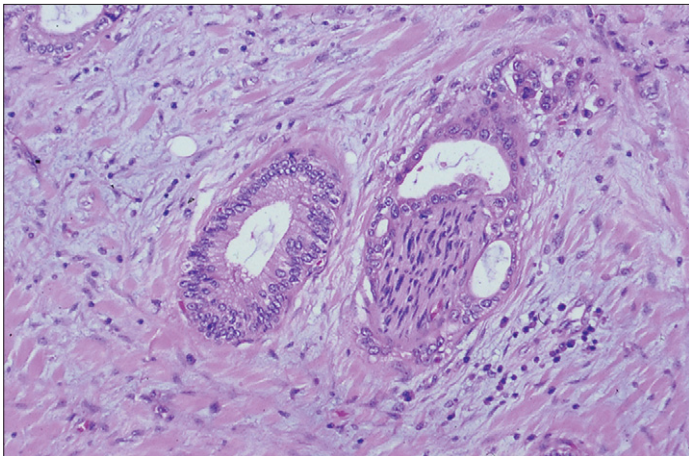
**Figure 7.42 Fibrolamellar variant of hepatocellular carcinoma.** The lamellar pattern of fibrosis is apparent in this photomicrograph. Clusters of fairly uniform, large eosinophilic tumor cells are separated by the fibrosis (hematoxylin and eosin).



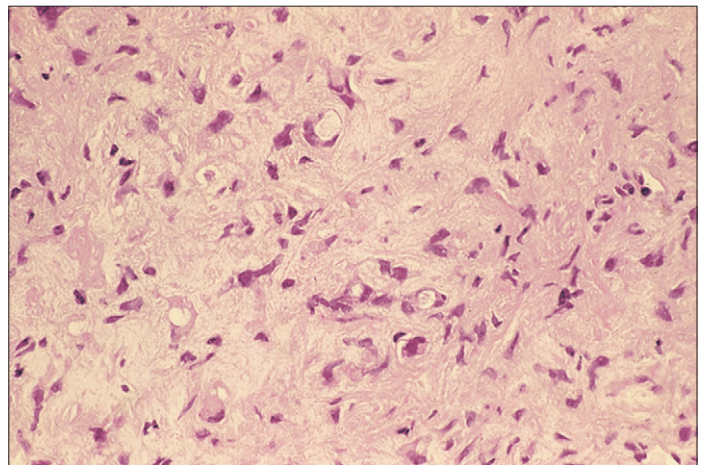
**Figure 7.45 Angiosarcoma.** The malignant nature of the endothelial cells is apparent in this tumor (hematoxylin and eosin).



**Figure 7.43 Cholangiocarcinoma.** The tumor grows in glandular structures in abundant desmoplastic stroma (hematoxylin and eosin).



**Figure 7.44 Cholangiocarcinoma.** Perineural invasion is a frequent finding (hematoxylin and eosin).



**Figure 7.46 Epithelioid hemangioendothelioma.** This unusual neoplasm consists of dendritic-type cells, some of which contain intracellular lumina. The tumor is embedded in a hyalinized stroma (hematoxylin and eosin).



### Lymphoma

When primary in the liver, lymphoma may present as a mass lesion, or as portal-based nodules of tumor cells. Some lymphomas are associated with chronic HCV. Hodgkin cells may involve the liver; Hodgkin's is also a rare but known cause of duct paucity and hepatic granulomas.

### Extrahepatic malignancy with presentation as liver disease

Non-tumor lesions in liver biopsy may be paraneoplastic; some are suggestive of extrahepatic malignancy. Hemophagocytosis is commonly associated with lymphoma. The varieties of lesions that may be associated with Hodgkin disease are discussed above.

## TRANSPLANT PATHOLOGY

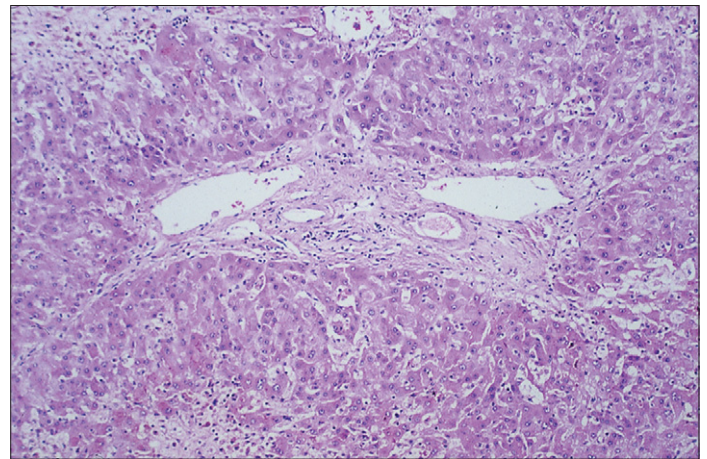
### Orthotopic liver transplantation

Biopsy of the donor liver before the transplant procedure may be indicated for pretransplant evaluation of the donor organ and for prediction of post-transplant function. In the case of the former, the evaluation is most commonly a frozen section request for evaluation of donor disease or steatosis. Necrotizing granulomas, malignancy, or evidence of chronic liver disease are criteria for exclusion in some centers; the presence of more than 30% steatosis has been correlated with subsequent dysfunction or non-function of the allograft.

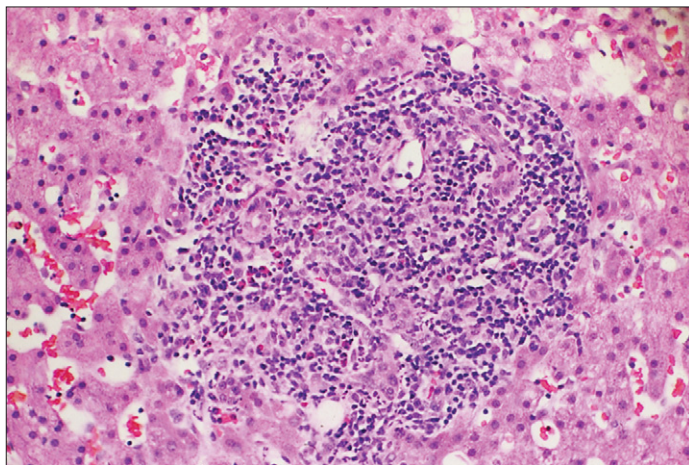
Liver biopsy remains the 'gold standard' for post-transplant evaluation as biochemical assays do not adequately discriminate immune-mediated, technical (biliary and vascular), or systemic complications that may cause allograft dysfunction. Post-transplant biopsies done at predetermined protocol intervals may be useful in detecting clinically inapparent graft injury. Rejection remains a leading cause of allograft dysfunction. Terminology currently in use for rejection reflects both pathogenesis and potential reversibility. Acute rejection, a cell-mediated entity, is characterized by mixed portal infiltrates with lymphocytes and eosinophils, bile duct injury, and endothelial inflammation (endotheliitis) of terminal hepatic and portal veins (Fig. 7.47). Acute rejection is potentially responsive to increased immuno-

suppression. Ductopenic rejection usually evolves from acute rejection and may include a component of secondary ischemia from immune injury of the biliary vascular supply. The portal tracts become devoid of bile ducts and inflammation, and features of chronic cholestasis and ischemia may be seen in the lobules (Fig. 7.48). Hyperacute rejection, a rare complication in liver transplantation, is caused by preformed antibodies usually in the ABO blood group system and is not responsive to medical intervention.

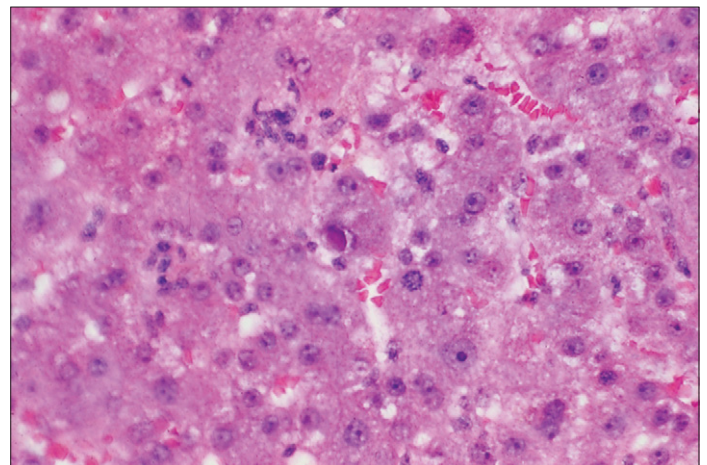
Table 7.11 shows the common causes of allograft dysfunction and their respective histologic lesions. Figures 7.49 and 7.50 are illustrations of the most common opportunistic infections of allograft patients: cytomegalovirus and herpes virus. Recurrent disease and post-transplant lymphoproliferative disorder (Fig. 7.51) are important considerations in the later time period. At any time post-transplant, acute rejection, pathologic



**Figure 7.48 Chronic allograft rejection.** In ductopenic rejection, the portal tracts are nearly devoid of inflammation. Absence of the interlobular bile duct is apparent in this portal tract in which only the hepatic artery and portal vein branches are seen (hematoxylin and eosin).



**Figure 7.47 Acute allograft rejection.** On high power, the triad of acute rejection is seen: mixed chronic inflammation with eosinophils and occasional polymorphonuclear leukocytes, bile duct infiltration and damage, and subendothelial inflammation of the portal vein, endotheliitis (hematoxylin and eosin).



**Figure 7.49 Post-transplant opportunistic infection.** Cytomegalovirus infection, a common post-transplant opportunistic infection, may involve the allograft liver as well as other organs. The enlarged infected cells contain the characteristic intranuclear inclusion; frequently, a small cluster of acute inflammatory cells is present in adjacent parenchyma (hematoxylin and eosin).

<b>Liver transplant pathology</b>	
<i>Diagnostic considerations</i>	<i>Morphologic findings</i>
<b>Rejection</b>	
Hyperacute	Hemorrhagic necrosis or polymorphonuclear infiltrates; IgM, complement in vessel walls
Acute	Triad: mixed chronic inflammation with eosinophils in portal tracts; bile duct infiltration, injury; portal or central endotheliitis; zone 3 necrosis, dropout in severe rejection
Early chronic rejection	Degenerative changes in a majority of interlobular bile ducts or loss of bile ducts in less than 50% of portal tracts; foam cells in the sinusoids; zone 3 necrosis, dropout common
Late chronic rejection	Loss of interlobular bile ducts in $\geq 50\%$ of portal tracts; zone 3 ballooning, cholestasis, or dropout; foam cells in the sinusoids; foam cell arteriopathy
Preservation-related changes	Zone 3 hepatocellular ballooning $\pm$ canalicular cholestasis; subcapsular necrosis
<b>Vascular thrombosis</b>	
Outflow compromise	Zone 3 congestion or red blood cell extravasation into cords
Venous or arterial inflow compromise	Zone 3 hepatocellular ballooning; less commonly, ischemic infarct
Arterial thrombosis	As above $\pm$ bile duct ischemic change or necrosis
Biliary obstruction	Acute cholestasis with portal edema, proliferating ductules, and polymorphonuclear leukocytes
Transmission of donor disease	Excess iron pigment (hemochromatosis); zone 1 PAS-d globules ( $\alpha_1$ -antitrypsin globules); chronic hepatitis (HCV); malignancy
Recurrence of original disease	Viral hepatitis; HBV surface and core antigens, HDV antigens may be demonstrated with immunohistochemistry; HBV and HCV recurrences may be seen as fibrosing cholestatic hepatitis
	Malignancy: hepatocellular carcinoma; cholangiocarcinoma
	Primary biliary cirrhosis, Autoimmune hepatitis
	Steatohepatitis, alcoholic or nonalcoholic
Post-transplant lymphoproliferative disorder	Atypical lymphoid infiltrate, monoclonal B- or T- cell proliferation demonstrated by flow cytometry, DNA gene rearrangement studies on fresh tissue
<b>Opportunistic infections</b>	
CMV	Microabscesses; intranuclear and intracytoplasmic inclusions in hepatocytes, biliary epithelium, or sinusoidal lining cells
HSV	Non-zonal 'punched-out' necrosis; ground-glass viral inclusions at the periphery of the necrotic foci
EBV	Sinusoidal and portal atypical lymphocytic inflammation
Fungal	Necrosis with organisms demonstrated by GMS or other fungal stains
Drug-induced hepatotoxicity	As in non-transplant liver pathology
CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HSV, herpes simplex virus; IgM, immunoglobulin M; PAS-d, diastase-treated periodic acid-Schiff; GMS, Gomori methenamine silver.	

**Table 7.11 Liver transplant pathology.**

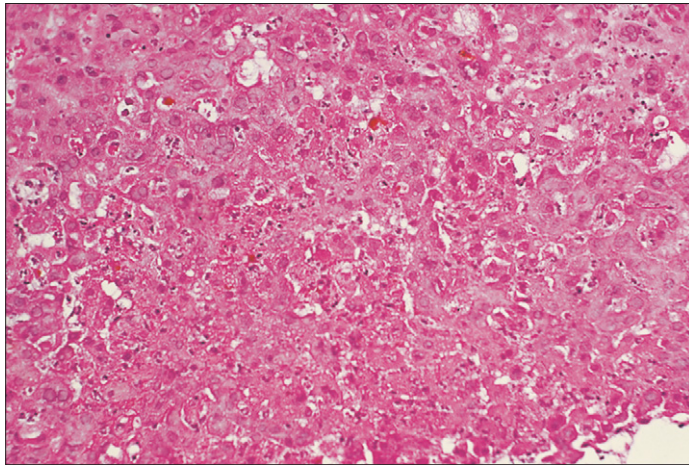
findings related to surgical complications, the transmission of clinically unknown donor disease, drug hepatotoxicity, and hepatic manifestations of sepsis may be seen. Finally, because the vascular supply to the biliary ductal system derives entirely from the hepatic artery, bile duct injury and related morphologic changes may be related to either the biliary anastomosis or to hepatic artery insufficiency from occlusion or immune-mediated injury. This change is more common in the later post-transplant period.

### Recurrence of viral hepatitis

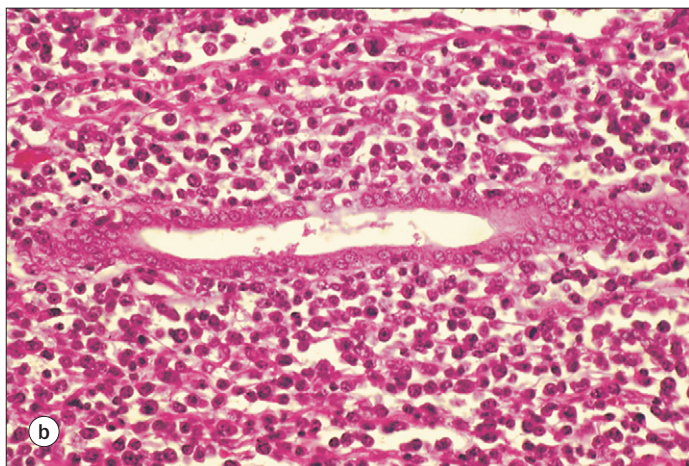
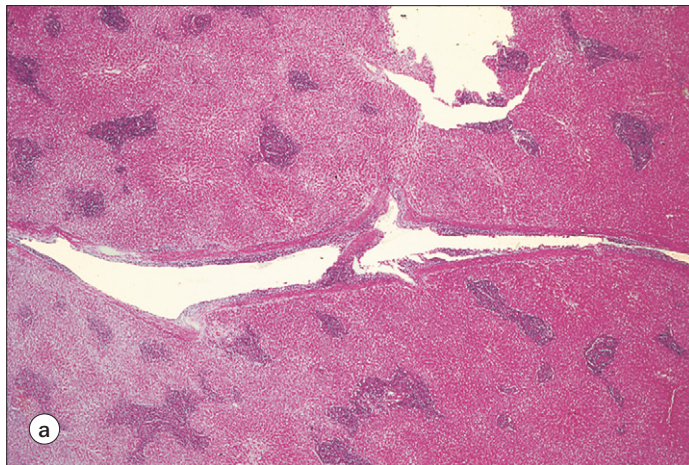
A major challenge occurs in distinguishing allograft injury caused by a recurrence of viral hepatitis B or hepatitis C infection from

rejection. In some cases, there may be an overlap of findings and more than one process may be present. Ground-glass change in hepatocytes and immunohistochemical stains for HBV antigens are helpful in documenting the presence of HBV. Infiltration of bile ducts by lymphocytes may be seen in either acute rejection or infection with HCV; portal inflammation greater than acinar inflammation and endotheliitis favors the former, whereas ballooning of hepatocytes, steatosis, acidophil bodies and lymphoid aggregates are more indicative of the latter. Eosinophils may be seen in both processes. In some cases, serial biopsies are necessary for determining the causes of allograft dysfunction.





**Figure 7.50 Post-transplant opportunistic infection.** Herpes virus infection of the allograft liver shows similar histologic features as seen in the non-allograft liver: non-zonal 'punched-out' necrosis of hepatic parenchyma. On the borders of these foci, hepatocytes with the ground-glass nuclear features of herpes simplex virus inclusions are present (hematoxylin and eosin).

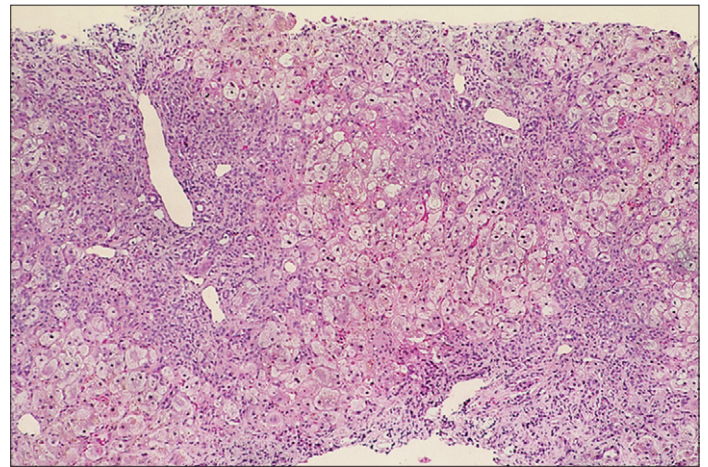


**Figure 7.51 Post-transplant lymphoproliferative disorder.** (a) Post-transplant lymphoproliferative disorder is a process that may also lead to marked cellular expansion of portal tracts (hematoxylin and eosin). (b) High-power examination of the involved portal tracts shows that the infiltrate consists of atypical, monomorphic lymphoid cells. The bile duct is surrounded but not destroyed by the infiltrate. Further phenotypic and genotypic characterization of the infiltrate is necessary to determine the clonality of the process (hematoxylin and eosin).

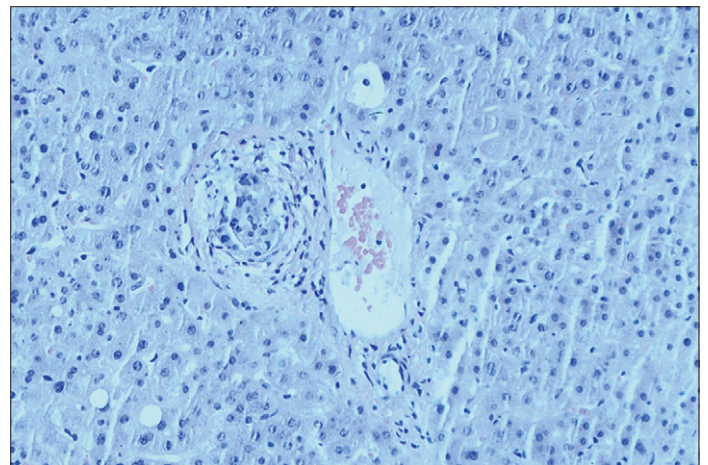
In addition, both recurrent HBV and HCV may cause severe graft dysfunction in the form of fibrosing cholestatic hepatitis, characterized by clinical jaundice and graft deterioration. The histopathologic features include hepatocellular ballooning, marked ductular proliferation, periportal fibrosis and cholestasis. Abundant intracytoplasmic viral antigens are readily demonstrated in HBV fibrosing cholestatic hepatitis (Fig. 7.52).

## LIVER BIOPSY IN BONE MARROW TRANSPLANTATION

Liver dysfunction following bone marrow transplantation (BMT) may be attributable to processes that occur in the non-BMT setting, such as infection and drug toxicity, to processes that are unique to the post-BMT clinical setting, or to a combination of problems. Veno-occlusive disease is a serious, often fatal complication, with characteristic clinical findings. Rarely is



**Figure 7.52 Recurrent hepatitis B.** This biopsy is an example of a variant of recurrent hepatitis B infection known as fibrosing cholestatic hepatitis. There is panacinar hepatocyte ballooning; the portal tracts are expanded by chronic inflammation and ductular proliferation. In these cases, diffuse immunoreactivity with hepatitis B virus surface and core antigens will be present (hematoxylin and eosin).



**Figure 7.53 Graft versus host disease.** Acute graft versus host disease may show significant damage to the interlobular bile ducts. As illustrated, the changes in the ductal epithelium include loss of nuclear polarity and cytoplasmic vacuolization. Inflammation is relatively mild (hematoxylin and eosin).



liver biopsy needed for the diagnosis, but the lesions are as described above (Fig. 21). Acute graft versus host disease (GVHD) usually occurs between 30 and 100 days; after 100 days, the differential diagnostic considerations include chronic GVHD as well as recurrent disease and viral hepatitis from transfusion. Biopsies of the liver for acute GVHD may show

single cell necrosis, ballooning, or lymphocytic infiltration and epithelial damage of the bile duct epithelium similar to that seen in acute allograft rejection; the latter is characteristic (Fig. 7.53). Chronic GVHD is usually characterized by bile duct injury and loss with minimal hepatocellular necrosis; a distinct form of chronic GVHD also shows lobular hepatitis.

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## SECTION II: EVALUATION OF PATIENTS WITH LIVER DISEASE

### CHAPTER

# 8

## Imaging of the Liver

B. Kirke Bieneman and Adrian M. Di Bisceglie

### INTRODUCTION

Accurate imaging of the liver plays a critical role in diagnosis of diseases of the liver and biliary tree. In addition, interventional radiology has become an important discipline in the management of liver disease and its complications. Thus close cooperation between clinicians and radiologists is important in providing optimal patient care. Multiple techniques are currently available for imaging the liver. These individual modalities have unique advantages and limitations inherent to their design and physical properties of image acquisition. Over the past few years, dramatic improvements in technology have improved the quality of cross-sectional imaging in the abdomen and, specifically, the liver. Therefore, clinicians are often faced with many choices of which diagnostic modality to request for the evaluation of a given abnormality.

This chapter discusses the imaging techniques available, their indications, advantages and limitations. Information is provided on the imaging features of several selected liver disease processes with recommendations regarding the approach to imaging these patients.

### IMAGING MODALITIES

The techniques currently available to image the liver include ultrasound, scintigraphy, computed tomography (CT) and magnetic resonance imaging (MRI). The choice of a particular technique is usually dictated by the clinical question and is sometimes affected by issues of cost.

#### Ultrasound

Ultrasound acquires images of the body by transmitting sound waves through tissues and sampling the waves that return to the transducer. Tissues propagate these waves at different speeds based on their acoustic impedance and reflect the waves at different angles based on the angle of incidence of the sound wave to the individual tissue. This property allows specific localization and characterization of different tissues. Unfortunately, these properties are very complex and can cause numerous artifacts. Therefore, experience and expertise are critical for optimal performance and interpretation of these examinations in order to prevent misdiagnoses.

The unique advantages of ultrasound include the ability to perform the examination in real time with multiplanar capability and the absence of radiation. Improvements in transducer technology have led to the development of higher frequency transducers, which offer improved spatial resolution and are currently available for endoscopic or intraoperative use. This allows proximity to the region of interest and the formation of

extremely high-resolution images. When combined with Doppler techniques, information on blood flow direction and velocity can be obtained. This information can be displayed as numeric values with statistical analysis or as color representing velocity of blood flow superimposed on the anatomic gray scale images. The recent introduction of intravenous contrast agents for ultrasound examination has enhanced the accuracy of assessment of blood-flow dependent abnormalities and has provided increased lesion conspicuity for biopsy or interventional radiologic treatment.

The main limitation of ultrasound in imaging of the abdomen in general and the liver and biliary tree in particular, relates to the restriction of sound wave penetration through certain tissues, such as bowel gas, bone, and fatty tissues that obscure visualization beneath them.

Its widespread availability, cost and versatility make ultrasound a reasonable initial screening tool for most hepatobiliary disorders. Specifically, ultrasound is useful when evaluating the size of the liver and the parenchyma for diffuse infiltrative disorders that can alter the normal echotexture. Ultrasound is also useful for detection of certain mass lesions, dilatation of the bile ducts and evaluation of vascular anatomy, patency and hemodynamics. Intraoperative ultrasound is commonly used to identify hepatocellular carcinoma (HCC) or hepatic metastases at the time of surgical resection, allowing optimal removal of tumor burden.

#### Scintigraphy

Images of the liver are obtained by injecting intravenous radiopharmaceutical agents that are taken up by the liver. These radiopharmaceutical agents are unstable and emit electromagnetic radiation (radioactive decay), which can be detected by scintillation cameras. Images are usually acquired in a coronal projection (planar acquisition); however, specialized cameras allow individual slices to be reconstructed in various planes [single photon emission computed tomography (SPECT)]. Radiolabeled technetium colloid is taken up by cells of the reticuloendothelial system and, as such, provides a means of imaging the spleen and estimating splenic size.

The advantages of scintigraphic studies include the administration of physiologically targeted radiopharmaceutical agents. Many radiopharmaceutical agents are available for imaging the liver, and the choice of the agent depends on the suspected abnormality. For example, technetium sulfur colloid is taken up by the reticuloendothelial system and produces a liver/spleen scan that can evaluate the size and configuration of the liver or detect focal mass lesions, which appear as photopenic or 'cold' lesions within the parenchyma. Tagged red blood cells are blood pool agents that can be used to evaluate perfusion to specific

lesions in the liver. Agents that are taken up by the hepatocytes and excreted into the biliary system can evaluate the function and excretion of the liver parenchyma and for obstruction of the biliary tract.

The application of these targeted radiopharmaceutical agents allows relatively high sensitivity for detection of small concentrations of the radiopharmaceutical agents. The major limitations to nuclear medicine studies include the lack of spatial resolution, which relates to several factors, including the use of radiopharmaceutical agents as the energy source, diffuse vascular distribution of the agent, sparse and dispersed  $\gamma$  emissions, scatter of the emitted energy, and low-resolution imaging devices. Efforts to improve spatial resolution using SPECT have not significantly changed the current approach to imaging of the liver.

Prior to the widespread availability of CT, MRI and ultrasound, scintigraphy played a major role in diagnosis of liver disease. However, over the last two decades, scintigraphy has played a steadily decreasing role in the evaluation of the liver. Nonetheless, there are certain situations where scintigraphy is still utilized. These include characterization of mass lesions in the liver, such as confirmation of hemangioma using tagged red blood cell scan and adjunctive imaging for the confirmation of focal nodular hyperplasia (FNH) using technetium sulfur colloid. Parenchymal perfusion, function and excretion can be evaluated and may be useful in the evaluation of liver transplant patients. Evaluation for acute cholecystitis and biliary dyskinesia is commonly performed with technetium-labeled iminodiacetic acid scans.

### Computed tomography

CT images are acquired by rotating an ionizing radiation source around the patient and reconstructing images based on attenuation differences. Two basic acquisition techniques are currently available. Dynamic incremental scanning is performed by rotating the radiation source around the patient at a selected collimation (thickness) with acquisition of a single slice. Subsequently, the table is then advanced at a predetermined level (gap) and the next slice is obtained. With the development of 'slip-ring' technology, images can be obtained by continuous rotation of the radiation source and continual table advancement, thus producing a volume acquisition with a 'screw-like' appearance. This technique is referred to as spiral or helical CT. The recent development of multidetector row CT scanners or 'multislice' CT have allowed faster scanning at much thinner slice thickness with the ability to reconstruct the original CT data into three-dimensional models or additional imaging planes such as sagittal and coronal.

In order for abnormalities to be identified by CT, the technique requires having differences in attenuation between normal and abnormal areas within the hepatic parenchyma. These differences may be minimal unless there is evidence of bone destruction or formation of gas. Radiopharmaceutical agents have been developed to improve lesion conspicuity. In particular, iodinated contrast agents have been developed that are rapidly excreted and have negligible protein binding. When these agents are injected intravenously, they penetrate capillaries and are distributed extracellularly. The result is increased parenchymal contrast, allowing improved differentiation of normal tissue from pathology.

The liver is a unique organ in that it has a dual blood supply (portal and hepatic arterial). Various imaging protocols have been evaluated to exploit this pathophysiology and improve lesion conspicuity. Because approximately 80% of the liver perfusion is received from the portal venous system, and 20% from the hepatic arterial system, peak liver enhancement is seen during the portal venous phase (usually in the range of 60–90 s). Because of the rapidity of spiral CT, a complete examination of the liver can be obtained during the peak enhancement period. Also, software is now available that allows automatic acquisition to begin when peak enhancement occurs, thus reducing the number of suboptimal scans secondary to variability in hemodynamics. Tumors and other pathologic conditions have usually altered perfusion and, therefore, appear as an area of low attenuation within the liver during the portal venous phase. However, because of biologic variations in hemodynamics, some lesions have similar attenuation to normal liver parenchyma. Because most tumors are supplied by the arterial system, there has been an emphasis on imaging the liver during the early phase when arterial flow predominates. This technique of scanning during the arterial and portal venous phase is referred to as 'multiphasic' imaging. By utilizing this technique, certain hypervascular tumors with increased blood flow may be demonstrated as areas of high attenuation on the early arterial phase. This technique has been particularly useful in evaluating HCC and some hypervascular metastases. Rapid intravenous infusion of contrast also allows examination of hepatic vasculature, particularly the intra- and extrahepatic portal vein and intrahepatic branches of the hepatic veins.

The advantages of CT include the rapidity of acquisition and the ability to visualize the entire abdomen and pelvis. The technique is not operator-dependent; therefore, errors in acquisition of pertinent information are reduced. Extremely high-resolution images can be obtained as thin as 1 mm in thickness. With volume acquisition, images can be reconstructed at overlapping intervals and reformatted in various planes. With new software techniques, three-dimensional images can be produced allowing the clinician a new perspective of the spatial locations. Images obtained during the arterial phase of injection can be reconstructed, producing arteriographic images known as CT angiography, and potentially obviate the performance of invasive arteriography.

The principal limitations of CT are related to the acquisition of data in the transverse plane. When organs have borders with complex shapes, adjacent tissues of discrepant density can be included in the individual voxel (volume element) that are averaged in density and appear as one erroneous density in the imaging pixel (picture element). These tissues of adjacent different density can lead to partial volume averaging artifacts, which can obscure tissue boundaries and mimic pathology. With the ability to reconstruct and reformat data with helical CT, this limitation may be reduced. Another major limitation includes the use of iodinated contrast, which is associated with renal tubular injury and is contraindicated in patients with pre-existing renal insufficiency or in individuals with contrast allergy.

### Magnetic resonance imaging

MRI is done by placing the patient in a uniform magnetic field and then applying and sampling repetitive radiofrequency



waves. When the patient is placed inside the magnetic field, the hydrogen nuclei inside the body align themselves with the magnetic field vector. To create an image, a radiofrequency (RF) pulse must be applied. This RF pulse is generated from a coil and causes the nuclei to be rotated out of alignment. When the RF pulse is terminated, the nuclei attempt to realign with the magnetic field. During this process of 'relaxation', a signal is produced in the coil. This signal is then processed and created into an image. Different tissues and pathologies have various relaxation times. By varying the intervals of pulse application and time to listen to the signal ( $T_1$  and  $T_2$  weighting), these tissues and disorders can be differentiated.

MRI technology has dramatically improved over the last several years. Improvements in magnetic coil design have led to the development of smaller dedicated coils that can be used for specific body parts, improving spatial resolution, signal to noise, and smaller field of views, thus optimizing the examination. Improvements in the magnet have led to stronger, faster magnets with marked reduction in scan time, which now allows examination of the liver in a single breath hold. Improvements in software design have led to improved tissue contrast and lesion conspicuity. Three-dimensional images of the biliary tract [magnetic resonance cholangiography (MRC)] can now be obtained rapidly without the injection of contrast media. With faster scans and newer software, non-invasive angiography or venography by MRI (MRA or MRV) is becoming widely used. The ability to identify normal flow direction and velocity, patency, and anatomy is currently being utilized in the work-up of abdominal disease and gives valuable information regarding portal venous hemodynamics.

As with CT, various pharmaceutical agents have been evaluated as contrast agents to improve identification and characterization of lesions. The most widely used agents are the gadolinium chelates, which are extracellular agents. Other agents, such as iron oxide, which are taken up by the reticuloendothelial system, and hepatoselective agents, such as mangafodipir trisodium (MN-DPDP) and other gadolinium-based agents, are undergoing extensive research. Preliminary data suggest that these agents may be useful in improving the detection and characterization of lesions.

The advantages of MRI include the inherent tissue contrast without the use of intravenous contrast and the ability to change various parameters to optimize the examination for a specific clinical question. Images can be obtained in coronal, sagittal, axial, or oblique planes, and three-dimensional volume acquisitions can be performed that can then be reconstructed in various planes of view. Gadolinium-enhanced MRI can be performed in individuals who cannot undergo contrast-enhanced CT secondary to iodine allergy or impairment in renal function.

Although MRI is very robust, the technique does have several limitations. Current costs restrict its use to that of a problem-solving modality. Motion artifacts, which are most notable in the upper abdomen, are becoming less of a limitation with faster scanning techniques. The physical geometry of the bore of the magnet restricts scanning larger patients and can lead to claustrophobia in others. Because of this limitation, vendors are now producing magnets with larger bores or an 'open' design. This improved access to the patient also allows the performance of MRI-guided biopsies and surgical procedures. Finally, patients with contraindications, such as pacemakers, early generation

cerebral aneurysm clips, or certain other ferromagnetic devices, are restricted from undergoing an MRI examination.

MRI can confirm fatty infiltration of the liver, diffuse infiltrative processes (including hemochromatosis), and contribute to evaluation of vascular anatomy and patency. The role of MRC is growing and has become a clear alternative to endoscopic retrograde cholangiopancreatography (ERCP) in some instances. Its usefulness in detecting common bile duct stones and stenoses and primary sclerosing cholangitis is now well documented.

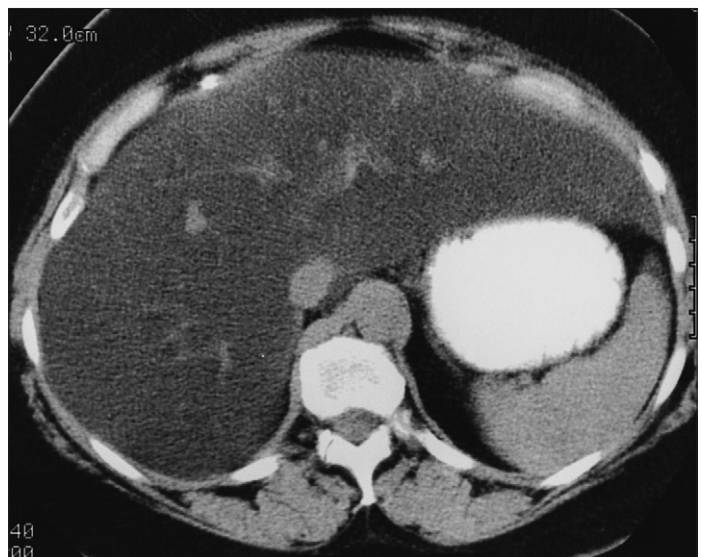
## IMAGING OF SELECTED DISEASES

### Diffuse diseases of the liver

Several disease processes can cause diffuse infiltration of the liver parenchyma and may present with similar clinical signs and symptoms. The diagnosis is usually confirmed by liver biopsy; however, cross-sectional imaging may allow identification and characterization of these diseases and potentially prevent an invasive biopsy. In this section, we discuss the more commonly occurring diffuse diseases of the liver and their radiographic appearance.

Hepatic steatosis has multiple etiologies and may present in a variety of radiographic patterns, including diffuse, nodular, geographic, or focal areas of infiltration. On sonography, fatty infiltration leads to increased echogenicity with decreased visualization of the intrahepatic vessels, reduced through transmission. However, this increased echogenicity is non-specific and may be seen due to hepatitis or hepatic fibrosis. On unenhanced CT scans, steatosis presents as areas of low attenuation. Criteria have been established to compare attenuation values of the liver in relation to the spleen. On unenhanced CT scans, liver attenuation less than the spleen is diagnostic for steatosis (Fig. 8.1).

Occasionally, focal fatty infiltration or the focal absence of fat can mimic a space-occupying mass. MRI is useful for the confirmation of hepatic steatosis in this setting. Fat demonstrates



**Figure 8.1 Hepatic steatosis.** Unenhanced computed tomography scan demonstrates markedly lower attenuation of the liver relative to the spleen, which is consistent with fatty infiltration.

high signal intensity on  $T_1$ -weighted images; however, because of the relative sparse quantity deposited in the liver in relation to the rest of the body, the majority of hepatic steatosis will not demonstrate high signal and, therefore, is difficult to detect on conventional spin echo sequences. The most sensitive technique for demonstration of fatty infiltration is chemical shift imaging. Fat and water 'rotate' at different rates in a magnetic field, and, by changing various parameters, images can be obtained when fat and water are either in phase (together) or out of phase (separated). Areas with small quantities of fat within an individual voxel will show a relative decrease in signal on the opposed phase images compared with the in-phase images. By comparing these two images, fatty infiltration can then be confirmed (Fig. 8.2). Other techniques, such as inversion recovery sequences, have also been utilized. MR spectroscopy (MRS) has been shown to be equivalent to CT in quantification of fat, and these two modalities may be used to serially follow a patient for regression or progression.

### Cirrhosis

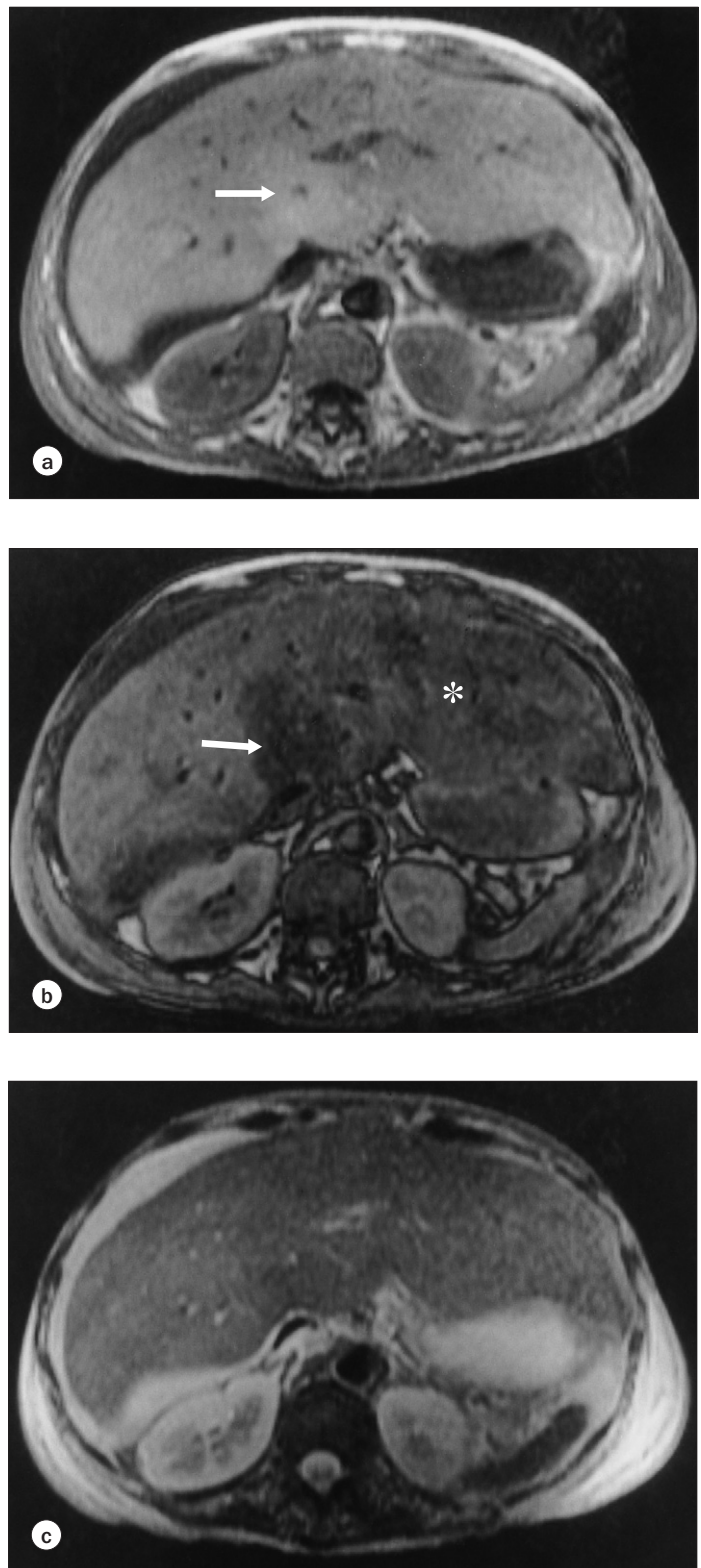
Cirrhosis is caused by irreversible hepatic fibrosis with destruction of the underlying hepatic architecture. The early radiographic changes of cirrhosis may consist only of fatty infiltration. However, as the disease progresses and the fibrosis increases, certain morphologic changes appear. The liver becomes small with a nodular contour; there is atrophy of the right lobe and medial segment of the left lobe; and hypertrophy of the caudate and lateral segment of the left lobe occurs. These changes are well demonstrated on ultrasound, CT and MRI (Fig. 8.3). By using parameters based on the ratio of individual lobe size, ultrasound has been shown to have a sensitivity and specificity of approximately 95% in the diagnosis of cirrhosis and CT, a sensitivity of 84%, and specificity of approximately 100%. Hemodynamic alterations identified on Doppler ultrasound in cirrhosis include flattening of the normal pulsatility of the hepatic vein wave form, increased pulsatility of the portal vein wave form, and alterations in directional flow in the portal vein.

Regenerating nodules are the hallmark of cirrhosis, some of them bigger or more prominent than others. Because of the inherent improved tissue contrast, these nodules may be more conspicuous on MRI. Extensive work has been done in Japan in an attempt to differentiate the spectrum of benign regenerating nodules, dysplastic or precancerous nodules, and early HCCs based on signal characteristics. However, there is an overlap and nodules that are detected should be monitored closely for the development of HCC.

### Iron overload

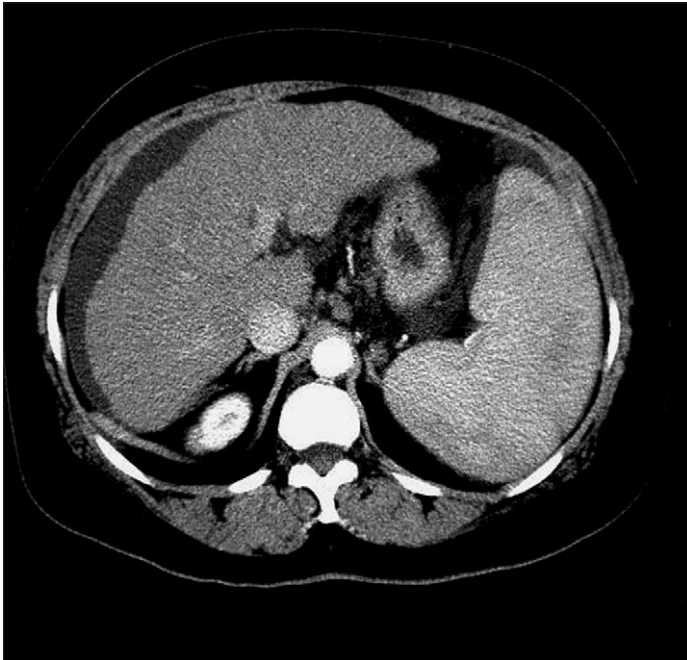
Iron deposition in the liver can be secondary to several etiologies, including hereditary hemochromatosis (HH), transfusional iron overload and segmental areas of deposition in cases of altered intrahepatic portal perfusion. MRI is inherently sensitive to iron deposition, which decreases the  $T_2$  and  $T_2^*$  relaxation times and produces areas of low signal on  $T_2$ -weighted and gradient echo images (Fig. 8.4).

The possibilities of obtaining a non-invasive diagnosis of iron deposition have led to several studies evaluating MRI in the diagnosis of HH with sensitivities and specificities higher than 90%. However, because of variations in magnet strength and parameters in these studies, no standardized data are currently



**Figure 8.2 Hepatic steatosis.** Demonstration of two areas of fatty infiltration of the liver on a magnetic resonance imaging scan. A  $T_1$ -weighted image (a) shows a focal area of higher intensity near the junction of the right and left lobes (arrow). Chemical shift images (b) show a lower signal intensity in this region and a more diffuse low signal in the left lobe (asterisk). This relative drop in signal intensity on chemical shift imaging confirms the diagnosis of fat. This  $T_2$ -weighted image (c) shows no abnormal areas of increased signal in these areas, demonstrating further evidence of the benign etiology.





**Figure 8.3 Cirrhosis.** Computed tomography scan showing a classic morphologic appearance of cirrhosis, consisting of a small liver with nodular surface contour and associated hepatic ascites. The spleen is also enlarged.

available and reproducible quantification parameters are lacking. Therefore, the diagnosis of significant iron overload is still best made by liver biopsy. MRI does allow the ability to monitor patients undergoing therapy by following serial signal intensities.

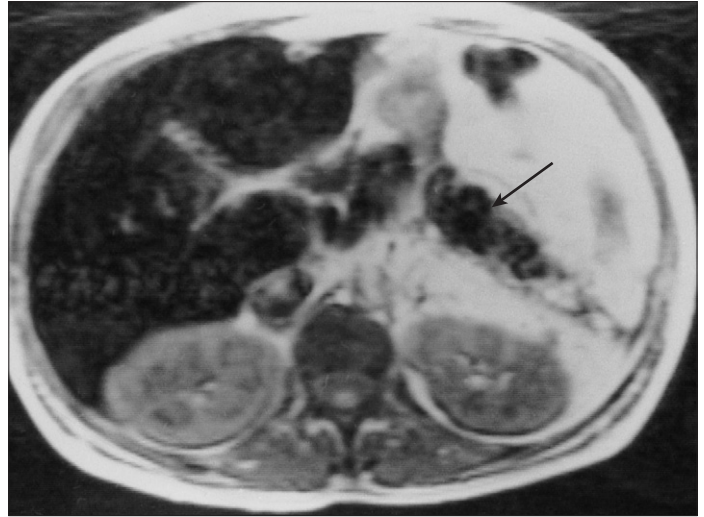
Other organs with increased iron will show a similar low signal, which helps to evaluate the overall distribution of iron deposition. This may help to distinguish hemochromatosis from other secondary causes, such as transfusional iron overload.

## MASSES

### Hepatic metastases

The liver is one of the most common organs affected by metastatic disease. Thus, the majority of research involving imaging of the liver has focused on the identification of metastases. Detection of small metastases has been challenging, because of variations in the biologic nature of various tumor types and even differences between individual lesions within a single histologic type. Much of this variation relates to the vascularity of the tumor and its relative blood supply from the arterial or portal venous systems. This physiology has led to the development and evaluation of different imaging protocols, with imaging the liver in different times during hepatic enhancement, as discussed previously.

The accuracy of the various imaging techniques is widely debated in the literature and appears to be institutionally biased. The major problem is the lack of a 'gold standard' in comparing these modalities. Because of its availability, cost, accepted sensitivity and ability to screen the entire chest, abdomen and pelvis, CT should be the initial study performed in assessing intra-abdominal metastases, even though it is not the most sensitive technique. Because most patients with metastases have multiple



**Figure 8.4 Hemochromatosis.** T<sub>1</sub>-weighted magnetic resonance image demonstrates marked low signal intensity in the liver and spleen. For comparison, the liver should be a similar intensity as the adjacent renal cortex.

lesions, the accuracy of CT in identifying patients with hepatic metastases is higher than 80%. However, for detection of individual lesions, the sensitivity is much less.

MRI is probably more sensitive than CT, however, results vary and are determined by coil selection, pulse sequence protocol and the types of magnets utilized. At the present time, MRI should be utilized when there are confusing findings on CT, when there is a contraindication to contrast-enhanced CT, or in the setting of diffuse fatty infiltration, which can limit detection of a lesion on CT.

CT arterial portography (CTAP) is the most sensitive technique, excluding intraoperative ultrasound. In patients who are considered to be surgical candidates, either CTAP or MRI (or a combination of the two studies) should be performed because of their improved lesion detection. MRI is complimentary to CTAP, thus allowing possible differentiation of perfusion anomalies from metastases with a combined sensitivity of approximately 97%.

The radiographic appearance of hepatic metastases varies and is often non-specific. Certain features may suggest the presence of metastases, including the demonstration of calcification that is seen most often with mucinous carcinoma, such as metastatic colon cancer. Low-density areas may correlate with areas of necrosis. The morphologic pattern of contrast enhancement or signal patterns demonstrating heterogeneity or rings is a useful finding that distinguishes solid neoplasms from cysts or hemangiomas (Fig. 8.5).

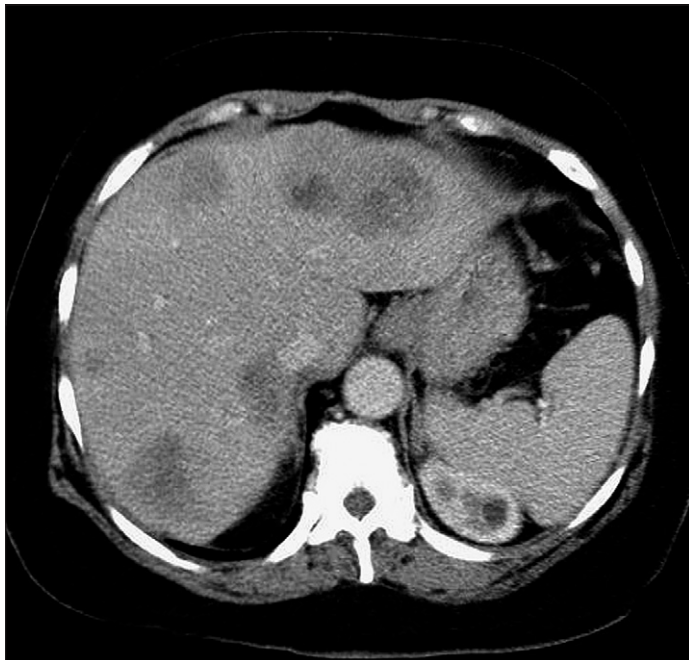
### Primary liver tumors

#### Hepatocellular carcinoma

HCC frequently occurs in association with chronic liver disease and cirrhosis (Figs 8.6 and 8.7). Detection may be more difficult than for metastases because HCC typically occurs in the presence of chronic hepatitis, cirrhosis or steatosis. Common clinical scenarios in which HCC is diagnosed include:

- screening patients with chronic liver disease, and
- preoperative evaluation for patients undergoing partial hepatectomy or liver transplantation.





**Figure 8.5 Hepatic metastases.** Computed tomography scan. The contrast-enhanced image reveals multiple hypodense lesions within the liver, consistent with relatively hypovascular metastases.

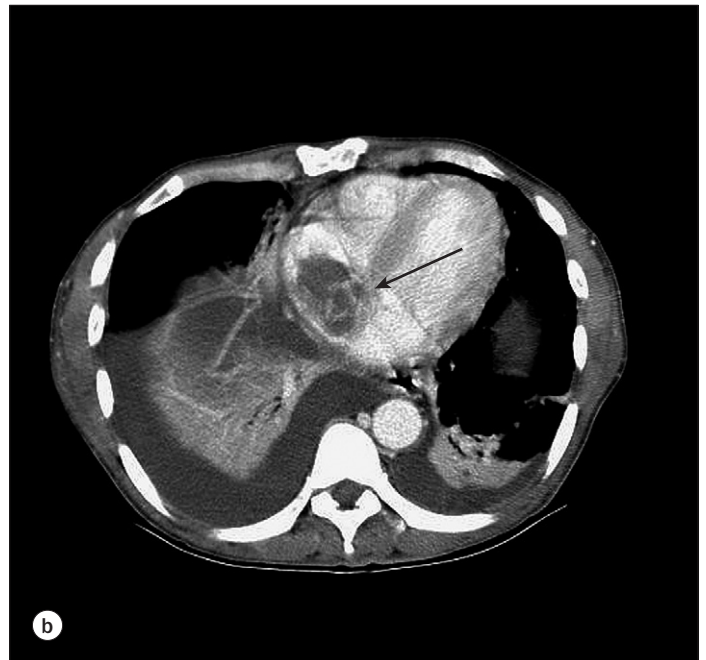
With regard to screening and surveillance, ultrasound has been the best studied modality, in part because of lower cost and wide availability. Many of the studies describing screening by ultrasound have come from Japan and other parts of Asia. In



**Figure 8.7 Chemoembolization.** The non-contrast axial image following chemoembolization reveals a dense concentration of lipiodol within the tumor.

the USA and some other developed Western countries, body habitus (in particular obesity) may limit the effectiveness of ultrasound in detecting HCC. There has been little direct comparison of ultrasound with CT or MRI in the setting of screening, but MRI is probably too expensive while CT carries risks associated with radiation exposure which may need to be repeated many times over many years.

A wide range of accuracy of the individual imaging techniques has been reported in the literature. For routine screening and



**Figure 8.6 Vascular invasion by hepatocellular carcinoma (HCC).** (a) Contrast-enhanced computed tomography (CT) scan. Enlarged right hepatic lobe of HCC is present with adjacent inferior vena cava invasion. Residual crescentic enhancement is present in the remaining patent portion of the inferior vena cava. (b) Contrast-enhanced CT at the level of the right atrium. A large area of HCC extends into the right atrium. The arrow indicates the non-enhancing tumor thrombus in the right atrium

follow-up of patients with chronic liver disease, a combination of ultrasound, CT and  $\alpha$ -fetoprotein levels have been shown to have a moderate sensitivity of approximately 84% in detecting small HCC; however, a significant number of individual nodules can be missed. Other studies have been shown to have fairly low sensitivity of CT in the identification of HCC. The addition of arterial phase images on CT and MRI protocols has allowed detection of additional lesions that would not be visible on the portal venous phase and conventional MR pulse sequences. MRI may provide further information when differentiating HCC from regenerating nodules.

In patients who are being evaluated for hepatectomy or transplantation, identification of individual lesions is critical; therefore, the most sensitive techniques should be used. CT and MRI are commonly used to diagnose and stage HCC in patients with cirrhosis. A commonly used system for non-invasive diagnosis of HCC has been proposed by a group of experts under the auspices of the European Association for the Study of the Liver (EASL). Under these criteria, HCC can be reliably diagnosed in a cirrhotic individual if there are two consecutive imaging studies of the liver which show a hypervascular lesion >2 cm in diameter or one imaging technique with a hypervascular lesion >2 cm combined with a serum  $\alpha$ -fetoprotein level >400 ng/mL. The choice of CT or MRI appears to be based on local institutional expertise.

### **Cavernous hemangioma**

Hemangiomata are exceedingly common and are present in 15–20% of the adult population. These lesions are commonly found on imaging of the liver. When these lesions are detected in an individual with a malignancy, they may mimic metastases. Because of the frequency of this dilemma, several studies have been done to evaluate the sensitivity and specificity of CT, MRI and tagged red blood cells in differentiating hemangiomas from metastases.

The imaging features of hemangioma reflect the histology and hemodynamics of the tumor, which is characterized by a cavernous collection of blood spaces. Blood flow is very slow and almost undetected, except for peripheral sites of entry. With intravenous contrast administration, hemangiomas demonstrate a classic pattern of peripheral nodular enhancement, centripetal filling and uniform enhancement with retention of the blood pool product (Fig. 8.8).

Using the aforementioned criteria, CT has a sensitivity and specificity of 80% and almost 100%, respectively. The appearance of hemangioma on MRI is that of a well-defined lesion with a very strong signal (equivalent to fluid) on heavily T<sub>2</sub>-weighted images (Fig. 8.9). Enhancement with gadolinium is similar to that seen on a CT scan. Using these criteria, the specificity is similar to tagged red blood cell scans and is higher than 95%. MRI, although more expensive than tagged red blood cell scans in the confirmation of hemangioma, does offer some advantages. MRI can detect smaller lesions that are slightly less than 1 cm, whereas tagged red blood cell scans using SPECT techniques can only detect lesions on the order of 1–1.5 cm. MRI offers improved visualization of lesions near larger vessels, which may be subtle on tagged red blood cell scans because of the blood pool activity within the vessel. MRI also allows identification of other unsuspected lesions that would be missed by performing tagged red blood cell scans.



**Figure 8.8 Hemangioma.** Computed tomography demonstration of the classic early enhancement appearance showing nodular discontinuous peripheral enhancement (arrow).



**Figure 8.9 Hemangioma.** Magnetic resonance imaging appearance of a hemangioma consists of a well-defined high signal intensity mass on T<sub>2</sub> imaging (arrow).

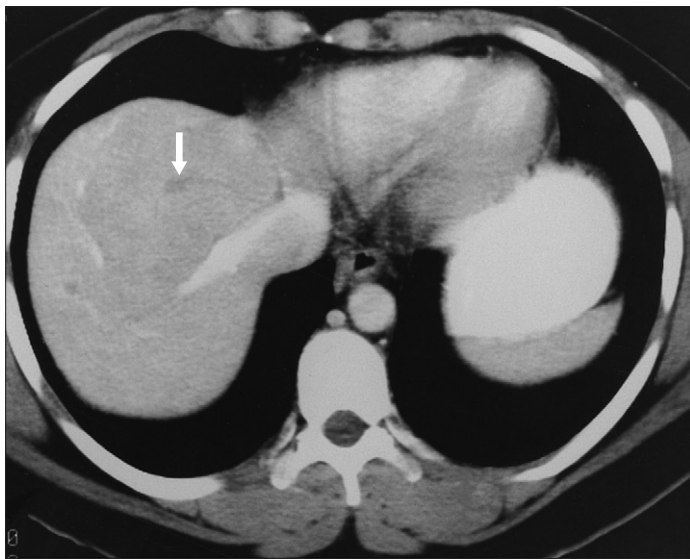
### **Miscellaneous tumors**

The sensitivity of CT in detecting other primary tumors of the liver is quite high; however, many of the features are non-specific and do not allow definitive characterization. Certain tumors may have features that can suggest the diagnosis. For example, hepatic adenomas may contain areas of fat, necrosis and hemorrhage. The presence of a central scar suggests the diagnosis of FNH (Fig. 8.10) although this feature can sometimes be seen in fibrolamellar HCC and hepatic adenomas.

### **VASCULAR ABNORMALITIES**

Ultrasound can provide exquisitely sensitive information regarding the hemodynamics of the liver, such as vascular patency, velocity and directional flow, and should be the initial study of





**Figure 8.10 Focal nodular hyperplasia.** An enhanced computed tomography scan demonstrates an isodense mass in the dome of the liver with a central scar, which is characteristic of focal nodular hyperplasia.

choice when evaluating suspected vascular abnormalities. Ultrasound has a sensitivity of approximately 87% in the diagnosis of Budd-Chiari syndrome. Characteristic findings include the absence of the normal hepatic veins with areas of stenoses, webs, or thrombus. Abnormal Doppler wave forms may consist of absent or reversed flow in hepatic vein tributaries or the inferior vena cava, turbulent flow, or continuous flow. Ultrasound is 100% sensitive and 93% specific for the diagnosis of main portal vein thrombus and has been shown to be 97% sensitive for the diagnosis of significant arterial vascular disease in post-liver transplant patients.

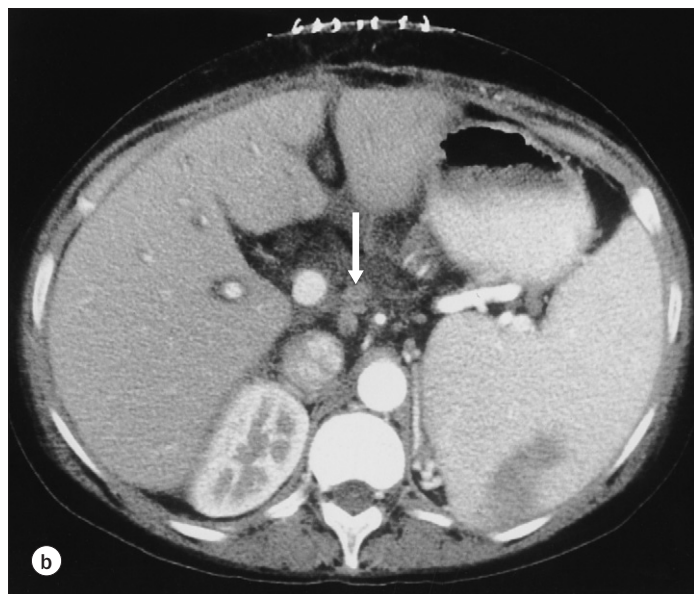
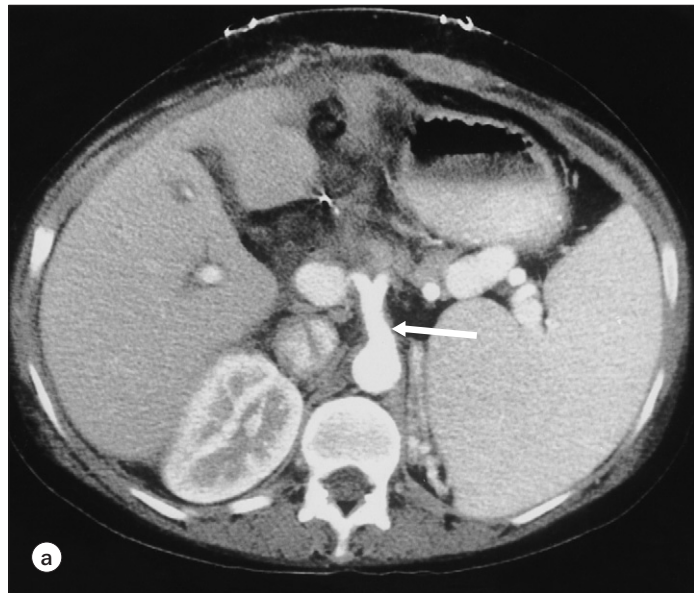
MRI, with its ability to image flowing blood and determine the direction and velocity of flow, is generally complementary to ultrasound in the evaluation of hepatic vessels. However, MR should be used in select cases in which ultrasound is non-diagnostic or confusing. Because of its expanded field of view, MRI also shows associated collateral vessels throughout the abdomen.

With the rapid, high-resolution images obtained with helical CT, intravenous enhanced spiral CT scans can exquisitely demonstrate vascular anatomy and patency, especially of the arterial system (Figs 8.11 and 8.12). Delayed images during the portal venous phase can evaluate the venous structures; however, occasionally flow defects within these vessels can mimic thrombus.

## BILIARY ABNORMALITIES

### Gallstones

The primary modality that should be used to confirm the presence of cholelithiasis is ultrasound. Sonography has a sensitivity of greater than 95% in the diagnosis of cholelithiasis and can detect approximately 70–80% of choledocholithiasis. Gallstones within the distal part of the common bile duct are slightly more difficult to detect secondary to overlying bowel gas. On ultrasound, gallstones appear as echogenic structures

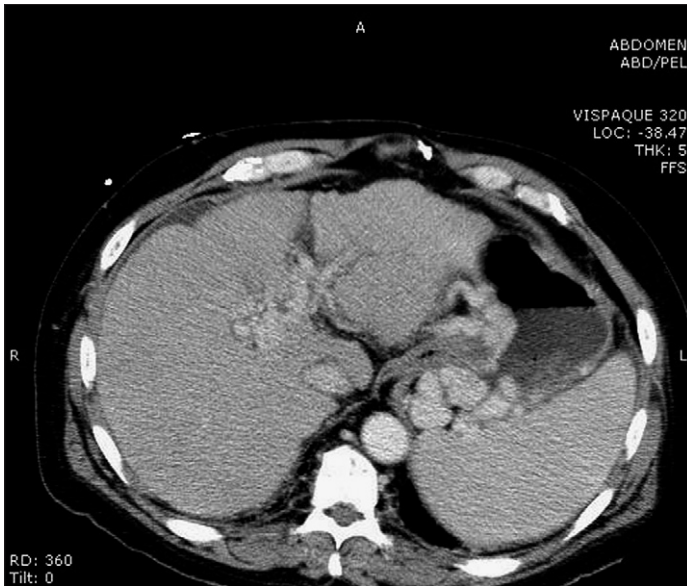


**Figure 8.11 Hepatic artery thrombosis.** The hepatic artery was unable to be identified by ultrasound in this patient who is status post-liver transplant. Axial images from a computed tomography angiogram show the celiac axis (a, arrow) and a thrombus (b) within the proximal hepatic artery (arrow).

with posterior acoustic shadowing. Because of the numerous possible pitfalls in diagnosis, meticulous technique and knowledge of these drawbacks are a necessity. To allow distention of the gallbladder and improve detection of gallstones, patients should be asked to fast for at least 4 to 6 h before the examination.

When using a high-resolution technique, CT can detect a significant number of calcified or dense gallstones because of the contrast between the calcium and bile. However, many gallstones demonstrate similar attenuation to bile, thus decreasing their rate of detection. Intravenous contrast agents that are excreted in the bile have been found to increase contrast between the stone and surrounding bile and improve detection; however, because of numerous side-effects, these agents are not widely





**Figure 8.12 Cavernous transformation of the portal vein.** Severe portal hypertension or occlusion of the portal vein may result in the development of large adjacent collaterals, evidenced by cavernous transformation of large veins in the upper abdomen related to the spleen and portal vein.

used in clinical practice. MRC has been shown to be useful in the diagnosis of gallstones with a sensitivity for detecting choledocholithiasis of 70–100% (Fig. 8.13).

### Acute cholecystitis

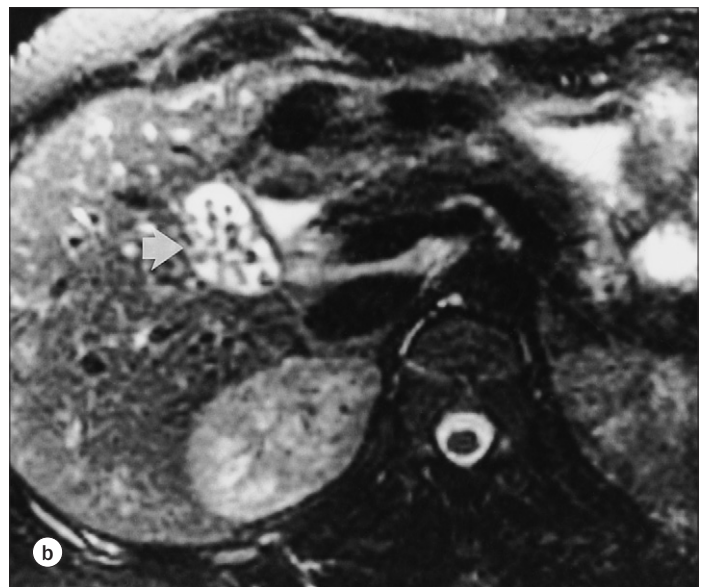
In patients with suspected acute cholecystitis, sonography or hepatobiliary scintigraphy should be the initial imaging study performed. An advantage of ultrasound over scintigraphy is that ultrasound allows a generalized screening of the right upper quadrant, which may detect other abnormalities that mimic

acute cholecystitis. Findings of acute cholecystitis on ultrasound include the presence of gallstones with focal tenderness over the gallbladder (sonographic Murphy sign), gallbladder distention, gallbladder wall thickening, and air within the wall or lumen of the gallbladder, internal membranes and pericholecystic fluid. The most reliable sign is the presence of gallstones and the positive sonographic Murphy sign with a positive predictive value of 92% and a negative predictive value of 95%.

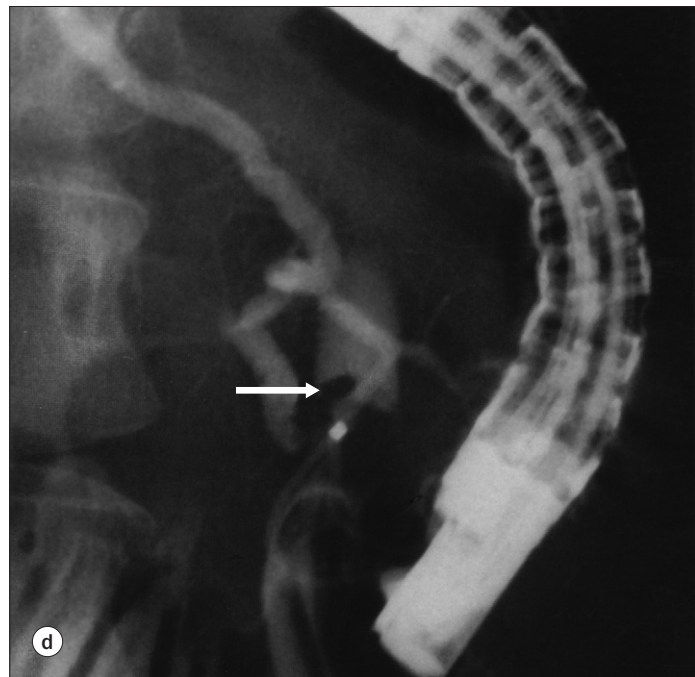
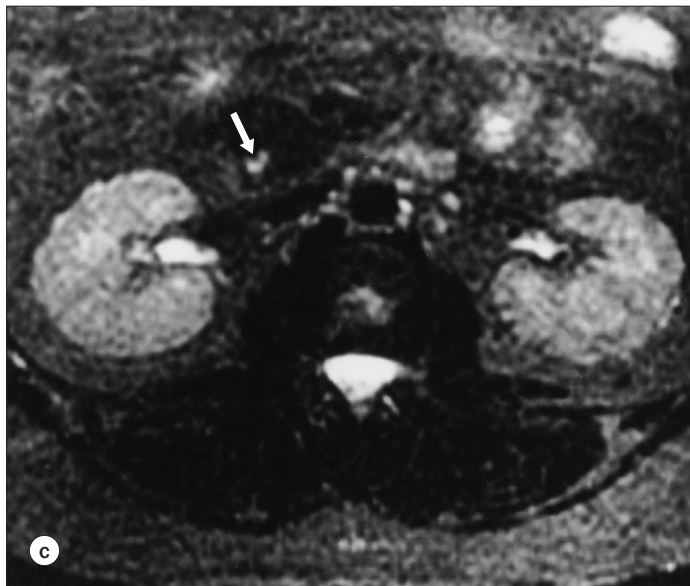
In acute cholecystitis, hepatobiliary scintigraphy (technetium-labeled iminodiacetic acid scans) demonstrates lack of visualization of the gallbladder secondary to obstruction of the cystic duct. Hepatobiliary scintigraphy has a high sensitivity and specificity of more than 95%. Occasionally, patients with acute cholecystitis may have non-specific clinical findings and be referred for CT examination. The CT findings are similar to those found on ultrasound, including gallbladder wall thickening, pericholecystic stranding, gallbladder distention, pericholecystic fluid, subserosal edema, high attenuation bile and sloughed membranes (Fig. 8.14). In one study CT allowed detection of approximately 50% of cases of acute cholecystitis; however, the absence of findings does not exclude the diagnosis, and further imaging with ultrasound or scintigraphy should be performed.

### Biliary obstruction

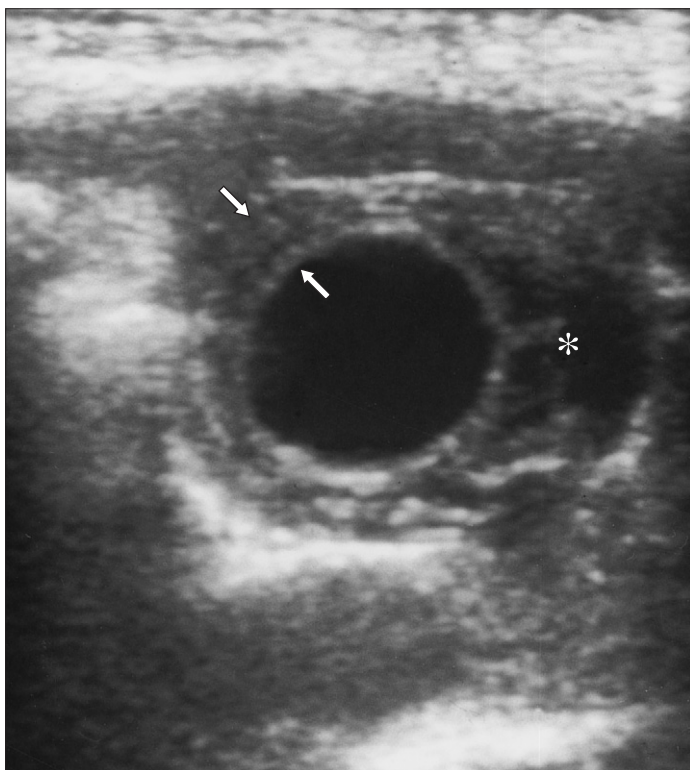
Biliary obstruction can be caused by many conditions, including extrinsic or intrinsic masses, inflammation and strictures. The evaluation of individuals with suspected biliary obstruction should begin with right upper quadrant sonography. Ultrasound allows detection of bile duct dilatation and can usually determine the site of obstruction in more than 90% of the cases and the cause in more than 70% of the cases. CT may provide useful information when the ultrasound is inconclusive or incompletely evaluates or stages the abnormality detected. MRC is a non-invasive technique to obtain cholangiographic images and the detection of bile duct strictures.



**Figure 8.13 Magnetic resonance cholangiography.** (a) A normal-appearing magnetic resonance imaging (MRI) cholangiogram. (b) Cholelithiasis. Note the appearance of gallstones on the MRI. The typical gallstone is a low signal intensity structure seen within the high-intensity bile, such as in the gallbladder (arrow).



**Figure 8.13 Magnetic resonance cholangiography.** (Cont'd) (c) Choledocholithiasis. A tiny, 2–3 mm, low-signal intensity structure is seen on the axial images from a magnetic resonance cholangiogram in the distal common bile duct (arrow). (d) Gallstone. This tiny gallstone was confirmed on subsequent endoscopic retrograde cholangiopancreatography.



**Figure 8.14 Acute cholecystitis.** An ultrasound demonstrates a greatly thickened gallbladder wall (arrows) with intramural fluid (asterisk). These findings combined with a positive 'sonographic Murphy sign' are diagnostic of acute cholecystitis.

## OTHER IMAGING TECHNIQUES

Conventional arteriography of the liver was previously done in preparation for surgery but is now more frequently done as part of a therapeutic intervention, such as with chemoembolization, to assess arterial stenoses after liver transplantation or to embolize sources of hemorrhage within the liver. Angiography has also been used to enhance the sensitivity of CT. Because of the wide variation of tumor enhancement and the less than satisfactory results for individual lesion identification utilizing dynamic bolus techniques, other methods to administer contrast at angiography with selected cannulation of the hepatic artery [CT arteriography (CTA)] or by the superior mesenteric artery and splenic artery (CTAP) are commonly performed. These techniques offer improved contrast and sensitivity in individual lesion detection; however, due to variations in the portal venous perfusion, they are plagued by the problem of perfusion defects that can cause false-positive diagnoses of lesions.

Lipiodol CT is a technique in which an oily contrast medium derived from poppy seed oil is infused into the hepatic artery by using angiographic techniques. This oily material is retained selectively by HCC tissues which can be more readily seen when a CT scan of the liver is performed some days after the lipiodol is infused.

$^{18}\text{F}$ -fluorodeoxyglucose (FDG) uptake allows estimation of glucose metabolism of cells using positron emission tomography (PET). Normal liver contains a relative abundance of glucose 6-phosphatase (G6P) and lower levels of hexokinase (HK) while tumor cells tend to have increased HK activity but little, if any, G6P. This difference in metabolism results in increased accumulation of FDG in cholangiocarcinoma, HCC and metastatic liver tumors, potentially allowing differentiation between normal tissue and tumor on PET images. While PET of extra-hepatic



solid malignant tumors has become standard, this approach has not achieved its potential with regard to liver tumors. PET appears to have its highest sensitivity for cholangiocarcinoma but is able to detect only about 50% of HCCs, although it may be useful in identifying extra-hepatic metastases of HCC in some cases. The value of combining PET imaging and CT (so called PET-CT) has not yet been evaluated for liver disease.

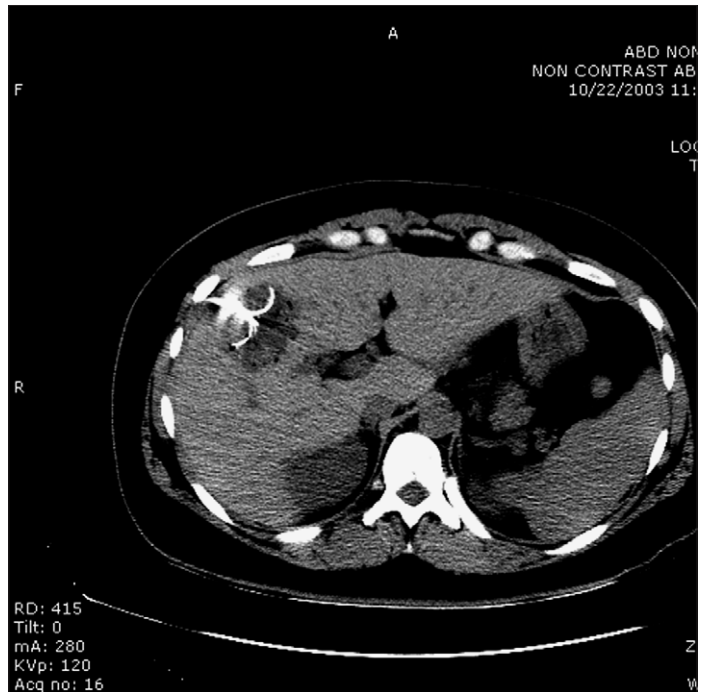
## INTERVENTIONAL RADIOLOGY AND THE LIVER

The techniques in hepatic imaging are constantly improving as are the minimally invasive methods that interventional radiologists can offer for the treatment of liver disease and tumors. The procedure of transjugular intrahepatic portosystemic shunt (TIPS) placement for the management of portal hypertension and as an adjunct in acute variceal hemorrhage is widely accepted (Fig. 8.15). Newer techniques are being increasingly used in academic medical centers for the management of unresectable liver tumors including both primary liver tumors and metastases. Percutaneous ablation methods for hepatic malignancy and metastatic cancer include alcohol or acetic acid ablation and RF ablation (Figs 8.15 and 8.16). Both methods utilize imaging guidance with ultrasound or CT scanning and placement of a needle or probe to destroy the region of tumor. Ethanol or acetic acid injection causes destruction of tissue by protein denaturation and RF ablation employs a probe for continuous deposition of heat into the tissue to produce thermal coagulation necrosis (Fig. 8.17). Cryoablation is currently being tested as an MRI compatible method of tumor destruction using interventional MRI machines.

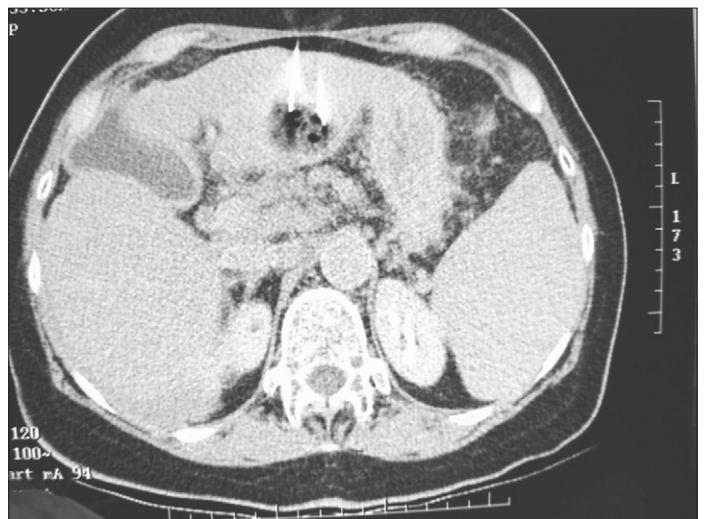
Most types of hepatic malignancy respond poorly to systemic chemotherapy. Chemoembolization or transcatheter arterial chemoembolization (TACE) may be used to shrink or slow the advancement of unresectable hypervascular liver tumors including HCC, carcinoid tumors, breast cancer metastases or less



**Figure 8.15 Venogram of the hepatic vein.** This is the first step in preparation for performing transjugular intra-hepatic shunt (TIPS).



**Figure 8.16 Radiofrequency ablation (RFA) of hepatocellular carcinoma.** Non-enhanced computed tomography showing RFA needle in position with tines deployed.



**Figure 8.17 Ethanol injection.** Contrast-enhanced computed tomography showing needles in position within a hepatocellular carcinoma in left lobe of the liver. Note the presence of air in the lesion after injection.

commonly ocular melanoma. The main goal of chemoembolization is to achieve a highly localized concentration of chemotherapeutic agents in the liver tumor with an associated ischemia that provides lasting tumor shrinkage. The procedure is often repeated up to three times for maximization of tumoricidal effect. Patients are typically chosen based on lesion size and tumor type consistent with hypervascularity. Non-vascular lesions such as metastatic adenocarcinoma of the colon exhibit a less substantial effect from chemoembolization due to the reduced ability to deliver concentrated chemotherapeutic agents into the tumor.



Consultation with an interventional radiologist is advised when referring patients for percutaneous ablation or chemoembolization, as lesion size and tumor type, conspicuity on

imaging and location within the liver play significant roles in the type of treatment performed and the ability to provide lasting tumor destruction.

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## SECTION III: CLINICAL MANIFESTATIONS OF LIVER DISEASE

### CHAPTER

# 9

## Portal Hypertension and Gastrointestinal Hemorrhage

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### INTRODUCTION

Variceal bleeding, a direct consequence of portal hypertension, is the most lethal complication of cirrhosis and accounts for approximately one-third of all deaths among patients with chronic liver disease and cirrhosis. Optimal management of portal hypertension and variceal hemorrhage requires a clear understanding of the pathophysiology of portal hypertension, its pathogenesis and the natural history of varices as well as the risk factors associated with their bleeding.

Portal hypertension is defined as the elevation of hepatic venous pressure gradient (HVPG) to greater than 5 mmHg. Portal hypertension is caused by a combination of two hemodynamic processes:

- increased intrahepatic resistance to the passage of blood flow through the liver, and
- increased splanchnic blood flow secondary to vasodilatation within the splanchnic vascular bed.

Hence, numerous vasoconstrictors and vasodilators can have an impact on portal pressure and act therapeutically by decreasing both portal hypertension and intravariceal pressure.

Portal hypertension is responsible for the two major complications of cirrhosis – variceal bleeding and ascites. These two complications account for a large proportion of the 32 000 deaths and 20 million days of work loss related to cirrhosis annually in the USA. Of these complications, variceal hemorrhage is more immediately life threatening and is associated with a 20–30% mortality with each episode of bleeding.

### PATHOPHYSIOLOGY OF PORTAL HYPERTENSION

#### Portal venous anatomy

The portal venous system normally drains blood from the stomach, intestines, spleen, pancreas and gallbladder. The portal vein is formed by the confluence of the superior mesenteric and splenic veins (Fig. 9.1). The superior mesenteric vein drains deoxygenated blood from the small bowel, the head of the pancreas, the ascending colon and part of the transverse colon whereas the splenic vein drains the spleen and the pancreas and is joined by the inferior mesenteric vein, which brings blood from the transverse and descending colon as well as the superior two-thirds of the rectum. Thus, the portal vein normally receives blood from almost the entire gastrointestinal tract.

#### Classification of portal hypertension

The causes of portal hypertension are usually classified into prehepatic, intrahepatic and posthepatic. Prehepatic causes are those affecting the venous system before it enters the liver, such

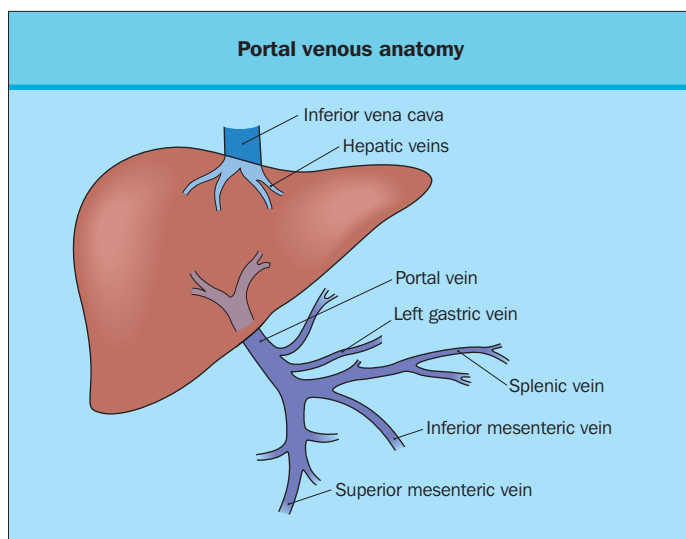


Figure 9.1 Portal venous anatomy.

as thrombosis of the portal vein. Intrahepatic causes represent the main group, with cirrhosis accounting for the vast majority of cases. Posthepatic causes encompass those affecting the hepatic veins and venous drainage to the heart. (e.g. Budd-Chiari syndrome).

A more precise classification emerges when the intrahepatic anatomy of the venous connections is taken into account. The portal vein divides and branches throughout the liver, eventually forming portal venules at the lobular level. These connect to the central veins via the hepatic sinusoids. The pressure in the portal venules is the same as that in the systemic venous circulation. There is a normal gradient of approximately 3 mmHg across the liver from portal (7 mmHg) to hepatic (4 mmHg) venous systems.

In cases of portal hypertension, a high wedged hepatic venous pressure (WHVP) suggests an intrahepatic block at the level of the sinusoids (flow being obstructed between a portal venule and central vein, with a resulting high pressure in the venule), whereas a normal WHVP reflects normal pressure in the portal venule and thus a presinusoidal cause of portal hypertension, such as disease around the portal tracts or outside the liver. Thus, portal hypertension can be reclassified as presinusoidal (both prehepatic and intrahepatic), sinusoidal (intrahepatic) and postsinusoidal (intrahepatic and posthepatic) (Table 9.1).

This classification has immediate clinical implications: for example, in presinusoidal portal hypertension (both pre- and intrahepatic) where the liver function is well preserved and liver



Causes of portal hypertension
<b>Presinusoidal portal hypertension</b> Extrahepatic causes Portal vein thrombosis Cavernous transformation of the portal vein Extrinsic compression of the portal vein Arterioportal fistula Massive splenomegaly
Intrahepatic causes Schistosomiasis Sarcoidosis Primary biliary cirrhosis Idiopathic portal hypertension (non-cirrhotic portal hypertension)
<b>Sinusoidal portal hypertension</b> Cirrhosis Alcoholic hepatitis Vitamin A toxicity
<b>Postsinusoidal portal hypertension</b> Veno-occlusive disease Budd-Chiari syndrome Hepatic venous or inferior vena cava web Restrictive heart disease Constrictive pericarditis Severe congestive heart failure

Table 9.1 Causes of portal hypertension.

failure and hepatic encephalopathy are infrequent following variceal hemorrhage. Nevertheless, many hepatic causes of portal hypertension produce pathologic changes that cause a block at both the presinusoidal and sinusoidal levels and often, therefore, the distinction is academic. In contrast, there are clear therapeutic implications for differentiating hepatic venous outflow obstruction (posthepatic) from other causes of portal hypertension.

Anatomy and classification of gastroesophageal varices

The tributaries of the portal vein also communicate with veins draining the systemic venous circulation. Such portosystemic communications exist primarily in five locations:

- at the cardia via the intrinsic and extrinsic veins of the region,
- in the anal canal via anastomoses between the superior and middle hemorrhoidal veins,
- in the falciform ligament via recanalization of the para-umbilical veins draining the abdominal wall,
- the splenic venous bed and the left renal vein, and
- the retroperitoneum.

Of these, the gastroesophageal collaterals have the greatest clinical significance and allow portal blood flow to return to the heart when flow through the portal system is obstructed.

The veins draining the esophagus may be classified as intrinsic, extrinsic and venae comitantes of the vagal nerve. The intrinsic veins are comprised of a subepithelial and submucosal plexus, which run along the length of the esophagus. These drain via perforating veins into an extrinsic plexus of veins surrounding the esophagus. In the cervical region and superior mediastinum, they drain into the inferior thyroid and brachiocephalic veins, whereas in the posterior mediastinum they drain into the

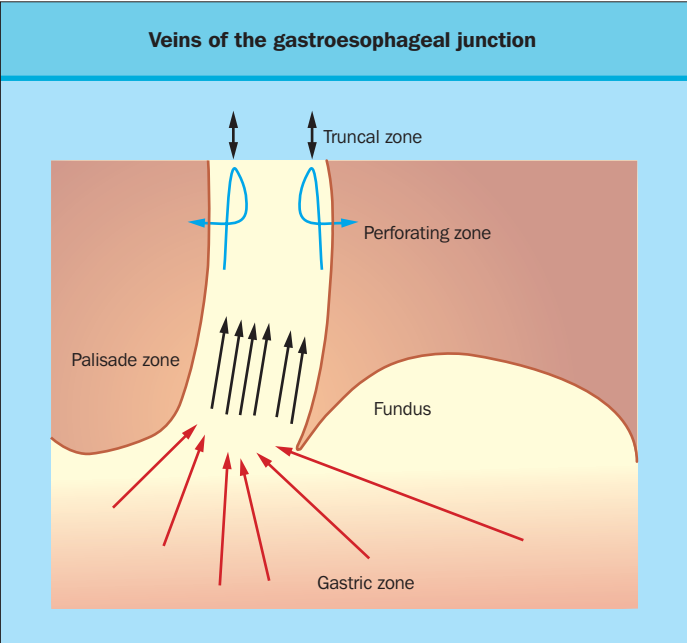


Figure 9.2 Veins of the gastroesophageal junction. The venous anatomy allows subdivision of the region into four zones: the gastric, palisade, perforating and truncal zones. Modified from Vianna in McIntyre et al (91) by permission of Oxford University Press.

azygos vein. The extrinsic venous plexus around the abdominal part of the esophagus drains into the left gastric vein.

The intrinsic veins of the gastroesophageal junction are divided into four well-defined zones (Fig. 9.2):

- The *gastric zone* is a 2–3 cm zone with its upper border at the gastroesophageal junction and consists of veins arranged radially, in the submucosa and lamina propria.
- The *palisade zone* commences at the gastroesophageal junction and extends cranially for 2–3 cm and is a direct extension of the veins of the gastric zone, which runs in ‘palisades’ or packs of longitudinally arrayed veins in the lamina propria. When portal pressures rise and impede drainage of the veins in the gastric zone into the left gastric vein, flow is directed via the veins of the palisade zone into the esophagus and eventually into the azygos vein.
- The intrinsic veins drain into the extrinsic veins primarily in the *perforating zone* via the valved perforating veins that normally allow only unidirectional flow. However, flow through these veins increases in subjects with portal hypertension, the valves become incompetent and allow bidirectional flow. The perforating zone extends 2–3 cm cranially from the palisade zone.
- The *truncal zone* is an 8–10 cm zone extending upward from the perforating zone. The intrinsic veins consist of three or four large venous trunks in the submucosa, which communicate with an irregular polygonal venous plexus in the submucosa. Blood in these veins flows from a cranial to a caudal direction and drains via the perforating veins into the extrinsic veins.

When portal hypertension occurs, the impediment to portal flow is partly compensated for by an increase in flow through portosystemic communications, especially the intrinsic veins around the gastroesophageal junction, which dilate and become

varicose veins. Such gastroesophageal varices occur in four basic patterns:

- varices in the fundus of the stomach,
- gastric and palisade zone varices,
- varices in the perforating zone, and
- paraesophageal varices that involve the extrinsic esophageal veins.

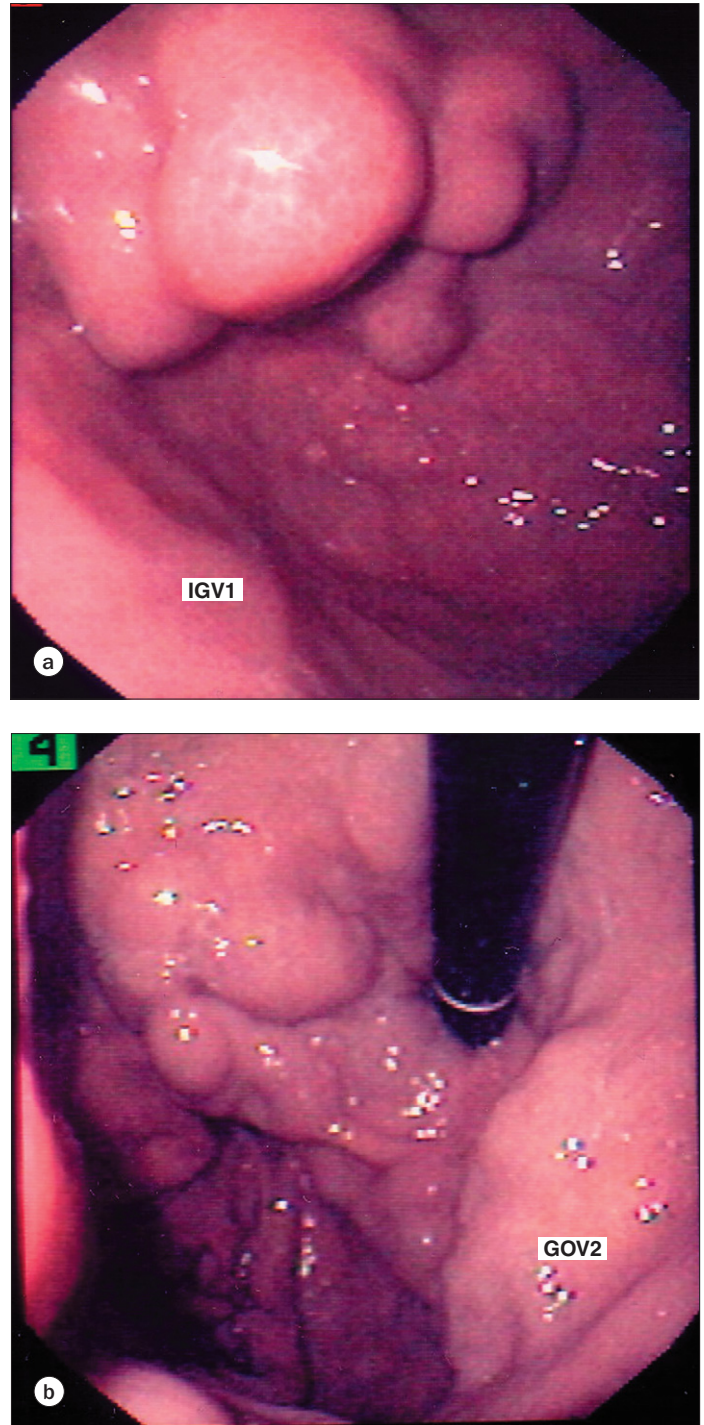
Clinically, varices are classified by their location as esophageal or gastric varices. Esophageal varices are graded by their size (Table 9.2).

In contrast, gastric varices are classified primarily by their location (Fig. 9.3). As agreed at the Baveno Consensus Conference, they are classified as follows: gastroesophageal varices (GOV, gastric varices in continuity with esophageal varices) or isolated gastric varices (IGV) (Table 9.2).

GOV1 are formed by a communication between the deep submucosal veins in the gastric zone with a branch of the left gastric vein. These varices allow blood from the left gastric vein to drain via palisade zone veins into the systemic circulation. GOV1 varices are invariably associated with large esophageal varices. In contrast, only 50% of GOV2 are associated with large esophageal varices. IGV1 are usually associated with either segmental portal hypertension (splenic vein thrombosis) or the presence of spontaneous collaterals from the splenic vein to the renal vein, which feed these varices. Approximately 50% of cases of ectopic varices, including IGV2, are associated with portal hypertension. The mechanism underlying the predilection for varices to develop at sites away from the gastroesophageal junction when the portal vein is occluded remains unknown.

Gastrointestinal manifestations of portal hypertension	
Manifestation	Comments
<b>Varices</b>	
1. Esophageal	
F1	Small, straight
F2	Enlarged, tortuous, <1/3 of lumen
F3	Large, coil-shaped, >1/3 of lumen
2. Gastroesophageal (GEV)	
GEV-1	Along lesser curve, 2–5 cm
GEV-2	Along greater curve and fundus
3. Isolated gastric (IGV)	
IGV-1	Isolated in the fundus
IGV-2	Limited in the corpus, antrum, pylorus
4. Ectopic varices	
Duodenum, small bowel, colorectum	
<b>Portal hypertensive gastropathy</b>	
PHG	Fundus and corpus of the stomach
GAVE	Limited to the antrum
GAVE, gastric antral vascular ectasia; PHG, portal hypertensive gastropathy	

**Table 9.2 Gastrointestinal manifestations of portal hypertension**



**Figure 9.3 Type I isolated gastric varices (IGV1) as a cluster of varices in the fundus (a) and cardia (b) of the stomach.**

The pressure in the portal vein is a direct product of portal inflow and outflow resistance. Thus:

$$\text{portal pressure} = \text{portal venous inflow} \times \text{outflow resistance} \quad (\text{eq. 1})$$

Portal pressure is determined by hepatic venous catheterization and measurement of the free hepatic venous pressure (FHVP) and WHVP and calculation of the HVPG:

$$\text{HVPG} = \text{WHVP} - \text{FHVP} \quad (\text{eq. 2})$$

WHVPs reflect sinusoidal pressures and, in the absence of portal vein thrombosis, reflect portal vein pressures. The free hepatic venous pressure corrects for the effects of intra-abdominal pressures. Portal hypertension usually occurs when the resistance to flow through the portal venous bed is increased. As mentioned earlier, this may result from an obstruction to portal venous flow before it enters the hepatic sinusoids (presinusoidal portal hypertension, e.g. portal vein thrombosis), in the hepatic sinusoids (sinusoidal portal hypertension, e.g. cirrhosis), or beyond the hepatic sinusoids (postsinusoidal portal hypertension, e.g. hepatic vein thrombosis). In patients with cirrhosis, the increase in portal pressures is further compounded by a secondary increase in portal flow that results from splanchnic arteriolar dilatation.

A principal hemodynamic consequence of portal hypertension is diversion of portal flow to the systemic circulation via porto-systemic communications that dilate to form varices. These varices, especially in the intrinsic veins around the gastro-esophageal junction, are likely to rupture bringing them to clinical attention.

The likelihood of variceal rupture is determined by its wall tension, which may be defined as:

$$\text{wall tension} = (\text{transmural pressure gradient}) \times \text{radius of varix/width of variceal wall (w)} \quad (\text{eq. 3})$$

The transmural pressure gradient is the product of flow and resistance through the varix and is defined as:

$$P_1 - P_2 = Q \times R \quad (\text{eq. 4})$$

where  $P_1$  and  $P_2$  are the pressure within and outside the varix respectively,  $Q$  is the blood flow per unit time, and  $R$  is the resistance to flow through the varix. The resistance to flow may be calculated by Poiseuille formula and expressed as:

$$R = 8\eta l / \pi r^4 \quad (\text{eq. 5})$$

where  $l$  is the length of the varix,  $\eta$  is related to blood viscosity (Reynold number), and  $r$  is the radius of the vessel. Interposing equations 4 and 5 in equation 3, variceal tension may be redefined as:

$$\text{Wall tension} = Q \times (8\eta l / \pi r^4) \times \eta w \quad (\text{eq. 6})$$

Consequently, based on equation 6 above, long, large varices with high flow rates and a thin wall are most likely to rupture and bleed. Conversely, decreasing collateral flow, resistance to flow, or increasing wall thickness should decrease the risk of variceal rupture. Variceal flow is decreased primarily by decreasing portal pressures. This is accomplished in most cases by inducing splanchnic arteriolar constriction, which reduces portal inflow (see equation 1) and forms the physiologic basis for most forms of pharmacologic treatment of portal hypertension. Whereas decreasing resistance to flow through the varices should decrease wall tension also, the results are less predictable due to an associated increase in collateral flow that negates the beneficial effects of decreased collateral resistance. This may explain some of the interindividual variability in hemodynamic response to pharmacologic treatment.

## NATURAL HISTORY AND CLINICAL FEATURES OF PORTAL HYPERTENSION

The three primary features of portal hypertension are gastro-esophageal varices, ascites and hypersplenism. The latter two topics are dealt with elsewhere.

### Esophageal varices

Endoscopic screening among cirrhotics has shown that approximately one-third have varices. An additional 5–15% of those with cirrhosis without varices develop varices de novo each year. It is estimated that most cirrhotic individuals (~75%) will develop varices during their lifetime and that about one-third of all patients with esophageal varices will experience variceal hemorrhage. Several factors predict the risk of bleeding from esophageal varices (Table 9.3)

It is both intuitively as well as clinically obvious that varices cannot bleed if they do not develop. Several studies have now shown that at HVPG values less than 12 mmHg, varices do not form and therefore do not bleed. Varix formation as identified by endoscopy is invariably associated with a HVPG greater than 12 mmHg. However, the precise level of portal pressure above 12 mmHg does not correlate well with the likelihood of bleeding. An important implication of these observations is that if the HVPG is maintained below 12 mmHg, the risk of variceal hemorrhage can be minimized. Another corollary of these findings is that occurrence of varices may be prevented by decreasing the portal pressure below 12 mmHg before the development of varices. Clinical trials designed to test this hypothesis are currently under way.

The portal pressure determines the degree of portosystemic shunting and is an indirect marker of variceal flow and transmural pressure gradient. Traditionally, intravariceal measure-

Predictive factors for variceal bleeding
Child class
HVPG >12 mmHg
Intravariceal pressure
Size of varix
Location of varix
Endoscopic stigmata (various color signs)
Red wales
Hematocystic spots
Diffuse erythema
Bluish color
Cherry-red spots
White nipple
Alcohol consumption (in alcoholic cirrhosis)
Tense ascites
HVPG, hepatic venous pressure gradient.

**Table 9.3 Predictive factors for variceal bleeding**



ment necessitated puncture of the varices and, therefore, was not considered clinically useful. Recently, non-invasive measurement of variceal pressure has become technically feasible by attaching a pressure gauge to the distal end of an endoscope. The gauge is a small chamber covered by a latex membrane and perfused by nitrogen. When applied over a varix, the pressure needed to perfuse the gauge equals the pressure inside the varix. More than 70% of patients with an intravariceal pressure higher than 16 mmHg experienced hemorrhage within 1 year of measurement whereas only 9% of patients with pressure between 13 and 14 mmHg bled during the same period. If these data are corroborated by other clinical trials, it is likely that this technique will be incorporated in clinical practice.

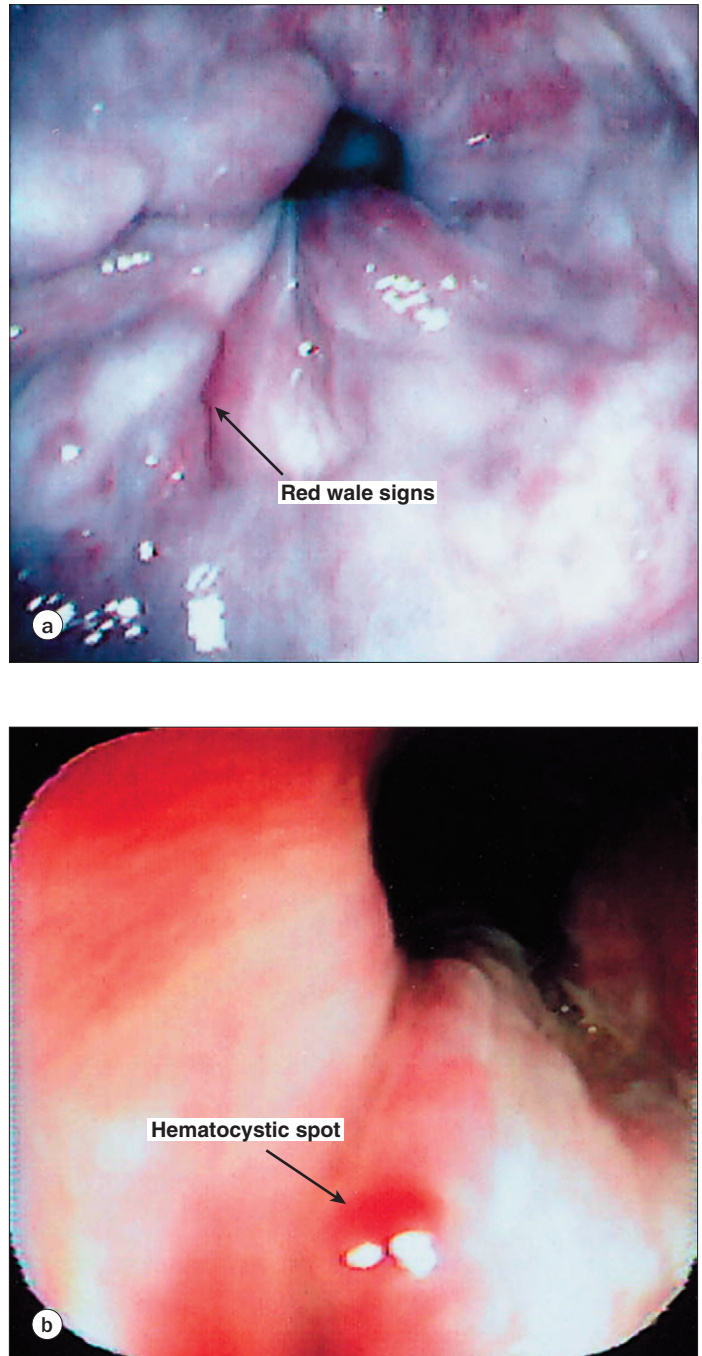
The likelihood of variceal rupture is also directly related to the varix diameter because the wall tension is directly related to its radius. Varices at the gastroesophageal junction and palisade zone are located more superficially within the wall of the esophagus. Consequently, they have a thinner coat and are most likely to bleed.

Several endoscopic features of esophageal varices have been shown to predict the risk of hemorrhage. Together, these are often referred to as 'red signs' due to their color (**Fig. 9.4**). These findings include *red wale marks* (longitudinal red streaks on varices that resemble red corduroy wales), *cherry red spots* (discrete red cherry-colored spots that are flat and overlie varices), *hematocystic spots* (raised discrete red spots overlying varices and resemble 'blood blisters') and *diffuse erythema* (denotes a diffuse red color of the varix).

Patients with more advanced liver failure are more likely to experience variceal hemorrhage. Thus, with increasing bilirubin and prothrombin time and decreasing albumin levels, the risk of bleeding increases significantly. Tense ascites is also an important risk factor for variceal hemorrhage.

All of the risk factors described above have been used to estimate the probability of variceal hemorrhage with relative precision. The North Italian Endoscopic Consortium (NIEC) has proposed an index by combining Child class, size of varices and the presence or absence of 'red markings'. This index stratifies cirrhotic patients with varices into groups according to their risk of bleeding. Thus, in a patient with Child class C cirrhosis and large varices with red signs, the risk of hemorrhage is more than 75% within 1 year. In contrast, another patient with Child class A cirrhosis and small varices has a less than 10% likelihood of bleeding. Of note, the risk of bleeding decreases over time following their identification, and most bleeding episodes occur within the first 3 years after identification of varices.

After the onset of active hemorrhage, bleeding stops spontaneously in approximately 50% of the individuals. Patients with large spurting varices and those with Child class C cirrhosis are most likely to experience exsanguinating hemorrhage without specific intervention. The period following cessation of active bleeding is usually subdivided into two periods, based on the risk of rebleeding. The risk of rebleeding is extremely high in the period immediately after hemostasis is achieved and decreases over the next 6 weeks to baseline values. The risk factors for such early rebleeding include large varices, age of greater than 60 years, renal failure, and severe initial bleeding as determined by a hemoglobin level of less than 8 g/dL at admission. Also, overly aggressive volume replacement may cause rebound portal hypertension and precipitate early rebleeding. The long-term



**Figure 9.4 Endoscopic stigmata of recent/imminent variceal hemorrhage.** (a) Red wale sign ; (b) hematocystic spots.

course after an index bleed is characterized by recurrent variceal hemorrhage, liver failure and death. The risk of recurrent bleeding is related to the degree of liver failure, continued alcoholism, variceal size, renal failure and the presence of hepatocellular carcinoma (HCC). Overall, approximately 70% of untreated individuals rebleed and die within 1 year of their index bleeding.

### **Gastric varices: natural history and prediction of hemorrhage**

The prevalence of gastric varices in patients with cirrhosis appears to be higher than in those presenting with bleeding

compared with those with no prior variceal bleeding. The likelihood of bleeding from gastric varices depends on their location. Although GOV1 constitute more than 70% of gastric varices, only 10% of GOV1 ever bleed. In contrast, about 80% of IGV1 experience hemorrhage even though they comprise only 8% of all gastric varices. Bleeding from IGV1 occurs at lower portal pressures than those seen in non-bleeding subjects with esophageal varices. Furthermore, bleeding from such varices is more severe and the risk of encephalopathy is higher than in patients with bleeding esophageal varices. Overall, gastric varices bleed less frequently but more severely than do esophageal varices. Recently, the risk of bleeding from IGV1 was shown to correlate with the size of varices (>10 mm), Child class and the presence of a red spot on the varices. Based on these findings, a prognostic index was calculated:

Prognostic index (PI) = 0.53 Child class + 0.78 varix size + 0.72 red spot

where Child class A = 0; B = 1; C = 2; the varix size scored as small (<5 mm) = 0, medium (5–10 mm) = 1, and large (>10 mm) = 2; and red spot scored as absent = 0 and present = 1. The risk of bleeding correlated directly with the prognostic index.

Esophageal variceal sclerotherapy causes spontaneous obliteration of 60% of GOV1. In those where GOV1 persists for more than 6 months after esophageal variceal obliteration, the risk of rebleeding is substantially greater than those where varices disappear (28 versus 2%). GOV2 are not affected by esophageal sclerotherapy. Contrary to some earlier misconceptions, only 10% of patients undergoing sclerotherapy for esophageal varices develop new gastric varices.

### Portal hypertensive gastropathy

Portal hypertensive gastropathy (PHG) is a frequent endoscopic finding in patients with portal hypertension. Endoscopically, this gastric mucosal lesion appears as a mosaic pattern with red markings as well as cherry-red, brown, and black spots. To date important elements that define the natural history of PHG such as incidence, progressive behavior, bleeding rates, mortality associated with bleeding as well as influence of sclerotherapy, have not been well documented. For example, the reported incidence of PHG ranges from less than 10 to almost 100% and bleeding rates from 4 to 40%; even in mild PHG the probability of bleeding ranges from 0 to 15%. Possible explanations for this variability in the literature include differences in: the patient populations studied, the time when lesions appeared, lack of uniform endoscopic criteria, a universally accepted classification system as well as prior endoscopic intervention for esophago-gastric varices. In recent years, some of these issues have been addressed and settled, particularly with the recently introduced classification of PHG. There are two types of portal gastropathy: the classical PHG involving the corpus and fundus and the gastric antral vascular ectasia (GAVE), which is limited to the antrum and pylorus. Furthermore, PHG can be transient (it may disappear), persistent (remains stable) or progressive (from mild to moderate to severe). In general, bleeding from PHG is relatively uncommon and rarely severe, usually manifested as melena and decreasing hemoglobin.

## THERAPEUTIC STRATEGIES

### Pharmaceutical agents

#### Non-selective $\beta$ blockers

Portal venous inflow is perhaps the most important determinant of portal pressure and it is mainly dependent on mesenteric arteriolar resistance.  $\alpha$ -adrenergic agonists constrict mesenteric arterioles and decrease portal blood flow, whereas  $\beta$ -adrenergic agonists cause splanchnic vasodilation and increase portal venous blood inflow. Conversely,  $\beta$ -adrenergic antagonists block the vasodilatory effects mediated via the  $\beta$  receptors, thus allowing unopposed  $\alpha$ -adrenergic effects resulting in mesenteric arteriolar constriction and a decrease in portal inflow. In addition,  $\beta$  blockers may also increase portocollateral resistance, thus decreasing collateral blood flow. Finally, portal pressure is further reduced by the  $\beta$  blockade reduction of the cardiac output. Currently,  $\beta$  blockers are the only pharmaceutical agents recommended for prophylaxis against the first variceal bleeding. Propranolol and nadolol are the two most studied  $\beta$ -adrenergic antagonists.

After either an oral or intravenous dose of propranolol, the hepatic venous pressure gradient decreases by 9–31%. This results primarily from a drop in portal pressures as measured by the wedged hepatic pressure with a smaller contribution from increased free hepatic venous pressure noted after  $\beta$ -blocker administration. At higher doses, a decrease in heart rate and cardiac output result in a reduction of portal pressures.

An important aspect of the pharmacodynamic effects of propranolol on portal pressures is the heterogeneity in response: up to 50% of patients show a less than 10% decrease in portal pressures following acute administration. In the long term, the portal hypotensive effects are maintained in most subjects. Tachyphylaxis does, however, occur in some patients and is clinically important, because only those with a sustained decrease in portal pressures may benefit from  $\beta$  blockade.

$\beta$ -blocker therapy is associated with a large number of side-effects and, in clinical trials of  $\beta$  blockers for portal hypertension, up to 20% of patients have had to discontinue treatment due to intolerable side-effects. The most clinically important side-effects involve bronchoconstriction, development of heart failure and erectile dysfunction. Many patients also complain of non-specific deterioration in mental function. Despite the increase in renin activity and decrease in renal flow, renal dysfunction only occurs occasionally after propranolol administration. It is, however, worthy of note that the administration of  $\beta$  blockers has not been shown to impair the hemodynamic response to acute blood loss. Thus,  $\beta$  blockers are contraindicated in chronic obstructive pulmonary disease (COPD), heart failure, atrioventricular block and peripheral arterial insufficiency. In contrast, sinus bradycardia and diabetes mellitus are currently only relative contraindications.

#### Nitrovasodilators (nitrates)

An important mediator of the vasodilated state seen in patients with cirrhosis is nitric oxide (NO). The 'nitrovasodilator' group of drugs activates NO pathways and produce vasodilation in their target vascular beds. At usual pharmacologic doses, these agents are primarily vasodilators and decrease postsinusoidal resistance and thus a decrease in portal pressure. The cardiac output falls in patients with a normal myocardial length-tension

relationship due to a decrease in venous return. With increased doses, arterial dilatation and a decreased cardiac output produce hypotension and reflex splanchnic vasoconstriction. Finally, nitrate-mediated baroreceptor reflexes in the pulmonary capillary bed also trigger splanchnic vasoconstriction and contribute to their portal hypotensive action.

Acute administration of either isosorbide mononitrate (ISMN) or dinitrate (ISDN) cause up to 40% decrease in HVP, 30% decrease in portal flow, and 15% decrease in azygous flow. The effects of ISMN are more predictable than those of ISDN and ISMN is the nitrate of choice when this class of drugs is used for treatment of portal hypertension. Tachyphylaxis does occur with repeated doses and the long-term effects on portal pressure are variable.

### **Vasopressin (pitressin) and its analogs**

Vasopressin causes a marked vasoconstriction of mesenteric arterioles and decreases portal venous inflow, thereby reducing portal pressures. In addition, it reduces portocollateral blood flow and variceal pressure. Vasopressin is administered intravenously, initially as a bolus injection of 0.4 U, followed by an infusion of 0.4–1.0 U/min. Unfortunately, the potent vasoconstricting effects of vasopressin are not limited to the splanchnic arterial bed but also include the systemic circulation. Therefore, the risk of serious complication such as cerebrovascular accidents, myocardial ischemia and infarction, arrhythmias, peripheral vascular ischemia and intestinal ischemia is considerable. Vasopressin has also been found to reduce the fibrinolytic state, and theoretically, can worsen bleeding diathesis. Moreover, recent studies have shown that the portal hypotensive effect of vasopressin in non-bleeding cirrhotics is attenuated when variceal bleeding occurs.

Glypressin (terlipressin) is a synthetic analog of vasopressin and is composed of three glycyl residues attached to the N-terminal of lysine-vasopressin. Cleavage of these residues releases the drug in a slow and sustained manner at the vascular smooth muscle. The result is immediate vasoconstriction followed by prolonged hemodynamic effects. Like vasopressin, it causes portal and portocollateral blood flow reduction and parallel decrease in portal and variceal pressures. Clinical studies have shown that terlipressin has less frequent side effects than vasopressin, even when vasopressin is combined with nitroglycerin (NTG). Furthermore, terlipressin, unlike vasopressin, does not activate fibrinolysis and has a longer biological activity that allows its administration even every 4–6 h. Terlipressin is not yet available in the USA.

### **Somatostatin and its analogs**

Somatostatin is a decapeptide (14 amino acids) named for its growth hormone-inhibiting properties. It has a very short half-life and disappears within minutes after a bolus injection. Octreotide is a synthetic octapeptide (eight amino acids), which by a shared four amino acid segment retains most of the effects of somatostatin but with a longer half-life. The affinity of octreotide for the somatostatin  $S_2$  receptors is three to tenfold higher than the natural hormone. Thus, octreotide mimics part of the effects of somatostatin.

## **Endoscopy**

### **Endoscopic injection sclerotherapy**

Endoscopic injection sclerotherapy (EIS) was the first endoscopic therapeutic modality to revolutionize our approach to bleeding varices. During the past two decades EIS has been extensively evaluated not only for the control of active variceal bleeding but also for the long-term prevention of recurrent variceal hemorrhage. Only esophageal varices and gastric varices in direct continuity with esophageal varices are treatable by EIS. The technique involves the injection of a sclerosant agent via the endoscope aiming at the obliteration of varices. Both intra- and paravariceal injections have been associated with good outcome. The outcome is also similar regardless of the type of sclerosant used, the volume injected, or frequency of sessions.

The injection of the sclerosant agent causes necrosis of esophageal tissues and mucosal ulcers. Small ulcers appear within the first few days in most patients and large ulcers develop in approximately 50%. Many patients have transient retrosternal discomfort after sclerotherapy. Patients who develop large ulcers are at risk of bleeding, delays in further sclerotherapy treatments, and the development of esophageal strictures, weeks to months later. Esophageal perforation is a rare complication. The incidence of symptomatic strictures is approximately 15%.

### **Endoscopic variceal band ligation**

Endoscopic variceal ligation (EVL) was developed in an effort to find an effective means of treating esophageal varices endoscopically with fewer complications than sclerotherapy. The concept was based on experience of treating hemorrhoids with rubber band ligation. EVL works by capturing all or part of a varix resulting in occlusion from thrombosis. The tissue then necroses and sloughs off in a few days to weeks, leaving a superficial mucosal ulceration, which rapidly heals. EVL avoids the use of sclerosant and thus eliminates the deep damage to the esophageal wall that occurs after EIS. Since its inception in 1986 advances in the technique have led to its routine use in the care of patients with esophageal varices. One of the biggest advances was the development of multiple band ligators (such as the Saeed Six Shooter and Speedband), which have simplified and improved the safety of EVL.

EVL devices are placed on the tip of standard endoscopes. The device has a soft sheath portion that fits over the tip of the endoscope and a hard plastic portion. There is also a separate hard plastic part over which the bands are stretched, ready to be deployed. The original (and still available) banding device (Stiegmann-Goff ligator) requires the use of an overtube because, after each band is deployed, the endoscope needs to be removed, the device reloaded, and put back on the endoscope before esophageal intubation and then advanced using the shaft of the endoscope as a guide, or passed separately after the initial intubation. The multiple band ligating systems have made use of the overtube less necessary since 4–10 bands can be deployed without having to remove the endoscope. This is helpful in patients who are receiving follow-up treatments for known varices. However, for actively bleeding patients, the overtube still offers advantages that need to be considered even when using the multiple ligating device. In particular, an overtube helps protect against aspiration.

The complications of EVL are substantially less than with EIS, presumably because of the shallower tissue injury



(cumulative complication rate of 11 versus 25%). In contrast to EIS, fever and mediastinitis do not occur with EVL. Post-procedure pain is uncommon. Esophageal dysfunction after EVL is less than EIS as determined by motility studies. Transient bacteremia has been documented after EVL, but this is not significant to warrant any special precautions and appears to be much lower with EVL than with EIS (3.3 versus 17% in one series). Pulmonary infections are uncommon and appear to occur with equal frequency with both EVL and EIS. Esophageal ulcers develop at each ligation site as the banded tissue necroses and sloughs off. These ulcers are limited to the mucosa as compared to sclerotherapy-related ulcers, which are often deeper and are thus prone to causing mediastinal inflammation and/or esophageal wall scarring. Significant bleeding from postligation ulcers is rare. If these ulcers do bleed, however, standard endoscopic methods for controlling ulcer bleeding are usually effective.

### Transjugular intrahepatic portosystemic shunting

Transjugular intrahepatic portosystemic shunting (TIPS) involves creating an artificial communication between the hepatic and portal veins angiographically, using a transjugular approach. The tract is maintained patent, initially by dilatation and subsequently by the deployment of an expandable metal stent. This connection via a stent allows the diversion (shunting) of blood flow from a high pressure vascular bed (portal) to a lower pressure vascular bed (hepatic veins/inferior vena cava), thus facilitating the return of blood to the heart and decompressing the portal hypertension. The ability of TIPS to produce sinusoidal and portal decompression without the risk of open surgery has led to a resurgence of interest in mechanical portal decompression as a treatment of portal hypertension and its complications. Thus, TIPS has been used both in the control of acute variceal bleeding, for the prevention of recurrent variceal bleeding and in the management of refractory ascites.

A transjugular intrahepatic portosystemic shunt can be successfully placed in more than 90% of patients. The duration of the procedure, which is done under conscious sedation, is approximately 1–2 h in most medical centers. In experienced hands, the procedure-related complication rate is less than 10%. However, complications associated with TIPS continue to expand as more of these procedures are performed every year. The Model for End-Stage Liver Disease (MELD) scoring system was originally based on estimating the risk of mortality among patients undergoing TIPS but has become much more widely accepted and is now used to prioritize patients for liver transplantation in the USA. The model uses serum bilirubin, creatinine and prothrombin to calculate a risk score and accurately identifies which patients are too ill to undergo TIPS. Maintenance of the patency of the TIPS has confronted hepatologists since its inception. Recurrent stenosis of the stent not only requires multiple reinterventions but also leads to recurrent variceal bleeding and poor survival. Recently, an expandable polytetrafluoroethylene (ePTFE)-covered endoprosthesis has become available for TIPS implantation. This ePTFE-covered stent graft minimizes transmural permeation of bile and mucin (which are common causes of patency loss) and reduces tissue ingrowth into the graft, resulting in much improved patency rates after correct placement. Initial data from clinical studies are very encouraging and it is anticipated

that with further improvement in stent design and materials, as well as insertion techniques, the patency problem will be effectively solved.

### Balloon tamponade

Balloon tamponade is an effective way to achieve short-term control of bleeding varices. Three main balloons have been used:

- the Sengstaken-Blakemore tube with a 250 cc gastric balloon, an esophageal balloon and a single gastric suction tube;
- the Minnesota tube, which is a modified Sengstaken-Blakemore tube with an esophageal suction port above the esophageal balloon; and
- the Linton-Nachlas tube, which has a single 600 cc gastric balloon.

It is apparent that the latter is effective only for gastric varices.

One of the main problems with these tubes is the high risk of rebleeding following deflation of the balloon. In addition, balloon tamponade is associated with significant complications, the most lethal of which is esophageal rupture. Major complications have been observed in approximately 15% of patients, occurring more frequently where tubes were inserted by relatively inexperienced staff. Thus, these tubes should only be used in settings in which experienced staff is available. The tubes should be used cautiously in patients with respiratory failure, cardiac arrhythmias or a hiatus hernia. The airway must be protected in all patients receiving such treatment due to their failure to clear oral secretions and high risk for aspiration. Because of the above considerations, the only current role of this treatment modality is to temporarily stabilize the patient so that a more definitive treatment can be instituted.

### Surgery

Two basic types of operations can be done for the control of variceal hemorrhage – shunt operations (decompressive surgery) and non-shunt surgery. Shunt operations can be categorized as either non-selective (those that decompress the entire portal tree and divert all flow away from the portal system, such as portacaval shunts) and selective (those that compartmentalize the portal tree into a decompressed vascular system while maintaining sinusoidal perfusion via a hypertensive superior mesenteric portal compartment, such as a distal splenorenal shunt).

Non-shunt operations generally include either esophageal transection (in which the distal esophagus is transected and then stapled back together) or devascularization of the gastro-esophageal junction. One of the most popular non-shunt operations is the Sugiura procedure. The surgery, which is achieved in two operative stages, within a 6–8 week interval between the stages, involves extensive esophagogastric devascularization, esophageal transection to interrupt submucosal varices, vagotomy and pyloroplasty, and splenectomy. Recently, a new surgical approach to fundal variceal bleeding was described. The surgery includes a fundectomy and periesophagogastric devascularization. This method offers an alternative operative method to cirrhotic patients with variceal hemorrhage from gastric fundic varices.

### Orthotopic liver transplantation

Although primarily a treatment for end-stage liver disease, liver transplantation can correct all complications associated with decompensated cirrhosis including portal hypertension and variceal bleeding. In practical terms, however, transplantation is

an option only for patients ready for transplantation at a recognized transplant center. Many patients with variceal hemorrhage, such as unreformed alcoholics, are not suitable candidates for liver transplantation. The use of orthotopic liver transplantation (OLT) is limited further by the paucity of available donor livers and the high procedure costs.

## THERAPEUTIC OPPORTUNITIES

### Primary prevention of variceal bleeding

According to the recent Veterans Affairs (VA) Hepatitis Resource Centers' recommendations, three major factors identify accurately patients at high risk of bleeding from varices: large variceal size, red wale markings on the varices, and severe liver failure, as defined by the Baveno III Consensus conference. Thus, patients with these features can be considered targets for strategies to prevent the first episode of bleeding.

### Pharmacologic measures

Pharmacologic therapy with non-selective  $\beta$  blockade remains the treatment of choice for primary prevention of variceal hemorrhage. A total of 10 placebo-controlled clinical trials of propranolol or nadolol have been reported and reviewed extensively. Comparisons across trials are confounded by the differences in patient populations studied as well as treatment regimens used. Despite the heterogeneity between trials, several general observations can be made. In the first year of follow-up (the period of greatest risk of bleeding), those treated with  $\beta$  blockers had a lower risk of variceal hemorrhage than did those treated with placebo (0–18% versus 12–30%) (Fig. 9.5). These effects were even more pronounced after 2 years of follow-up. Also, there was a decrease in variceal hemorrhage-related mortality. However, these beneficial effects translated into an overall survival advantage in only one study.

Meta-analyses of the data suggest that non-selective  $\beta$  blockade reduces the risks of initial variceal hemorrhage by 45% and bleeding-related deaths by 50%. The factors associated with failure of treatment included younger age, large variceal size, advanced liver failure and lower doses of propranolol. On the

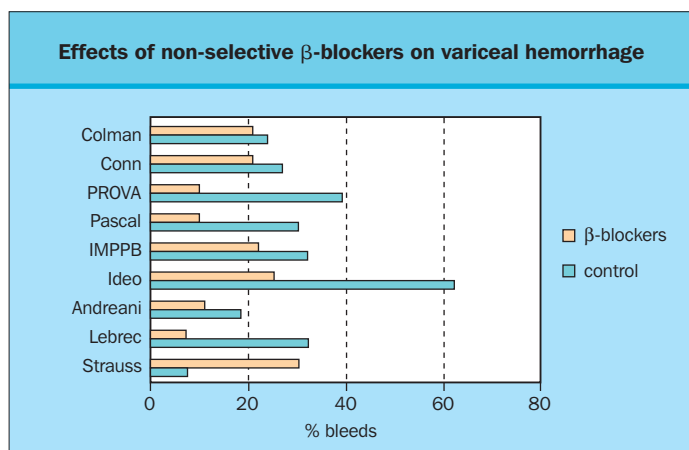
other hand, good compliance with treatment was an important predictor of a good outcome. It has also been shown that an important predictor of a favorable outcome is the degree of portal decompression achieved. Those patients who are able to sustain a drop in hepatic venous pressure gradient by 25% or to levels below 12 mmHg over time did not bleed and had a significantly prolonged survival. It has been suggested, therefore, that repeated measurements of HVPG be used to guide pharmacologic therapy of portal hypertension. However, the cost effectiveness of such an approach remains to be demonstrated and routine measurement of HVPG is not available at most community hospitals.

An important study prospectively randomized 118 patients to receive either ISMN (20 mg t.i.d.) or propranolol (to the maximum tolerated dose). Although no differences were noted during initial analysis 29 months after entry into the study, an increased mortality especially in those older than 51 years of age (72 versus 48%) was noted in those receiving ISMN after 7 years of follow-up (Fig. 9.6).

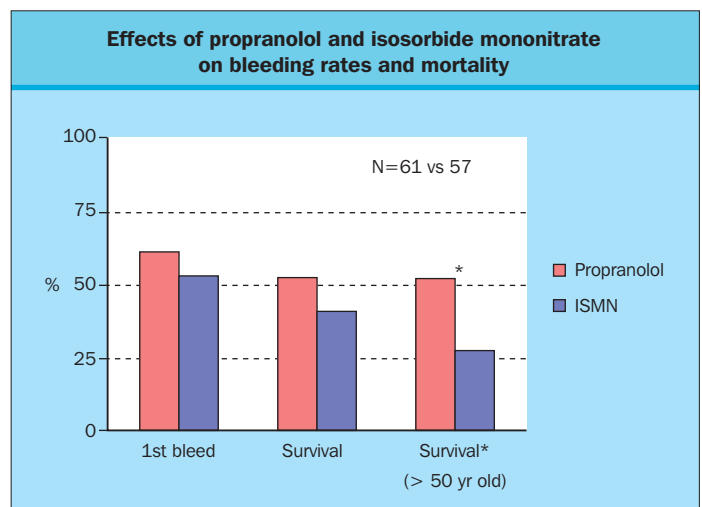
Potential explanations for the increased mortality in those receiving nitrates include worsening of the hyperdynamic circulatory state and a decrease in effective circulating volume, increased sodium retention and tissue hypoxia with increased lactate levels in cirrhotic individuals. At this time, the data do not support a role for nitrates as monotherapy for the primary prophylaxis of variceal hemorrhage. It has been shown that a combination of nitrates and  $\beta$  blockers is more effective than  $\beta$  blockers alone for the prevention of variceal hemorrhage (Fig. 9.7). However, this does not translate to a survival advantage.

### Endoscopic therapy

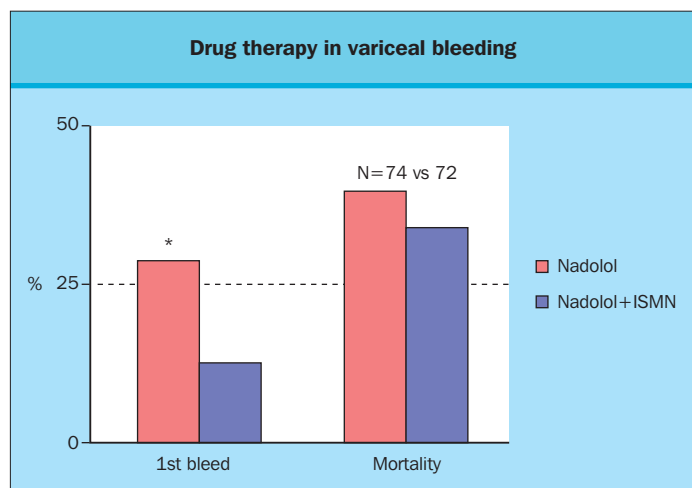
In recent years, various endoscopic therapeutic modalities have been proposed for the prevention of the initial variceal hemorrhage in patients with cirrhosis. The observation that EIS is an effective therapeutic modality for bleeding esophageal varices has led to a large number of randomized controlled trials



**Figure 9.5 Effects of non-selective  $\beta$ -blockers on variceal hemorrhage.** Effects on rates of variceal hemorrhage in clinical trials of  $\beta$  blockers for primary prophylaxis of variceal hemorrhage. Modified from Sanyal et al (97).



**Figure 9.6 Effects of propranolol and isosorbide mononitrate on bleeding rates and mortality.** Used for primary prophylaxis of variceal hemorrhage. Modified from Angelico et al (97).



**Figure 9.7 Drug therapy in variceal bleeding.**  $\beta$  blockers versus a combination of  $\beta$  blocker plus nitrates for the primary prevention of (first) variceal bleeding.

evaluating its role in primary prophylaxis. Unfortunately, the efficacy of prophylactic sclerotherapy remains unclear because most of the published studies have produced conflicting results. These clinical trials are characterized by significant heterogeneity in the patient population, the use of sclerotherapy for acute bleeding, and particularly, the baseline bleeding risk ranging from 0 to 75%. Although some earlier studies found sclerotherapy beneficial for both preventing first bleeding and reducing mortality, more recent studies have failed to confirm these observations. In fact, a VA cooperative multicenter study, one of the largest trials ( $n = 81$ ) of prophylactic sclerotherapy, was terminated prematurely due to the higher mortality in the sclerotherapy arm than the sham therapy arm (32 versus 17.4%). Thus, EIS either alone or in combination with  $\beta$  blockers is not recommended because of its high complication rates and the availability of other less invasive and more cost-effective therapeutic modalities.

In recent years, the observation that EVL obliterates and eradicates esophageal varices as effectively as EIS but with fewer complications, has led to an explosion of clinical trials comparing EVL in the primary prophylaxis of variceal bleeding.

A recent meta-analysis of five randomized controlled trials that included more than 600 patients concluded that compared with the no treatment arm, prophylactic EVL significantly reduced the risk of first variceal bleeding, bleeding-related mortality, and all-cause mortality. More interestingly, the reduction in the risk of the initial bleeding remained when the analysis included only the four trials in which EVL was compared to  $\beta$  blockade. In contrast, bleeding-related mortality as well as all-cause mortality did not differ between EVL and the  $\beta$ -blocker arms. The results clearly show that in patients with cirrhosis, EVL is as effective and as safe as non-selective  $\beta$  blockade in the prevention of the first variceal hemorrhage.

For the time being, the selection of EVL over  $\beta$  blockers in primary prophylaxis should be very judicious, taking into account the cost, inconvenience and risks associated with variceal band ligation. Furthermore, EVL may not be a permanent solution and varices may recur after initial eradication.

Several studies have shown a significant rate of variceal recurrence, following the initial EVL, thus necessitating continued patient surveillance.

### Other measures

Currently, neither TIPS nor surgery have a role for primary prevention of variceal bleeding.

### Treatment of active bleeding

Acute variceal hemorrhage is the most ominous complication of cirrhosis with portal hypertension and accounts for about one-third of all deaths related to cirrhosis. The outcome of an episode of active hemorrhage depends upon the control of active bleeding and avoidance of major complications associated with bleeding and its treatment. Establishing the correct diagnosis is also important. Other causes of upper gastrointestinal bleeding in patients with liver disease include peptic ulcer disease, Mallory-Weiss tear, PHG and GAVE, esophagitis and gastroduodenitis. Moreover, any of these lesions is more likely to bleed in the presence of portal hypertension and may indeed bleed briskly. The frequency with which varices account for upper gastrointestinal (UGI) bleeding in patients with cirrhosis is uncertain. Varices appeared to be the source of bleeding in 50–90% of cirrhotic patients with UGI bleeding in various reports, although a variety of other causes may also be responsible. In a recent multicenter prospective study among 465 cirrhotics with acute UGI bleeding, 72% bled from varices.

There are two distinct phases in the course of variceal hemorrhage: an acute phase; and a later phase in which there is a high risk of recurrent bleeding. The acute phase starts with the onset of active hemorrhage. Only 50% of patients with variceal hemorrhage stop bleeding spontaneously; this is quite different from the more than 90% spontaneous cessation rate in patients with other forms of UGI hemorrhage. Patients with Child class C cirrhosis and large, actively spurting varices are less likely to achieve spontaneous hemostasis.

Following cessation of active hemorrhage, there is a period of approximately 6 weeks in which there is a high risk of recurrent hemorrhage. The greatest risk is within the first 48–72 h, and over 50% of all early rebleeding episodes occur within the first 10 days. Risk factors for early rebleeding include age greater than 60 years, renal failure, large varices, and severe initial bleeding as defined by hemoglobin below 8 g/dL at admission. Survival during this period mirrors the risk of rebleeding and is directly related to the outcome of bleeding.

The risk of bleeding and of death in patients who survive 6 weeks is similar to that in cirrhotic patients of equivalent severity who have never bled. Approximately 52% of those who survive 2 weeks after a variceal bleed reach 1-year survival. Survival appears to have increased slightly in the past two decades largely due to a decrease in short-term mortality.

### General principles of management

Acute variceal hemorrhage can be torrential, constituting a medical emergency that may culminate in death if bleeding is not controlled promptly. Hence, adequate resuscitation remains the single most important step in the management of these patients, before diagnostic or therapeutic interventions (Table 9.4).



**General medical management of variceal hemorrhage**

## Volume resuscitation:

- keep hemoglobin 9–10 g/dL
- maintain urine output of at least 50 cc/h

## Correct hematologic status:

- packed red cells to replace blood loss
- platelet transfusions if platelet counts  $\leq 50\,000/\text{mm}^3$
- fresh frozen plasma for severe coagulopathy (consider recombinant factor VII)

## Sepsis:

- blood cultures and diagnostic paracentesis of ascites
- prophylactic intravenous antibiotics (switch to oral when feasible)

## Electrolytes:

- monitor for hypercalcemia, hypomagnesemia and hypophosphatemia (in alcoholics)
- monitor for hypocalcemia if large amounts of EDTA-containing blood are used

## Management of encephalopathy and mental status changes:

- thiamine for alcoholic patients
- monitor for and manage alcohol withdrawal
- clear the gastrointestinal tract of blood and control bleeding

## Maintain cardiopulmonary functions:

- protect airway (particularly with severe bleeding or mental status changes)
- optimize volume status

## Renal function:

- monitor urine output and serum creatinine levels
- aseptic technique for urinary bladder catheterization

EDTA, ethylenediaminetetraacetic acid

**Table 9.4 General medical management of variceal hemorrhage****Pharmacologic measures**

Drug therapy has inherent advantages because it is readily available and its administration does not require specially trained personnel. Pharmacologic treatment is usually employed to stop or decrease variceal bleeding, pending further endoscopic treatment, although drug monotherapy has been reported to be effective in active variceal bleeding. The rationale for pharmacologic therapy is to lower the increased portal pressure and thereby the intravariceal pressure. This is achieved by either decreasing portal venous blood flow or by decreasing intrahepatic or portosystemic resistance.

Vasopressin has been used in the management of acute variceal hemorrhage for the last three decades. Pooled data from a number of studies have shown significant beneficial effect of vasopressin in reducing failure to control bleeding. Vasopressin can achieve initial hemostasis in 60–80% of patients, but has only marginal effects on early rebleeding episodes and does not improve survival from active variceal hemorrhage. The benefit of bleeding cessation may be counterbalanced by enhanced mortality due to extrasplanchnic vasoconstrictive properties and resultant myocardial, cerebral and bowel ischemia.

The systemic vasodilator NTG has been concurrently administered in an attempt to avoid the adverse effects related to vasopressin-induced systemic vasoconstriction. It is recommended that, when used, intravenous vasopressin should be combined with intravenous NTG. Several clinical trials have shown that glypressin (terlipressin) is at least as effective as somatostatin and more effective than placebo.

A number of clinical trials have compared somatostatin or octreotide to either vasopressin or placebo in the management of active bleeding. Most of these studies have demonstrated that somatostatin is more effective for controlling bleeding than placebo or vasopressin and has fewer side-effects than vasopressin. Although the role of octreotide is less well established, it is the current drug of choice in the USA because of its easy availability compared to somatostatin. Interestingly, despite their widespread use, neither drug has a clearly established benefit on mortality. The current view of most hepatologists is that octreotide should only be used as an adjunctive therapy to endoscopic variceal sclerotherapy or band ligation in the control of acute variceal hemorrhage.

Endoscopic treatment is currently the intervention of choice for acute variceal bleeding. Furthermore, endoscopy can provide valuable diagnostic information. Several trials have shown that EIS is highly effective in the control of acute bleeding and prevention of early rebleeding in more than 85–90% of patients. Both esophageal as well as GOV1 and GOV2 are amenable to treatment by sclerotherapy. However, gastric varices do not lend themselves to this modality of treatment. Clinical trials have shown sclerotherapy to be superior to balloon tamponade alone, vasopressin alone, and a combination of vasopressin and balloon tamponade for control of active hemorrhage, prevention of early rebleeding, reduction of complication rates, and improved survival.

During the past decade, EVL has emerged as an alternative endoscopic modality to sclerotherapy because of the relatively high rate of complications associated with EIS. A number of controlled trials have shown that EVL controls bleeding in approximately 85–100% of patients.

Emergency endoscopic therapy fails to control bleeding in 10–20% of patients. In case of failure to control bleeding or early rebleeding, a prompt decision for rescue therapy should be made (i.e. no more than two sessions of endoscopic therapy) as these patients are at high risk of both exsanguination and other complications related to active bleeding. Most patients will have already received a trial of pharmacologic treatment by the time a diagnosis of failed endoscopic treatment is established. In such patients, a second attempt at endoscopic hemostasis may be made (e.g. band ligation for failed sclerotherapy). However, if bleeding is not quickly and effectively stopped, more definitive therapy must be instituted immediately with balloon tamponade or some type of portosystemic shunt. Once the patient is stabilized by whatever means, a more definitive therapy such as TIPS or surgery should be instituted immediately. Factors affecting risk of continued bleeding or recurrent bleeding are shown in Table 9.5.

**Transjugular intrahepatic portosystemic shunt and emergency surgery**

In the early clinical TIPS studies, hemostasis was achieved in the vast majority of patients with refractory variceal hemorrhage despite endoscopic treatment. TIPS could be performed in a reasonable period of time in most patients with low procedure-related morbidity or mortality. However, interpretation of the efficacy of TIPS in the setting of active hemorrhage was confounded by the heterogeneous patient populations and failure to define the nature of active bleeding.

<b>Factors affecting risk of continued bleeding or recurrent bleeding</b>
<b>Factors associated with failure to control acute variceal hemorrhage</b> <ul style="list-style-type: none"> <li>– Spurting varices</li> <li>– Child-Pugh score</li> <li>– Hepatic venous pressure gradient</li> <li>– Infection</li> <li>– Portal vein thrombosis</li> </ul>
<b>Factors associated with early rebleeding</b> <ul style="list-style-type: none"> <li>– Severity of initial bleeding</li> <li>– Overly aggressive volume resuscitation</li> <li>– Infection</li> <li>– Hepatic venous pressure gradient</li> <li>– Complications of endoscopic treatment</li> <li>– Renal failure</li> </ul>
<b>Factors associated with late rebleeding</b> <ul style="list-style-type: none"> <li>– Child-Pugh score</li> <li>– Variceal size</li> <li>– Continued alcohol use</li> <li>– Hepatocellular carcinoma</li> </ul>

**Table 9.5 Factors affecting risk of continued bleeding or recurrent bleeding**

The data are difficult to combine due to heterogeneity of patients studied, different types of operations used, and varying protocols used for supportive management. However, the following general conclusions can be made:

- Both portal decompressive surgery and esophageal transection are highly effective in achieving hemostasis.
- Emergency portacaval shunts are more likely to thrombose than elective shunts. In addition, portacaval shunts alter vascular anatomy, complicate future liver transplant surgery and are associated with an approximately 40–50% incidence of encephalopathy.

Large-scale, randomized prospective trials are needed to establish the roles of surgery and TIPS in average risk subjects with active hemorrhage who have failed endoscopic and medical treatment. At present, TIPS is the procedure of choice in such patients who are poor risks for surgery; the relative roles of TIPS and surgery in those who are good candidates are uncertain. The ideal patient for surgical therapy is one with well-preserved liver function who fails emergent endoscopic treatment and has no complications from the bleeding or endoscopy. The choice of surgery is usually dependent on the training and expertise of the surgeon.

### **Acute bleeding from gastric varices**

Bleeding gastric varices can be technically difficult to treat. They frequently rebleed despite initially successful endoscopic therapy; as a result, endoscopic treatment should not be used outside of randomized studies. Successful hemostasis and obliteration of gastric varices has been reported with intra-variceal injection of sclerosants, absolute alcohol, thrombin and cyanoacrylate. Cyanoacrylate injection appears to be more effective and safer than band ligation or alcohol injection.

At present, patients with bleeding intragastric varices should be treated with octreotide (or somatostatin) and balloon

tamponade followed by either TIPS or surgery. Emergency TIPS appears to be as effective for short-term control of bleeding gastric varices as esophageal varices. However, TIPS may be less effective than surgery in patients with bleeding gastric varices who have spontaneous splenorenal collaterals.

### **Prevention and treatment of recurrent variceal bleeding**

The probability of recurrent variceal bleeding, following control of the acute hemorrhage, is approximately 30% at 6 weeks and ultimately close to 75%. The corresponding mortality rate is about 33%. The causes of death include recurrent variceal bleeding, liver failure, hepatic encephalopathy and progressive ascites and infections. These points underscore the importance of preventing recurrent hemorrhage, sustaining liver function, maintaining an ascites-free state, and avoiding infections to achieve prolonged survival. At this time, OLT is the only treatment which achieves all of these objectives and prolongs long-term survival with any degree of certainty.

Currently, there are two main areas of intervention for the prevention of recurrent variceal bleeding:

- at the 'local' level, i.e. the obliteration of the target organ (varices), which can be achieved either by EIS or EVL, and rarely by surgical devascularization procedures; and
- at the 'central' level, i.e. by lowering the portal pressure below the threshold (12 mmHg) that is known to be associated with variceal bleeding.

The latter can be accomplished either pharmacologically ( $\beta$  blockers) or by mechanical intervention with creation of a portosystemic shunt radiologically (TIPS) or surgically.

### **Pharmacological therapy**

Studies on the pharmacological prevention of recurrent variceal hemorrhage have focused on the use of  $\beta$  blockers and, to a lesser extent, nitrates. Several clinical trials have compared  $\beta$  blockers and placebo for the prevention of recurrent variceal hemorrhage. With a few exceptions, an overall improvement in rebleeding rate was noted, but a survival advantage was shown in only one study. Meta-analysis of these data suggests that the risk of bleeding is decreased by approximately 40% while the risk of death is decreased by 20%. Propranolol has also decreased recurrent bleeding (35 versus 62% at 1 year) from portal hypertensive gastropathy.

The presence of HCC, poor compliance and continued alcohol use are risk factors for recurrence of variceal hemorrhage in patients treated with propranolol. Although acute hemodynamic changes after initiation of propranolol do not predict long-term outcome, the presence of a sustained portal hypotensive effect at 1 month after initiation of therapy appears to be an important predictive factor. Thus, measurement of portal hemodynamics may be an important prognostic element of treatment. However, facilities for routine measurement of portal pressures are not widely available and the cost-effectiveness of these measurements for guidance of medical therapy remains to be established. As a general rule, the optimal candidate for management with  $\beta$  blockers is one with well-compensated alcoholic liver disease who is likely to be compliant with therapy.

Several studies have suggested that the combination of  $\beta$  blockers and nitrates improves the hemodynamic response

and may markedly lower the rate of rebleeding, possibly due to a decrease in outflow resistance.

### Endoscopic treatment

Endoscopic sclerotherapy has been extensively evaluated for the long-term prevention of recurrent variceal hemorrhage. Compared to placebo, it decreases both the risk of rebleeding and death. However, it remains controversial whether these effects represent the benefits of repeated episodes of sclerotherapy or the beneficial effects of sclerotherapy for episodes of active hemorrhage. Only esophageal varices and gastric varices in direct continuity with esophageal varices are treatable by endoscopic sclerotherapy.

EVL was developed as an alternate endoscopic treatment over the past 15 years. The technical aspects and complications of EVL have been described above. A number of studies have shown that the relative efficacy of EVL for the management of recurrent variceal hemorrhage is superior to EIS in reducing the rebleeding rate, the overall mortality, the death rate due to rebleeding, and the development of esophageal strictures. Variceal obliteration is achieved in similar proportions of patients with both techniques but the number of treatments to achieve obliteration is lower with ligation.

The Practice Parameters Committee of the American College of Gastroenterology and the VA Hepatitis Group, recommend EVL not only as the endoscopic treatment of choice for the long-term management of variceal hemorrhage but also to eliminate completely EIS as an alternate endoscopic therapy.

### TIPS in the prevention of recurrent variceal bleeding

Despite the use of  $\beta$ -adrenergic blockade and endoscopic therapy to prevent rebleeding, rates remain as high as 30–50%. A number of clinical trials have evaluated the efficacy of TIPS versus endoscopic therapy for the prevention of recurrent variceal hemorrhage. Most studies demonstrated a significant decrease in the risk of rebleeding in patients undergoing TIPS. Unfortunately, despite reduction of rebleeding rates, TIPS is often associated with worsening liver function, hepatic encephalopathy and long-term shunt dysfunction (Table 9.6). Moreover, TIPS improves neither overall mortality nor bleeding-related mortality rates when compared to endoscopic therapy.

Thus, the superiority of TIPS for prevention of rebleeding is offset by its failure to improve survival, increasing morbidity due to the development of liver failure and encephalopathy, the frequent occurrence of TIPS stenosis, and possibly increased cost. For these reasons, TIPS is used primarily as a salvage therapy for those with recurrent bleeding despite an adequate trial of endoscopic and pharmacologic treatment. It should be emphasized that rebleeding after TIPS may still occur and is mainly due to the recurrence of portal hypertension. Such recurrence may result from the thrombosis, retraction or kinking of the stent or from concurrent right heart failure (Table 9.7).

### Surgery

Surgical options for recurrent variceal hemorrhage at present are principally used in patients with continued bleeding despite endoscopic and/or pharmacotherapy. Despite its demonstrated efficacy in decreasing recurrent variceal hemorrhage, enthusiasm for surgery has declined over the last two decades due in part to

Transjugular intrahepatic portosystemic shunt (TIPS): contraindications, side-effects and complications	
Side-effects and complications	Contraindications
<b>Technical</b> Continued variceal bleeding Persistent gastric varices Stent displacement Kinking Retraction	<b>Absolute</b> Severe congestive heart failure Polycystic liver disease
<b>Stent-related</b> Stenosis Thrombosis Hemobilia Hemolysis Infection	<b>Relative</b> Active intra- or extrahepatic infection Poorly-controlled encephalopathy Hypervascular liver tumors Portal vein thrombosis Severe hepatic failure
<b>Porto-systemic shunting</b> Hepatic decompensation Encephalopathy	

**Table 9.6 Transjugular intrahepatic portosystemic shunt (TIPS): contraindications, side-effects and complications.**

Causes of gastrointestinal bleeding after transjugular intrahepatic portosystemic shunt (TIPS)
Continued variceal bleeding
<b>Stent malfunction</b> Early (usually <6 weeks) Thrombosis (usually occurs early) Kinking Retraction Displacement Late (usually >6 weeks) Stent stenosis
<b>Hemobilia</b>
<b>Persistent gastric varices associated with spontaneous splenorenal collaterals</b>

**Table 9.7 Causes of gastrointestinal bleeding after transjugular intrahepatic portosystemic shunt (TIPS)**

the more easily used techniques of endoscopic therapy. However, despite these considerations, surgery remains a valuable form of treatment for the prevention of recurrent variceal hemorrhage, especially in those who experience recurrent bleeding despite adequate endoscopic treatment and who are not candidates for OLT.

While the choice of surgery is usually dictated by the surgeon's training and expertise, non-shunt operations are typically considered in patients with unmanageable bleeding due to extensive portal, superior mesenteric and splenic venous thrombosis in whom a shunt operation is not technically feasible while shunt surgery (both non-selective and selective) has similar long-term rates of rebleeding and survival. Survival is most closely linked



to Child class immediately prior to surgery. Distal splenorenal shunts (DSRS) are associated with a number of advantages: they do make subsequent OLT less complicated in contrast to portacaval shunts, the risk of hepatic encephalopathy may be somewhat less, and liver function is relatively preserved due primarily to maintained portal perfusion.

### Orthotopic liver transplantation

Liver transplantation is the only treatment which corrects both the portal hypertension and liver failure. It can be performed even in those with active hemorrhage. The presence of a previous portacaval shunt does not affect survival after OLT although it lengthens the time taken for surgery. A previously placed transjugular intrahepatic portosystemic shunt can be easily removed with the explant at the time of transplant in most instances; in occasional patients, however, the stent extends into the right atrium or superior mesenteric veins, rendering the surgery much more difficult. Patient survival ranges from 80 to 90% at 1 year to 60% at 5 years after OLT. The value of transplantation as a sole treatment for prevention of recurrent hemorrhage is limited by the frequently long waiting period before an organ is available; during this time, all of the factors contributing to mortality remain operative.

## OVERALL SUMMARY AND RECOMMENDATIONS

### For primary prevention of variceal bleeding

All patients with cirrhosis should undergo an initial diagnostic endoscopy to document the presence of varices and to determine the risk of variceal bleeding. The high-risk group includes all patients with medium- and large-sized varices (F2 or F3) with or without red wale signs. In patients with small varices, the risk of bleeding is so small that prophylactic therapy would not be cost-effective. Instead, these patients should undergo repeat screening endoscopy, 1 to 2 years after the index procedure, because of the risk of varix development and enlargement over time. In those patients, in whom varices are not present at the initial esophagogastroduodenoscopy, endoscopic follow-up should be performed at 2–3-year intervals. Non-selective  $\beta$  blockade with propranolol or nadolol is the recommended primary prophylaxis for the prevention of initial variceal hemorrhage. Drugs such as clonidine and nitrates can be considered but are not ‘standard of care’.

During the past decade, EVL has emerged as a sound and promising therapeutic modality for primary prophylaxis of variceal hemorrhage and represents an alternative for those patients at risk of bleeding in whom  $\beta$  blockade is contraindicated or is associated with intolerable side-effects.

### For treatment of active variceal bleeding (Fig. 9.8)

Initial therapy of variceal bleeding is aimed at hemodynamic resuscitation, prevention and treatment of complications, and supportive measures. Somatostatin or octreotide may be started in the emergency room in those patients in whom a high index of suspicion for variceal hemorrhage exists. In those with significant hemorrhage or when a variceal source is suspected, early endoscopy is essential to establish the diagnosis and to initiate specific treatment. Bleeding esophageal varices are treated by either sclerotherapy or band ligation. If active bleeding persists

or recurs within 48 h, bleeding may be controlled by surgery or TIPS. The choice of the procedure depends upon the clinical condition of the patient and the expertise available. When bleeding occurs more than 48 h after initial endoscopic treatment, a second attempt at controlling hemorrhage by endoscopy is reasonable before proceeding to salvage therapy.

### For secondary prevention of variceal bleeding (Fig. 9.9)

Candidates for secondary prophylaxis are all survivors of a variceal bleed, have had no evidence of hemorrhage for at least 24 h, and in whom pharmacological therapy for control of acute variceal hemorrhage has been discontinued. The optimal approach to the prevention of recurrent variceal hemorrhage in patients with cirrhosis is uncertain. One approach is illustrated in Figure 9.5. The goal of long-term treatment is prevention of:

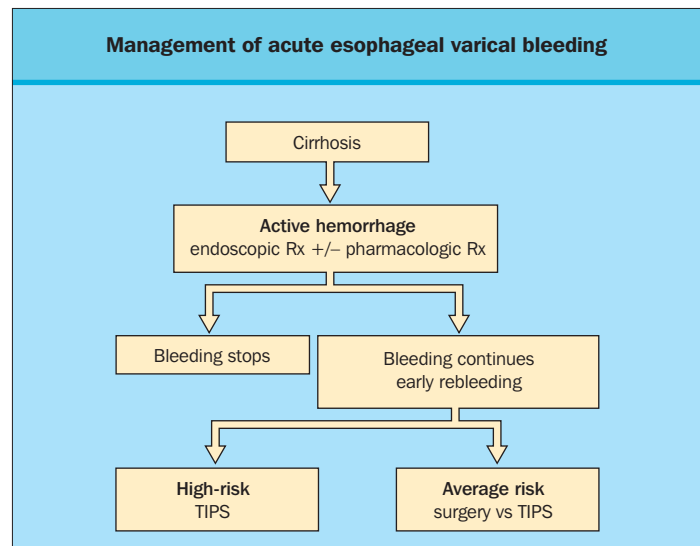


Figure 9.8 Management of acute esophageal variceal bleeding.

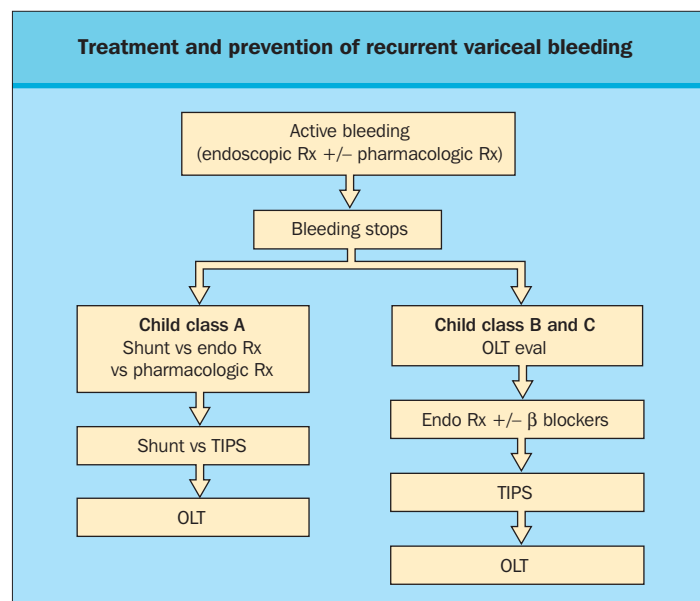


Figure 9.9 Treatment and prevention of recurrent variceal bleeding.

rebleeding, hepatic decompensation and death. OLT is the only treatment that reliably achieves all three objectives. Thus, all survivors of a variceal bleed should be evaluated for liver transplantation. Those with Child class B or C should be listed for transplantation unless a contraindication exists; listing for those with Child class A cirrhosis should be individualized.

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While waiting for transplantation, endoscopic band ligation is the initial treatment of choice in most individuals. Some patients with Child A cirrhosis who are compliant with therapy may be treated with propranolol or nadolol, while others may benefit from surgical portal decompression.

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## SECTION III: CLINICAL MANIFESTATIONS OF LIVER DISEASE

### CHAPTER

# 10

## Ascites, Hyponatremia, Hepatorenal Syndrome and Spontaneous Bacterial Peritonitis

Andrés Cárdenas, Pere Ginès and Vicente Arroyo

The natural course of patients with cirrhosis is frequently complicated by the accumulation of fluid in the peritoneal or pleural cavities and interstitial tissue. This is caused by an abnormal regulation of extracellular fluid volume, which is commonly associated with alterations in renal function (especially sodium retention) and, less commonly, solute-free water retention and renal vasoconstriction. These are responsible for fluid accumulation in the form of ascites, dilutional hyponatremia and hepatorenal syndrome respectively. Ascites is the most common complication of cirrhosis and poses an increased risk for infections, renal failure and mortality. Patients with cirrhosis and ascites have a poor prognosis and it is estimated that nearly 50% of these patients will die within approximately 2 years without liver transplantation. Dilutional hyponatremia and hepatorenal syndrome occur later and also carry a poor prognosis. Other than these abnormalities in renal function, cirrhotic patients with ascites may develop spontaneous bacterial peritonitis, an infection of ascitic fluid without any intra-abdominal source of infection.

### ASCITES

#### Definition

Ascites is defined as the presence of fluid in the peritoneal cavity. The most common cause of ascites is portal hypertension secondary to cirrhosis, which accounts for over 80% of patients with ascites. Although the presence of ascites is highly suggestive of cirrhosis, a definitive diagnosis should always be established by clinical or exploratory findings or, if necessary, by liver biopsy.

#### Pathogenesis of ascites

The formation of ascites in cirrhosis is the consequence of a combination of abnormalities in renal function, causing sodium retention with subsequent expansion of extracellular fluid volume, and alterations in portal and splanchnic circulation, which facilitate the accumulation of fluid in the peritoneal cavity by increasing lymph formation. Sodium retention is the earliest alteration of renal function observed in patients with cirrhosis. As sodium is retained iso-osmotically in the kidney (i.e. 135 mEq of sodium for 1 l of water retained), it causes fluid accumulation and expansion of the extracellular fluid volume, which results in ascites or edema formation. Sodium retention in cirrhosis is caused mainly by abnormally increased tubular reabsorption of sodium that occurs both in the proximal and distal tubules. The main effectors are hyperaldosteronism and enhanced renal sympathetic nerve activity, although other intra-renal and extrarenal sodium-retaining mechanisms may be involved

#### Factors involved in sodium retention in cirrhosis

Hyperaldosteronism
Increased renal sympathetic nerve activity
Reduced renal perfusion
Renal resistance to natriuretic peptides
Increased tubular sensitivity to aldosterone

**Table 10.1** Factors involved in sodium retention in cirrhosis.

(Table 10.1). Although many patients with ascites have an impaired ability to excrete solute-free water, this disorder is not required for the development of ascites. The only renal excretory function abnormality needed for the development of ascites is sodium retention.

Portal hypertension is another key pathogenic factor in the development of ascites in cirrhosis. The increased intrahepatic vascular resistance causes not only a rise in hydrostatic pressure in the portal venous system but also a marked vasodilatation of the splanchnic arteries, which results in an increased portal venous inflow. Both abnormalities cause marked changes in the splanchnic microcirculation that result in an enhanced splanchnic lymph production. In the splanchnic arterial bed, development of vasodilation contributes to portal hypertension by increasing flow and inducing changes in the microcirculation that predispose to increased filtration of plasma. Several vasodilator factors, in particular nitric oxide, are responsible for this vasodilatory effect. Splanchnic vasodilation by decreasing effective arterial blood volume leads to stimulation of central baroreceptors thereby triggering the activation of vasoconstrictor systems. Aside from changes in splanchnic hemodynamics, patients with ascites develop a hyperdynamic state characterized by reduced systemic vascular resistance and arterial pressure, increased cardiac output, and activation of vasoconstrictor systems (Table 10.2). These hemodynamic changes play an important role in sodium retention by causing a baroreceptor-dependent activation of vasoconstrictor and antinatriuretic systems, mainly the renin-angiotensin-aldosterone system and the sympathetic nervous system leading to the development of sodium and water retention, as well as renal vasoconstriction.

The theory that best explains the pathogenesis of sodium retention and ascites formation in cirrhosis is the *Arterial Vasodilatation Theory* (Fig. 10.1). According to this hypothesis,



sodium retention is the consequence of a homeostatic response raised by an underfilling of the arterial circulation secondary to arterial vasodilatation in the splanchnic vascular bed. The underfilling of the arterial circulation is sensed by arterial and cardiopulmonary receptors and results in hypervolemia by activating antinatriuretic factors. The retained fluid compensates initially the disturbance in the arterial circulation and suppresses the activation of sodium-retaining mechanisms. However, as the vasodilatation in the splanchnic circulation causes more marked arterial underfilling, the retained fluid does not adequately fill the intravascular compartment, mainly because fluid is leaking

continuously into the peritoneal cavity and the sodium-retaining mechanisms become permanently activated. This theory also provides an explanation for the pathogenesis of dilutional hyponatremia and hepatorenal syndrome (see later).

Clinical features

The main clinical symptom of patients with ascites and edema is an increase in abdominal girth often accompanied by lower extremity edema. In most cases ascites develops insidiously over the course of several weeks. Patients must have approximately 1500 mL of fluid for ascites to be detected reliably by physical examination. In patients with a large amount of ascites, respiratory function and physical activity may be impaired. Dyspnea in these patients may occur as a consequence of increasing abdominal distention and/or accompanying pleural effusions. Other common manifestations of patients with ascites may include anorexia, malaise, weakness, malnutrition and decreased muscle mass. Increased intra-abdominal pressure may also favor the development of abdominal hernias in patients with cirrhosis and long-standing ascites.

Patients are classified according to the degree of ascites. Patients with grade 1 ascites are those in whom ascites is detected only by ultrasonography. Patients with grade 2 ascites (moderate ascites) are those in which ascites causes moderate symmetrical distention of the abdomen. Patients with grade 3 ascites (large ascites) have increased amounts or tense ascites causing marked abdominal distention (Fig. 10.2). Patients with refractory ascites are those that do not respond to high doses of diuretics or develop side-effects that preclude their use (see later).

Pleural effusion is common in patients with ascites. When the effusion is large (i.e. greater than 500 mL) it is defined as hepatic hydrothorax. This complication of cirrhosis is relatively uncommon with an estimated prevalence among cirrhotic patients

Hemodynamic abnormalities in splanchnic and systemic circulation in patients with cirrhosis and ascites	
Splanchnic circulation	Systemic circulation
Increased pressure in the portal venous system	Increased cardiac output
Vasodilatation of splanchnic arteries	Reduced systemic vascular resistance
Increased portal venous inflow	Reduced arterial pressure
Increased intestinal capillary pressure	Increased plasma volume
Increased splanchnic lymph flow	Overactivity of vasoconstrictor systems
Development of portocollateral circulation	Increased natriuretic peptide levels

Table 10.2 Hemodynamic abnormalities in splanchnic and systemic circulation in patients with cirrhosis and ascites.

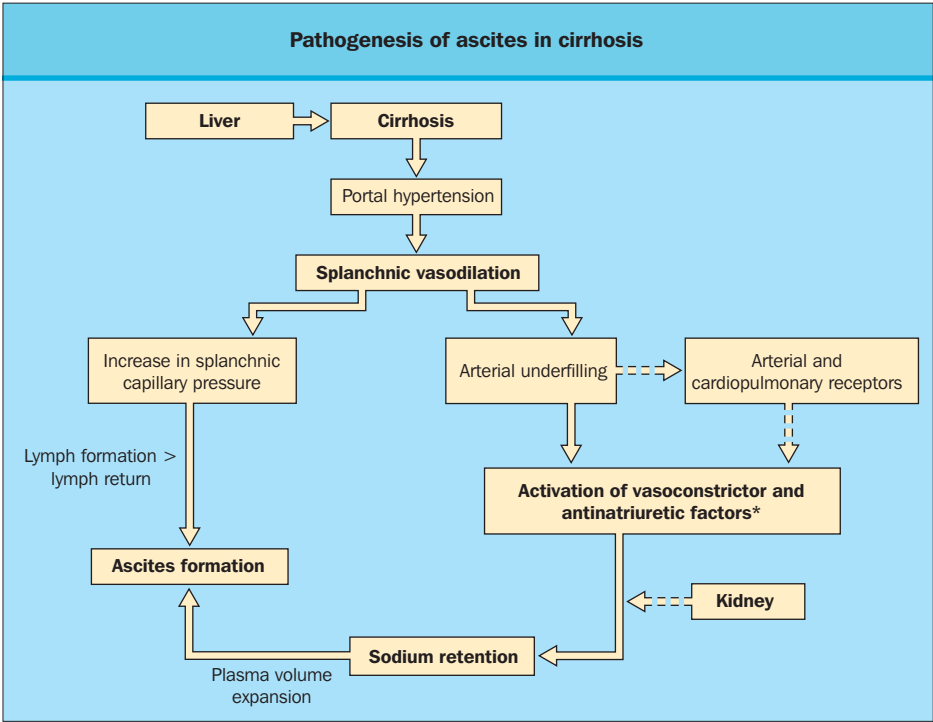


Figure 10.1 Pathogenesis of ascites in cirrhosis. As proposed by the arterial vasodilatation theory. \*Antinatriuretic factors are the renin–angiotensin–aldosterone system and the sympathetic nervous system.



**Figure 10.2 Patient with advanced cirrhosis and tense ascites.** Note the decreased muscle mass in the patient's arms and chest.

of 5–10%. The effusion is usually mild or moderate and more frequent on the right side. In some cases, pleural effusions recur after therapy with diuretics and constitute the main manifestation of the disease. These cases are usually caused by the existence of anatomic defects in the diaphragm that create a communication between the peritoneal and pleural cavities.

### Diagnostic approach to the patient with ascites

#### General evaluation (Table 10.3)

The general evaluation of the patient with cirrhosis is dealt with in Section II but the issues pertinent to patients with ascites are outlined in Table 10.3. The abdominal ultrasound is important to screen for hepatocellular carcinoma and evaluate patency of the portal venous system. Upper gastrointestinal endoscopy is performed to assess the presence of esophageal or gastric varices or portal hypertensive gastropathy. Percutaneous liver biopsy, if needed, can be performed after resolution of ascites because its presence increases the risk of complications. In patients with coagulation disturbances or intractable ascites, liver biopsy may be performed using a transjugular approach.

#### Evaluation of ascitic fluid

A diagnostic paracentesis of approximately 30–40 mL of fluid is required in all patients presenting with ascites de novo, requiring hospitalization, and those with any evidence of clinical deterioration such as fever, abdominal pain, gastrointestinal bleeding, or hepatic encephalopathy. Basic parameters to be determined in ascitic fluid are cell count, culture in blood culture bottles (10 mL of fluid injected at the bedside), albumin and total protein. The protein concentration of ascitic fluid is usually low in cirrhotic patients, with 60% of patients having an ascitic fluid protein concentration lower than 10 g/L. The difference between serum and ascites albumin is greater than 1.1 g/dL; values lower than 1.1 g/dL suggest a cause of ascites other than cirrhosis. The protein concentration of ascitic fluid is an important predictive factor in cirrhosis, because patients with ascitic protein concentrations lower than 10 g/L have a higher probability of developing spontaneous bacterial peritonitis and shorter survival expectancy than do patients with protein concentrations higher than 10 g/L (Fig. 10.3). The ascitic fluid red blood cell count is usually low

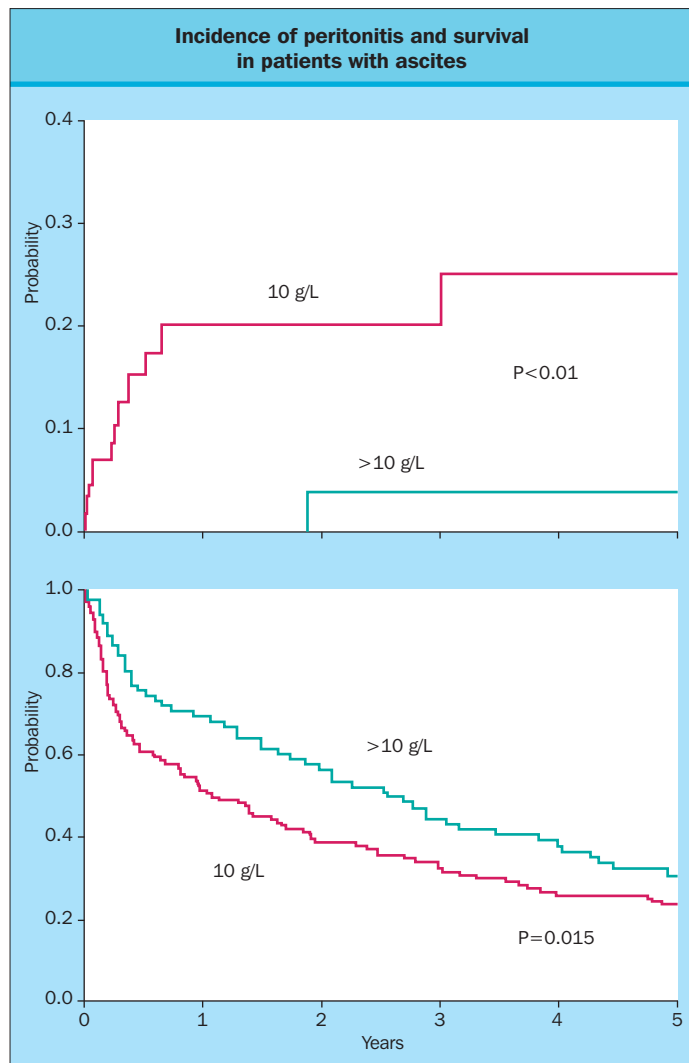
Evaluation of patients with cirrhosis and ascites	
<i>General evaluation</i>	
Complete history and physical examination	
Arterial blood pressure, heart rate and pulse oxymetry	
Standard hematology, coagulation, liver tests and $\alpha$ -fetoprotein	
Abdominal ultrasonography and Doppler flow (including the kidneys)	
Upper gastrointestinal endoscopy	
Liver biopsy (selected cases)	
<i>Evaluation of ascitic fluid</i>	
Total protein and albumin measurement	
Cell count	
Culture in blood culture bottles	
<i>Evaluation of renal function*</i>	
24 h urine sodium	
Diuresis after water load (5% dextrose i.v., 20 mL/kg) <sup>†</sup>	
Serum electrolytes, serum blood urea nitrogen and serum creatinine	
Urine sediment and protein excretion	
<i>Evaluation of circulatory function*</i>	
Arterial pressure	
Plasma renin activity and plasma norepinephrine concentration <sup>‡</sup>	
*Renal and circulatory function should preferably be assessed with the patient maintained on a low-sodium diet without diuretic therapy.	
<sup>†</sup> May provide important information when an accurate estimate of prognosis is required.	
<sup>‡</sup> In clinical research setting.	

**Table 10.3 Evaluation of patients with cirrhosis and ascites.**

(<1000 cells/mm<sup>3</sup>), although bloody ascites (>50 000 red blood cells/mm<sup>3</sup>) may be seen in some patients. In bloody ascites a correction factor of 1 polymorphonuclear (PMN) cell/250 red blood cells is recommended. A superimposed hepatocellular carcinoma should be excluded in the latter patients. The ascitic fluid white blood cell count is less than 500/mm<sup>3</sup> in most patients, and mononuclear cells predominate (>75%). As discussed later, a high number of polymorphonuclear cells (>250 cells/mm<sup>3</sup>) in the ascitic fluid indicates infection of the ascitic fluid, particularly spontaneous bacterial peritonitis (SBP).

#### Evaluation of renal function

The evaluation of renal function in patients with cirrhosis and ascites is important in the assessment of prognosis and response to therapy. The evaluation should be performed while on low-sodium diets and at least 4 days after withdrawal of diuretics. Parameters of interest include 24 h urine volume and sodium excretion, urine volume after a water load of 5% dextrose (20 mL/kg), serum electrolytes and serum creatinine. A strong prognostic marker of cirrhotic patients with ascites is the ability to handle water by means of a water load test. In this test a



**Figure 10.3 Incidence of spontaneous bacterial peritonitis and survival in cirrhotic patients.** Probability of developing spontaneous bacterial peritonitis (top) and survival (bottom) in cirrhotic patients with ascites according to the protein concentration in ascitic fluid. Modified from Llach et al (92) by permission of Wiley-Liss, Inc.

patient receives 20 mL/kg of intravenous 5% dextrose and following this, the renal excretion of water is measured. An impaired ability to excrete the water load (urine volume of <8 mL/min) is associated with a poor prognosis. In patients with renal failure (serum creatinine >1.5 mg/dL), tests of urine sediment, 24 h urine protein, and a renal ultrasound should also be performed.

### Evaluation of circulatory function

Evaluation of circulatory function provides prognostic information in patients with cirrhosis and ascites. The evaluation of circulatory function should include the measurement of arterial pressure in conditions of bed rest, low-sodium diet, and without diuretic therapy. Measurement of plasma renin activity and plasma norepinephrine concentration as an index of the activity of the renin-angiotensin system and sympathetic nervous system, respectively, also provide important prognostic information but are only used in a clinical research setting.

### Treatment

All patients should be evaluated for liver transplantation. Early referral is advocated due to the short survival expectancy patients have once they develop this complication. The objective of the treatment of ascites or edema in cirrhosis is to reduce the patient's discomfort due to abdominal or leg swelling. Besides, the decrease in the amount of fluid in the peritoneal cavity reduces the risk of complications related to abdominal hernias, such as incarceration or rupture. Consequently, therapeutic measures in these patients should be oriented to reduce the amount of ascites and edema and to prevent their reaccumulation after therapy.

The initial step in the management of cirrhotic patients with ascites or edema is sodium restriction, because the amount of extracellular fluid retained within the body depends on the balance between the sodium ingested in the diet and the sodium excreted in the urine. As long as the sodium excreted is lower than that ingested, patients will accumulate ascites or edema. Conversely, when sodium excretion is greater than intake, ascites or edema will decrease. The reduction of sodium content in the diet to 70–90 mEq/day (approx. 1.5 g of salt) causes a negative sodium balance and loss of ascites and edema in patients with mild sodium retention (5–20% of the whole population of patients with ascites). In patients with moderate or marked sodium retention, such dietary sodium restriction is not sufficient by itself to achieve a negative sodium balance, but it may slow the accumulation of fluid. These patients need diuretic therapy.

Patients with *moderate ascites* (grade 2 ascites) should be treated with sodium restriction and diuretics. As plasma aldosterone concentration is usually increased in patients with ascites and plays an important role in the increased tubular sodium reabsorption, spironolactone, a drug that competes with aldosterone for the binding to the mineralocorticoid receptor in the collecting tubular epithelial cells of the kidney, is the diuretic of choice in these patients. Other diuretics acting in the distal tubules, such as amiloride or triamterene, are less effective. Spironolactone is initially given at a dose of 50–100 mg/day and increased as needed every 7 days up to a maximum dose of 400 mg/day. Loop diuretics, especially furosemide (20 mg/day initially and increased up to 160 mg/day) which acts by inhibiting the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  cotransporter in the loop of Henle, may be given in combination with spironolactone to increase the natriuretic efficacy. Loop diuretics should not be used as single therapy in patients with cirrhosis, because they may put the patients with mild sodium retention at risk for volume depletion and prerenal failure and are less effective than spironolactone in patients with marked sodium retention. The combination of starting furosemide 20–40 mg/day and spironolactone 100 mg is another option and doses may be increased in a stepwise fashion every 7–10 days by doubling doses (ratio of 40 mg:100 mg); furosemide up to 160 mg/day and spironolactone up to 400 mg/day. The response to diuretic therapy in cirrhotic patients should be evaluated regularly by measuring body weight, urine volume and sodium excretion. Diuretics should be given at the minimum effective dose to achieve a maximum weight loss of approximately 0.5 kg/day in patients with ascites without edema and 1 kg/day in patients with ascites and edema. An inadequate sodium restriction is a common cause of failure of diuretic therapy, especially in non-hospitalized patients who usually eat more salt than inpatients. This situation should be



<b>Treatment strategy for patients with moderate (grade 2) ascites</b>
1. Begin with low-sodium diet (approx. 80 mEq/day) and spironolactone (50–200 mg/day as a single dose). Monitor body weight daily and urine sodium weekly. Ideal weight loss should be 500 g/day in patients without peripheral edema and 1000 g/day in patients with peripheral edema
2. Low doses of loop diuretics (furosemide, 20–40 mg/day) may be used in combination with spironolactone to increase the natriuretic effect particularly in patients with leg edema. Patients should be monitored closely to prevent excessive diuresis
3. If no response is seen, check compliance with treatment and low-sodium diet. Increase the dose of diuretics stepwise every 7 days up to 400 mg/day of spironolactone and 160 mg/day of furosemide
<i>Maintenance therapy</i>
1. Maintain sodium restriction and reduce the diuretic dosage approximately by 50%
2. If ascites or edema does not recur, increase sodium intake slowly and progressively and maintain a low dose of diuretics

**Table 10.4 Treatment strategy for patients with moderate (grade 2) ascites.**

suspected when body weight and ascites do not decrease despite a high urine volume and natriuresis. The proposed treatment strategy for patients with moderate ascites is outlined in **Table 10.4**.

Complications of diuretic therapy in patients with cirrhosis are common and include electrolyte disturbances (hyponatremia and hypo/hyperkalemia), renal impairment, hepatic encephalopathy, gynecomastia and muscle cramps. Hyponatremia occurs more frequently in patients with marked renal dysfunction and during treatment with high doses of loop diuretics. A rise in serum potassium levels is observed in most patients treated with spironolactone, particularly in those who have renal impairment. In some cases dose reductions may be necessary. Hypokalemia usually does not develop unless patients are treated with loop diuretics alone. Gynecomastia can be very painful and cause significant discomfort. A switch to amiloride is usually effective and tamoxifen has been reported to be successful in this situation. The impairment of renal function during diuretic therapy is due to intravascular volume depletion and is usually rapidly reversible after therapy is discontinued. Although aggressive diuretic therapy has been described as a precipitating factor to hepatorenal syndrome, there is no convincing evidence to support this relationship.

Patients with *large ascites* (grade 3, whether tense ascites or not), particularly those requiring hospitalization, are initially best managed with therapeutic paracentesis. This treatment is a more effective and less costly approach to initial management than prolonged bed rest and conventional diuretic therapy. In this approach, either all or part (4–6 L) of fluid is removed by a specially designed peritoneal needle using strict aseptic techniques. Albumin should be infused intravenously, at a dose of 6–8 g/L of ascitic fluid removed, to avoid circulatory dysfunction caused by a decrease in effective intravascular volume after

<b>Treatment strategy for patients with large (grade 3) ascites</b>
1. Total paracentesis plus intravenous albumin (8 g/L of ascites removed)
<i>Maintenance therapy</i>
1. Low-sodium diet (approx. 80 mEq/day) associated with diuretic therapy
2. If the patient was not taking diuretics before the development of severe ascites, start with spironolactone (200 mg/day as a single dose) with or without loop diuretics (furosemide, 40 mg/day) and then adjust the dose to maintain the patient with mild or no ascites or edema. Check body weight daily and urine sodium weekly. Closely monitor the patient during the first weeks of therapy
3. If the patient was taking diuretics before the development of severe ascites, start with a dose slightly higher than the dose taken before paracentesis
4. If ascites or edema increases, check compliance with treatment and the low-sodium diet. Increase the dose of diuretics stepwise every 7 days up to 400 mg/day of spironolactone and 160 mg/day of furosemide
5. If ascites or edema does not recur, a balance should be maintained between sodium intake and diuretic therapy

**Table 10.5 Treatment strategy for patients with large (grade 3) ascites.**

paracentesis. This circulatory disorder is almost inevitable if patients are treated with paracentesis without plasma volume expansion, and this is of clinical relevance due to the greater risk of recurrence of ascites and impaired survival. Synthetic plasma expanders (e.g. dextran-70, saline or hemacel) are less effective than albumin in the prevention of this disorder when more than 5 L of ascites are removed. Because paracentesis does not modify the pre-existing sodium retention, maintenance diuretic therapy in conjunction with sodium restriction should be instituted to avoid recurrence of ascites. The recommended treatment strategy for large or tense ascites is outlined in **Table 10.5**.

Approximately 10% of patients with ascites are refractory to treatment with diuretics. In refractory ascites, sodium excretion cannot be achieved either because patients do not respond to high doses of diuretics (spironolactone 400 mg/day and furosemide 160 mg/day) or because they develop side-effects (hyperkalemia, hyponatremia, hepatic encephalopathy or renal failure) that preclude their use (**Table 10.6**). Treatment options include repeated large-volume paracentesis plus plasma expansion with albumin or transjugular intrahepatic portosystemic shunts (TIPS). TIPS is a non-surgical method of portal decompression that acts as a side-to-side portocaval shunt that reduces portal pressure and decreases ascites and diuretic requirements in these patients.

Therapeutic paracentesis is the most accepted initial therapy for *refractory ascites*. Patients require paracentesis regularly and the majority may be treated as outpatients. This therapeutic approach is easy to perform and inexpensive. TIPS, although very effective in relieving ascites frequently may be complicated by obstruction of the prosthesis. Clinical trials comparing TIPS versus repeated paracentesis show that TIPS is associated with a lower rate of ascites recurrence, but a higher rate of hepatic

**Definition and diagnostic criteria for refractory ascites in cirrhosis proposed by the International Ascites Club**

**Diuretic-resistant ascites:** Ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of a lack of response to sodium restriction and diuretic treatment.

**Diuretic-intractable ascites:** Ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of the development of diuretic-induced complications that preclude the use of an effective diuretic dosage

**Requisites:****1. Treatment duration**

Patients must be on intensive diuretic therapy (spironolactone 400 mg/d and furosemide 160 mg/d) for at least 1 week and on a salt-restricted diet of less than 80 mmol/day

**2. Lack of response**

Mean weight loss of <0.8 kg over 4 days and urinary sodium output less than the sodium intake

**3. Early ascites recurrence**

Reappearance of grade 2 or 3 ascites within 4 weeks of initial mobilization

**4. Diuretic-induced complications**

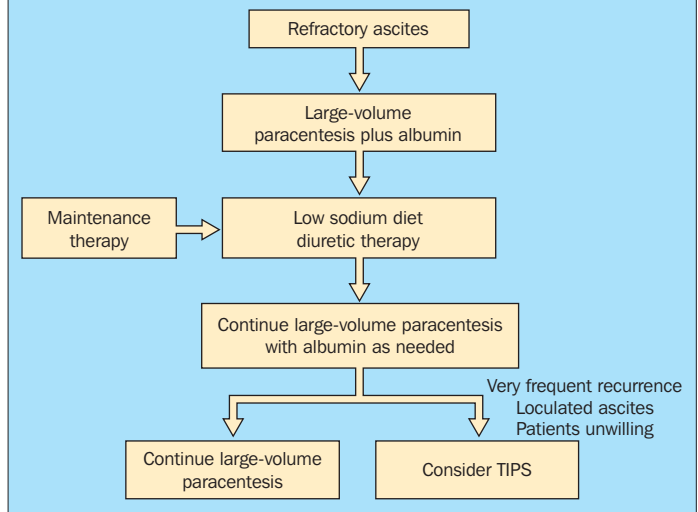
Diuretic-induced hepatic encephalopathy is the development of encephalopathy in the absence of any other precipitating factor. Diuretic-induced renal impairment is an increase of serum creatinine by >100% to a value >2 mg/dL in patients with ascites responding to treatment. Diuretic-induced hyponatremia is defined as a decrease of serum sodium by >10 mmol/L to a serum sodium of <125 mmol/L. Diuretic induced hypo- or hyperkalemia is defined as a change in serum potassium to <3 mmol/L or >6 mmol/L despite appropriate measures.

**Table 10.6 Definition and diagnostic criteria for refractory ascites in cirrhosis proposed by the International Ascites Club.**

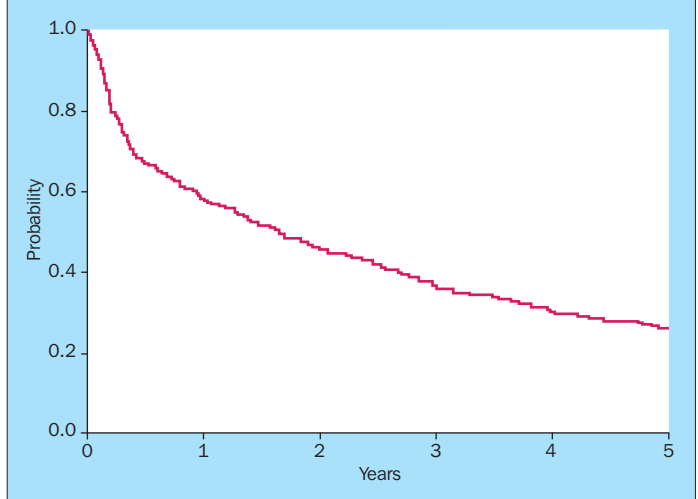
encephalopathy. There is also conflicting evidence in regards to survival because some studies have shown survival benefit with TIPS whereas others have not shown any difference in survival. The cost of treating patients with refractory ascites with TIPS is much higher than the cost of repeated paracentesis plus albumin. Therefore large-volume paracentesis appears to be the treatment of choice because of its wider applicability, lower cost and less side-effects when compared to TIPS. TIPS placement should be evaluated on a case-by-case basis and probably reserved for younger patients with preserved liver function in the absence of a history of hepatic encephalopathy, or those unwilling to undergo repeated paracentesis. **Figure 10.4** outlines a proposed therapeutic strategy for the treatment of refractory ascites.

**Prognosis**

The prognosis of patients with cirrhosis and ascites is poor, with less than 50% of patients surviving 2 years after the onset of ascites (**Fig. 10.5**). Long-term survivors (>10 years) are very uncommon. Factors known to be associated with a poor prognosis are those that manifest abnormalities in renal function and systemic hemodynamics (**Table 10.7**). In patients with ascites, these parameters are better predictors of prognosis than are liver tests or the Child-Pugh score. The prognosis can also be

**Treatment strategy for patients with refractory ascites**

**Figure 10.4 Treatment strategy for patients with refractory ascites.** Initial treatment is large volume paracentesis with albumin (8 g/L tapped). Maintenance treatment is continued with low sodium diet of approximately 80 mEq/L and diuretics if urinary sodium is >30 mEq/L under diuretic therapy. Transjugular intrahepatic portosystemic shunt (TIPS) should be considered in patients with preserved hepatic function, no hepatic encephalopathy, either with loculated fluid, or those unwilling to have repeated paracentesis.

**Survival of cirrhotic patients with ascites**

**Figure 10.5 Survival of cirrhotic patients with ascites.**

estimated by observing the patient's response to therapy. When a satisfactory response is observed with only dietary sodium restriction and small doses of diuretics, then the outlook is much better than if intensive diuretic therapy or frequent paracentesis are needed. The decision to perform a liver transplantation should not be delayed until patients are in very poor condition (i.e. refractory ascites or hepatorenal syndrome).

Adverse prognostic factors in cirrhosis with ascites
<b>Exploratory findings*</b>
Absence of hepatomegaly
Poor nutritional status
Previous ascites
Low arterial pressure
Esophageal varices
<b>Liver tests</b>
High serum bilirubin
Low serum albumin
<b>Renal tests</b>
Dilutional hyponatremia
Low urine sodium
Increased serum creatinine
Reduced water excretion after water load
<b>Circulatory abnormalities</b>
Low arterial blood pressure
High plasma renin activity
High plasma aldosterone
High plasma norepinephrine
*Measurements of renal and hormonal function should be obtained after a minimum of 4 days on a low-sodium diet and without diuretics.

**Table 10.7** Adverse prognostic factors in cirrhosis with ascites.

## DILUTIONAL HYPONATREMIA

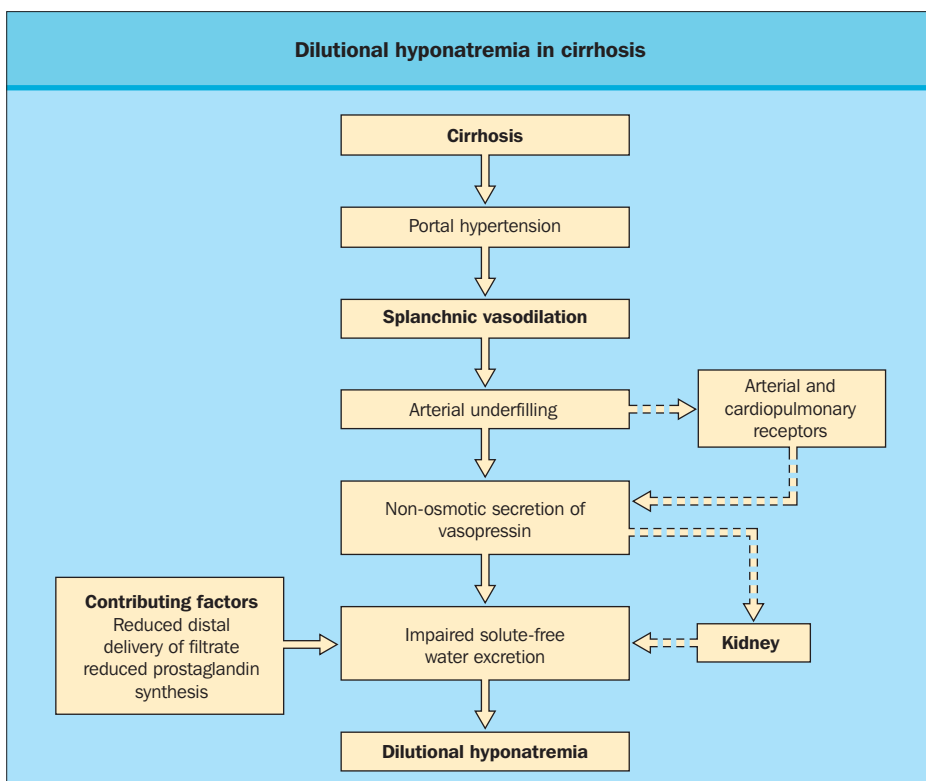
### Definition

Dilutional hyponatremia in cirrhotic patients is defined as serum sodium  $<130$  mmol/L (mEq/L) in the presence of ascites and/or edema. This type of hyponatremia is usually caused by an inability of the kidneys to excrete solute free water and occurs in the setting of increased total-body water and dilution of extracellular fluid volume. It is associated with sodium retention and increased total-body sodium and should be distinguished from true hyponatremia caused by sodium depletion, which although less common, may develop in cirrhotic patients on high doses of diuretics.

### Pathophysiology

The pathogenesis of solute-free water retention in cirrhosis involves several mechanisms, including a reduced delivery of filtrate to the ascending limb of the loop of Henle, the diluting site of the nephron, reduced renal synthesis of prostaglandins (particularly prostaglandin  $E_2$ ), and increased secretion of arginine vasopressin (AVP) (Fig. 10.6). The latter is probably the most important factor in the disturbed renal water handling in cirrhosis because:

- plasma AVP levels are commonly increased in cirrhotic patients and correlate closely with the reduction in solute-free water excretion;
- in experimental animals there is a close chronologic relationship between the appearance of solute-water retention and AVP hypersecretion;
- Brattleboro rats (rats with a congenital deficiency of AVP) with cirrhosis do not develop solute-free water retention;

**Figure 10.6** Dilutional hyponatremia in cirrhosis.

Proposed pathogenic mechanisms. There is a non-osmotic activation of arginine vasopressin due to decreased arterial blood pressure that activates baroreceptors and stimulates a hypothalamic release of the hormone.



Major causes of dilutional hyponatremia in cirrhotic patients with ascites
Spontaneous
Induced
Excessive administration of hypotonic i.v. fluids (dextrose)
Diuretics
Non-steroidal anti-inflammatory drugs
Therapeutic paracentesis without plasma expansion

**Table 10.8 Major causes of dilutional hyponatremia in cirrhotic patients with ascites.**

- kidneys from cirrhotic rats with ascites show increased gene expression of aquaporin-2, the AVP-regulated water channel; and
- the administration of specific antagonists of the tubular effect of AVP ( $V_2$  antagonists) restores the impaired solute-free water excretion in experimental and human cirrhosis.

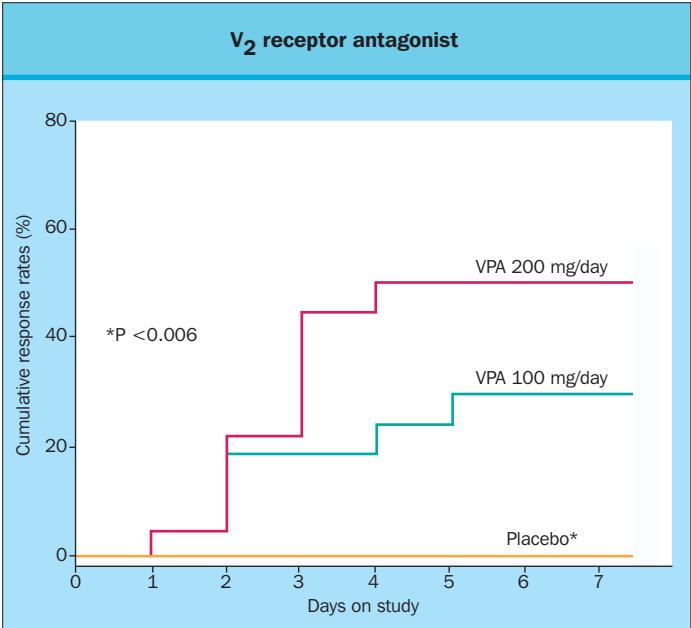
The increased plasma AVP levels in cirrhosis are secondary to an enhanced hypothalamic release owing to a non-osmotic stimulus. The mechanism of this nonosmotic hypersecretion is probably hemodynamic, because plasma AVP levels are suppressed by maneuvers that increase effective arterial blood volume (Fig. 10.6).

**Prevalence and clinical features**

The prevalence of dilutional hyponatremia in hospitalized cirrhotic patients with ascites is approximately 30%. Besides solute-free water retention, most patients with hyponatremia have avid sodium retention and renal vasoconstriction with reduced renal blood flow and glomerular filtration rate (GFR) and also marked activation of vasoconstrictor systems.

It should be noted that a normal serum sodium concentration in a cirrhotic patient with ascites should not be interpreted as indicative of a normal renal capacity to excrete solute-free water, because a large proportion of patients with ascites have moderate impairment in their capacity to excrete solute-free water. These patients are able to maintain a normal serum sodium concentration when their water intake is kept within normal limits, but they may develop dilutional hyponatremia when water intake is increased. A common example is hyponatremia observed in hospitalized cirrhotic patients who are maintained with intravenous (i.v.) 5% dextrose in an amount that exceeds their urine output. Other causes of dilutional hyponatremia are shown in Table 10.8.

In some patients, dilutional hyponatremia is asymptomatic, but in others it may be associated with clinical symptoms similar to those found in dilutional hyponatremia of other etiologies, including anorexia, difficulty in mental concentration and lethargy. In most cases it may be difficult to establish whether these symptoms are caused by hyponatremia, the underlying liver disease, hepatic encephalopathy or associated conditions. Therefore, the clinical implications of dilutional hyponatremia are not well known and deserve investigation.



**Figure 10.7  $V_2$  receptor antagonist.** Time to response after administration of the  $V_2$  receptor antagonist VPA-985 at two different doses to patients with cirrhosis and ascites and dilutional hyponatremia. Response was defined as normalization of serum sodium to  $\approx 136$  mmol/L. Mean time to response was 4.8 days in the 200 mg group and 5.7 days in the 100 mg group. There was no effect in the placebo group. Reproduced from Gerbes et al (03) by permission of the American Gastroenterological Association.

**Treatment**

If patients are taking diuretics these should be discontinued. No pharmacologic therapy is yet available for dilutional hyponatremia, and the only therapeutic measure that may improve or stop the progressive decrease in serum sodium concentration is water restriction. In patients with serum sodium levels of less than 130 mmol/L (mEq/L), fluid restriction to 1000 mL/day prevents the progressive decrease in serum sodium concentration but does not correct hyponatremia in most cases. The administration of hypertonic saline solutions is not recommended, because it invariably leads to further expansion of extracellular fluid volume and accumulation of ascites and edema. Pharmacologic therapy for dilutional hyponatremia is promising. Phase II clinical studies show that antagonists of the  $V_2$  receptor of AVP in the distal collecting duct increase solute-free water excretion and improve serum sodium concentration in hyponatremic patients with cirrhosis and ascites (Fig. 10.7). Phase III studies are being conducted with the aim of learning more about the efficacy and safety of these drugs which, if approved for clinical use, are likely to be very useful in managing these patients.

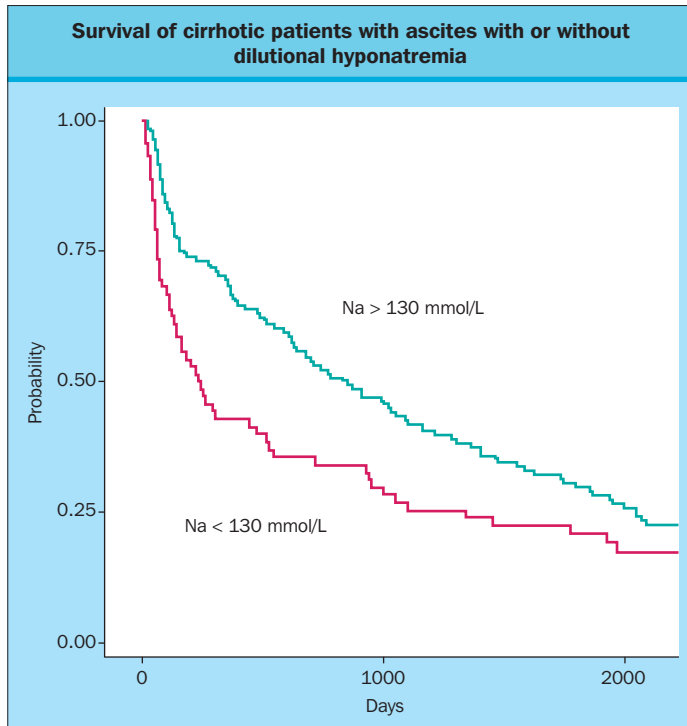
**Prognosis**

Spontaneous dilutional hyponatremia is a late event in the natural history of cirrhosis with ascites. The median survival time of patients with dilutional hyponatremia is much reduced compared with those patients who do not have hyponatremia (Fig. 10.8). In patients without overt renal failure, the presence of dilutional hyponatremia is a predictive factor for the development of hepatorenal syndrome.

## HEPATORENAL SYNDROME

### Definition

Hepatorenal syndrome (HRS) is a unique form of functional renal failure without identifiable renal pathology that occurs in approximately 10% of patients with advanced cirrhosis or acute

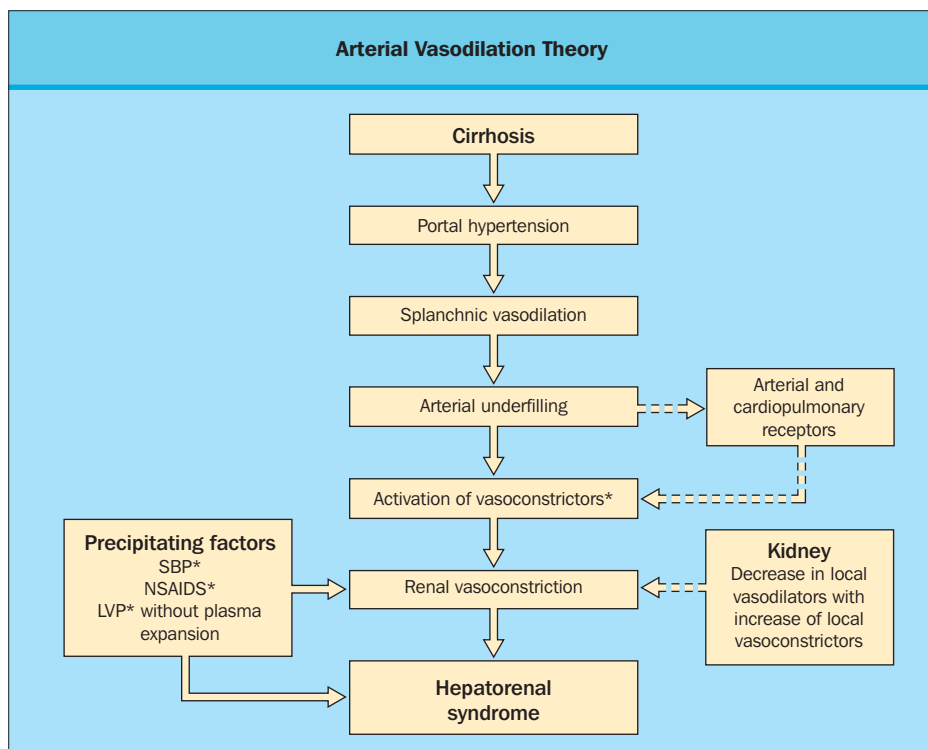


**Figure 10.8** Survival of cirrhotic patients with ascites with and without dilutional hyponatremia.

liver failure. HRS is characterized by impaired renal function and marked disturbances in the arterial circulation and activity of vasoactive systems. In the renal circulation there is a marked increase in vascular resistance, whereas total systemic vascular resistance is reduced and leads to arterial hypotension. This reduction in systemic vascular resistance is mainly caused by an arterial vasodilatation of the splanchnic circulation.

### Pathophysiology

The pathophysiologic hallmark of HRS is renal vasoconstriction. The kidneys are structurally intact. The mechanism of this vasoconstriction is poorly understood and possibly multifactorial, involving increased vasoconstrictor and reduced vasodilator factors that act on the renal circulation. The most accepted theory on the pathogenesis of HRS (*Arterial Vasodilation Theory*) proposes that renal hypoperfusion represents the extreme manifestation of an underfilling of the arterial circulation secondary to a marked vasodilatation of the splanchnic area (**Fig. 10.9**). This arterial underfilling would result in a progressive baroreceptor-mediated activation of vasoconstrictor systems (i.e. renin-angiotensin and sympathetic nervous systems) that would cause vasoconstriction not only in the renal circulation but also in other vascular beds (lower and upper extremities). The splanchnic area would escape the effect of vasoconstrictors and a marked vasodilation would persist, probably because of the existence of very potent local vasodilator stimuli. In early phases following the development of ascites, renal perfusion would be maintained within normal or near-normal levels despite the overactivity of vasoconstrictor systems by an increased synthesis/activity of renal vasodilator factors. The development of renal hypoperfusion leading to HRS would occur either as a result of a maximal activation of vasoconstrictor systems that could not be counteracted by vasodilator factors, decreased



**Figure 10.9** Arterial vasodilation theory.

Pathogenesis of hepatorenal syndrome as proposed by the Arterial Vasodilation Theory.

\*Renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system (SNS), and arginine vasopressin. SBP, spontaneous bacterial peritonitis; NSAIDs, non-steroidal anti-inflammatory drugs; LVP, large volume paracentesis.

activity of vasodilator factors, and/or increased production of intrarenal vasoconstrictor factors. An alternative theory proposes that renal vasoconstriction is the result of a direct relationship between the liver and the kidney, without any relationship with disturbances in systemic hemodynamics. The link between the liver and the kidney would be either a liver vasodilator factor, the synthesis of which would be reduced as a consequence of liver failure, or a hepatorenal reflex causing renal vasoconstriction. However, there is little or no evidence supporting this latter sequence of events in humans with cirrhosis.

Clinical features and diagnosis

Two different types of HRS, which likely represent distinct expressions of the same pathogenic mechanism, exist. *Type 1 HRS* is characterized by 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 or a 50% reduction of the initial 24 h creatinine clearance to a level lower than 20 mL/min in less than 2 weeks. Renal failure in these patients is often associated with progressive oliguria, marked sodium retention and dilutional hyponatremia. Serum creatinine levels in HRS are usually lower than values observed in patients with acute renal failure without liver disease due to a reduced muscle mass and low endogenous production of creatinine in cirrhosis. Patients with type 1 HRS are usually in a very severe clinical condition and show signs of advanced liver failure. In approximately half of the cases, this type of HRS develops spontaneously without any identifiable precipitating factor, whereas in the remaining patients it occurs in close chronologic relationship with some complications or therapeutic interventions [e.g. bacterial infections, particularly spontaneous bacterial peritonitis, administration of non-steroidal anti-inflammatory drugs (NSAIDs), large-volume paracentesis without plasma expansion]. *Type 2 HRS* is characterized by moderate and stable reduction of GFR with serum creatinine levels around 1.5–2.5 mg/dL that does not meet the criteria proposed for type 1. Unlike type 1 HRS, type 2 HRS usually occurs in patients with relatively preserved hepatic function and patients have a better outcome. The main clinical consequence of this type of HRS is refractory ascites.

There is no specific test for the diagnosis of HRS. The diagnosis of HRS is based on the exclusion of other common causes of renal failure that may occur in patients with cirrhosis (Table 10.9) and demonstration of an increased serum creatinine

(>1.5 mg/dL in the absence of diuretic therapy). Criteria for the diagnosis of HRS are shown in Table 10.10. Although a cut-off value of serum creatinine of 1.5 mg/dL may seem low, cirrhotic patients with ascites with serum creatinine above 1.5 mg/dL have a GFR below 30 mL/min, which represents only a quarter of the normal GFR for healthy subjects of the same age. The low serum creatinine values relative to the reduction in GFR are probably related to a reduced endogenous production of creatinine caused by the poor nutritional status of cirrhotic patients. Most cases of HRS have urine sodium below 10 mEq/L and urine osmolality above plasma osmolality because of the preservation of tubular function. Nevertheless, some patients may have high urine sodium and low urine osmolality, similar to what occurs in acute tubular necrosis. Conversely, cirrhotic patients with acute tubular necrosis may have low urine sodium and high osmolality. For these reasons, urinary indices are not considered major criteria for the diagnosis of HRS.

Prevention

HRS can be prevented in two clinical settings. First, in patients with SBP the administration of albumin (1.5 g/kg at diagnosis of infection and 1 g/kg 48 h later) prevents the circulatory dysfunction and subsequent development of HRS. The incidence of HRS in patients with SBP receiving albumin together with antibiotic therapy is in the order of 10%, compared with an incidence of 30% in patients not receiving albumin. Most importantly, the administration of albumin improves survival in these patients. Second, in patients with acute alcoholic hepatitis the administration of pentoxifylline, an inhibitor of tumor necrosis factor (400 mg t.i.d. orally for 28 days) also prevents the development of HRS and improves survival in this setting.

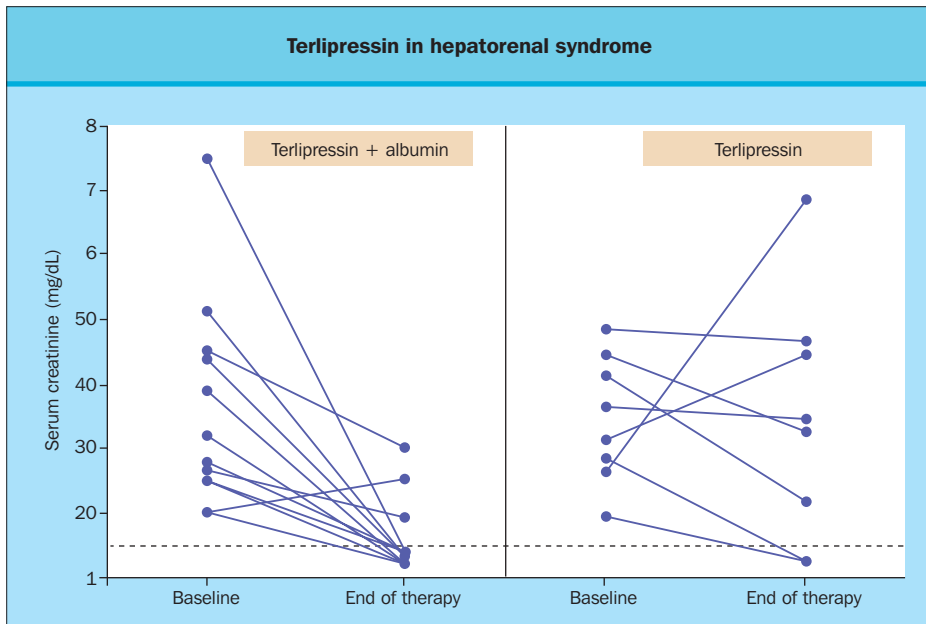
Common causes of renal failure in cirrhosis other than hepatorenal syndrome
Prerenal failure due to volume depletion (diuretics, vomiting, diarrhea)
Acute tubular necrosis after hypovolemic shock
Glomerulonephritis associated with hepatitis B and C
Nephrotoxicity (non-steroidal anti-inflammatory drugs, aminoglycosides)

Table 10.9 Common causes of renal failure in cirrhosis other than hepatorenal syndrome.

Diagnostic criteria of hepatorenal syndrome proposed by the International Ascites Club
Major criteria*
Serum creatinine greater than 1.5 mg/dL
Exclusion of shock, ongoing bacterial infection, volume depletion and use of nephrotoxic drugs
No improvement in creatinine despite stopping diuretics and volume repletion with 1.5 L of saline
No proteinuria or ultrasonographic evidence of obstructive uropathy or parenchymal renal disease
Minor criteria
1. Urine volume lower than 500 mL/day
2. Urine sodium lower than 10 mEq/L
3. Urine osmolality greater than plasma osmolality
4. Urine red blood cells less than 50 per high power field
5. Serum sodium concentration lower than 130 mEq/L
(* ) Only major criteria are necessary for the diagnosis of hepatorenal syndrome.

Table 10.10 Diagnostic criteria of hepatorenal syndrome proposed by the International Ascites Club.





**Figure 10.10 Terlipressin in hepatorenal syndrome.** The effects of terlipressin and albumin compared to terlipressin alone in the treatment of type 1 hepatorenal syndrome. Reproduced from Ortega et al (02) by permission of Wiley-Liss, Inc.

## Treatment

Effective therapies for HRS include liver transplantation, vasoconstrictors with plasma expansion and TIPS. Liver transplantation is the best treatment for suitable candidates with HRS because it offers a cure to the diseased liver and the HRS. However, a significant proportion of patients die before transplantation can be done because of their extremely short survival time. Therefore, liver transplantation should be indicated whenever possible, before the development of HRS. The presence of HRS is associated with increased morbidity and early mortality after transplantation. Patients with ascites who are more likely to develop HRS are those with greatly reduced urine sodium ( $<10$  mmol/L or mEq/L), dilutional hyponatremia, arterial hypotension and marked activation of renin-angiotensin and sympathetic nervous systems.

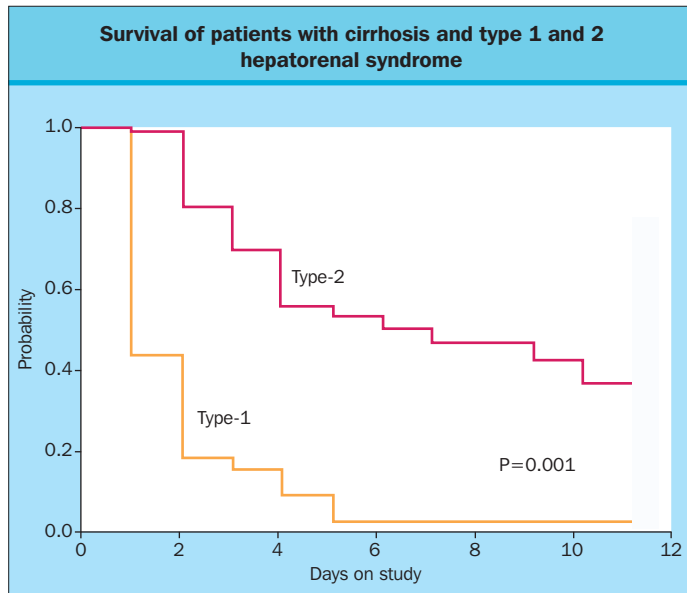
Renal vasodilator drugs such as dopamine and prostaglandin analogs were used in the past to treat HRS. However, this approach has been largely abandoned due to poor results, side-effects and lack of data confirming benefit. In contrast, systemic vasoconstrictors such as intravenous vasopressin analogs (terlipressin),  $\alpha$ -adrenergic agonists (midodrine) plus octreotide, or intravenous noradrenaline, and all of them in association with intravenous albumin, are effective in the treatment of HRS.

Intravenous terlipressin along with albumin as a plasma expander is associated with a significant improvement of renal function and normalization of serum creatinine in approximately 75% of patients treated for at least 5 days (Fig. 10.10). Administration of midodrine, an  $\alpha$ -adrenergic agonist in association with octreotide, a glucagon inhibitor, and albumin has also proved efficacious in HRS. Finally, noradrenaline in combination with albumin expansion also improves renal function in HRS. After stopping therapy, which typically lasts between 5 and 15 days, recurrence is uncommon. Due to limited information and the possibility of ischemic side-effects, treatment with vasoconstrictors should probably be restricted to patients with type 1 HRS. The main end point of therapy is a reduction of serum creatinine below 1.5 mg/dL. This ensures

that suitable transplant candidates can undergo transplantation without renal failure. Successful therapy probably improves the outcome after transplantation. Patients that successfully respond to treatment before transplantation have the same outcome as those that did not have HRS at the time of transplantation. Non-transplant candidates may also benefit by reducing readmissions, morbidity and mortality but this remains to be proved. The recommended doses and duration of vasoconstrictor therapy are summarized in Table 10.11.

Recommendations for using vasoconstrictors in type 1 hepatorenal syndrome	
1.	Treat for a maximum of 15 days
2.	End point of treatment is reduction of serum creatinine below 1.5 mg/dL
3.	Recommended medications and doses are:
A.	Terlipressin 0.5–1 mg i.v. every 4 h; increase dose every 2–3 days up to 2 mg/4 h when there is no decrease in creatinine
B.	Midodrine 7.5 mg orally three times daily with an increase to 12.5 mg three times daily and octreotide 100 $\mu$ g subcutaneously three times daily with an increase to 200 $\mu$ g three times daily if creatinine not decreasing
C.	Noradrelanine 0.5–3 mg/h continuous i.v. infusion
4.	Intravenous albumin infusion (20–40 g daily)
5.	Monitor for side-effects such as chest pain, distal ischemia, abdominal pain
6.	Avoid in patients with cardiac disease, peripheral vascular disease, and/or cerebrovascular disease, due to the potential risk of ischemic events

**Table 10.11 Recommendations for using vasoconstrictors in type 1 hepatorenal syndrome.**



**Figure 10.11** Survival of patients with cirrhosis and type 1 and 2 hepatorenal syndrome. Reproduced from Ginés P et al (03) by permission of Elsevier.

TIPS may improve renal function in type 1 HRS. Although the use of vasoconstrictors and albumin followed by TIPS is effective in a subset of patients, experience is limited and most patients with HRS have advanced cirrhosis that would preclude them from receiving a TIPS. Although uncontrolled studies suggest that TIPS improves prognosis in types 1 and 2 HRS, the real impact on survival remains to be assessed. Conventional hemodialysis is not routinely recommended in HRS unless there is volume overload, severe hyperkalemia or severe metabolic acidosis. Albumin dialysis [molecular adsorbent recirculating system (MARS)] has shown efficacy in one study, but its possible beneficial effects need to be confirmed in further studies.

### Prognosis

The prognosis of patients with HRS is very poor. Type 1 HRS is the complication with the worst prognosis for cirrhotic patients. The median survival time of these patients with HRS without therapy is less than 2 weeks, which is a survival time shorter than that of patients with acute renal failure of other etiologies. The combination of several factors, including renal failure, advanced liver failure, and, in some cases, associated infections, accounts probably for this extremely poor outcome. The median survival of patients with type 2 HRS is usually several months, which is a longer survival time than those with type 1 but shorter than patients with ascites without renal failure (Fig. 10.11).

## SPONTANEOUS BACTERIAL PERITONITIS (SBP)

### Definition

SBP is a common and severe complication of cirrhotic patients with ascites characterized by spontaneous infection of ascitic fluid without an intra-abdominal infection. The prevalence of SBP in hospitalized patients with ascites ranges from 10 to 30%. Cirrhotic patients with hydrothorax may also develop a sponta-

neous infection of the pleural fluid, known as spontaneous bacterial empyema.

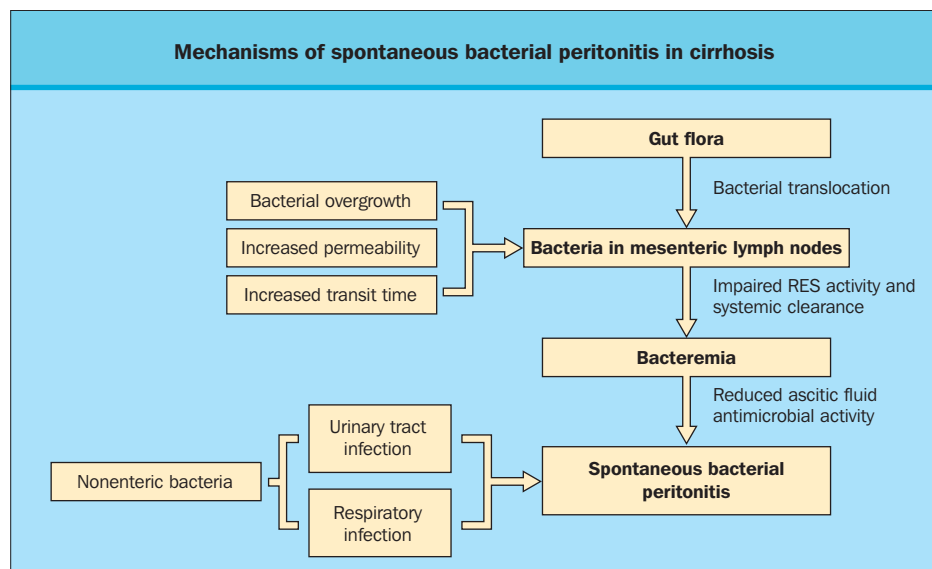
### Pathophysiology

The isolation of aerobic Gram-negative bacteria in most episodes of SBP suggests that the gastrointestinal tract is the source of bacteria. Although the pathogenesis of SBP is not completely understood, it is generally accepted that it involves three major steps:

- passage of bacteria from the intestinal lumen to the systemic circulation;
- bacteremia secondary to the impairment of reticuloendothelial system (RES) phagocytic activity, and
- infection of ascites caused by poor opsonization and defective bactericidal activity of the ascitic fluid.

Studies in experimental animals with cirrhosis suggest that bacterial translocation (i.e. passage of bacteria from the intestinal lumen to mesenteric lymph nodes) is the mechanism by which bacteria from the intestinal lumen reach the systemic circulation. Bacterial translocation may increase under several circumstances, such as gastrointestinal bleeding and hemorrhagic shock. This may explain, at least in part, the high incidence of infections caused by enteric bacteria in cirrhotic patients with gastrointestinal hemorrhage. The reduced phagocytic activity of the RES (a system that removes bacteria from the circulation) is another important pathogenic factor in the development of SBP. Cirrhotic patients with reduced activity of the RES are highly predisposed to develop SBP, whereas this infection is rarely seen in patients with normal activity of the RES. Finally, a reduced antimicrobial activity of the ascitic fluid also plays a very important role in the development of SBP. Patients with reduced antimicrobial activity of ascitic fluid have a greater risk of developing ascitic fluid infection than those with normal antimicrobial activity of the ascitic fluid. As the antimicrobial activity of ascites correlates with total ascitic fluid protein concentration, patients with low ascites protein content (<10 g/L) have a greater risk of developing SBP compared with patients with higher ascites protein concentration (Fig. 10.3, top).

The most plausible theory on the pathogenesis of SBP is shown in Figure 10.12. The initial step involves translocation of bacteria from the gut flora to mesenteric lymph nodes. This translocation would occur either spontaneously or as a consequence of some precipitating events (i.e. gastrointestinal hemorrhage). An increased permeability related to gut edema, intestinal bacterial overgrowth, or increased transit time may facilitate bacterial translocation. The bacteria then reach the systemic circulation through the lymphatic system. In cases of SBP that are not caused by bacteria from enteric origin, the bacteria would reach the systemic circulation from other areas (i.e. respiratory or urinary tracts or skin). In patients with normal activity of the RES, bacteria are efficiently removed from the circulation, but persistent bacteremia develops in patients with impaired activity of the RES. Subsequently, bacteria may reach the ascitic fluid through a hematogenous route. The development of SBP depends on the opsonic and bactericidal activity of ascitic fluid. Infection of ascites does not usually occur in patients with good antimicrobial activity of the ascitic fluid. In contrast, patients with reduced antimicrobial activity of the ascitic fluid are at high risk of developing SBP. A similar mechanism may explain the development of spontaneous bacterial empyema.



**Figure 10.12 Mechanisms of spontaneous bacterial peritonitis in cirrhosis.**  
RES, reticuloendothelial system.

### Clinical features and diagnosis

The clinical spectrum of SBP is quite variable and ranges from a full-blown picture of peritonitis to complete absence of symptoms (Table 10.12). A very high degree of clinical suspicion is required to make a diagnosis, because only a relatively low percentage of patients show the typical features of an acute peritoneal infection with fever, chills, diffuse abdominal pain, rebound tenderness and reduced bowel sounds. Fever may be the only clinical sign in a large proportion of patients. In other cases, the infection is manifested by hepatic encephalopathy or septic shock. In 10% of cases, SBP may be totally asymptomatic; therefore the diagnosis relies on the examination of peritoneal fluid. Renal failure develops in one-third of patients with SBP likely due to an accentuation of the splanchnic vasodilation with decreased effective arterial blood volume and activation of the vasoconstrictor systems. The development of renal failure may occur despite the resolution of the infection and is associated with a poor prognosis. The administration of albumin in the setting of SBP decreases the incidence of renal failure and improves survival in these patients (see later). Patients who survive an episode of SBP are at high risk of developing recurrent episodes of SBP during follow-up. As many as 70% of patients who survive 1 year after the first episode of SBP will develop a new episode of infection within this period. Aerobic Gram-negative bacteria are responsible for nearly 80% of cases and *Escherichia coli* accounts for most of them. Aerobic Gram-positive bacteria, mostly *Streptococcus viridans*, *Staphylococcus aureus* and *Enterococcus faecalis* are isolated in approximately 20% of cases. Anaerobic and microaerophilic organisms, although very abundant in gut flora, rarely cause SBP.

The diagnosis of SBP is based on examination of ascitic fluid. A diagnostic paracentesis should be performed routinely in all cirrhotic patients admitted to hospital with ascites and in those hospitalized patients in whom an infection is suspected because of fever, abdominal pain, shock, hepatic encephalopathy or renal impairment. The diagnosis of SBP is made when the fluid sample has a PMN count greater than 250/mm<sup>3</sup>. The use of reagent strips or 'urine dipsticks' seems to be useful for obtaining a rapid

**Clinical signs or symptoms at presentation in a series of 161 cirrhotic patients with spontaneous bacterial peritonitis**

Clinical signs or symptoms	Percentage of patients
Abdominal pain	80
Fever	70
Hepatic encephalopathy	58
Hypoperistalsis	32
Diarrhea	19
Septic shock	16
Gastrointestinal bleeding	15
Vomiting	13
No symptoms	10

**Table 10.12 Clinical signs or symptoms at presentation in a series of 161 cirrhotic patients with spontaneous bacterial peritonitis.**

diagnosis of SBP. A drawback of using these strips is that there is no cell count number or differential, therefore it is prudent to also obtain a concomitant cell count and differential. The inoculation of 10 mL of ascitic fluid into blood culture bottles immediately after paracentesis significantly improves the rate of positive ascitic fluid cultures compared to standard diagnostic methods.

Despite the use of sensitive methods of ascitic fluid culture, between 20 and 40% of episodes of SBP diagnosed by high PMN count are culture-negative. This condition, usually referred to as culture-negative SBP, or culture-negative neutrocytic ascites, should be considered SBP and treated accordingly (Table 10.13). Bacterascites is a different condition that consists of a positive ascitic fluid culture with a normal PMN count and represents probably a colonization of ascitic fluid by bacteria without inflammatory response.



Recommendations for the management and prevention of spontaneous bacterial peritonitis (SBP)	
Therapy	
1.	Start with third-generation cephalosporins (i.e. cefotaxime 2 g/12 h i.v. or ceftriaxone 1 g/24 h i.v.) immediately after diagnosis of SBP
2.	Infuse albumin (1.5 g/kg at diagnosis of the infection and 1 g/kg 48 h later)
3.	Maintain antibiotic therapy until disappearance of signs of infection or for at least 5 days. Patients should be evaluated daily to assess signs of infection and at least one follow-up paracentesis should be performed to determine polymorphonuclear cell count. If there is a reduction of less than 25% of polymorphonuclear cells compared to the basal levels, the presence of a secondary peritonitis and/or bacteria resistant to antibiotic therapy should be suspected and treatment changes made accordingly
4.	After resolution of infection, start long-term norfloxacin 400 mg/day p.o.

**Table 10.13 Recommendations for the management and prevention of spontaneous bacterial peritonitis (SBP).**

The main condition to be considered in the differential diagnosis of SBP is peritonitis secondary to gut perforation or acute inflammatory disorders (i.e. cholecystitis, appendicitis and diverticulitis) which are also associated with an increased PMN count in ascitic fluid. Although secondary peritonitis is less common than SBP, the differentiation between the two conditions is very important, because surgical treatment is usually required for secondary peritonitis. The ascitic fluid in cirrhotic patients with secondary peritonitis is usually characterized by ascitic fluid total protein >1 g/dL, glucose less than 50, and lactate dehydrogenase >225 mU and more than one organism in the Gram stain or culture. Nevertheless, the existence of perforation or abscesses causing secondary peritonitis should be confirmed by appropriate methods, such as abdominal X-ray, ultrasound, or computed tomography (CT) scan.

### Treatment

Empirical antibiotic therapy should be initiated in patients with a PMN count in ascitic fluid greater than 250/mm<sup>3</sup> before the detection of the causative organisms by ascitic fluid culture. The antibiotics of choice as initial empirical treatment for cirrhotic patients with SBP are third-generation cephalosporins for at least 5 days owing to their broad antibacterial spectrum, high efficacy, and few side-effects. Although cefotaxime has been the drug more commonly used, other cephalosporins, such as ceftriaxone, have similar efficacy. In randomized comparative studies, cefotaxime has been shown to be more effective than other types of antibiotics, such as aztreonam or aminoglycosides plus ampicillin. Amoxicillin-clavulanic acid also seems effective, but large comparative studies between these antibiotics and third-generation cephalosporins are lacking. Ofloxacin is as effective as intravenous cefotaxime in terms of resolution of infection and survival and has the additional advantage of oral administration and low cost. Nevertheless, i.v. antibiotics should be used in very sick patients (e.g. septic shock) or in those with

complications that may impair oral absorption of drugs (e.g. gastrointestinal hemorrhage or ileus). Therapy modification depends on results from cultures. Responses to therapy include frequent clinical evaluation and repeat diagnostic paracentesis 2–3 days after beginning antibiotics. If patients are not responding (worsening infection or no decrease in PMN count) antibiotic therapy should be reviewed. Patients should be treated until the complete disappearance of all signs of infection (fever, abdominal pain, normalization of blood PMN count) and decrease of PMN count in ascitic fluid below 250/mm<sup>3</sup>. Recommendations for the management of SBP are summarized in Table 10.13.

### Prognosis

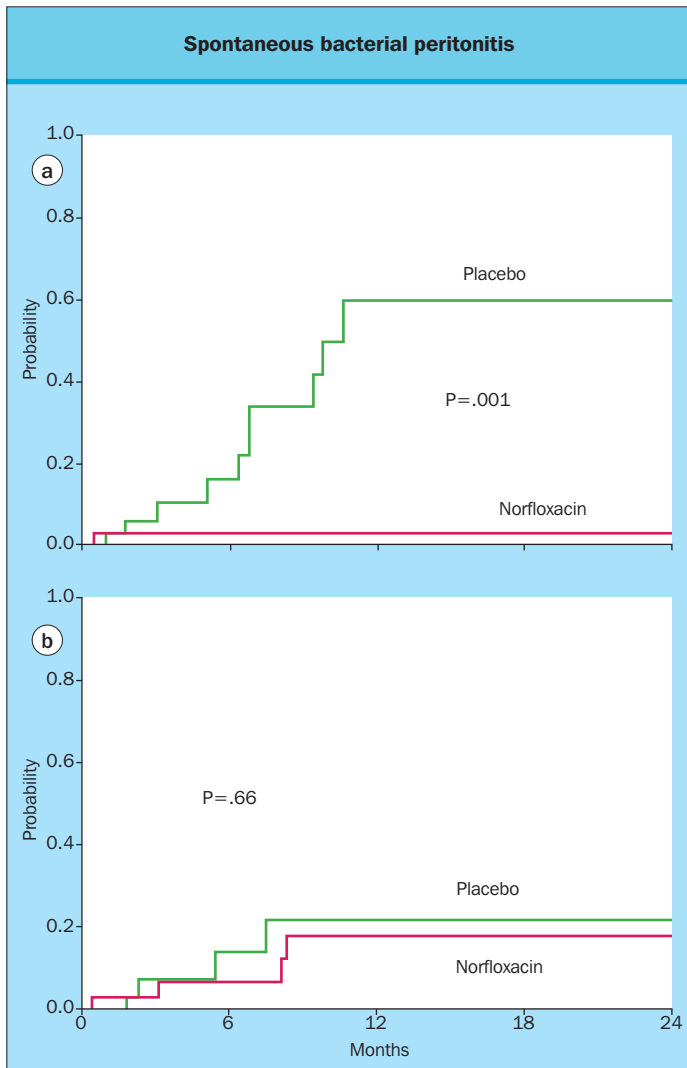
SBP resolves in approximately 90% of cases if treated with the above regimens. Factors associated with a lack of response to therapy are renal impairment, high blood PMN count, hospital acquired SBP, and high aspartate aminotransferase (AST) levels. The most important predictor of survival in patients with SBP is the development of renal failure during the infection. Before the introduction of albumin expansion at diagnosis, hospital mortality was near 30%, mainly due to renal failure. The administration of albumin at a dose of 1.5 g/kg at the diagnosis of the infection and 1 g/kg 48 h later prevents renal failure and significantly reduces mortality in patients with SBP from 30 to 10% during the infection episode.

Recurrence is common, approximately 70% in 1 year, and constitutes a major cause of death in these patients. The 1-year survival probability is only 40% and therefore patients should be evaluated for liver transplantation once they have recovered from SBP. The main causes of death in these patients are recurrent episodes of SBP, liver failure and gastrointestinal hemorrhage.

### Prevention

Because most episodes of SBP are caused by Gram-negative bacteria present in the normal intestinal flora, the approach used for the prophylaxis of SBP has been based on the administration of antibiotics, usually quinolones such as norfloxacin or ciprofloxacin, that produce a selective decontamination of the gastrointestinal tract, with elimination of aerobic Gram-negative bacteria without affecting aerobic Gram-positive bacteria and anaerobes. The efficacy of this approach has been demonstrated in patients with gastrointestinal hemorrhage and patients who have recovered from the first SBP episode. This policy has been endorsed by a panel of experts in an International Consensus Conference on SBP.

In patients with gastrointestinal hemorrhage, the short-term (7 days) administration of norfloxacin markedly reduces the incidence of SBP or bacteremia as compared with patients not receiving prophylactic antibiotics. Long-term norfloxacin administration is very effective in the prevention of SBP recurrence (secondary prophylaxis). Patients treated with norfloxacin have a lower probability of developing SBP recurrence caused by Gram-negative bacteria compared to that of patients not receiving norfloxacin, while the risk of developing SBP by Gram-positive bacteria remains unchanged (Fig. 10.13). Whether this decrease in SBP recurrence rate results in an improved survival has not been assessed but appears likely on the basis of the high mortality rate associated with SBP. Antibiotic prophylaxis (norfloxacin, ciprofloxacin or trimethoprim-sulfamethoxazole) also appears to be effective in the prevention of SBP in patients with low



**Figure 10.13 Spontaneous bacterial peritonitis.** Probability of the recurrence in patients treated chronically with norfloxacin (400 mg/day) as secondary prophylaxis and patients treated with a placebo. (a) Episodes caused by Gram-negative bacteria. (b) Episodes caused by Gram-positive bacteria or culture-negative bacteria. Reproduced from Ginès et al (90) by permission of Wiley-Liss, Inc.

### Prevention of spontaneous bacterial peritonitis (SBP) in patients with cirrhosis and ascites

Patients with gastrointestinal hemorrhage:

1. Norfloxacin 400 mg/12 h orally or per gastric tube, for 7 days
2. Alternative regimes include systemic antibiotics (ciprofloxacin, amoxicillin-clavulanic acid, ofloxacin)

Patients with ascites with a previous episode of SBP:

1. Norfloxacin 400 mg/day indefinitely
2. Consider liver transplantation

Patients with ascites without a previous episode of SBP:

1. Low ascitic fluid protein concentration (< 10 g/L): no consensus on the use of prophylactic antibiotics

**Table 10.14 Prevention of spontaneous bacterial peritonitis (SBP) in patients with cirrhosis and ascites.**

ascitic fluid protein (<10 g/L), who are at high risk of developing the first episode of SBP (primary prophylaxis), but no definitive conclusions can be obtained from published studies because either they include a low number of patients, have a short follow-up (6 months) or are not placebo controlled. Recommendations for prophylaxis of SBP are summarized in Table 10.14.

In recent years, Gram-negative bacteria resistant to quinolones have been isolated with an increasing frequency from fecal flora of patients under chronic quinolone therapy. In some patients, this colonization of intestinal flora by resistant bacteria may result in bacterial infections and it may well be that in years to come more episodes of microorganisms resistant to quinolones may complicate the clinical course of cirrhotic patients with previous episodes of SBP.

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# SECTION III: CLINICAL MANIFESTATIONS OF LIVER DISEASE

## CHAPTER

# 11

# Hepatic Encephalopathy

Andres T. Blei

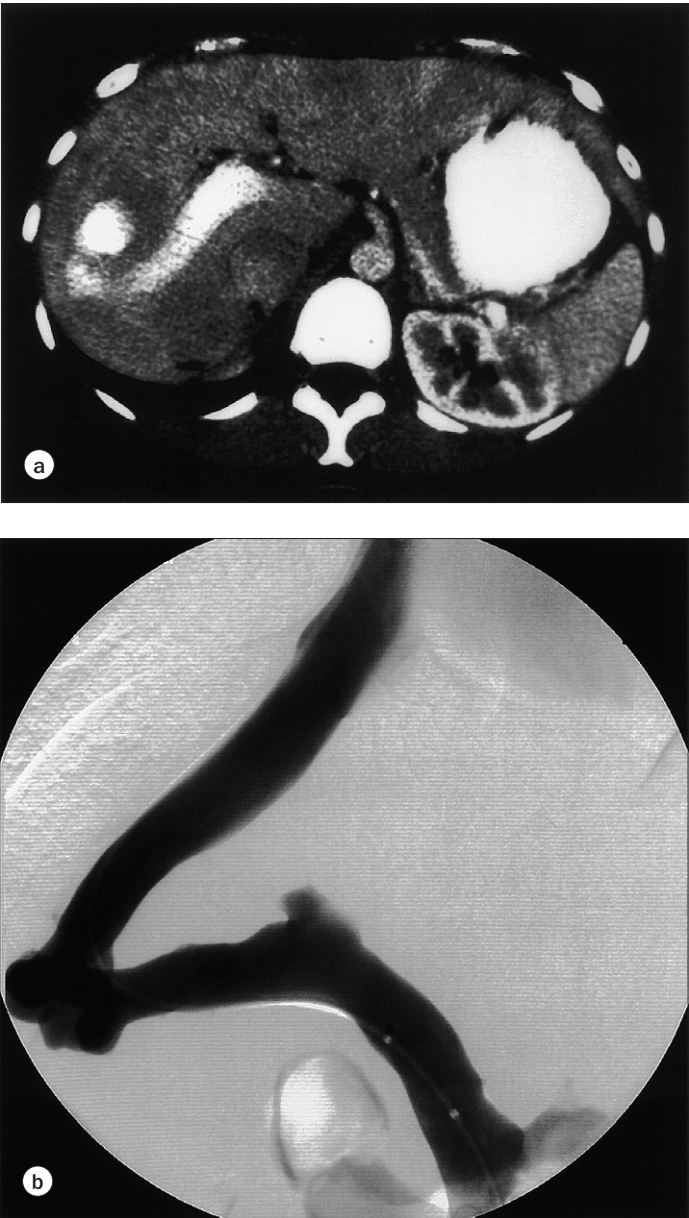
Hepatic encephalopathy can be broadly defined as the alterations in mental state and cognitive function that occur in liver failure. The course of both acute and chronic liver failure can be complicated by the development of neurologic dysfunction. In acute liver injury, encephalopathy portends a poor prognosis and death can occur as a result of neurologic complications. In patients with cirrhosis, encephalopathy is a cause of major morbidity, and when chronic, can be an incapacitating condition. The latter is especially problematic in subjects awaiting liver transplantation, in whom chronic hepatic encephalopathy is increasingly noted.

### CLASSIFICATION

A classification of hepatic encephalopathy should encompass the clinical setting in which symptoms occur. A recent consensus has been published which addresses three main clinical situations: acute liver failure, portal-systemic bypass and chronic liver failure (Table 11.1). Additional divisions within chronic liver failure in different clinical situations are also included.

Cases with isolated portal-systemic shunting are rare and are mainly due to congenital alterations of the portal circulation. A survey from Japan revealed more than 100 such cases, with variable clinical presentation, including some, where symptoms become manifest after the age of 50. A case of a portal vein–

hepatic vein connection is illustrated in Figure 11.1, in whom neuropsychologic testing and magnetic resonance (MR) spectroscopy of the brain shared the same features with the



**Figure 11.1** A case of “endogenous TIPS”. (a) Spiral abdominal computed tomography image demonstrating a massively enlarged portal vein (PV) running toward the hepatic vein (HV). (b) Portal venous system injection performed from the right transjugular approach. Portal venogram showing a direct connection from the PV and the HV. Reproduced from Crespin et al (2000) by permission of Blackwell.

Proposed nomenclature of hepatic encephalopathy			
HE type	Nomenclature	Subcategory	Subdivisions
A	Encephalopathy associated with acute liver failure		
B	Encephalopathy associated with portal-systemic bypass and no intrinsic liver disease		
C	Encephalopathy associated with cirrhosis and portal hypertension/or portal-systemic shunts	Episodic HE	Precipitated Spontaneous Recurrent
		Persistent HE	Mild Severe Treatment dependent
		Minimal HE	

**Table 11.1** Proposed nomenclature of hepatic encephalopathy.

brain of chronic liver disease. These cases highlight an important concept: development of portal-systemic shunts as a result of portal hypertension is a major contributor to encephalopathy in chronic liver disease.

While a decrease in functional hepatic mass also contributes to the process of encephalopathy, the term 'portal-systemic encephalopathy' is commonly used as a synonym for 'hepatic encephalopathy'. However, it should be best reserved for the variable disturbance of consciousness and impairment of intellectual function that result from extensive portal-systemic shunting or after a decompressive procedure for portal hypertension.

## NEUROPATHOLOGY

A striking feature of the examination of the brain of patients dying in hepatic coma is the paucity of anatomic findings. Microscopic studies show hyperplasia of astrocytes of the cerebral cortex, lenticular and dentate, diencephalic and other brainstem nuclei. Morphometric studies demonstrate enlargement in size and increase in the number of astrocyte nuclei. The presence of Alzheimer type II astrocytes is a common and characteristic feature of the neuropathologic examination (Fig. 11.2). The change is noted in brains fixed by immersion. In experimental models, where the brain is fixed via perfusion, astrocyte swelling can be seen. Loss of glial fibrillary acidic protein, a key component of intermediate filaments in astrocytes, accompanies such astrocyte swelling.

Astrocytes comprise approximately a third of the brain's cellular mass. They have small cellular bodies and extend their foot processes to contact adjacent neurons, capillaries and axonal processes. Viewed for some time as a simple anatomical support for the other structures, their varied physiologic importance has been increasingly recognized. They are involved in the control of the extracellular concentration of ions and neurotransmitters, providing a stable milieu for neuronal function. They are the

sole brain cell that contains glutamine synthetase, which under normal conditions catalyzes the use of ammonia to amidate glutamate for the formation of glutamine. The marked increase in brain glutamine seen in hepatic encephalopathy highlights the functional importance of astrocytes in the process that leads to alterations in mental state.

Neuronal involvement is rare. Cortical atrophy may represent the effects of other insults, including chronic alcohol consumption. A patchy laminar necrosis of the cerebral cortex can be seen in hepatocerebral degeneration, a condition that arises from long-standing portal-systemic shunting. However, the main pathology in this condition is centered on the striatum, where polymicrocavitation can be seen. This lesion is similar to the one observed in Wilson disease, and hence the term 'acquired hepatolenticular degeneration' that has also been coined for this condition. Demyelination is exceptional; long-standing portal-systemic shunting can be associated with spinal demyelination and the development of spastic paraparesia. In addition, patients with hepatic encephalopathy are at increased risk for central pontine myelinolysis.

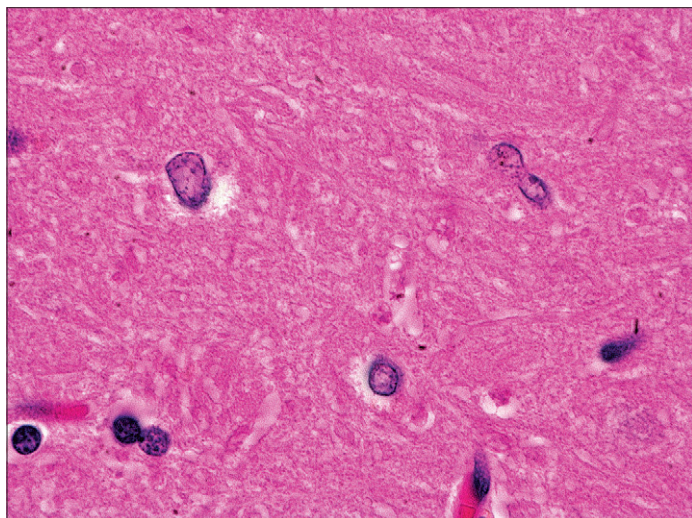
## PATHOGENESIS

An explanation for the mechanisms responsible for hepatic encephalopathy has been sought for many decades. Studies in humans are limited by the difficulties in studying brain neurochemistry, though newer techniques such as MR spectroscopy and positron-emission tomography have provided new insights. Work in experimental models of acute liver failure and portal-systemic shunting have also provided valuable insights.

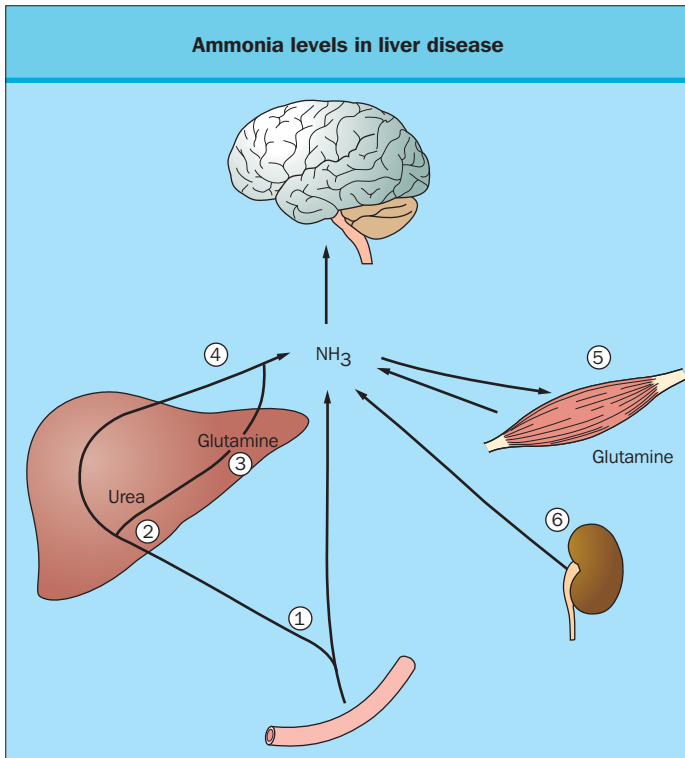
The search for the pathogenesis of hepatic encephalopathy has focused on deleterious neurotoxins that escape hepatic removal and then access the brain. Clinical clues point at the nature of such toxins: of intestinal origin, as witnessed by the aggravation of encephalopathy with constipation and the presence of portal-systemic shunts; nitrogenous in origin, as a large protein load can precipitate encephalopathy. Intestinal bacteria are related to the development of encephalopathy, as changes in the intestinal flora can improve an abnormal mental state in chronic liver disease. Finally, the toxin(s) must be metabolized by the liver and have a high first-pass extraction; its concentration in the hepatic effluent should be tightly regulated to prevent an altered mental function.

*Ammonia* meets all the above-mentioned criteria. In the splanchnic territory, ammonia originates from the action of glutaminase on glutamine in the mucosa of the small bowel, from the degradation of luminal protein and from the urease-containing activity of intestinal bacteria. In the liver, it is utilized for urea synthesis in periportal hepatocytes and for the formation of glutamine in last rim of perivenous hepatocytes. The dual metabolizing capacity of the liver allows for tight control of ammonia efflux. Indeed, portal venous ammonia levels are 10-fold higher than levels seen in the hepatic vein effluent.

Administration of  $^{13}\text{N}$ -labeled ammonia has allowed examination of the disposition of ammonia in humans. Conversion to glutamine in peripheral muscle accounts for approximately 50% of ammonia metabolism, followed by liver, kidney and to a much lesser extent (7%) brain. In the latter, uptake is a linear function of its arterial concentration and is highest in the cerebral cortex.



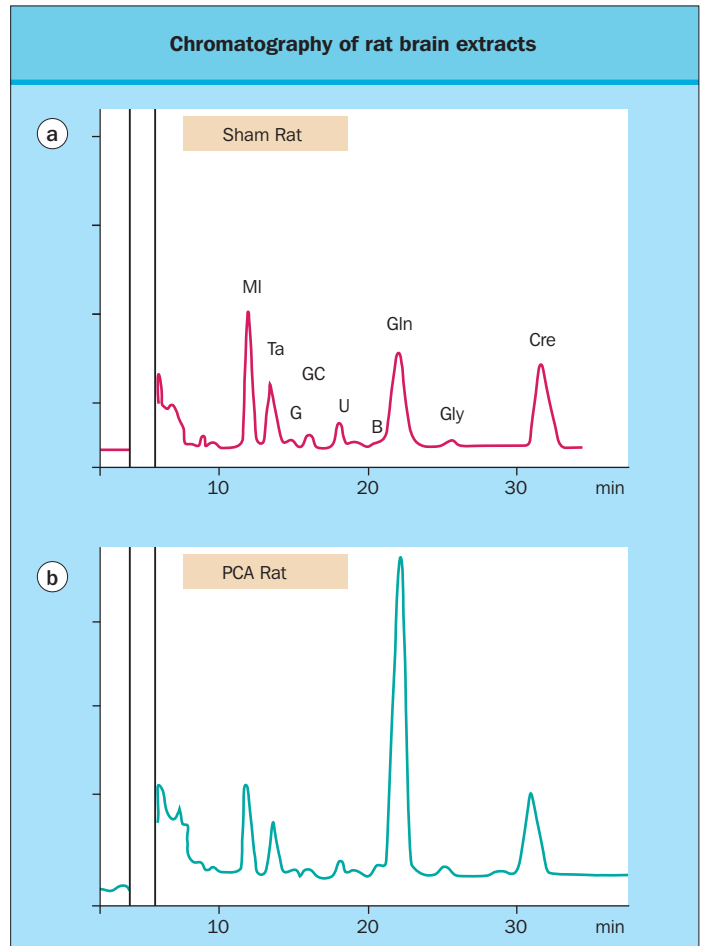
**Figure 11.2 The Alzheimer type II astrocyte.** This is characterized by an enlarged pale nucleus and is seen in patients with cirrhosis who die in hepatic coma. (Courtesy of Dr Ellen Bigio, Dept of Pathology, Northwestern University Feinberg School of Medicine.)



**Figure 11.3 Ammonia levels in liver disease.** Several factors combine to raise ammonia levels in liver disease. As the hepatic extraction of ammonia is high, > 0.9, portal-systemic shunting (1) will be a major factor. Hepatic metabolism of ammonia, via the formation of urea [periportal area (2)] or glutamine [perivenular hepatocyte (3)], may be impaired. Intrahepatic shunts (4) are present in cirrhosis. Metabolism of ammonia in muscle (5) becomes an important alternative pathway; loss of muscle mass may reduce the formation of glutamine. Renal vein ammonia (6) rises as a consequence of primary respiratory alkalosis. Reproduced from Kaplowitz (92) by permission of Lippincott, Williams & Wilkins.

In liver disease, the disposition of ammonia is affected at multiple levels (Fig. 11.3):

- Bowel motility is slower in established cirrhosis, a situation that favors the generation of ammonia in both the small and large bowel.
- Portal hypertension results in the development of extrahepatic and intrahepatic collaterals, shunting higher portal ammonia into the systemic circulation. A decrease in functional liver mass also results in a higher output of splanchnic ammonia to the systemic circulation, as seen in acute liver failure and in the later stages of the course of chronic liver disease. Portal-systemic shunting can by itself cause hepatic atrophy, a phenomenon well documented in the experimental animal but also present in human disease.
- In liver disease, muscle becomes an important site for ammonia detoxification, especially in acute liver failure. An increase in the activity of muscle glutamine synthetase can be seen in experimental models. As a result of this uptake, arterial levels are higher than corresponding venous samples, especially in acute liver failure. In cirrhosis, however, the arteriovenous difference in plasma ammonia is less predictable. Loss of muscle mass, seen with malnutrition and especially in patients with ascites, can adversely affect the ability to remove ammonia.



**Figure 11.4 Chromatography of rat brain extracts.** High performance liquid chromatography chromatograms of brain extracts from (a) a sham-operated rat and (b) a rat that underwent portacaval anastomosis (PCA). MI, myo-inositol; Ta, taurine; G, glycerophosphoethanolamine; U, urea; B, betaine; Gln, glutamine; Gly, glycine; Cre, creatine. Note the marked increase in the glutamine peak in the PCA animal. The sum of organic osmolytes is similar in both groups. Reproduced from Cordoba et al (96) by permission of Wiley-Liss, Inc.

- Ammonia is generated in the kidney for the production of titratable acidity. While it can be eliminated in the urine, the effectiveness of this route is limited in view of its reabsorption, favored in liver disease by the presence of respiratory alkalosis.
- Entry of gaseous ammonia ( $\text{NH}_3$ ) into the brain is thought to occur by diffusion, as the blood–brain barrier is viewed as impermeable to ionized  $\text{NH}_4^+$ . Recent studies suggest the presence of specialized  $\text{NH}_4^+$  channels. Brain glutamine levels markedly increase, as seen with MR spectroscopy. Multiple functional consequences arise within brain:
  - Swelling of astrocytes, driven by the osmotic effects of glutamine, affects their function at multiple levels, including the re-uptake of ions and neurotransmitters. Osmotic compensation is demonstrated by the reduction in the level of myoinositol, as seen in MR spectroscopy in humans and by direct measurements in the experimental animal (Fig. 11.4). Ammonia induces the mitochondrial permeability transition in isolated astrocytes; oxidative and nitrosative stress can also be detected in animal models.



- Increased transport of neutral amino acids across the blood–brain barrier, a process that allows extrusion of some of the increased glutamine from brain. In the case of tryptophan, this amino acid is a precursor of serotonin; an increased serotonergic tone may contribute to the symptomatology of hepatic encephalopathy.
- Inhibition of the activity of the Krebs cycle by decreasing the activity of  $\alpha$ -ketoglutarate dehydrogenase. This alteration can result in anaerobic glycolysis with an accumulation of brain lactate.
- Variable effects on cerebral perfusion.
- In neurons, ammonia can inhibit chloride extrusion from cortical neurons, affecting thus postsynaptic inhibitory potentials.

A wide range of disorders that affect mental state are associated with hyperammonemia, including diseases with hepatic microvesicular steatosis, such as Reye syndrome and sodium valproate toxicity, urea cycle enzyme defects and breakdown of urea by urease-producing bacteria colonizing intestinal urinary conduits. All share clinical features with the hyperammonemia of liver disease.

### Synergism

In the early 1970s, a number of other compounds arising from bacterial metabolism in the colon were shown to induce encephalopathy in experimental animals. Fatty acids (especially octanoic acid), phenols and mercaptans (derivatives of methionine) potentiate the neurotoxicity of ammonia. The mechanisms by which these toxins may induce encephalopathy is unclear; among proposed pathways are inhibition of mitochondrial electron transfer (mercaptans) and  $\text{Na}^+/\text{K}^+$  ATPase activity (octanoic acid).

### False neurotransmitter hypothesis

This theory was proposed in the late 1970s. Weak neurotransmitters, such as octopamine and phenylethanolamine, would replace established neurotransmitters such as dopamine and noradrenaline. The source of these neurotransmitters could be twofold:

- Octopamine can be generated from the activity of intestinal bacteria on protein. Increased plasma levels and facilitated entry into the brain would increase cerebral levels of this false neurotransmitter.
- They could also be generated within the brain from aromatic amino acid precursors. An abnormal plasma amino acid pattern is seen in liver disease, with elevated plasma aromatic amino acids (as a result of decreased hepatic uptake) and a reduction in branched-chain amino acids (the consequence, in part, of peripheral insulin resistance). Entry of aromatic amino acids into the brain is favored via exchange with brain glutamine.

The importance of this mechanism in the development of encephalopathy appears to have lessened in light of the overall lack of significant benefit from the use of therapeutic branched-chain amino acid solutions.

### GABAergic neurotransmission

GABA ( $\gamma$ -aminobutyric acid), generated in the brain from the decarboxylation of glutamate, is the main brain inhibitory neurotransmitter. Ligation of GABA to its receptor increases

the permeability to chloride, resulting in hyperpolarization and a decrease in neurotransmission. In 1982, intestinally generated GABA was proposed as a mediator of encephalopathy. In view of the lack of conclusive evidence for an effect of GABA itself, 6 years later emphasis was made on a role for endogenous benzodiazepine ligands. Binding of benzodiazepine to its receptor facilitates GABA effects on its own receptor, hence promoting GABAergic neurotransmission.

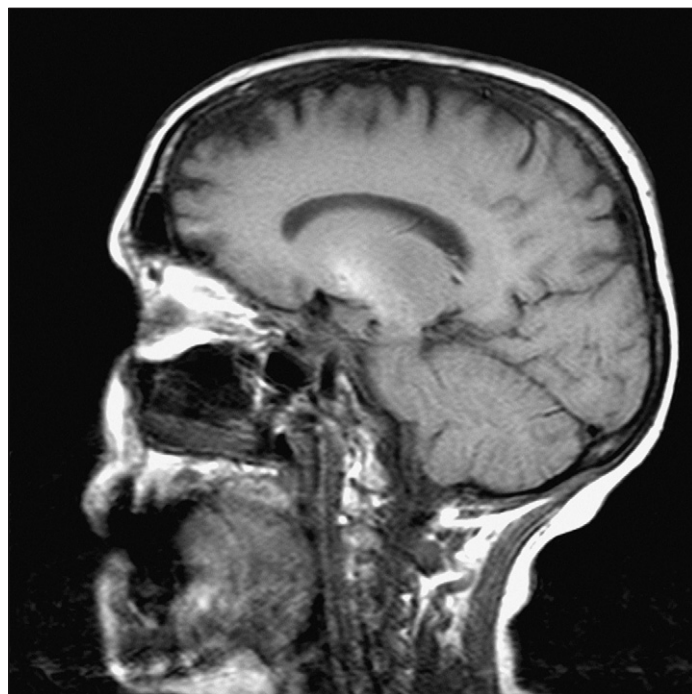
Now, the importance of this hypothesis has decreased. The nature of the endogenous benzodiazepine ligand remains obscure. A therapeutic experience with flumazenil, a benzodiazepine receptor antagonist, has not been clinically convincing. Moreover, direct effects of ammonia on GABAergic neurotransmission may exist.

### Infection

Increasingly, evidence is mounting that the presence of infection plays an important role in the development of hepatic encephalopathy. In acute liver failure, the development of the systemic inflammatory response and/or the presence of infection via positive cultures is associated with worsening of the mental state. In patients with cirrhosis, resolution of infection will improve cognitive function in spite of similar ammonia levels. The role of circulating cytokines may be of special importance, as they can result in intracerebral signals via different pathways. This is an area of active research at this time.

### Manganese

Neuropathologic changes in the basal ganglia of patients with cirrhosis have been highlighted. Radiologically, a hyper-resonant globus pallidus is a frequent finding in patients with cirrhosis and portal-systemic shunting (Fig. 11.5). Measurements in human brain tissue have shown elevated manganese levels in basal ganglia.



**Figure 11.5 Sagittal plane: T<sub>1</sub>-weighted image (500/18).** The image shows increased intensity in the globus pallidus. Reproduced from Crespin et al (00) by permission of Blackwell.

Manganese intoxication can result in loss of dopamine receptors in this area and development of Parkinsonian features. In liver disease, manganese accumulates as a result of both portal-systemic shunting and decreased biliary excretion. An explanation for the selective deposition of manganese in subcortical nuclei is not forthcoming at this time. The impact of its removal has not been assessed clinically, in view of the lack of a specific chelator.

## CLINICAL MANIFESTATIONS

### Encephalopathy in acute liver failure

Patients with acute liver failure may develop changes in mental state within 1 week (hyperacute), 1 month (acute) or up to 6 months after the development of jaundice (subacute). Although patients can quickly evolve from a state of somnolence to coma, excitation and mania can be seen in the early stages of evolution, a symptom seldom seen in chronic encephalopathy. The Glasgow Coma Scale may be useful to monitor the evolution of patients with severe encephalopathy.

Brain edema is a unique complication seen in patients with acute liver failure and severe encephalopathy. It is not the result of a generalized breakdown of the blood–brain barrier, as seen in vasogenic edema. Rather, cytotoxic mechanisms predominate, with predominant swelling of the gray matter. Brain edema can be reproduced in animal models of acute liver failure, where a complex pathogenesis has emerged. It includes osmotic alterations in the brain, triggered by the accumulation of glutamine in astrocytes, a metabolic effect, with an increase in brain lactate, as well as hemodynamic changes, namely cerebral hyperemia. The increase in cerebral blood flow, also seen in humans with the disease, may be the result of intracerebral vasodilatory signals, whose nature has not been yet identified.

Brain edema can also be reproduced in the experimental animal after the administration of ammonia. Clinically, arterial ammonia levels above 200  $\mu\text{mol/L}$  have been associated with cerebral herniation. Such hyperammonemia in urea cycle enzyme defects is also associated with brain edema and when uncontrolled, can result in death from cerebral herniation. Rather than ammonia itself, it is the product of its metabolism, glutamine, which has emerged as the key factor in the pathogenic sequence that leads to brain swelling.

A rise in intracranial pressure can be clinically silent or result in changes in oculovestibular reflexes and decerebrate posturing. Myoclonic seizures have been reported. Abnormalities in pupillary reflexes may herald the development of temporal lobe herniation.

### Precipitant-induced encephalopathy in cirrhosis

This presentation is the most commonly seen form of encephalopathy in clinical practice. A wide range of precipitating events may be seen (Table 11.2). Many of them result in an increased ammonia load. However, other factors do not implicate such a load. For example, the mechanisms by which infection triggers encephalopathy are complex, and include hemodynamic and metabolic effects. The brain in liver disease is more susceptible to the effects of sedatives and caution should be exercised in their use.

Early stages of encephalopathy may be subtle. Family members note a change in personality, with dull speech and a sullen

#### Precipitating factors in hepatic encephalopathy

Gastrointestinal bleeding
Uremia, dehydration
Sedatives
Dietary protein
Infection
Constipation
Hypokalemia, hyponatremia

Table 11.2 Precipitating factors in hepatic encephalopathy.

#### Wake/sleep patterns in liver patients

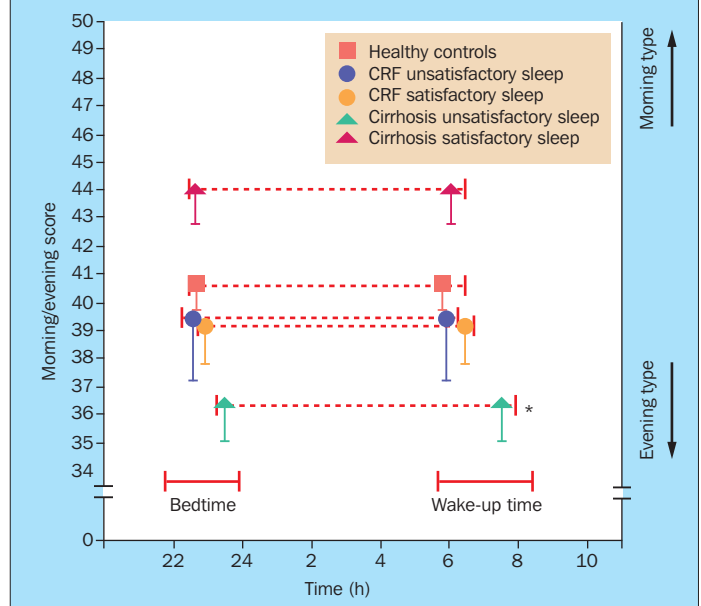


Figure 11.6 Wake/sleep patterns in liver patients. Bedtime and wake-up time of patients with cirrhosis, chronic renal failure (CRF) (grouped by sleep complaints), and healthy controls are plotted against the values of Horne-Osberg questionnaire (chronotypology profile). Results expressed as means  $\pm$  SE. \*Cirrhosis patients with unsatisfactory sleep show a delayed bedtime, a delayed wake-up time, and a lower score (evening type) in this questionnaire compared with their satisfactory sleep counterparts ( $P < 0.05$ ). Reproduced from Cordoba et al (98) by permission of Wiley-Liss, Inc.

attitude. The ability to perform mental and physical tasks decreases and assessment by a close relative provides better information than the one offered by the patient. Errors in motor activity reflect difficulties in recognizing the shape and position of objects. Altered sleep–wake patterns are characterized by difficult or late onset of sleep at night with sleepiness during the day (Fig. 11.6).

Overt encephalopathy has been classified in four stages: I, subtle changes; II, lethargy, confusion; III, stupor; IV, coma. Several physical signs can be detected during this evolution.

- Asterixis represents the failure of muscle groups to maintain their posture. It is traditionally elicited by asking patients to outstretch and fix their forearms while retroflexing their wrists; a 'flap' reflects a sudden forward movement of the wrist. It can be elicited from other muscle groups, such as the tongue, or in debilitated patients by requesting the examiner's fingers be squeezed. As it requires voluntary cooperation, it can not be elicited in advanced stages of encephalopathy. Asterixis is not a specific sign, as it can be seen in other metabolic encephalopathies, such as uremia and hypercapnia, as well as in the intoxication from certain drugs.
- Fotor hepaticus is difficult to describe: a pungent odor can be detected in the exhaled breath of such patients. Volatile sulfur-containing compounds, such as mercaptans, may account for this finding.
- Long-tract neurologic signs can be confusing, especially at the time of the first encounter. Unilateral hyper-reflexia, Babinski sign and ankle clonus can lead to an erroneous diagnosis. The rapid appearance and disappearance of such long-tract signs argues against a focal intracerebral event.

### Diagnosis

A search for the precipitating event should be initiated. Evidence for blood in the stool, infected ascitic fluid or orthostatic changes in vital signs are clues to the nature of the precipitant. Standard laboratory testing is critical to detect uremia and hypokalemia. Respiratory alkalosis is common, with patients hyperventilating as a result of toxin-induced stimulation of the respiratory center.

While measurement of plasma ammonia is preferentially obtained from arterial blood, venous samples can also provide the needed information: a clue to the origin of an unknown encephalopathy. Once the diagnosis has been established, repeat measurements of ammonia do not provide additional information to that furnished by an evaluation of mental state and should be discouraged. Technical aspects of measurement are important. Samples should be promptly assayed and the laboratory should be familiar with possible sources of contamination.

The electroencephalogram (EEG) shows characteristic changes. The normal background of 9–12 cycles/s is replaced by slower waves, including theta waves (seen in earlier stages of encephalopathy), triphasic waves and  $\delta$  waves (two to three cycles/s). Frequency analysis may provide an integrated assessment of EEG abnormalities. Other neurophysiologically-based tests include visual and auditory evoked potentials. Recently, the critical flicker frequency test has been shown to discriminate patients with lesser degrees of encephalopathy.

A differential diagnosis arises in patients with alcoholic cirrhosis. Alcohol withdrawal is characterized by agitation and tremor, but not asterixis. Wernicke encephalopathy is suggested by the presence of a disconjugate gaze in the setting of a confusional state. Exclusion of chronic meningeal infection (tuberculosis, fungi) and subdural hematomas may require lumbar puncture and radiologic examination of the brain. Brain atrophy is common in cirrhosis, but this is a non-specific finding.

### Persistent encephalopathy

This can be mild, severe or treatment-dependent, as noted in Table 11.1. It carries a poor prognosis, as recognized in the classic Child criteria. It may be the result of poor hepatic function, in

which case appropriate candidates should be considered for liver transplantation. Severe encephalopathy may also be seen in patients with acute-on-chronic liver failure, where short-term prognosis is poor and transplantation can be problematic due to the presence of multi-organ failure.

Chronic encephalopathy may also reflect, in part, the presence of a large spontaneous portal-systemic collateral, most often a splenorenal communication. A clinical clue to the latter is a patient with chronic encephalopathy and relative preservation of liver function. Encephalopathy can also arise from the construction of a portal-systemic shunt, either radiologically [in the case of transjugular intrahepatic portal-systemic shunt (TIPS)] or surgically. The latter is less frequently performed nowadays, but performance of a selective distal splenorenal shunt may reduce the incidence of post-surgical encephalopathy. TIPS, which represents non-selective portal decompression, results in encephalopathy in 30–40% of individuals, with severe symptoms in <5% of subjects. Such patients may require radiologic occlusion of the shunt.

Hepatocerebral degeneration describes an entity appearing many years after a surgical portacaval anastomosis or extensive spontaneous shunting in which varying degrees of tremor, ataxia, choreoathetosis and disturbances of gait are noted. The mood can become jovial, inappropriate to the limitations of lifestyle imposed by the neurologic picture. Varying degrees of dementia may be observed. Spastic paraparesis has also been described.

### Minimal encephalopathy

Patients with cirrhosis may appear intellectually appropriate, exhibit a normal neurologic examination and yet have evidence of cognitive defects elicited by neuropsychologic testing. Patients likely to have such changes exhibit some degree of hepatic functional impairment (Child class B) and have esophageal varices. An elaborate psychologic evaluation indicates a predominant alteration in attention. A psychometric hepatic encephalopathy score has been proposed using five tests, including the Reitan trail-making test parts A and B, digit symbol test, line drawing test and serial dotting test.

The impact of minimal encephalopathy on daily activities has been shown to affect selected areas of function: sleep, work capacity and recreation. A recent study describes impairment in the ability to drive an automobile. In the absence of clear guidelines on the matter, it may be prudent to have patients at risk, especially those with a prior history of encephalopathy, to undergo a neuropsychologic evaluation and therapy instituted if results are abnormal.

## THERAPY OF HEPATIC ENCEPHALOPATHY

There are basically three strategies for therapy:

- control of precipitating factors;
- changes in the nature of dietary protein;
- removal of nitrogenous products, mainly through effects on the intestine.

### Control of precipitating factors

Control of the precipitating factor may be the single most important therapeutic measure in patients with acceptable liver function. Correction of diuretic-induced hypokalemia and/or dehydration will improve the encephalopathic picture. Systemic



treatment of infection with antibiotics may also affect the gut flora. Reversal of encephalopathy due to narcotics and sedatives may be possible with specific antagonists. Prevention of encephalopathy after upper gastrointestinal bleeding can be accomplished with vigorous bowel cleansing.

### **Diet**

While animal-based protein is implicated in the precipitation of encephalopathy, dietary protein restriction should be abandoned. Patients with cirrhosis are hypercatabolic and require a higher dietary protein content (1–1.5 g/kg). Vegetable-based protein will have a cathartic effect and result in decreased ammoniogenesis. A casein-based diet is also beneficial.

Many studies have examined potential benefits of oral and intravenous formulations of branched-chain amino acids. Normalization of the abnormal ratio of aromatic/branched-chain amino acids is difficult to achieve. Numerous trials in different clinical situations have yielded variable results; meta-analysis has been complicated by marked differences in study design. The product is expensive and the benefit over standard measures may only be marginal. It is reserved for patients intolerant to the minimum amount of required protein.

### **Removal of nitrogenous products from the intestine**

#### **Use of non-absorbable carbohydrates**

Non-absorbable disaccharides, lactulose and lactitol, reach the colon as they are not metabolized in the small bowel. Once in the colon, these disaccharides are metabolized, mainly by anaerobic bacteria, to acetic and lactic acid, resulting in colonic acidification. Such changes in pH favor the passage of ammonia into the intestinal lumen where it is used by bacteria as an energy source. A cathartic effect ensues, contributing to the elimination of nitrogenous products.

A recent analysis of the evidence supporting the use of such disaccharides has pointed at the weak support for their use. However, fault may also lie in the design of the original studies, as precipitant-induced encephalopathy was included and mental state in this situation may improve simply by removing the precipitating factor. There is a body of evidence indicating several mechanisms by which such drugs may help encephalopathy, supporting their continued use, especially in chronic encephalopathy.

Doses of lactulose range from 15 to 30 mL, two to four times a day. The goal is to obtain two to three soft bowel movements a day. Improvement of encephalopathy is not immediate; patients may require 48–72 h before a marked change occurs. Administration of lactulose enemas (300 mL in a total of 1 L volume) may be useful to hasten the process in comatose patients. In areas of the world with a high prevalence of lactose malabsorption, this disaccharide may be used for oral treatment, at a considerably lower cost.

If diarrhea develops, the drug should be stopped to be re-initiated later. Patients may complain of flatulence and cramping and the sweet syrupy taste of lactulose may not be palatable to all patients. Persistence of diarrhea may result in excessive loss of hypotonic colonic water. This leads to hypernatremia, with changes in mental state associated with hyperosmolarity. Lack of awareness of this possibility may result in an inappropriate increase in lactulose dosage.

Lactitol, a more palatable non-absorbable disaccharide, has been tested with success in Europe; another benefit over lactulose appears to be its quicker onset of action.

### **Antibiotics**

A reduction of aerobic, urease-splitting bacterial flora is the rationale for the use of neomycin. Such treatment has also been recently criticized for the lack of supportive evidence in clinical trials, in consonance with criticisms on non-absorbable disaccharides. Clinical experience suggests that 2–4 g/day in divided doses may be effective. A minimal amount is absorbed, and long-term use requires monitoring of renal and auditory function. The combination of lactulose and neomycin is effective in reducing ammonia levels; although neomycin reduces the aerobic intestinal flora, lactulose is mainly split by *Bacteroides fragilis* and other anaerobes.

In patients with renal dysfunction, other oral antibiotics such as vancomycin may be used; metronidazole has been postulated to be as effective as neomycin. As hepatic metabolism of metronidazole is impaired, doses should be reduced and started at 250 mg twice daily. A recent study compared the effects of rifaximin, a non-absorbable antibiotic, and lactulose in the management of precipitant-induced encephalopathy. Similar efficacy was seen in both groups.

### **Removal of nitrogenous products by other organs**

In children with urea cycle enzyme deficiencies, alternative pathways for hepatic nitrogen excretion have been delineated. These consist of the administration of a drug (sodium benzoate or sodium phenylbutyrate), conjugation with an amino acid (glycine or glutamine) and excretion in the urine (as hippurate or phenylacetylglutamine). A trial with sodium benzoate in cirrhosis showed equivalence with lactulose in precipitant-induced encephalopathy.

Ornithine-aspartate has been used outside the USA to treat hepatic encephalopathy. Transamination of these amino acids can increase the availability of glutamate and enhance the production of glutamine in muscle. Provision of ornithine can also improve rates of urea synthesis. Zinc, a cofactor of all five enzymatic reactions in the urea cycle, can also be administered (as acetate or sulfate). Improvement in encephalopathy is especially noted in patients with malnutrition.

### **Treatment of chronic intractable encephalopathy**

Patients with chronic encephalopathy pose major problems in management. Once the previously discussed measures have been exhausted, four therapeutic possibilities arise:

- Use of bromocriptine, a dopamine antagonist, has yielded conflicting results in patients with chronic encephalopathy. Rationale for its use centers on the 'false neurotransmitter' hypothesis, with correction of supposed deficiency of dopaminergic neurotransmission. Doses of up to 15 mg/day may be tried before attempting other alternatives.
- A portacaval shunt may be either occluded with the use of a balloon technique or closed at surgery, with a simultaneous esophageal transection to prevent variceal rebleeding. Radiologic occlusion of a TIPS catheter may improve intractable encephalopathy.

- In a suitable candidate, liver transplantation may offer a radical solution. Recent reports document clear improvement in a few patients with hepatocerebral degeneration after this procedure, patients who had a short duration of their chronic symptomatology. However, poor outcomes may also be possible and more definitive data are needed.
- An abdominal colectomy, a measure of last resort, may provide relief in patients in whom other treatments are not possible. This procedure is rarely employed, as major abdominal surgery is by itself riskier in patients with liver failure.

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## CONCLUSION

Hepatic encephalopathy develops in patients with liver failure. It carries a poor prognosis in both acute and chronic liver disease. Recognition of precipitating events is critical for its management. There is a lack of specific medications directed at the abnormal brain neurochemistry. Rather, most of the current available therapies are directed at improving ammonia removal in the gut, muscle, kidney and/or liver.

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## SECTION III: CLINICAL MANIFESTATIONS OF LIVER DISEASE

### CHAPTER

# 12

## Malnutrition in Cirrhosis

Arthur J. McCullough

### INTRODUCTION

The liver is primarily a metabolic organ that orchestrates a complex array of physiologic and biochemical processes, including the regulation of protein and energy metabolism (PEM). Consequently, it is not surprising that patients with advanced liver disease commonly have malnutrition. Increasing information suggests that PEM itself may accelerate deteriorating liver function and adversely affects clinical outcome.

However, the nutritional management of patients with liver disease remains a much discussed but controversial area of clinical hepatology. At a strategic level, there can be little doubt that adequate nutritional sustenance is a fundamental component to the survival and sense of well-being in these patients. However, the logistics of identifying patients in need of nutritional therapy as well as choosing the type, duration and quantity of nutritional supplements remain elusive and ill defined. In addition, questions persist regarding the cost-effectiveness of nutritional therapy, its effect on quality of life, and the definition of the appropriate goals and outcomes to be achieved from such therapy in patients.

The importance of this concept has led to the development of guidelines for the nutritional management of patients with liver disease. The rationale for aggressive nutritional therapy in these patients is based on promising, but nascent clinical information and a number of nutritional axioms relative to chronic liver disease. First, malnutrition is common, but often underdiagnosed. Second, these patients have alterations in energy metabolism and nutritional status that mimic starvation. Third, many of the complications associated with chronic liver disease (such as hepatic encephalopathy, ascites and sepsis) occur *pari passu* with a negative nitrogen state, such as occurs before and immediately after liver transplantation. Finally, there is the expectation that correction of malnutrition may improve the clinical outcome of these patients.

Most clinicians employ a common sense approach to feeding patients with liver disease, whereas some investigators advocate the aggressive use of nutritional supplements and specialized formulations. The aim of this more aggressive approach is to correct pre-existing PEM and to stimulate hepatic regeneration. Aggressive nutritional therapy may, in fact, benefit certain types of patients with liver disease and emphasize the need for a greater understanding of the etiologic factors responsible for PEM so that the specific role of nutrition in the diagnosis, prognosis and therapy of chronic liver disease can be improved and better defined.

### DEFINITION

Protein energy malnutrition can be defined as the loss of protein stores (both visceral and muscle/somatic) and fat mass. Two forms are described: marasmus (combined protein energy malnutrition) and kwashiorkor (hypoalbuminemic) malnutrition. The former is an insufficient amount of both protein and calories resulting in the loss of muscle and fat mass with relative sparing of visceral proteins, such as albumin and fibrinogen. The latter is an insufficient amount of predominantly protein that results in diminished protein stores that occurs to a greater extent in visceral proteins as compared to muscle proteins.

In addition to these more classical forms of malnutrition it is also important to realize that over nutrition in the form of obesity is occurring more frequently in patients with liver disease. Obesity – defined as a body mass index (BMI)  $\geq 30$  – poses specific and important issues regarding the nutritional management of these patients, as well as a potential etiologic factor for the development of advanced liver disease *per se* and contributes to the development of cirrhosis in patients with liver injury from alcohol and/or hepatitis C.

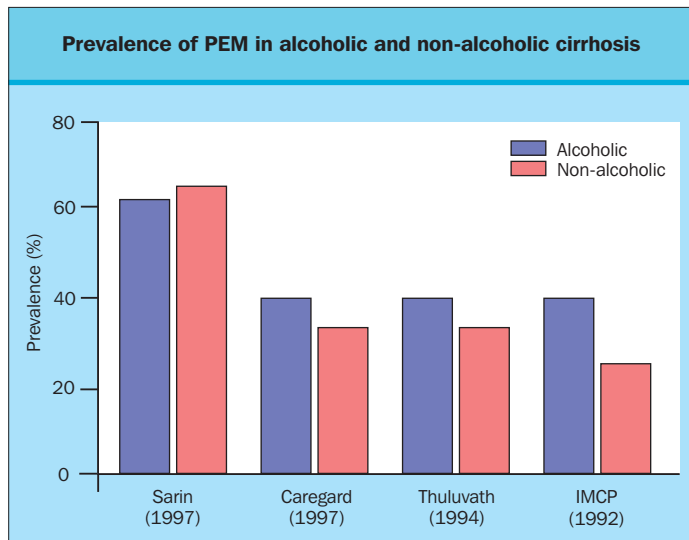
### PREVALENCE

#### Protein energy malnutrition

PEM is uncommon in the precirrhotic stages of liver disease, except in the setting of extrahepatic biliary obstruction. The prevalence of PEM in cirrhosis ranges between 10 and 100% and may correlate with the severity of disease defined by the CTP (Child-Turcotte-Pugh) - score. In one study, PEM was reported to be present in 20% of patients with compensated cirrhosis (Child's A) and in 50–60% of patients with decompensated cirrhosis (Child's C). The large range in reported prevalence rate may depend on how nutritional assessment is performed.

Although most of the earlier studies concentrated on patients with alcoholic liver disease (which reported weight loss, nausea and vomiting to occur in 60%, 55% and 87% of such patients, respectively), the prevalence of PEM is high in all forms of cirrhosis. **Figure 12.1** shows four studies that have measured nutritional status in both alcoholic and non-alcoholic cirrhosis. These studies ascertain that the prevalence of PEM is high and almost identical in these two different types of cirrhosis. Based on anthropometric measurements of the lowest fifth percentile of a control population, the prevalence of PEM varied between 34–62% and 27–67% in these studies for alcoholic and non-alcoholic cirrhosis, respectively. There is also a high prevalence





**Figure 12.1** Prevalence of protein and energy malnutrition in alcoholic and non-alcoholic cirrhosis. This is shown for four available studies.

of PEM in patients awaiting liver transplantation with estimates ranging between 18 and 100% with the two most detailed studies showing that moderate or severe PEM occurred in 70–80% of these patients. As listed in **Table 12.1**, disease-specific patterns of PEM have also been shown in patients with cirrhosis awaiting liver transplantation. Therefore, clinicians need to be aware that PEM is very common in these patients, especially because it has prognostic significance.

### Obesity

The prevalence of obesity is approximately 30% of the adult population in the USA and its prevalence is increasing at an alarming rate worldwide. Obesity is associated with non-alcoholic fatty liver disease (NAFLD), which is a spectrum of histologic liver disease ranging from steatosis alone to its most severe form – non-alcoholic steatohepatitis (NASH). Obesity has also been reported as a risk factor for the development of cirrhosis in alcoholic and viral related liver disease.

Although the prevalence of obesity is unknown in patients with liver disease, obesity has been evaluated in patients awaiting transplantation. While the definition of obesity differed among the studies, it appears that moderate or severe obesity is present in 7–32% of patients at the time of transplantation.

## PATHOPHYSIOLOGY OF MALNUTRITION

There are multiple factors (as listed in **Table 12.2**) involved in the etiology of malnutrition which accelerates significantly after the development of cirrhosis and portosystemic shunting. Many of the factors can be treated successfully when nutritional status is recognized and treated as a priority.

### Nutrient intake

Inadequate dietary intake has been described in 27–87% of patients with both alcoholic and non-alcoholic liver disease has been attributed to anorexia, nausea, early satiety (especially in the presence of ascites) dysgeusia (possibly related to trace metal

Pattern of malnutrition in chronic liver disease			
	Muscle wasting	Loss of fat stores	Synthetic function
Alcohol	+++	+	++
Viral	++	++	+
Primary biliary cirrhosis	+++	+++	+
Primary sclerosing cholangitis	++	+	+
+, ++, +++, mild, moderate, or severe abnormalities respectively.			

**Table 12.1** Pattern of malnutrition in chronic liver disease.

and/or vitamin deficiency) and unpalatable sodium and protein restricted diets. In hospitalized patients, the deficient intake is often iatrogenic and related to diagnostic and therapeutic procedures. The importance of these observations are emphasized by recent work which demonstrated that a regular oral diet can improve PEM in cirrhotic patients.

### Absorption

Some studies also have demonstrated PEM in cirrhotics despite normal dietary intake. Therefore, other factors must be involved.

Although malabsorption is not usually considered a major cause of PEM in cirrhosis, moderate steatorrhea (<12 g daily) occurs in both cholestatic and non-cholestatic liver disease. It has been reported to occur in as many as 40% of cirrhotic patients and may exceed 30 g daily in 10% of cirrhotic patients. This malabsorption has been attributed to decreased intestinal bile acids, pancreatic insufficiency and a diminished capacity for long chain fatty acid absorption. These abnormalities are more severe in cholestatic liver disease. Celiac sprue occurs more frequently in primary biliary cirrhosis; while inflammatory bowel disease and exocrine pancreatic abnormalities are more common in primary sclerosing cholangitis. The use of oral lactulose or neomycin for hepatic encephalopathy may also exacerbate nutrient malabsorption in all forms of liver disease.

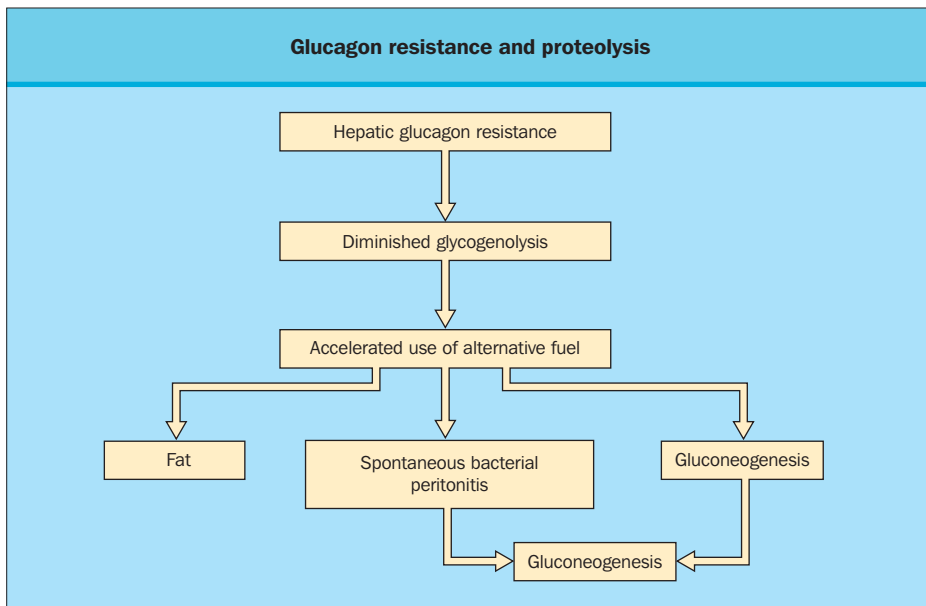
### Alcohol

Although alcohol is recognized as a direct hepatotoxin, it also has profound effects on protein and energy metabolism as recently reviewed elsewhere. From a practical approach, these adverse effects can be avoided by abstinence from alcohol use.

### Protein metabolism

Underlying pathophysiologic factors cause loss in both fat and protein stores. After an overnight fast, cirrhotics have an increased rate of protein breakdown that is not suppressed normally in response to feeding. Additionally, because of alterations in energy metabolism, suggested to be due to glucagon resistance and diminished glycogenolysis (**Fig. 12.2**), amino acids are oxidized for energy, especially the ketogenic amino acid leucine. This irreversible loss of nitrogen is also associated with an increased rate of gluconeogenesis. Recent evidence also suggests that

Etiologic factors of malnutrition in cirrhosis		
Non-hospitalized patients	Inadequate diet*	Quantitative iatrogenic – (protein, fluid/salt restriction)
	Malabsorption*	Pancreatic and bile salt deficiency Enteropathy
	Anorexia, nausea and vomiting	
	Effects if alcohol toxicity on energy and protein	
	Altered protein and energy metabolism	Amino acid oxidation Accelerated fat oxidation and gluconeogenesis Increased protein breakdown Decreased protein synthesis
Hospitalized patients	Fasting status*	Diagnostic testing Gastrointestinal bleeding Altered neurologic status
	Purgation and neomycin toxicity*	
	Unpalatable diets*	
	Stressful complications*	
*Indicates factors that can be currently altered or treated		

**Table 12.2 Etiologic factors of malnutrition in cirrhosis.****Figure 12.2 Hepatic glucose production in cirrhosis.** A hypothesis that decreased hepatic glucose production caused by hepatic glucagon resistance leads to the use of alternative fuels and increased proteolysis in cirrhosis.

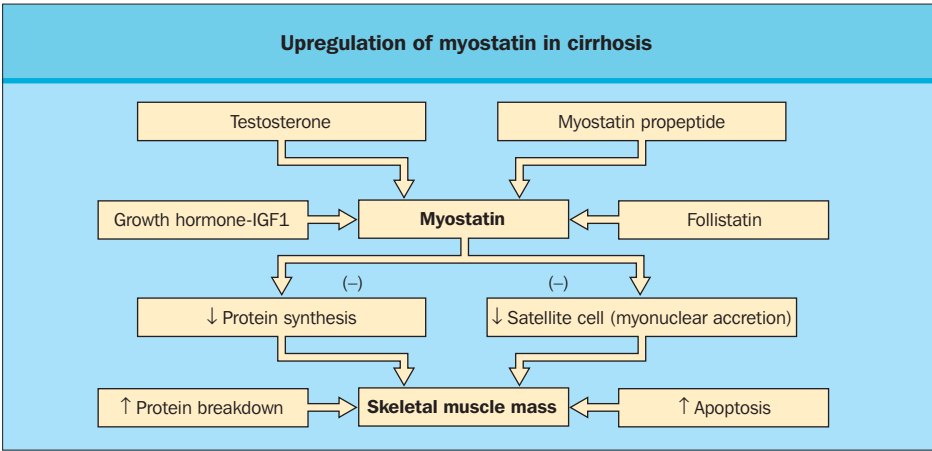
increased myostatin and decreased insulin like growth factor (which stimulates muscle protein synthesis) may also cause skeletal muscle atrophy in patients with cirrhosis (**Fig. 12.3**).

Regardless of the cause of the altered protein status of cirrhotic patients, their protein requirements are increased. According to recommended dietary allowance (RDA) recommendations, healthy individuals have an average requirement of 0.6 g/kg/day. Allowing for two standard deviations and suboptimal composition of dietary protein in a mixed diet, the RDA recommendation is

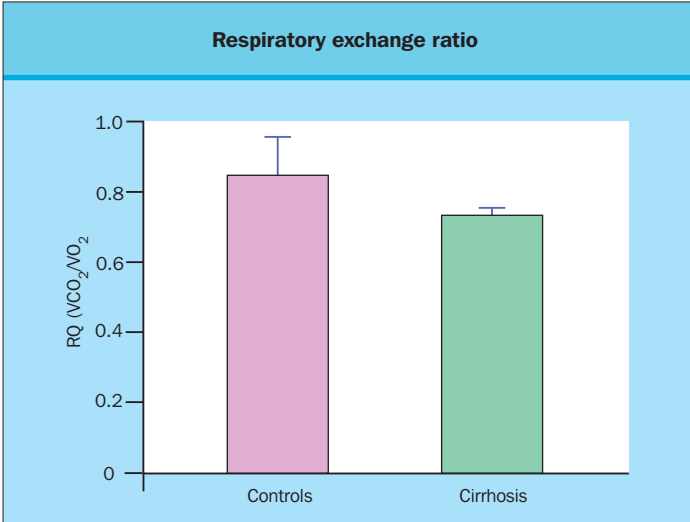
for 0.8 g/kg/day on a mixed diet. When adding the two standard deviations, this recommendation is increased to 1.2–1.3 g/kg/day in cirrhosis, based on an average need of 0.8 g and a standard deviation of 0.2 g.

### Energy

Although highly variable, when corrected for lean body mass, resting energy expenditure has been shown to be increased in cirrhotics. When measured energy expenditure is compared with



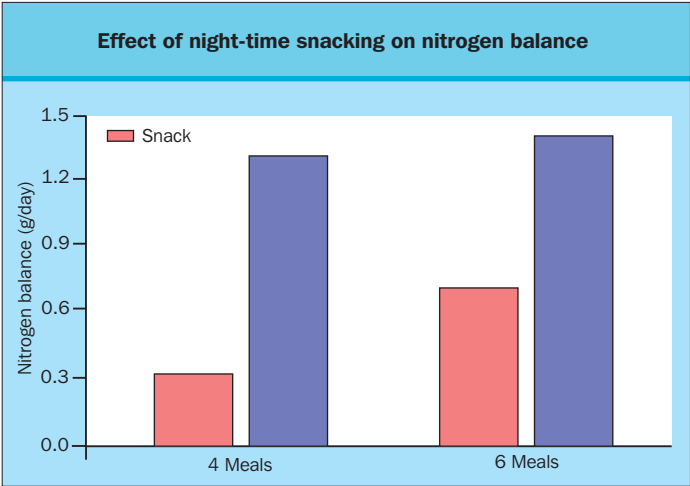
**Figure 12.3 Upregulation of myostatin in cirrhosis.** Myostatin is a newly described protein that is likely to be upregulated in cirrhosis (based on animal studies). This protein suppresses skeletal muscle synthesis by multiple mechanisms.



**Figure 12.4 Respiratory exchange ratio.** The respiratory exchange ratio [referred to as the respiratory quotient (RQ)] is shown as the aggregated data from 7 published trials for controls and patients with cirrhosis.  $\text{VCO}_2$ ,  $\text{CO}_2$  production;  $\text{VO}_2$ , oxygen consumption.

predicted energy expenditure, 30%, 20% and 50% of cirrhotics have high, low, or normal metabolic rates. Patients with hyper-metabolism have increased rates of morbidity and mortality after liver transplantation. Therefore, a clinician can tailor specific energy requirements for individual patients using simple calculations.

Regardless of the rate of energy expenditure, the preferred fuel substrate is clearly altered in cirrhosis. Cirrhotics have a decrease in the respiratory quotient, which is the rate of carbon dioxide production divided by the rate of oxygen consumption. Cirrhotics obtain 75% of their calories from fat after an overnight fast compared with 35% for controls, who would take 48–72 h of starvation to obtain the low respiratory quotient levels that cirrhotics obtain after only 12–18 h (Fig. 12.4). In addition, amino acids are oxidized for energy and converted to glucose via gluconeogenesis. Therefore, cirrhosis should be considered a disease of accelerated starvation with early recruitment of alternative fuels. Consequently, food should not be withheld



**Figure 12.5 The effect of a night-time snack on nitrogen balance.**

from cirrhotic patients for any extended period, and frequent enteral feedings need to be encouraged. Cirrhotic patients given an evening snack to supply energy during the sleeping hours have been shown to maintain greater positive nitrogen balance (Fig. 12.5).

### PROGNOSIS

Table 12.3 lists results from a number of studies, which suggest a causal relationship between malnutrition and survival in cirrhosis. Malnutrition has prognostic value in patients undergoing liver transplantation and other surgical operations. Similar observations have been made in non-surgical liver disease patients. Malnutrition predicts impaired immunity and increased susceptibility to infection in patients hospitalized for cirrhosis and ascites. The disappearance of ascites following placement of a peritoneo-venous shunt improves nutritional status and immunity. Malnutrition has also been shown to correlate with poor hepatic function and severity of disease in cirrhotic patients. These collective data suggest that the recognition and treatment of malnutrition are important considerations in the management of patients with cirrhosis, especially in those situations listed in Table 12.3.

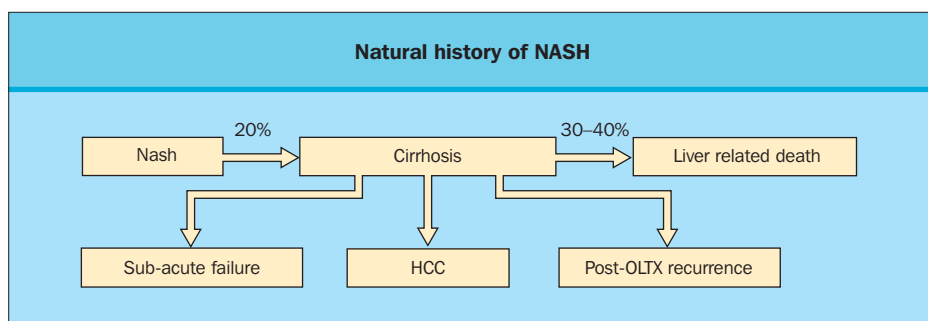


Recent evidence indicates that obesity associated NASH is also associated with a poor prognosis (**Fig. 12.6**). Cirrhosis develops in 15–25% of NASH patients. Once developed, 40% of these patients may succumb to liver-related death over a 10-year period, the mortality rate being similar to or worse than cirrhosis associated with hepatitis C. NASH is also now considered the major cause of cryptogenic cirrhosis. Patients with NASH-associated cirrhosis can also experience decompensation into subacute liver failure, progression to hepatocellular carcinoma (HCC), and can have reoccurrence post-transplantation. In contrast, steatosis alone is reported to have a more benign clinical course, although progress of fibrosis in cirrhosis has occurred in 3% of these patients with steatosis alone.

Most, but not all studies show the importance of nutritional status on graft and/or patient morbidity and mortality in the transplant patient. Early data showed malnutrition adversely affected survival. Another recent well-performed study of patients awaiting liver transplantation showed that PEM as well as hypermetabolism predicted decreased survival after transplantation, whereas the Child-Pugh score did not. These collective data suggest that recognition and treatment of PEM should be an important consideration before liver transplantation.

<b>Protein energy malnutrition as a poor prognostic factor in cirrhosis</b>	
<i>Condition or procedure</i>	<i>Clinical outcome measured</i>
1. Cirrhosis	1. Survival/ immunodeficiency
2. Cirrhotic patients undergoing abdominal surgery	2. Survival/ postoperative complications
3. Hospitalized patients with ascites/cirrhosis	3. Survival
4. Alcoholic hepatitis	4. Survival/liver function
5. Liver transplantation	5. Patient/graft survival
6. Hepatectomy	6. Postoperative complications
7. Obesity and cirrhosis	7. Post-transplant multiorgan failure
8. Obesity associated NASH	8. Cirrhosis
NASH, non-alcoholic steatohepatitis.	

**Table 12.3 Protein energy malnutrition as a poor prognostic factor in cirrhosis.**



**Figure 12.6 Natural history of non-alcoholic steatohepatitis (NASH).** HCC, hepatocellular cancer; OLTx, orthotopic liver transplantation.

There is much variability among transplant centers regarding the importance of obesity as a risk factor for patients undergoing liver transplantation. Graft and patient survival are similar in patients post-transplantation. Nonetheless, many transplant physicians believe that obesity is a significant enough risk factor for liver transplantation to place certain restrictions on transplantation in obese patients. These concerns appear most relevant in obese patients with a body mass index (BMI – calculated by dividing weight in kilograms by height in meters squared) of 35 or greater. Obese patients with a BMI greater than 35 compared with patients with a BMI less than 30 required more intra-operative blood transfusions, had an increased number of wound infections, and were more likely to die of multiorgan failure, even though these two groups had similar 3-year survivals. Therefore, it seems appropriate to study and monitor closely patients with severe obesity. However, based on the available information, obese and non-obese patients have similar long-term outcomes after liver transplantation.

## NUTRITIONAL ASSESSMENT

Because PEM has prognostic value and influences clinical decisions regarding nutritional therapy, accurate methods of assessing nutritional status are vital.

Nutritional assessment can be difficult in patients with chronic liver disease. Parameters of common use in clinical practice should diagnose protein (kwashiorkor or hypoalbuminemic) or combined protein calorie (marasmic) forms of malnutrition (**Table 12.4**). Fluid excess is universal in end-stage liver disease and may be present even in earlier stages of disease. This fluid excess causes weight-to-height parameters (such as percentage of ideal body weight or BMI) to underestimate both the prevalence of chronic liver disease, which itself causes alterations in visceral protein synthesis, cellular immunity, and total lymphocyte count independent of PEM. This causes an overestimation of the degree but not necessarily the prevalence of PEM. In contrast, markers of muscle mass (creatinine height index and midarm muscle circumference) and fat mass (triceps skin fold thickness) are less influenced by either alcohol or chronic liver disease. When performing a creatinine height index estimation, creatinine clearance should be measured to confirm the precision of the creatinine height index.

In addition to these anthropometric and laboratory parameters, bioelectrical impedance analysis (BIA) has been used to estimate total lean body mass rather than just skeletal mass and is logistically convenient and relatively inexpensive. Because this method is inaccurate in cirrhotic patients with ascites or edema

Measurements used to assess nutritional status					
	Protein	Malnutrition Protein/calorie	Alcohol toxicity	Liver disease	Renal function
Visceral proteins	X		X	X	
Lymphocyte count	X	X	X	X	X
Cellular immunity	X	X	X	X	X
% Ideal body weight		X		X	X
Anthropometry		X		(?)	(?)
Creatinine height index		X		(?)	X
SGA		X		X	?
BIA				X	
DEXA				X	
BIA, bioelectrical impedance analysis; DEXA, bone density; SGA, subjective global assessment.					

Table 12.4 Measurements used to assess nutritional status.

Nutrition assessment in advanced liver disease		
History	Physical examination	Laboratory tests
Weight loss > 10% of usual weight	Wasting of skeletal muscle mass (temporal muscles)	Visceral proteins, albumin, pre-albumin and retinol-binding
Loss of appetite	Skin/hair	Vitamin and mineral levels
Decrease of energy level	Acrodermatitis (zinc deficiency) Dry rash (vitamin A deficiency) Oral mucosa (vitamin B deficiency) Peripheral neuropathy (thiamine deficiency) Hyper-reflexia (magnesium deficiency)	Total lymphocyte count, vitamin and mineral levels

Table 12.5 Nutrition assessment in advanced liver disease.

and has some imprecision even in those without ascites or edema, it may be best used to serially monitor the response to nutritional feeding. Dual-energy X-ray absorptiometry (DEXA) is a non-invasive method with minimal radiation that provides accurate measurements of total body bone mineral, fat, and fat free soft tissue mass. The accuracy of this method is also influenced by fluid retention as with BIA.

Subjective global assessment is an evaluation of PEM based predominantly on the following clinical findings: the presence of edema, muscle wasting, subcutaneous fat loss, glossitis and cheilosis. As a practical method for estimating PEM, it compares favorably with standard measures in patients without liver disease. Subjective global assessment in cirrhotic patients especially helps evaluate patients prior to liver transplantation. Estimation of handgrip strength may also be important.

Despite the limitations of these standard methodologies, more sophisticated measurements of body cell mass (BCM) provide evidence for PEM in chronic liver disease. The BCM, which comprises the central energy-expending mass of working tissue, has been found to be decreased in both alcoholic and non-alcoholic cirrhosis by three different measurements:

- total body potassium;
- intracellular water; and
- total body protein.

Therefore, nutritional assessment is useful in all types of cirrhosis, especially when a composite score emphasizing anthropometry, handgrip strength and creatinine height index is performed and combined with overall clinical judgment including subjective global assessment.

## CLINICAL FEATURES

### Medical history

The duration, severity and etiology of liver disease are pertinent to PEM in patients with liver disease (Table 12.5). Other disease processes, such as renal failure, cardiac and pancreatic disease in alcoholism, and diabetes and cardiac disease in hemochromatosis, should also be evaluated. Factors that may limit nutrient ingestion and absorption, such as anorexia, early satiety, vomiting and diarrhea should be identified. If possible, dietary recall should estimate macronutrient and micronutrient intake and the consumption of alcoholic beverages. Because cirrhotic patients

Guidelines for nutritional therapy					
	Protein (g/kg/day)	Energy (kcal/kg/day)	Energy substrate %CHO %fat		Nutritional goal
1. Hepatitis (acute or chronic*)	1.0–1.5	30–40	67–80	20–33	Prevent malnutrition Enhance regeneration
2. Cirrhosis (uncomplicated)	1.0–1.2	30–40	67–80	20–33	Same as 1.
3. Cirrhosis (complicated) <sup>†</sup>					
a. Malnutrition	1.2–1.8	40–50	72	28	Restore normal nutritional status
b. Cholestasis	1.0–1.5	30–40	73–80	20–27	Prevent malnutrition Treat fat malabsorption <sup>‡</sup>
c. Encephalopathy <sup>§</sup>					
Grade 1 or 2	0.5–1.2	25–40	75	25	Provide nutritional needs without precipitating encephalopathy
Grade 3 or 4	0.5	25–40	75	25	
4. Liver transplant					
a. Peritransplant	1.2–1.8 1.0	30–50	70–80	20–30	Restore normal nutritional status
b. Post-transplant		30–35	>70	≤30	Retain and maintain ideal body weight
<p>*There are no convincing data indicating any extraordinary vitamin or trace metal requirements in liver disease. A typical substitution solution for parenteral feeding is provided. Vitamins: (10 cc/L) – ascorbic acid (20 mg/mL), vitamin A (660 IU/mL), vitamin D (40 IU/mL), thiamine HCL (0.6 mg/mL), riboflavin phosphate (0.72 mg/mL), pyridoxine HCl (0.8 mg/mL), niacinamide (8 mg/mL), D-pantothenic acid (3 mg/mL), vitamin E (2 IU/mL), biotin (12 mcg/mL), folic acid (0.08 mg/5 mL), vitamin B<sub>12</sub> (1 mcg/5 mL). Trace metals: (1 mL/L) – zinc chloride (10.42 mcg/5 mL, copper chloride (2.68 mcg/5 mL), manganese (1.44 mcg/5 mL), chromium chloride (0.012 mcg/5 mL)</p> <p><sup>†</sup>Ascites and sepsis are two complications which are not listed but which should be treated aggressively as part of nutritional therapy (see text).</p> <p><sup>‡</sup>Medium chain triglycerides may be necessary if fat malabsorption is present. Pancreatic enzymes are necessary if pancreatic insufficiency is present; especially in alcoholic cirrhosis and primary sclerosing cholangitis.</p> <p><sup>§</sup>Branched-chain amino acid (BCAA) formulations may be necessary. Available hepatic enteral formulations are 45% BCAA enriched and parenteral formulations are 36% BCAA enriched. This compares to standard formulations for preparations which contain 26% BCAA. For outpatients, diets high in vegetable protein or casein hydrolysates may be better tolerated than standard dietary protein in the protein-intolerant patient with cirrhosis.</p>					

**Table 12.6 Guidelines for nutritional therapy.**

often self-select a higher proportion of their dietary intake at breakfast, a full day's calorie count is required to obtain an accurate assessment.

### Physical examination

During physical examination one should look for signs of decompensation, such as mental status changes, asterixis, jaundice, ascites, leg edema and bruising. Weight should be recorded with specific attention to fluid overload. Signs of specific nutritional deficiency, such as acrodermatitis from zinc deficiency or pellagra from niacin deficiency, should be sought. Patients with cholestatic liver disease and steatorrhea may have skin excoriations related to pruritus, xanthomas due to hyperlipidemia, or dermatitis from essential fatty acid deficiency.

Evaluation of the oral mucosa, skin and hair are particularly important. In the oral mucosa, glossitis (raw tongue), atrophic tongue (slick tongue) and angular stomatitis are common findings and result from deficiencies in the B vitamins, iron and folate. Less common are gingivitis (vitamin C deficiency) and altered taste/smell (zinc deficiency).

On the skin, petechiae (vitamin C deficiency) and purpura (vitamin A and zinc deficiency) may be present. Hair may demonstrate sparseness (protein, zinc and biotin deficiencies),

follicular hyperkeratosis (vitamins A and C deficiency) or be corkscrewed and coiled (vitamin C deficiency).

## NUTRITIONAL REQUIREMENTS

### Nitrogen

In stable cirrhotics, the average protein requirement to maintain positive nitrogen balance is 0.8 g/kg/day, which is similar to that required in the normal population. Because the range of protein required is larger in cirrhotics than in controls, compensated cirrhotics should receive at least 1.2 g/kg/day. Although nitrogen balance is achieved at this amount, nitrogen retention continues to improve up to 1.8 g/kg/day. Even stable cirrhotics are protein deficient and this is an important management consideration. Cirrhotic patients undergoing surgery should receive protein doses between 1.2 and 1.5 g/kg/day (Table 12.6).

### Energy

Abnormalities in extracellular fluid levels in fat and non-fat body compartments, portosystemic shunting, and variability in energy expenditure between different body requirements make the prediction of energy requirements quantitatively unreliable in cirrhosis. Therefore, with knowledge that hypermetabolism



Vitamins in liver disease
<ul style="list-style-type: none"><li>Many complications associated with liver disease are manifestations of vitamin deficiencies. These include: macrocytic anemia (folate and vitamin B<sub>12</sub> deficiency); neuropathy (pyridoxine, thiamine or vitamin B<sub>12</sub>); confusion, ataxia and ocular disturbances (thiamine) and impaired adaptation to dark (vitamin A)</li><li>Pyridoxine phosphate (the major active form of pyridoxine) rather than pyridoxine itself may be required to correct pyridoxine deficiency; especially in alcoholics</li><li>The clinical response to thiamine is usually rapid and effective. However, the neuropathy may be irreversible and abnormalities in red cell transketolase may not improve</li><li>Deficiencies in vitamin B<sub>12</sub> and folic acid may develop faster in cirrhotic patients due to diminished hepatic storage</li><li>In the presence of active hepatic inflammation, fatty liver or hepatocellular cancer, normal or elevated levels of vitamin B<sub>12</sub> levels do not exclude a deficiency of this vitamin</li><li>Decreased serum levels of vitamin A do not necessarily reflect vitamin A deficiency. Zinc deficiency may be causing decreased transport out of the liver</li><li>In non-cholestatic liver disease, abnormal serum levels of fat-soluble vitamins should not be sought or treated in the absence of clinical or laboratory abnormalities, which indicate a functional vitamin deficiency. In cholestatic liver diseases, abnormal serum levels of the fat soluble vitamins may be treated even in the absence of clinical symptoms or laboratory abnormalities</li><li>Suggestions for use of vitamin supplements: (a) Water-soluble vitamins may be supplied with a multivitamin preparation; (b) fat-soluble vitamins should be used in a water-soluble form whenever possible. Vitamin A, Aquasol 1 capsule daily (50 000 IU), vitamin K, Synkayvite, 1 tablet daily (15 mg). Vitamin D treatment should be individualized by monitoring plasma 25-hydroxy vitamin D levels and serum and urinary calcium levels. Vitamin D<sub>2</sub> (ergocalciferol), 1.25 mg (50 000) U, 1 tablet three to four times a day, may be adequate. Vitamin E (D-<math>\alpha</math>-tocopheryl polyethelene glycol 1000 succinate (TPGS) 23 IU kg/day)</li></ul>

Table 12.7 Vitamins in liver disease.

may have adverse clinical sequelae, energy expenditure should be measured with indirect calorimetry, especially in hospitalized patients undergoing liver transplantation. If indirect calorimetry is not available, the daily resting energy expenditure for cirrhosis should be assumed as 25–30 kcal/kg body weight (based on ideal body weight if ascites and/or edema is present) for decisions regarding restorative or maintenance needs.

Energy is proportionally provided by carbohydrates and fat (Table 12.6). Two points are important:

- Insulin resistance is universal in cirrhosis irrespective of the type or severity of the disease. Glucose intolerance is not typically an important management issue, except hypoglycemia, which develops in up to 50% of cirrhotic patients during sepsis.
- Lipid formulations are very useful in cirrhosis because of their low water content and high caloric density. Initial concerns that lipids might precipitate encephalopathy have not been confirmed.

Nutritional considerations for specific alterations in liver disease	
Specific alterations	Implications for nutritional management
1. Accelerated starvation	1. Frequent feedings with night-time snack
2. Hepatic encephalopathy	2. Routine or prophylactic protein restriction should be discouraged
3. Hepatic metabolism of non-essential amino acids	3. Cysteine and tyrosine may become essential amino acids in cirrhosis
4. Salt restriction	4. Salt restriction should be balanced against palatability of the diet
5. Alcohol toxicity	5. Abstinence needs to be continually reinforced
6. Obesity	6. Gradual weight loss should be instituted

Table 12.8 Nutritional considerations for specific alterations in liver disease.

Vitamins

Approximately 40% of patients with non-alcoholic liver disease have fat soluble vitamin deficiencies (vitamin A and E); 8–10% have deficiencies in the B complex vitamins (nicotinic acid, thiamine, vitamin B<sub>12</sub>, riboflavin and pyridoxine) and 17% have folate deficiency. These abnormalities are related more to disordered hepatic function and diminished reserve rather than deficient dietary intake or malabsorption. However, in severe advanced cirrhosis, malabsorption may play a role in both fat soluble vitamins and the B complex vitamins. These vitamin deficiencies are more prevalent and severe in alcoholic than non-alcoholic liver disease. Specific issues regarding vitamins in liver disease are provided in Table 12.7.

SPECIFIC ALTERATIONS IN LIVER DISEASE (Table 12.8)

Accelerated starvation

Cirrhosis is a disease of accelerated starvation with early recruitment of alternative fuels. Cirrhotic patients should avoid any extended period of time without feeding. The advantage of a frequent feeding approach has been confirmed by nitrogen balance and indirect calorimetry measurements in cirrhotic patients.

Hepatic encephalopathy

Advanced acute or chronic liver failure often presents with a constellation of neuropsychiatric abnormalities known as hepatic encephalopathy. These mental status alterations may range from mild behavioral changes to deep coma. Subclinical hepatic encephalopathy is present in up to 70% of patients with cirrhosis and currently does not require any specific nutritional therapy. Overt encephalopathy is almost always associated with some precipitating event such as gastrointestinal bleeding, infection/sepsis, fluid and electrolyte imbalances, constipation, acid–base

abnormalities or the use of sedating drugs. In a minority of cirrhotic patients, hepatic encephalopathy may be precipitated by protein intake (especially animal protein) without any other precipitating factor. These patients are considered protein intolerant. More than 95% of cirrhotic patients can tolerate diets containing as much as 1.5 g/kg/day of mixed proteins. Therefore, true protein intolerance is rare except for fulminant hepatic failure, or the occasional patient with 'endogenous' chronic encephalopathy.

In those few cirrhotic patients with endogenous protein intolerance, branched-chain amino acid formulations are better tolerated than standard amino acid supplements and can achieve positive nitrogen balance. It should be emphasized, however, that there is no clearly proven benefit of branched-chain amino acids. Thus, standard amino acid formulations are suitable for the majority of patients with chronic liver disease.

### **Protein restriction**

There is no justification for the routine use of protein restriction as prophylaxis against precipitating hepatic encephalopathy. Available data indicate that the vast majority of cirrhotic patients and even patients with severe alcoholic hepatitis can tolerate large quantities of protein feeding (up to 1.75 g/kg/day).

### **Non-essential amino acids**

Administration of standard or specialized total parenteral nutrition solutions devoid of the non-essential amino acids tyrosine and cysteine may not achieve positive nitrogen balance in cirrhosis despite provisions of adequate amounts of essential amino acids. This observation emphasizes the observation that certain intrinsic liver functions may be rate limiting in cirrhosis such as the ability to synthesize cysteine from methionine and tyrosine from phenylalanine.

### **Salt restriction**

Although frequently necessary, sodium and consequently salt restriction significantly decreases the palatability of the diet and consequently may diminish food intake. In the hospitalized patient, very low 250–500 mg sodium (0.63–1.3 g salt) diets may be appropriate. In the non-hospitalized patient, every effort should be made to avoid salt restriction. In those patients with fluid overload who otherwise cannot be managed effectively, the least sodium restriction that is effective should be employed. A 2.5 g sodium (6.3 g salt) intake approximates a no added salt diet. This restriction can be tolerated by the motivated patient and is usually effective for fluid management in most patients and does not significantly limit calorie or protein intake.

### **Alcohol related alterations**

Alcohol has direct deleterious effects on muscle protein status independent from associated liver injury. Alcohol is known to inhibit meal stimulated hepatic protein production which is an important contributor to skeletal muscle and whole body protein synthesis in humans. In addition alcohol increases intestinal permeability which in turn initiates endotoxin/cytokine induced muscle proteolysis. Alcohol also has a caloric density of seven but produces less efficient energy per gram of nutrient than both carbohydrates and lipids. Further, it replaces the amount of other caloric sources.

### **Obesity**

Obesity is associated with hepatic steatosis which not only causes cirrhosis per se, but also is a risk factor in the development of cirrhosis in patients with alcohol and hepatitis C associated liver disease. It is also associated with multiorgan failure post-liver transplant.

## **NUTRITIONAL MANAGEMENT**

### **Goals**

Although early studies used survival as the primary outcome measure for determining the efficacy of nutritional therapy, more recently, improvement in nutritional status, infection rates, immune function, nitrogen balance and perioperative morbidity have been shown to improve with nutritional therapy in cirrhotic patients. Nutritional therapy is most effective when used for longer periods or in certain subgroups such as in those with severe malnutrition, chronic hepatic encephalopathy and decompensated liver disease. No reliable information is available regarding the cost-effectiveness of nutritional therapy or its effect on the quality of life of cirrhotic patients.

Regarding goals specific for liver disease, nutritional therapy needs to correct pre-existing PEM while simultaneously providing sufficient amino acids to encourage hepatic regeneration and normalization of function without precipitating encephalopathy. The clinical emphasis should be placed on supplying the basic requirements of nitrogen and calories. Overall recommendations are provided in Table 12.9.

### **Specific patient populations**

#### **Cirrhosis**

Enteral feeding improves liver function, encephalopathy and perhaps survival in severely malnourished cirrhotics and in patients with decompensated alcoholic liver disease. Nutrient intake is increased by enteral nutrition in all published studies to date and may in part be responsible for these benefits. Providing nutritional supplements (1000 cal and 34 g of protein – casein based) for 1 year to patients with complicated alcoholic cirrhosis resulted in higher protein and caloric intake to levels which simply met their required needs.

#### **Alcoholic hepatitis**

There have been eight published trials on the use of standard intravenous amino acid formulations as primary therapy for alcoholic hepatitis. The results are conflicting, but six of the eight studies showed improvement in either liver histology or function. One of these studies additionally showed a strong trend toward an improvement in mortality. Two of the studies concluded that supplemental amino acids were of no benefit. In one of these negative studies, the mortality was 3.3% in the 30 patients in whom positive nitrogen balance was achieved, but was 58% in those patients who remained in negative nitrogen balance despite nutritional therapy. A recent trial also reported enteral feedings to be equally as effective as corticosteroids.

#### **Fulminant hepatic failure**

Fulminant hepatic failure defined as the development of hepatic encephalopathy within 8 weeks of the onset of liver disease is a life-threatening illness associated with a rapid development of protein calorie malnutrition; even when what is calculated to be

Overall guidelines for the nutritional management of patients with liver disease	
• Assume protein calorie malnutrition is present in all patients	
• Assume an inadequate dietary intake, even in hospitalized patients	
• Qualitative stool fat should be done intermittently, especially in patients with alcoholic or cholestatic cirrhosis. If malabsorption is present; determine the cause and treat	
• Treatment with either neomycin or lactulose for hepatic encephalopathy may exacerbate malabsorption, which should be considered in nutritional management	
• Treat ascites aggressively to decrease energy expenditure	
• Diuretic therapy is preferred over large volume paracentesis for the management of ascites in order to minimize protein loss	
• Balance the need for sodium restriction with nutritional considerations and diet palatability	
• Nutritional assessment is useful in all types of cirrhotic patients	
• A composite score (emphasizing anthropometry and creatinine height index) combined with overall clinical judgment should be employed	
• The clinician should remember that all the methods for nutritional assessment in cirrhosis are influenced or potentially influenced by the presence of liver disease alone as well as abnormalities associated with liver disease such as renal failure, alcohol ingestion and expansion of the extracellular water compartment	
• Determine energy expenditure requirements with indirect calorimetry (if possible) in hospitalized patients or patients listed for liver transplantation. If energy expenditure requirements are estimated from prediction equations, calculate energy need based on ideal weight rather than actual weight if extracellular water (ascites/edema) is present	
• Multiple (five to six) small feedings with a carbohydrate-rich evening snack, which consists of approximately 10–15% of caloric needs, should be given. The need for breakfast feeding must also be stressed to the patient	
• For calories, complex rather than simple carbohydrates should be used. Lipids should supply 20–40% of caloric needs	
• Nutritional requirements may vary according to the specific type of patient and/or clinical situation as listed in Table 12.6	
• Severely malnourished or decompensated cirrhotics should be given oral or enteral supplements as recommended in Table 12.6	
• Long-term nutritional supplements may be necessary to provide recommended protein and caloric supplements	
• Patients with severe alcoholic hepatitis should be given supplemental standard protein 1.0 g/kg via an enteral or peripheral parenteral route	
• Perioperative nutritional therapy should be given to those cirrhotic patients with significant malnutrition as defined by weight loss of more than 10% or for anthropometry or creatinine height index less than 5% of predicted values	
• Post-liver transplant, patients need higher amounts of protein and energy (see Table 12.6)	
• Pediatric patients undergoing liver transplant may benefit from branched-chain amino acids	
• Cirrhotics should never be treated prophylactically with protein restriction to prevent hepatic encephalopathy.	
• Standard protein or amino acid mixtures should be supplied to meet the measured estimated nitrogen needs (as provided in Table 12.6)	
• Protein restriction should be implemented only if protein intolerance as manifested by encephalopathy occurs in the absence of precipitating factors is found	
• Protein restriction below the required amounts should not be continued for more than 3–4 days	
• Branched-chain amino acids should be given only if the required amount of standard feedings cannot be tolerated without precipitating hepatic encephalopathy	
• Monitor for hypoglycemia and treat aggressively with concentrated glucose solutions which may also decrease serum ammonia levels	
• Enteral feeding is the preferred route of feeding patients with insufficient oral intake. Enteral feeding tubes may be used even if non-bleeding varices are present.	
• Do not use any nutritional product devoid of cysteine or tyrosine as the only nitrogen source for any prolonged period of time. Be aware of clinically important issues related to vitamins listed in Table 12.7.	

Table 12.9 Overall guidelines for the nutritional management of patients with liver disease.



adequate amounts of calories and nitrogen are supplied. Up to four times the normal rate of protein breakdown accompanied by decreased hepatic amino acid oxidation leads to the accumulation of potentially toxic levels of certain amino acids (e.g. tyrosine, phenylalanine and methionine).

Hypoglycemia occurs commonly, and serum glucose levels must be maintained with concentrated glucose infused (20–40%) to avoid the risks of exacerbating cerebral edema. Lipid emulsions may be particularly useful in this setting.

Recent work suggests that administering at least 40–60 g of protein diminishes the rapid depletion of protein stores in patients with stages 1 and 2 encephalopathy. If necessary, the use of branched-chain amino acids may be necessary to achieve this goal in patients with more advanced stages of encephalopathy. However, the efficacy of branched-chain amino acids in this situation remains unproven. If encephalopathy should develop or worsen with protein feeding, the use of specialized formulations of branched-chain amino acids seems indicated.

### Weight loss

Serum aminotransferases almost always improve with weight loss in the obese patient (with as little as 5–10% decrease in body weight), but they are poor predictors of histology which does not always improve and may, in fact, worsen if weight loss occurs too rapidly. Gradual weight loss (1–2 lbs/week) with an overall goal of 10% weight loss over 6 months is recommended as a safe and effective clinical strategy especially in patients who are 30% overweight.

With success, further weight loss can be attempted, if indicated. Multiple interventions and strategies, including diet modifications, physical activity, behavioral therapy and pharmacotherapy with Orlistat, or a combination of these treatment modalities is recommended. The particular treatment modality should be individualized taking into consideration the BMI and presence of concomitant risk factors and other diseases. Given the lack of clinical trials in this area, these overall recommendations are a useful and safe first step for obese patients with NAFLD. The only prospective study using restrictive bariatric surgery in NAFLD patients was very effective in diminishing hepatic injury.

### Liver transplantation

There is no uniform approach among transplant centers regarding the management of nutrition in transplant patients. There is general agreement that malnutrition reflects the severity of chronic liver disease and should not be considered, in general, as an exclusion for patients receiving liver transplant. Most centers administer post-operative nutrition in a fashion similar to that given other patients after major gastrointestinal surgery. Therefore the recognition that malnutrition is a significant problem has been gaining momentum.

In the pretransplant patient, there are insufficient data upon which any specific recommendations can be based. Consequently, general principles of nutritional management as outlined in Tables 12.6 and 12.9 should be followed.

In the post-transplant patient, a number of benefits to nutritional therapy have been shown as demonstrated in **Table 12.10**. As compared to conventional therapy, early enteral feeding was shown to decrease the number of viral infections and obtain better nitrogen retention post-operatively. Both nasogastric and jejunostomy tubes have been used successfully in

these patients post-operatively. The available information also indicates that both enteral and parenteral nutrition are equivalent in their ability to deliver nutrients and improve nutritional status at the tenth postoperative day.

In contrast to these positive results, 1 year after follow-up, protein breakdown and lipolysis in these patients improved, but did not normalize, with improvement in total body water and total body fat but no change in body cell mass. An increasingly important nutritional problem which develops in the first 1–2 years after liver transplant is obesity which has been reported to occur in 30–70% of patients. Therefore, it is important to follow the nutritional status of these patients postoperatively and treat obesity aggressively if it develops.

## PRINCIPLES AND PRACTICAL IMPLEMENTATION

The nutritional support for patients with liver disease follows the general principles applicable to any other type of patient. However, there are a number of principles particularly relevant to liver disease patients with guidelines provided in Tables 12.6 and 12.9.

### Glucose requirement

Although insulin resistance and hyperglycemia are commonly observed in cirrhosis, hypoglycemia (<50 mg/dL) occurs in up to 50% of cirrhotic patients during episodes of stress. Therefore, serum glucose must be closely monitored in patients with fulminant or decompensated chronic liver disease.

### Route of nutrient administration

The least invasive route for nutritional supplementation is oral which should be tried first. If attempts at oral supplementation fail, enteral feedings can be administered via a small caliber nasogastric or nasojejunal tube. The presence of non-bleeding esophageal varices is not a contraindication to the use of enteral feeding tubes in these patients. However, feeding tubes should not be used if there is active esophageal variceal bleeding.

The placement of a gastrostomy or a jejunostomy tube in cirrhotics with ascites is not recommended due to the possible complications of peritonitis or ascitic fluid leakage. Unfortunately, this limits the potential of long-term enteral feeding in many of these patients.

Parenteral nutrition should be initiated only if nutritional requirements cannot be supplied orally or enterally in situations such as gastrointestinal bleeding, ileus, or after abdominal surgery. Because of its relatively low caloric density, peripheral parenteral

Benefits of nutritional therapy post-transplant
Improved nitrogen balance
Less time in the ICU (trend)
Lower hospital cost (trend)
Fewer viral infections with early enteral feeding which is equivalent to parenteral feeding
Both nasogastric and jejunostomy tubes have been used successfully

**Table 12.10** Benefits of nutritional therapy post-transplant.

Use of nutritional supplements and medications in NAFLD			
Possibly harmful		Possibly helpful	
Supplements	Medications	Supplements	Medications
St John's wort	Acetaminophen <sup>4</sup>	Vitamin E <sup>8</sup>	Betaine <sup>11</sup>
Ephedrine containing compounds	Tamoxifen <sup>5</sup>	MVI <sup>9</sup>	Ursodeoxycholic acid <sup>11</sup>
Excessive vitamin A <sup>1</sup>	Amiodarone <sup>5</sup>	SAMe <sup>10,11</sup>	Metformin <sup>11,12</sup>
Glucosamine <sup>2</sup>	Iron <sup>6</sup>	Milk thistle <sup>11</sup>	Statins <sup>11,13</sup>
Others <sup>3</sup>	Estrogen <sup>7</sup>		Thiazolidinediones <sup>11,12</sup>
<ol style="list-style-type: none"> <li>1. Vitamin A should not be used in excess of that contained in a daily multivitamin (MVI) which is 5000 IU.</li> <li>2. Since hexosamines in general cause insulin resistance, glucosamine should be used with some caution.</li> <li>3. All other herbs should be considered as possible causes of injury and should be avoided.</li> <li>4. Acetaminophen should be restricted to less than 2–3 g daily. Repeated or ongoing use of acetaminophen for longer than 3 days with daily doses above 1.5 g should be discouraged. Many over the counter (OTC) medications contain acetaminophen. Therefore the amount of acetaminophen in the OTC medications should be carefully sought.</li> <li>5. These drugs may cause hepatic injury that histologically looks similar to non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH). Therefore, the benefit risk of using these drugs in NAFLD/NASH should be carefully considered.</li> <li>6. Since iron may cause oxidative stress in the liver, iron supplements should only be used as per standard management for anemia. Transferrin saturation should not exceed 50%.</li> <li>7. Estrogens used as oral contraception pills (OCP) or as hormonal replacement therapy (HRT) do not have to be discontinued.</li> <li>8. Vitamin E should not be used at doses less than 400 IU daily.</li> <li>9. A daily multivitamin (MVI) with iron content &lt;20 mg should be used.</li> <li>10. SAMe = S-Adenosylmethionine.</li> <li>11. The use of these supplements or medications should not be encouraged. However, there are uncontrolled studies suggesting their benefit in NAFLD.</li> <li>12. This agent is approved for use in patients with type II diabetes.</li> <li>13. The use of the statins used as cholesterol lowering agents is not contraindicated (and in fact, may be beneficial) in NAFLD. However, baseline and interval measurements of liver tests should be performed.</li> </ol>			

Table 12.11 Use of nutritional supplements and medications in NAFLD.

nutrition cannot supply total nutritional requirements and is usually not a good choice in cirrhotic patients with sodium and water retention. Peripheral parenteral nutrition may be useful in supplementing enteral or oral feeding; especially for providing amino acids in severe alcoholic hepatitis. Central parenteral nutrition is preferred in most cirrhotic patients despite the risk of central vein catheter replacement in patients with coagulopathy and thrombocytopenia.

The amount of protein recommended for different types of liver disease patients in various situations is provided in Table 12.6. If these amounts of protein cannot be provided without precipitating hepatic encephalopathy, formulations enriched with branched-chain amino acids should be substituted for standard formulations. There are oral or enteral feedings (Nutrihep; – 50% branched-chain amino acid enriched and Hepatic Aid II – 46% branched-chain amino acid enriched) as well as intravenous formulations (Hepatamine and Hepatasol; both 36% branched-chain amino acid enriched) available. In addition to these formulations which have been tried in liver patients, there are also stress formulations (Freamine HBC and Aminosyn-HBC) both of which are approximately 45% branched-chain amino acid enriched. However, these stress formulations have not been evaluated in patients with liver disease.

### Dietary supplements

Many patients seek advice regarding the efficacy and safety of vitamins, herbs or other nutritional supplements. Unfortunately, there is insufficient information in this area to make sound

recommendations. General recommendations are provided in Table 12.11.

### Obesity

A number of different diets have been suggested, including: the American Heart Association healthy heart diet, the Diabetic Diet as recommended by the American Diabetes Association, a low glycemic diet, and diets enriched with omega 3 polyunsaturated fatty acids. However, the effect of these diets in NAFLD is unproven. Diets used to produce weight loss must always be individualized and related to the overall health status of the patient.

In general, patients should follow a well-balanced diet. One such diet is recommended by the National Cholesterol Education Program ([www.nhlbi.nih.gov/about/ncep](http://www.nhlbi.nih.gov/about/ncep)). This diet makes specific recommendations regarding total caloric intake, as well as the amount and type of fat and carbohydrate for patients who do not have to lose weight. If the patient has diabetes, specific recommendations have been made by the American Diabetes Association.

Overweight patients (BMI >25 kg/m<sup>2</sup> based on dry weight) should be given a diet with a goal of losing and sustaining an initial weight loss of 10% of body weight. The weight loss should be gradual and should not exceed 2 lbs/week. The National Heart, Lung and Blood Institute (NHLBI) guidelines for weight loss are provided in Table 12.12 as one typical diet that might be employed.

<b>Weight loss diet<sup>1</sup></b>	
<i>Nutrient</i>	<i>Recommended intake</i>
Calories <sup>2</sup>	Approximately 500– 1000 kcal/day reduction from usual state
Total fat <sup>3</sup>	30% or less of total calories
Saturated fatty acids <sup>4</sup>	8–10% of total calories
Monounsaturated fatty acids	Up to 15% of total calories
Polyunsaturated fatty acids	Up to 10% of total calories
Cholesterol <sup>4</sup>	<300 mg/day
Protein <sup>5</sup>	Approximately 15% of total calories
Carbohydrate <sup>6</sup>	55% or more of total calories
Sodium chloride	No more than 100 mmol/day (approx. 2.4 g of sodium or approx. 6 g of sodium chloride)
Fiber <sup>7</sup>	20–30 g/day
Calcium <sup>8</sup>	1000 –1500 mg/day
<ol style="list-style-type: none"> <li>1. This table provides guidelines for Step 1 weight loss as suggested in a monograph provided by the NHL BI, 2000 entitled 'The practice guide. Identification, evaluation and treatment of obesity in adults'.</li> <li>2. A reduction in calories of 500 to 1000 kcal/day will help achieve a weight loss of 1 to 2 pounds/week. Alcohol provides unneeded calories and displaces more nutritious foods. Alcohol consumption not only increases the number of calories in a diet but has been associated with obesity in epidemiologic studies as well as in experimental studies. The impact of alcohol calories on a person's overall caloric intake needs to be assessed and approximately controlled.</li> <li>3. Fat-modified foods may provide a helpful strategy for lowering total fat intake but will only be effective if they are also low in calories and if there is no compensation by calories from other foods.</li> <li>4. Patients with high blood cholesterol levels may need to use the Step II diet to achieve further reductions in low density lipoprotein cholesterol levels; in the Step II diet, saturated fats are reduced to less than 7% of total calories, and cholesterol levels to less than 200 mg/day. All of the other nutrients are the same as in Step 1.</li> <li>5. Protein should be derived from plant sources and lean sources of animal protein.</li> <li>6. Complex carbohydrates from different vegetables, fruits and whole grains are good sources of vitamins, minerals and fiber.</li> <li>7. A diet rich in soluble fiber, including oat bran, legumes, barley, and most fruits and vegetables, may be effective in reducing blood cholesterol levels. A diet high in all types of fiber may also aid in weight management by promoting satiety at lower levels of calories and fat intake. Some authorities recommend 20–30 g of fiber daily, with an upper limit of 35 g.</li> <li>8. During weight loss, attention should be given to maintaining an adequate intake of vitamins and minerals. Maintenance of the recommended calcium intake of 1000–1500 mg/day is especially important for women who may be at risk of osteoporosis.</li> </ol>	

**Table 12.12 Weight loss diet.**

## SUMMARY

Although more investigation is needed to study the effect of nutritional intervention in liver disease, clinicians need to recognize that malnutrition is virtually ubiquitous in all types of cirrhosis. It has prognostic significance, and its treatment has been shown to provide beneficial clinical outcomes. Therefore, it is important to identify malnutrition and treat it with increased amounts of conventional feedings while maintaining specialized formulations in only those patients who cannot tolerate standard dietary needs without precipitating encephalopathy.

Specific recommendations for the logistics of managing PEM in patients with liver disease are provided in Tables 12.6, 12.9, 12.11 and 12.12. The primary directive for nutritional therapy in cirrhosis is to use standard dietary methods to assure that nutritional requirements are provided and nutritional status is well maintained. The attainment of this strategy means that long-term outpatient management is even more important to these patients than when they are hospitalized for acute illness.



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## SECTION III: CLINICAL MANIFESTATIONS OF LIVER DISEASE

### CHAPTER

# 13

## Associated Systemic Conditions

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### INTRODUCTION

The liver is affected in many systemic diseases and, conversely, primary liver disease can affect every other organ system. Some of these associated conditions are specific to the underlying liver disorder, i.e. the presence of cryoglobulinemia in association with chronic hepatitis C virus infection or emphysema in patients with  $\alpha_1$ -antitrypsin deficiency. Associated conditions however, may not parallel the course of underlying liver disease and their diagnosis may precede or follow the recognition of a liver complaint. Many of these conditions result from alterations in hepatic blood flow and portal hypertension. Others may be based on the underlying pathogenesis of liver disease, whereas a final group may seemingly be bystanders to the hepatologic dysfunction. The extrahepatic manifestations of liver dysfunction may have dramatic clinical relevance. This chapter will focus on the systemic complications of liver disease in general.

### CARDIORESPIRATORY DYSFUNCTION

In evaluating patients with liver disease, up to 70% of patients may complain of dyspnea, while an abnormality of arterial blood gas analysis may be identified in as many as 45% of patients. The causes of pulmonary dysfunction include disorders not specifically related to liver disease per se, whereas a number of conditions exist which are directly attributable to the presence of liver disease and portal hypertension. Differentiation between processes resulting in either microvascular dilatation of the pulmonary vessels leading to hepatopulmonary syndrome (HPS), or arteriolar vasoconstriction leading to portopulmonary hypertension demonstrate that unique changes within the pulmonary circulation can occur in the context of liver disease. These syndromes significantly impact morbidity and mortality. Independent of these syndromes, other causes of pulmonary dysfunction are summarized in Table 13.1.

#### Hepatopulmonary syndrome

The term hepatopulmonary syndrome (HPS) has been given to the triad of liver disease, arterial hypoxemia and intrapulmonary vascular dilatation. In the original descriptions of the entity in juvenile cirrhosis, plastic casts of lung vasculature obtained from autopsy specimens showed both precapillary and capillary dilatations in addition to distinct anatomic arteriovenous communications. It was subsequently established that hypoxemia results from low ventilation to perfusion ratios (ventilation with excess perfusion) in the case of the precapillary/capillary dilatations and anatomic shunting (perfusion with no ventilation) in the case of direct arteriovenous communications. Thus, the response to breathing supplemental 100% inspired oxygen, which may

result in normalization of PaO<sub>2</sub> (arterial oxygen tension), is variable and dependent on the contributions of the vascular abnormalities. It is inappropriate therefore to consider all cases of hepatopulmonary syndrome as intrapulmonary shunting, since by definition shunts result in no improvement on administration of 100% inspired oxygen. As many as 40% of patients with cirrhosis have detectable intrapulmonary vasodilatation and between 8 and 15% of patients will develop sufficient changes in vascular tone to cause hypoxemia and functional limitation.

The importance of recognizing HPS in patients with liver disease is twofold. First, spontaneous regression is rare and most patients develop progressive shunting and have worsening gas exchange over time. Second, mortality when established is significant. Interestingly, moderate to severe HPS may be noted in patients with relatively well preserved synthetic hepatic function. Over time, it has also become apparent that liver transplantation is an effective long-term therapeutic option for HPS with over 80% of patients having regression of intrapulmonary shunting following surgery, albeit with an increased risk of morbidity and mortality in the perioperative period. Therefore, establishing the diagnosis preoperatively is important, so that the condition can be more effectively managed in the post-transplant period. Moreover, it is important to distinguish HPS from portopulmonary hypertension, since the response to liver transplantation is much less dramatic, particularly if advanced disease is unrecognized. Severe portopulmonary hypertension may be a contraindication to liver grafting.

Causes of pulmonary abnormalities in patients with liver disease	
Intrinsic cardiopulmonary disease	Congestive cardiac failure Chronic obstructive pulmonary disease Pneumonia Asthma Pulmonary fibrosis
Specific to liver disease	Emphysema in association with $\alpha_1$ -antitrypsin deficiency Fibrosing alveolitis in association with autoimmune liver disease Presence of large volume ascites/hepatic hydrothorax Hepatopulmonary syndrome Portopulmonary hypertension

**Table 13.1** Causes of pulmonary abnormalities in patients with liver disease.

Specific diagnostic criteria for hepatopulmonary syndrome and portopulmonary hypertension	
Hepatopulmonary syndrome	<p>Chronic liver disease*</p> <p><math>\text{PaO}_2 &lt; 70</math> mmHg or alveolar-arterial oxygen gradient <math>&gt; 20</math> mmHg<sup>†</sup></p> <p>Intrapulmonary vascular dilatations documented by either</p> <ul style="list-style-type: none"> <li>• demonstration of delayed shunt on contrast enhanced transthoracic echocardiogram or</li> <li>• brain uptake of <math>&gt; 6\%</math> following lung perfusion scanning with <math>^{99\text{m}}\text{Tc}</math> macroaggregated albumin</li> </ul>
Portopulmonary hypertension	<p>Portal hypertension</p> <p>Mean pulmonary artery pressure (MPAP) <math>&gt; 5</math> mmHg</p> <p>Pulmonary capillary wedge pressure (PCWP) <math>&lt; 15</math> mmHg</p> <p>Pulmonary vascular resistance (PVR) <math>&gt; 120</math> dynes/s/cm<sup>-5</sup></p>
<p>*Portal hypertension (cirrhotic or non-cirrhotic) has been reported in most cases.</p> <p><sup>†</sup>Some investigators suggest an alveolar-arterial oxygen gradient <math>&gt; 15</math> mmHg.</p> <p>PVR = (MPAP-PCWP)/cardiac output. This criterion helps to discern increased pulmonary artery pressures from high flow/hyperdynamic circulatory states (low PVR) caused by vasoconstriction (high PVR).</p>	

**Table 13.2** Specific diagnostic criteria for hepatopulmonary syndrome and portopulmonary hypertension.

No specific etiology of liver disease is known to increase the risk of developing HPS and the condition has been described in association with non-cirrhotic portal hypertension. In such cases, patients may have congenital hepatic fibrosis or nodular regenerative hyperplasia on liver biopsy. HPS has also been noted in conjunction with Budd-Chiari syndrome suggesting that the presence of both liver injury and portal hypertension contribute to the development of the condition. The majority of patients complain of progressive dyspnea, often worsening when erect. Although, the presence of spider nevi, clubbing and cyanosis has been described in patients with HPS, these features lack sensitivity in discriminating between patients with and without the condition. Pulse oximetry in contrast, is a useful non-invasive modality that screens for hypoxemia. Studies performed on patients with cirrhosis suggest that using a cut-off value of 94% would identify all patients with moderate to severe hypoxemia ( $\text{PaO}_2 < 60$  mmHg).

Diagnostic criteria for both HPS and portopulmonary hypertension are summarized in **Table 13.2**. Since no 'gold standard' diagnostic test for HPS exists, the diagnosis rests on documenting arterial oxygenation abnormalities resulting from intrapulmonary vasodilatation in the setting of liver disease. Intrapulmonary vasodilatation is most commonly demonstrated non-invasively. Contrast echocardiography (CE), and lung perfusion scanning are the commonest modalities used to determine whether shunts

are present or not. Two-dimensional transthoracic echocardiography involves the injection of microbubbles into the circulation (typically agitated saline). A positive test for intrapulmonary vasodilatation occurs when the delayed appearance of microbubbles is identified within the left heart chambers (usually third heartbeat following injection). The presence of an intracardiac shunt results in the immediate appearance of contrast within the right heart. Use of CE has demonstrated that up to 40% of cirrhotic patients may have some degree of intrapulmonary shunting even in the presence of normal blood gasses. The exact significance of this is unknown.

Radionuclide lung perfusion scanning with technetium-labeled macroaggregated albumin particles (MAA) ( $^{99\text{m}}\text{TcMAA}$ ) is commonly used to detect shunting. One advantage of radionuclide lung scanning over CE is its ability to identify the presence of HPS even in the presence of intrinsic lung disease. In addition, it is possible to quantify the degree of shunting present. The presence of HPS is characterized by the deposition of  $> 6\%$  of injected  $^{99\text{m}}\text{TcMAA}$  within the brain. This technique may be employed to assess either progression or resolution of the syndrome. However, its usefulness is limited by being less sensitive than CE and by its inability to evaluate cardiac function.

The diagnosis of HPS may be readily made when arterial hypoxia is moderate or severe and intrapulmonary vasodilatation is detected in the absence of intrinsic cardiopulmonary disease. However, diagnostic criteria for HPS in different studies have included a spectrum of severity of arterial blood gas (ABG) abnormalities from a widened alveolar-arterial oxygen gradient of 15–20 mmHg without hypoxemia reflecting mild defects, to frank hypoxemia reflecting severe oxygenation abnormalities ( $\text{PaO}_2 < 60$ –70 mmHg). Studies of response to 100% oxygen are frequently performed to assess the presence of HPS. Whereas complete correction of  $\text{PaO}_2$  (arbitrarily defined as a  $\text{PaO}_2 > 150$  mmHg) might be expected in patients with functional shunting, this is not identified in patients with anatomic shunts i.e. HPS. To complicate matters however, both functional shunts and anatomic shunts may coexist in patients with HPS and therefore the presence of a positive test (failure of  $\text{PaO}_2 > 150$  mmHg with 100% inspired oxygen) should mandate that the shunt be visualized either by use of high resolution computed tomography (CT) or by pulmonary angiography. Recent data suggests that the degree of dilatation noted on CT scan correlates with the severity of gas exchange abnormalities.

Pulmonary angiography, although highly specific, is not useful as a screening test. Two types of abnormality have been described; type 1, a diffuse spongy appearance of pulmonary vessels during the arterial phase and type 2, the presence of small arterio-venous communications. Occasionally it is possible to perform embolization of type 2 lesions and improve the response to 100% inspired oxygen.

Finally, large volume ascites, presence of hepatic hydrothorax, or intrinsic cardiopulmonary disease can also influence gas exchange and may coexist with HPS. Therefore, in some situations, defining the contribution of intrapulmonary vasodilatation to observed gas exchange abnormalities may be challenging and requires a systematic approach to such patients.

### Natural history

Mortality ranges from 41 to 63% over 2–3 years after diagnosis. After liver transplantation, patients with HPS have a lower survival



time compared with patients without the syndrome, especially in Child-Pugh class C.

### **Treatment**

Liver transplantation is the only proven therapy for HPS if the evaluated end points are based on either total resolution or significant improvement in gas exchange. Data from a multicenter database evaluating outcomes following liver transplantation in patients with either HPS or portopulmonary hypertension indicates that preoperative PaO<sub>2</sub> is the main determinant of survival, with a median PaO<sub>2</sub> of 55 ± 11 mmHg in survivors compared to 37 ± 8 mmHg in non-survivors. In many cases, it may take up to 1 year for gas exchange abnormalities to resolve following transplant. Complications include immediate postoperative deoxygenation requiring both prolonged mechanical ventilation and prolonged intensive therapy unit (ITU) stay. Other complications include worsening pulmonary hypertension and cerebral embolic bleeds. Use of nitric oxide and alteration of position (such as Trendelenburg) during mechanical ventilation to improve postoperative gas exchange has been utilized with some success.

In contrast, medical therapy has been less effective. Somatostatin, almitrine, indomethacin and plasma exchange have been unsuccessful. Aspirin occasionally improves arterial oxygenation in children with HPS. Administration of methylene blue, a substance that inhibits the effect of nitric oxide on guanylate cyclase may prevent vasodilatation acutely and improve oxygenation in HPS. Finally, garlic has been suggested be of benefit.

Recently, interest has focused on the use of transjugular intrahepatic portosystemic shunt (TIPS) in the management of HPS but the data are limited and contradictory. TIPS should be considered an experimental treatment for HPS and use should be confined to clinical trials.

### **Portopulmonary syndrome**

The association between pulmonary artery hypertension and portal hypertension has been termed portopulmonary hypertension. Defined as a mean pulmonary artery pressure (MPAP) >25 mmHg and a pulmonary capillary wedge pressure (PCWP) <15 mmHg occurring in the setting of portal hypertension and less commonly, an elevated transpulmonary gradient (MPAP–PCWP >10 mmHg) or elevated pulmonary vascular resistance are the applied definitions. These are summarized in Table 13.2. Autopsy series suggest a prevalence of pulmonary hypertension in patients with cirrhosis of 0.73% compared to a prevalence of 0.13% in patients without chronic liver disease. Recent data suggests that the prevalence of portopulmonary hypertension in patients referred for transplantation may be as high as 3.5–12.5%. Moreover, it is suggested that cirrhosis accounts for 7% of all pulmonary hypertension.

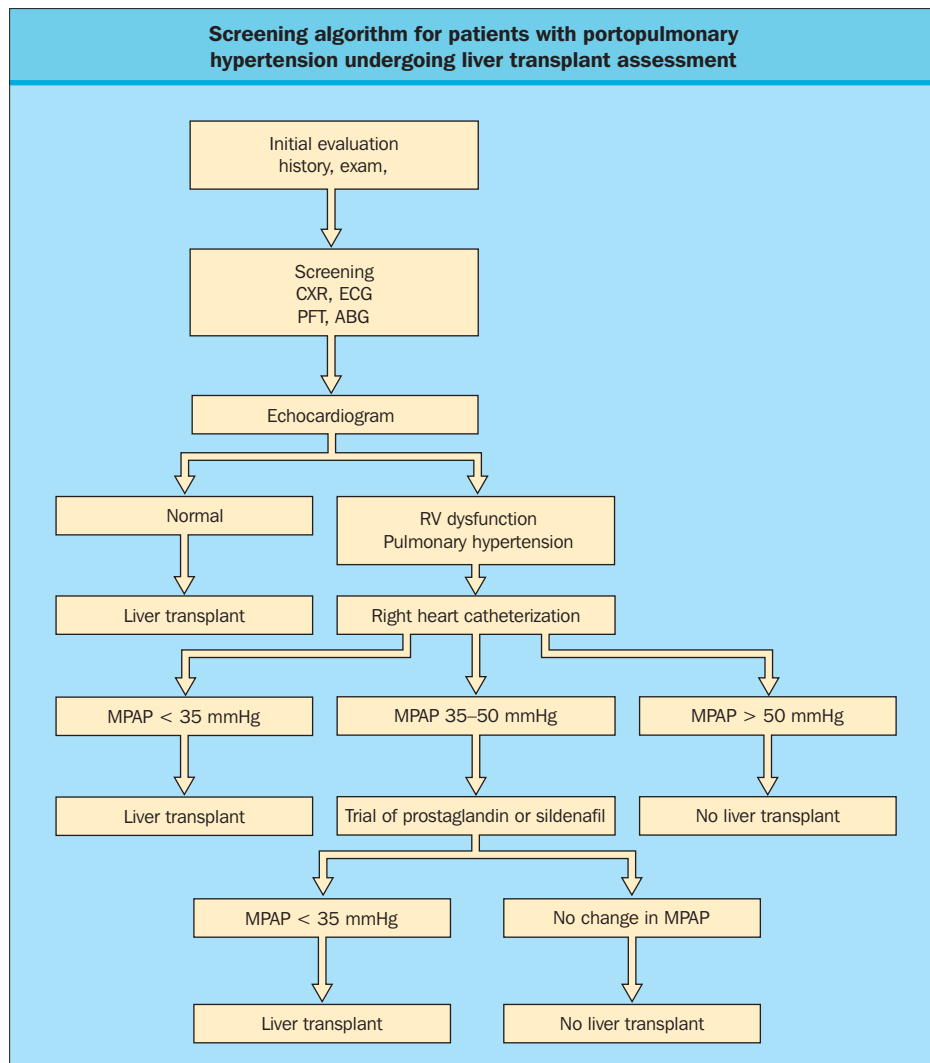
Patients with portopulmonary hypertension present with progressive symptoms that may begin with fatigue, dyspnea and peripheral edema. Commonest symptoms include dyspnea on exertion in 81–96%, syncope in 24–29%, chest pain in 15–24%, whereas commonest physical findings included a loud pulmonary heart sound in 65–82% and a systolic murmur in up to 69% of patients. Electrocardiographic abnormalities included signs of right heart strain in over 90% of patients. Men and women are equally affected.

The histologic findings in portopulmonary hypertension are similar to those found in primary pulmonary hypertension and include smooth muscle proliferation and hypertrophy which may be reversible, and concentric intimal fibrosis, necrotizing vasculitis and plexogenic arteriopathy which may be irreversible. The cause of these findings is not completely understood, but portopulmonary hypertension has been reported in the setting of portal hypertension with or without cirrhosis. The etiology is related to a complex interaction between the pulmonary endothelium and the hyperdynamic high-flow circulatory state, excess central volume and non-embolic pulmonary vasoconstriction. In primary pulmonary hypertension, abnormal endothelial function and growth and proliferation of myofibroblasts appear to play a central role. Altered production of endothelin-1 and serotonin, both potent vasoconstrictors, coupled with impaired production of vasodilators such as nitric oxide and prostacyclin appears to play a role. Although similar to primary pulmonary hypertension from a histologic standpoint, it is differentiated from it by the presence of a dramatically increased cardiac output.

Although symptoms are common in patients with portopulmonary hypertension and correlate with disease severity, a high index of suspicion is required since these symptoms are non-specific. Screening of portopulmonary hypertension is performed using chest radiography, electrocardiography and transthoracic echocardiography. Using Doppler echocardiography it is possible to estimate right ventricular systolic pressure in most patients. An elevated right ventricular systolic pressure >50 mmHg (normal, <30 mmHg) suggests the presence of portopulmonary hypertension and requires further evaluation in the form of right heart catheterization. In transplant candidates, two-dimensional echocardiography may overestimate the degree of pulmonary hypertension. The positive and negative predictive values of echocardiography for identifying clinically significant pulmonary hypertension (PASP >50 mmHg) are 37.5% and 91.9%, respectively in cirrhotic patients.

### **Therapy**

Unlike the limited therapeutic options available to treat HPS, a number of novel therapies exist to treat portopulmonary hypertension including liver transplantation. Data from a multicenter database indicates that the post-transplant mortality was 36%. There was no significant difference in measured hemodynamic parameters between survivors and non-survivors (MPAP in survivors was 45 ± 14 mmHg compared to 44 ± 8 mmHg in non-survivors). Pulmonary vascular resistance was measured at >250 dynes/s/cm<sup>-5</sup> in the majority of patients who died. The optimum pretransplant management of portopulmonary hypertension remains problematic in that mortality predominantly occurs as a consequence of right heart failure, but it is logical to suggest that earlier treatment with pulmonary vascular modulators (e.g. epoprostenol or sildenafil) followed by transplant may result in significant improvement in outcome. One of the difficulties in selecting candidates for transplantation is the prediction of post-transplant mortality in patients with more advanced degrees of pulmonary hypertension. Although some centers suggest that transplantation should not be offered to patients with a MPAP >35 mmHg, others report successful outcomes with MPAP in the 45–50 mmHg range. An algorithm for managing patients with portopulmonary hypertension is illustrated in **Figure 13.1**.



**Figure 13.1 Screening algorithm for patients with portopulmonary hypertension undergoing liver transplant assessment.** In patients with suspected portopulmonary hypertension, eligibility for liver transplantation is dependent on demonstrating a reversible component to the pulmonary hypertensive process or an absolute mean pulmonary artery (PA) pressure of <35 mmHg. Although liver transplant has been performed in patients with higher PA pressures, the results of transplantation are highly variable in that setting. Prostaglandin therapy is the most established form of treatment for reducing mean PA pressure in patients with portopulmonary hypertension. ABG, arterial blood gases; CXR, chest X-ray; ECG, electrocardiogram; MPAP, mean pulmonary artery pressure; PFT, pulmonary function tests; RV, right ventricle.

In primary pulmonary hypertension, the response to inhaled nitric oxide or intravenous epoprostenol is frequently measured and if a decrease in MPAP and pulmonary vascular resistance is measured, then administration of calcium channel blockers has been shown to prolong survival. Although no randomized data exists for patients with portopulmonary hypertension, similar strategies have been applied with some success. Alternative agents gaining acceptance in the treatment of primary pulmonary hypertension include the endothelin receptor antagonist bosentan that is approved for use in functional class III to IV pulmonary arterial hypertension. Sildenafil has been reported as being useful in the treatment of portopulmonary hypertension. The mechanism of this therapeutic intervention involves nitric oxide (NO), whose signaling is mainly mediated by the guanylate cyclase/cyclic guanylate monophosphate pathway. The effects of this second messenger system are limited by enzymatic degradation through phosphodiesterases (PDEs). This agent, despite oral application, displays characteristics of a pulmonary selective vasodilator.

### Cardiac dysfunction in cirrhosis

The cardiovascular effects of cirrhosis are wide ranging and encompass not only effects on the systemic and splanchnic circu-

lation but also on the heart itself. Whereas cardiac dysfunction as a consequence of specific liver diseases such as hereditary hemochromatosis or alcohol-related toxicity are well recognized, increasingly, patients are identified with primary cardiac dysfunction as a consequence of chronic liver disease. The entity known as cirrhotic cardiomyopathy is an example of this.

Patients with cardiac dysfunction and cirrhosis tend not to present with overt cardiac failure, presumably due to the reduction in afterload that accompanies the generalized vasodilation of advanced liver disease. However, they may be unable to cope with the increased stress placed on the heart by procedures such as TIPS insertion or liver transplantation, and therefore require careful evaluation.

### Cirrhotic cardiomyopathy

It was recognized as early as the 1960s that despite the presence of a normal resting cardiac index in patients with cirrhosis, an abnormal cardiac response to exercise existed. In patients with mainly alcoholic cirrhosis it could be shown that the expected rise in stroke index was either abrogated or did not occur at all. Other studies confirmed this and showed that left ventricular

ejection fraction diminished on exercise. Furthermore patients with cirrhosis were shown to be hyporesponsive to the cardiac glycoside ouabain.

These early studies were performed mainly on patients with alcoholic cirrhosis and alcohol was thought to be the major pathogenic factor in the development of this latent cardiomyopathy. Moreover, alcoholic cardiomyopathy typically presents with a dilated ventricle whereas the cirrhotic cardiomyopathy is characterized by ventricular hypertrophy.

In the last decade, the use of animal models and two-dimensional echocardiography has allowed a more detailed assessment of cardiac function at rest and under conditions of exercise. These studies have shown that whilst systolic function is well preserved at rest there is chronotropic and inotropic incompetence characterized by impaired increases in cardiac index, heart rate and ejection fraction on exercise compared to controls. These abnormalities correlated with severity of liver disease and presence or absence of ascites and changes were seen equally in patients with alcoholic liver disease and liver disease of other etiologies. The failure of the ejection fraction to increase, despite an increase in venous return, is suggestive of diastolic dysfunction. This may be assessed at echocardiography by examining the ratio of early maximal ventricular filling velocity to late diastolic or atrial velocity (E/A ratio). The presence of ventricular hypertrophy and left atrial dilation on echocardiogram supports diastolic dysfunction as the major abnormality in cirrhotic cardiomyopathy.

The degree of diastolic dysfunction is well demonstrated by studies in patients following TIPS. Following this procedure, pressures on the right side of the heart increase dramatically representing increased cardiac preload. Ordinarily this would lead to an increase in cardiac output but in patients following TIPS there is a sustained increase in pulmonary arterial wedge pressure and an increase in left atrial diameter reflecting limited left ventricular preload reserve.

Studies using brain natriuretic peptide (BNP) as a marker of high ventricular pressure reflecting cardiac dysfunction have shown that the levels of BNP correlate closely with the severity of cirrhosis as measured by Childs-Pugh class and also the degree of portal hypertension as measured by hepatic venous pressure gradient. The fact that there is no correlation with markers of the hyperdynamic circulation suggests that the cardiomyopathy is primary rather than an adaptive response to the hyperdynamic circulatory state.

The natural history of cirrhotic cardiomyopathy is largely unknown. However correction of a prolonged QT interval has been documented post-liver transplantation and the cardiodynamic changes seen post-TIPS resolve over time suggesting the heart is capable of remodeling if the underlying liver disease is reversed. Currently, no specific therapy can be recommended for patients with cirrhotic cardiomyopathy. Under the challenge of a significant stimulus such as liver transplantation, overt ventricular failure may develop. In such a case, standard treatment should be initiated as for non-cirrhotic heart failure. Recommendations on the use of specific inotropes remain uncertain, since the majority of data is based almost exclusively on experimental studies in animals. The relative lack of efficacy of  $\beta$ -agonists and cardiac glycosides point to drugs such as milrinone as the inotrope of choice if the clinical situation demands augmentation of the cardiac output.

### Ischemic heart disease and cirrhosis

Until recently, the prevalence of ischemic heart disease in patients with cirrhosis was thought to be low. However, since the advent of liver transplantation it has become clear that the incidence of coronary artery disease (CAD) may be higher in patients with end-stage liver disease than the general population. Coronary artery disease in cirrhosis is largely asymptomatic, yet patients with unrecognized CAD may develop significant ischemic complications both perioperatively and especially at reperfusion during liver transplant surgery. Furthermore, the course of CAD is accelerated post-transplant, for the most part due to the influence of immunosuppressive agents such as the calcineurin inhibitor class of drugs.

There is no generally accepted protocol for the identification of patients with CAD prior to liver transplantation. Widespread application of coronary angiography is not feasible because of cost, in addition to a very real risk of causing renal failure secondary to the contrast load. A number of studies have used dobutamine stress echocardiography as a non-invasive means of identifying patients with underlying coronary artery disease who may benefit from coronary angiography. However, the yield of stress echocardiography is low in asymptomatic individuals with no major risk factors; therefore, an alternative approach is to perform angiography on patients with a predefined number of risk factors prior to undergoing transplantation.

The identification of moderate-to-severe CAD need not be a contraindication to successful liver transplantation. Increasingly patients can be treated by percutaneous revascularization prior to liver transplantation or even undergo coronary artery bypass grafting (CABG) at the same time as liver transplantation assuming the left ventricular function remains intact. What is clear, however, is that cardiac surgery should not be undertaken lightly in isolation in patients with established liver disease as mortality rates of 30–50% have been reported for Child A or B cirrhosis.

### BONE DISEASE IN LIVER DISEASE

The presence of metabolic bone disease in patients with liver disease has been termed hepatic osteodystrophy. Historically, the major bone abnormality in patients with end-stage liver disease was thought to be osteomalacia. However, modern imaging methods and more detailed histomorphometric studies have revealed osteoporosis to be the most important abnormality of bone in patients with cirrhosis. Metabolic bone disease, however, is common and under-recognized as a cause of morbidity and mortality in both patients with chronic liver disease and in those who have undergone successful liver grafting. In addition, it has been long recognized that the clinical findings of clubbing and hypertrophic pulmonary osteopathy are associated with the presence of cirrhosis. The presence of hypertrophic osteoarthropathy illustrated in **Figure 13.2** is mainly attributable to platelet-derived growth factor and/or other additional growth factors derived from the lungs.

#### Osteoporosis

Osteoporosis is defined as a reduction in bone mass of greater than 2.5 standard deviations from the mean of a young adult population. The number of standard deviations away from this mean is defined as the T score. Therefore, a T score of  $-2.5$  defines





**Figure 13.2 Pulmonary osteodystrophy.** The periosteal reaction is classical of this condition, which is almost always associated with clubbing.

the presence of osteoporosis. Lesser degrees of bone loss (T scores between  $-1$  and  $-2.5$ ) are defined as osteopenia. Although there is no defined fracture threshold, the risk of fracture increases as the T score decreases. In the case of hip fractures, the relative risk is 2.6 per standard deviation decrease from normality.

Under normal conditions, there is a constant turnover of bone with new bone formation balancing that of resorption. In patients with chronic liver disease, an imbalance exists whereby the rate of bone resorption exceeds that of new bone formation. Most studies favor decreased formation of new bone as the major determinant of bone loss. Patients with cirrhosis demonstrate both a decrease in osteoid (unmineralized bone) thickness and defects in osteoblast activity. These abnormalities are not just seen in patients with cholestatic liver diseases such as primary biliary cirrhosis (PBC) or primary sclerosing cholangitis, but also in diseases of predominant hepatitic etiology such as autoimmune hepatitis or alcohol related liver disease.

The underlying cause of this impairment is unknown although a direct inhibitory effect of unconjugated bilirubin on osteoblast function has been observed. Osteoblast function is also directly affected by alcohol. Patients with alcohol related liver disease have lower levels of osteocalcin (a marker of bone formation). Following the complete withdrawal of alcohol, osteocalcin levels return to normal, thus indicating recovery of osteoblast function. Use of corticosteroids contributes significantly to the low bone mineral density seen in patients with autoimmune liver disease. Abnormalities in vitamin D metabolism and calcium absorption may be responsible, at least in part, for the accelerated rate of bone loss in patients with cholestatic liver diseases.

#### **Incidence and prevalence of hepatic osteodystrophy**

In controlled trials, the prevalence of osteoporosis in patients with a range of chronic liver diseases is up to 30%. The prevalence in PBC is particularly high and this may in part reflect the older female population that is a characteristic of this

disease. Some studies show significant correlation with the stage of PBC and bone loss. Moreover, patients with PBC may also have a higher rate of bone loss following menopause compared to controls, which may represent a predisposition to osteoporosis in these patients. The prevalence of osteoporosis in PSC may be higher due to concomitant use of steroids in patients with coexisting inflammatory bowel disease. Factors that predict osteoporosis in patients with chronic liver disease are a history of corticosteroid use, advanced age, female sex and reduced body mass index.

A point prevalence of fractures of up to 44% has been reported in patients with liver disease. Moreover, the fracture rate is twice that of controls and appears to correlate with the degree of liver dysfunction. Patients with PBC appear to be at particular risk with between 5 and 13% of patients developing new vertebral fractures within 2 years of follow-up.

#### **Post-transplantation bone disease**

As post-transplant survival continues to improve, the presence of osteoporosis has become a major cause of mortality and morbidity. Vertebral fracture rates as high as 30% have been reported in the first year after liver transplantation and can lead to severe pain and disability. The etiology of post-transplant bone disease is multifactorial and immunosuppressive regimens based on corticosteroids and calcineurin inhibitors clearly have a large part to play in its development. Most studies have demonstrated continued bone loss for the first 3–6 months following liver transplantation. This period of time tends to be that when doses of immunosuppression are highest and appears to correlate with liver graft function. A number of studies have demonstrated that bone mineral density (BMD) tends to return to pretransplant levels over the course of the first year post-transplant in patients with good graft function, but remains low in those patients with ongoing graft dysfunction.

This improvement in bone density is reflected by histologic studies which have shown increased osteoblastic activity and new bone formation as early as 3 months post-transplant. As noted previously, patients with cholestatic disorders have an increased rate of bone loss post-transplant compared to patients with other etiologies of liver disease but, again, this may seem artificially high due to the higher prevalence of older female patients in this group. Factors that predict bone loss post-transplant include poor nutrition, presence of cholestasis, alcohol use and importantly, the presence of pretransplant osteopenia.

#### **Osteomalacia and abnormalities of calcium and vitamin D metabolism**

Osteomalacia is defined as deficient mineralization of bone due to the deficiency of vitamin D. In children, this leads to characteristic skeletal abnormalities known as rickets. In adults osteomalacia has a varied clinical presentation ranging from the absence of symptoms to those of bone pain or proximal myopathy. Classically, the diagnosis of osteomalacia is made by histologic assessment of a bone biopsy. The biochemical abnormalities associated with osteomalacia such as low serum calcium, low phosphate, and raised serum alkaline phosphate may be present only singly or not at all making the diagnosis difficult on clinical grounds without a high index of suspicion. The classical radiologic abnormality of Looser zones or pseudofractures is pathognomic but occurs only rarely.

Osteomalacia due to liver disease was thought to be common and a major factor in the development of hepatic osteodystrophy. Despite quite marked abnormalities in calcium and vitamin D metabolism, histologic studies have shown that true osteomalacia is actually a rare event in chronic liver disease and is much more likely to be due to inadequate nutrition or lack of natural sunlight than due to liver disease per se.

Low levels of vitamin D have been demonstrated in a number of chronic liver diseases and some studies have suggested that there is impaired 25-hydroxylation of vitamin D in patients with chronic liver disease. Patients with cholestatic liver disease and to a lesser extent, patients with alcohol related liver disease may also malabsorb vitamin D and calcium. Despite this, there is no association between levels of calcium and vitamin D and severity of liver disease, and in patients with PBC, vitamin D supplementation alone did not prevent the development of osteopenia in PBC.

### Management of hepatic osteodystrophy

The optimal management of hepatic osteodystrophy depends on the timely appreciation of patients at risk, and the identification of those already suffering from osteoporosis. Dual X-ray absorptiometry (DEXA) is an accurate method of diagnosing osteoporosis or osteopenia in patients at risk. High-risk groups that should be evaluated with DEXA are those with a history of fragility, fracturing, or risk factors such as age, hypogonadism, corticosteroid use, low body mass index, cholestatic liver disease and those being evaluated for orthotopic liver transplantation.

Attention should be paid to modifiable risk factors in all patients such as nutrition, exercise, the cessation of smoking and avoidance of alcohol (**Table 13.3**). Calcium and vitamin D supplementation should be offered to all patients with evidence of osteopenia. Postmenopausal women with chronic liver disease may be offered hormone replacement therapy (HRT) in the form of estrogen, preferably by the transdermal route. Therefore, the more global risks and benefits of HRT should be evaluated for each individual patient prior to commencing therapy.

Patients with established osteoporosis and those with osteopenia receiving corticosteroids should receive therapy with a bisphosphonate. Bisphosphonates are effective at inhibiting the osteoclastic resorption of bone and are efficacious in treatment of osteoporosis in the general population. Studies in patients with chronic liver disease have shown cyclical etidronate is effective at reducing bone loss. Likewise alendronate has also been shown to be effective. For patients with established fractures who have pain as a major symptom, the administration of calcitonin can be effective in addition to the therapeutic options outlined in Table 13.3.

Patients undergo rapid bone loss in the first 3–6 months post-transplant. To minimize this bone loss, consideration should be given to optimizing nutrition and reducing the amount of time spent immobilized prior to transplant. For patients who have been diagnosed with osteoporosis, consideration should be given to the application of a steroid-sparing regimen to prevent further bone loss. It is reasonable to supplement calcium and vitamin D

Strategies of proven benefit in the management of metabolic bone disease in patients with chronic liver disease or following liver transplantation		
General measures	Exercise Stop smoking Increase intake of dairy products Avoidance/cessation of alcohol	
Pharmacologic intervention	Calcium and vitamin D	
	• Calcium	1200 mg/day
	• Cholecalciferol	10 µg/day*
	Bisphosphonates	
	• Alendronic acid	10 mg/day or 70 mg once weekly
	• Risedronate sodium	5 mg/day or 35 mg once weekly
	• Etidronate	400 mg/day for 14 days with CaCO <sub>3</sub> for 76 days
	Calcitonin	
	• Calcitonin	100 IU/day by injection (subcutaneous or intramuscularly) or 200 IU intranasal spray
	Hormone replacement therapy	
*Important to ensure adequate vitamin D prior to initiating bisphosphonate therapy		

**Table 13.3** Strategies of proven benefit in the management of metabolic bone disease in patients with chronic liver disease or following liver transplantation.

in all patients. Patients who remain on steroids for more than 3 months require careful monitoring and may benefit from bisphosphonate therapy. HRT may be beneficial in some post menopausal women. In keeping with the pretransplant setting, there is no reason why bisphosphonates cannot be used to treat osteopenia/porosis. Indeed, the use of intravenous pamidronate in individuals with established fractures following liver transplantation has been shown to be of significant clinical benefit.

## DISORDERS OF COAGULATION AND HEMOSTASIS

Disorders of coagulation are almost a universal finding in patients with chronic liver disease. The etiology of coagulopathy in most patients is multifactorial and may result from decreased synthesis of clotting factors or impaired clearance of anticoagulants. Moreover, patients may have overt thrombocytopenia and decreased platelet function. Finally, disseminated intravascular coagulation can be demonstrated in a number of patients. A summary of disorders of coagulation is given in Table 13.4.

### Changes in clotting factors in liver disease

Vitamin K is a cofactor for the synthesis of factors II, VII, IX and X. Vitamin K is required for the  $\gamma$ -carboxylation of glutamic acid residues in these factors and is vital for the correct functional interaction of these factors with phospholipid. As vitamin K is a fat soluble vitamin, it requires adequate levels of bile excretion for its absorption. Hence, in patients with severe cholestasis and bile duct obstruction, deficiency of vitamin K is a common cause of coagulopathy. Treatment of the deficiency with intravenous vitamin K quickly reverses the coagulopathy in such patients. Where the coagulopathy does not correct, it can be assumed that the impairment results from reduced synthetic capacity of the liver rather than vitamin K deficiency. Interestingly in cirrhosis small amounts of factors have been observed to

have abnormal  $\gamma$ -carboxylation; this may reflect deficiency of hepatic carboxylase.

When the synthetic capacity of the liver is impaired as in cirrhosis or acute liver failure, the first clotting factor to be affected is factor VII due to its short half-life. The other vitamin K dependent proteins are then affected by a degree that correlates with the amount of liver damage.

Factor V is synthesized in the liver independent of vitamin K. Decreases in factor V are more specific for actual parenchymal liver damage and have been used by some groups as a prognostic marker for the need for liver transplantation. In the presence of cholestasis, factor V levels may be raised or normal despite marked reductions in the vitamin K dependent factors. Other factors such as factor IX, XII, pre-kallikrein and high molecular weight kininogen are reduced in patients with chronic liver disease. Factor VIII levels are often increased in chronic liver disease. This may relate to either increased production by extrahepatic sites of synthesis, decreased clearance or release of factor VIII from damaged endothelial cells.

### Anti-thrombin III, protein C and protein S

These molecules are synthesized and cleared by the liver. Anti-thrombin III levels decrease in acute liver failure and cirrhosis due to decreased synthesis, clearance and transcapillary loss into other compartments such as into ascitic fluid. This would be expected to produce a procoagulant state but this does not occur probably due to the severe coexisting abnormalities in coagulation. Following liver transplantation, excess anti-thrombin III can be lost in the ascitic fluid from abdominal drains, and this may predispose to a prothrombotic state. Whether release of large amounts of anti-thrombin III into the circulation after peritoneovenous shunting procedures contributes to disseminated intravascular coagulation (DIC) is unknown.

Both protein C and protein S levels are also markedly reduced in liver disease with levels of protein S tending to be higher than levels of protein C. This reflects extrahepatic synthesis of this molecule.

### Thrombocytopenia and platelet function

Platelets may be reduced in number and function due to a number of different mechanisms in liver disease. Thrombocytopenia can be observed in most patients with acute liver failure and is present in up to 60% of patients with cirrhosis. In the absence of other contributory factors the platelet count rarely fall below 30/mm<sup>3</sup>. Splenic sequestration in cirrhosis accounts for most of the thrombocytopenia in chronic liver disease and up to 90% of the total platelet pool may be sequestered in the spleen. Platelet sequestration in the spleen is associated with the immune destruction of platelets mediated by platelet bound immunoglobulin G (IgG). Platelet count correlates with spleen size and has been used as a surrogate marker of both the presence of portal hypertension and esophageal varices.

Thrombopoietin is a cytokine synthesized within the liver that promotes thrombopoiesis and decreased levels of this cytokine have been demonstrated in patients with chronic liver disease and cirrhosis. Thrombopoietin levels increase after successful liver transplantation and this is associated with a partial recovery of the platelet count. Thrombocytopenia may occur in individuals with alcoholic liver disease due to a directly toxic effect of alcohol on the bone marrow and presence of coexistent folate deficiency.

Causes of coagulation defects in liver disease	
Factor deficiency	All except factor VIII Vitamin K deficiency
Fibrinolysis	Decreased clearance of tissue plasminogen activator Decreased hepatic synthesis of plasminogen activation inhibitor-1 Elevation in fibrin degradation products Activation by systemic sepsis
Disseminated intravascular coagulation	Activation of thrombin by tissue factor Consumption of platelets and clotting factors
Thrombocytopenia and platelet dysfunction	Hypersplenism Immune destruction Decreased thrombopoietin Drugs and alcohol Megaloblastic anemia Renal dysfunction

**Table 13.4 Causes of coagulation defects in liver disease.**



Prolongation of the bleeding time in patients with liver disease despite adequate circulating platelet numbers is suggestive of abnormal platelet function. Impaired aggregation to factors such as adenosine diphosphate (ADP) and arachidonic acid can be demonstrated in patients with liver disease. Local factors related to the vessel wall such as increased production of NO are also of importance. Finally, patients with advanced liver disease often have coexistent renal failure and impairment of platelet function in response to azotemia has been well characterized.

### **Disseminated intravascular coagulation**

DIC is the result of the uncontrolled formation of thrombin within the circulation. The characteristic findings in DIC are platelet microthrombi and fibrin deposition in vessels. This is associated with consumption of platelets and clotting factors resulting in a coagulopathy and bleeding diathesis. There has been much debate over whether or not true DIC exists in patients with liver disease because fibrin deposition in vessels has not been demonstrated. Despite this controversy, there is evidence of a low grade DIC characterized by increased levels of fibrin degradation products and shortened half-lives of prothrombin, plasminogen and fibrinogen in patients with chronic liver disease.

Patients with this low grade DIC are predisposed to develop severe DIC in response to surgery and sepsis. DIC has also been described in patients with acute liver failure, acute fatty liver of pregnancy and following procedures such as peritoneovenous shunting. The initial step in the pathogenesis of DIC is activation of thrombin by interaction of tissue factor and factor VIIa. DIC may also be triggered by the release of 'thromboplastin-like' material from necrotic liver and in the context of sepsis, tissue factor on circulating monocytes can also result in thrombin activation.

### **Dysfibrinogenemia and fibrinolysis**

Dysfibrinogenemia is a common finding in liver disease, being reported in the settings of acute liver failure, severe end-stage liver disease and hepatocellular carcinoma. Fibrinogen levels are usually normal, but when assessed in functional assays, activity is low. This is due to delayed polymerization of the fibrin molecule which causes a prolongation of the thrombin time.

Hyperfibrinolysis is also common in advanced liver disease and is associated with both gastrointestinal bleeding and bleeding at the sites of non-surgical and surgical trauma. The presence of hyperfibrinolysis in liver disease is suggested by shortened whole blood euglobulin clot lysis times, elevated levels of fibrin degradation products and typical appearances on thromboelastography. Hyperfibrinolysis in liver disease is mainly due to decreased clearance of tissue plasminogen activator (tPA). Tissue plasminogen activator catalyses the conversion of plasminogen to plasmin and the subsequent breakdown of fibrin clots. In health, circulating tPA is bound to its inhibitor plasminogen activator inhibitor-1 (PAI-1) which limits the effect of circulating tPA. High circulating levels of tPA have been described in cirrhotics especially those with alcoholic cirrhosis and this, coupled with the decreased hepatic synthesis of PAI-1, leads to activation of fibrinolysis. Conversely, in acute liver failure, levels of PAI-1 have been found to be high and this may explain the finding of hypofibrinolysis in acute liver failure.

Sepsis can have a profound effect on activation of the fibrinolytic system through the release of cytokines such as

tumor necrosis factor- $\alpha$ , and this may be the mechanism for the observed link between sepsis and gastrointestinal bleeding.

### **Evaluation of coagulopathy**

A number of standard laboratory tests are available to help in the evaluation of coagulopathy in liver disease. The standard coagulation profile includes the prothrombin time (PT) or international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen and platelet count. The PT is a measure of the activity of the extrinsic and common clotting pathways and is mainly dependent on the vitamin K dependent clotting factors (factors II, VII, IX and X). In early liver disease, decreased levels of factor VII are responsible for the prolongation of the PT, which may only be a few seconds beyond the normal range. In severe liver disease, the PT is more severely prolonged and reflects the severity of the underlying liver disease. A number of multivariate analyses have confirmed the usefulness of PT in a range of prognostic models, including the model for end-stage liver disease (MELD), Child-Pugh classification of disease severity in cirrhotic patients and the King's College criteria for defining mortality without liver transplantation in acute liver failure. Many centers, however, use the INR to describe the coagulation abnormality in liver disease. However, it must be remembered that the INR was developed for control of oral anticoagulant dosing and an INR value in liver disease may not represent the same degree of inhibition of coagulation as the same INR in a patient treated with oral anticoagulants.

The APTT measures the activity of the intrinsic and common coagulation pathways. The APTT is mildly abnormal in patients with vitamin K deficiency, with larger elevations noted in patients with chronic liver disease. Acute liver failure and DIC are associated with much greater prolongations in the APTT than chronic liver disease. Fibrinogen levels may be normal in chronic liver disease but as disease progresses the levels fall reflecting decreased hepatic synthesis and also loss of fibrinogen into ascites. Low levels of fibrinogen are seen in acute liver failure and DIC. Platelet numbers <30 000 should prompt a search for another cause such as DIC. Other tests such as D-dimer levels and fibrin degradation products are helpful in ruling out fibrinolysis or DIC, but there is considerable overlap between these conditions and diagnosis can be difficult. Measurement of specific coagulation factors is generally unhelpful apart from factor V which can be used as a marker of poor prognosis in acute liver failure.

It is generally accepted that static tests of coagulation such as PT, APTT and platelet counts may not be truly representative of the *in vivo* status of the coagulation system, because of the complex and multifactorial etiology of these patients. Moreover, it has been demonstrated that liver bleeding time following laparoscopic biopsy correlated poorly with standard markers of coagulation. Thromboelastography (TEG) has been used in liver transplantation for the rapid evaluation of coagulopathy. TEG measures the clotting dynamics of whole blood and provides information on the interaction between platelets, coagulation and fibrinolysis. TEG guided treatment of coagulopathy has been shown to decrease transfusion requirements in liver transplantation and in critically ill patients with liver disease in intensive care units.

### Treatment of coagulopathy

In general, there is no place for the routine correction of abnormal laboratory values unless clinical bleeding is present or an invasive procedure such as central line insertion or liver biopsy is planned. Vitamin K should be given intravenously to correct the component of the coagulopathy associated with vitamin K deficiency and is usually effective within a matter of hours.

Platelet infusions to correct thrombocytopenia are indicated in patients with platelet counts  $<50\,000/\text{mm}^3$  in the presence of active hemorrhage, or prior to invasive procedures. A unit of platelets can be expected to raise the platelet count by  $10\,000/\text{mm}^3$  although patients with splenomegaly or DIC may have a lesser increment in platelet count.

Fresh frozen plasma (FFP) is manufactured from single donor blood transfusions and stored at  $-30^\circ\text{C}$  until use. It is rich in all the clotting factors and is usually presented as a volume of between 250 and 300 mL. It is usually prescribed as a dose of 15 mL/kg which is often effective at correcting the PT to  $<3$  s prolonged. The effect is short lived and may last no longer than 12 h. Cryoprecipitate is formed by thawing FFP at  $4^\circ\text{C}$  and is rich in fibrinogen, factor VIII and von Willebrand factor. Cryoprecipitate is useful in patients with hypofibrinogenemia and bleeding, or in patients with massive bleeding. It does have an effect on both the PT and APTT, although not as great as FFP. It may also be of benefit in the treatment of coagulopathy if the patient is at risk of pulmonary edema, due to the smaller volume infused. The usual dose is between 5 and 10 units.

Antifibrinolytic agents such as tranexamic acid,  $\epsilon$ -aminocaproic acid and aprotinin are useful in situations of hyperfibrinolysis. These agents induce inhibition of plasminogen activation and plasmin activity. In liver transplantation, aprotinin and tranexamic acid have been shown to decrease blood transfusion requirements. Although helpful in hyperfibrinolysis, care must be taken not to apply these drugs in DIC as this may increase the risk of thrombosis.

Recombinant factor VII has been used to correct the coagulopathy of liver disease. This agent was initially developed to treat hemophilia A, and works by augmenting the initial step of the coagulation cascade, namely the interaction of factor VIIa and tissue factor. Theoretically, because tissue factor is required, augmentation of factor VIIa will enhance coagulation only at the site of tissue injury, thus avoiding systemic activation of coagulation. Recombinant factor VII has been shown to normalize the PT in patients with liver disease for a duration that is dose-related e.g. a dose of  $80\,\mu\text{g}/\text{kg}$  normalizes the PT for up to 12 h. Recombinant factor VII also has the added benefit of augmenting platelet function and has been used to correct the PT in patients prior to liver biopsy and intracranial pressure monitor insertion. Furthermore, recombinant factor VII has shown ability in small trials to arrest hemorrhage in bleeding esophageal varices, although rebleeding rates were not affected. In liver transplantation, a decreased need for blood and blood products has been demonstrated during surgery with the use of this agent.

Although, recombinant factor VII is clearly a useful addition to the options available for the correction of coagulopathy in patients with liver disease, it may have potential drawbacks. Theoretically, such a dramatic acceleration of the coagulation cascade could worsen DIC and lead to thrombosis. In addition, recombinant factor VII is expensive and therefore cannot be

Treatment of coagulopathy in liver disease		
General measures	FFP	10–15 mL/kg*
	Platelets	1–2 adult pools
	Cryoprecipitate	5–10 IU
Obstructive jaundice or prolonged cholestasis	Intravenous vitamin K	
Hyperfibrinolysis <sup>†</sup>	Tranexamic acid Aprotinin $\epsilon$ -Aminocaproic acid	
Disseminated intravascular coagulation	Treatment of underlying cause FFP/platelets/ cryoprecipitate	
FFP, fresh frozen plasma. For cases with life-threatening hemorrhage or where other measures have failed consideration can be given to the administration of recombinant factor VII at a dose of $80\,\mu\text{g}/\text{kg}$ . *Cryoprecipitate can be helpful in correcting the prothrombin time when there are concerns about volume overload. <sup>†</sup> Especially in liver transplantation.		

**Table 13.5 Treatment of coagulopathy in liver disease.**

recommended for the routine correction of hemostatic abnormalities in liver disease. Table 13.5 summarizes the agents used in the treatment of coagulopathy.

### THE HEMATOLOGIC CHANGES OF LIVER DISEASE

Disease of the liver may have profound effects on red cells, white cells and platelets that may impact upon therapy and prognosis of liver disease. Percutaneous liver biopsy for example, may be contraindicated by thrombocytopenia, while antiviral therapy for hepatitis C virus infection may be complicated by anemia, thrombocytopenia or neutropenia.

#### Anemia

Cirrhotic patients have impaired marrow responses to anemia and up to 75% of patients with chronic liver disease may exhibit mild to moderate anemia. Anemic patients may present with a normochromic, normocytic anemia similar to the anemia of chronic disease. In such patients, a decreased level of erythropoietin can be observed which is not present in patients with cirrhosis and no anemia. Moreover, chronic inflammation can cause decreased responsiveness of the bone marrow to erythropoietin and the elevated levels of pro-inflammatory cytokines seen in advanced liver disease may contribute to this hyporesponsiveness. Occasionally, the red cell mass may be normal and the anemia may be a result of pooling of red blood cells in the spleen (hypersplenism) or due to hemodilution secondary to the increase in plasma volume observed in cirrhosis.

Elucidating an underlying cause for anemia can be difficult in patients with liver disease for two reasons. First, the liver produces many of the proteins involved in the transport of hematinics, and second, liver damage may release hematinics into the circulation. Therefore, the standard laboratory tests of

hemopoiesis must be interpreted cautiously in the setting of acute or chronic liver disease. True iron deficiency occurs in chronic liver disease most often due to blood loss from the gastrointestinal tract and patients may bleed from either portal hypertensive sources or non-liver related gastrointestinal disorders.

Interpretation of serum ferritin levels in defining iron status is problematic in patients with liver disease. Elevated ferritin levels are commonly seen in patients with acute hepatic necrosis when levels may be massively elevated (10–50-fold increase from normal) due to release from damaged hepatocytes. In addition, ferritin is an acute phase protein and may be elevated in a number of inflammatory liver conditions. Interpretation of serum iron levels are inaccurate for similar reasons and use of the total iron binding capacity may be unhelpful since decreased synthesis of transferrin by the liver can lead to artificially high levels of transferrin saturation even in the context of iron deficiency. Therefore, it may be necessary to treat anemia on grounds of clinical suspicion following appropriate assessment and appraise the response to iron supplementation.

Commonly, patients with chronic liver disease due to alcohol misuse display abnormalities of folate metabolism due to decreased levels of absorption from the small intestine. Similarly, alcohol can interfere with the absorption of vitamin B<sub>12</sub>, fat, nitrogen, sodium, water, thiamine, and D-xylose all of which contribute to the malnutrition observed in such patients.

### **Hemolysis and changes in red cell morphology and membrane**

Hemolysis is a frequent complication of liver disease. Its presentation may be a life-threatening event such as that seen during an acute presentation of Wilson disease, or it may be related to the underlying disorder such as an autoimmune hemolysis complicating autoimmune liver disease. In many patients, a decreased red cell life span can be demonstrated, regardless of the etiology of the liver disease. The commonest site for the destruction of red cells is the spleen and hypersplenism accelerates this process, making the presence of hypersplenism a risk factor for hemolysis in its own right. In addition, chronic liver disease can cause changes in the phospholipid component of the red cell membrane, inducing changes in their shape, flexibility and osmotic fragility and therefore increasing the likelihood of destruction within the spleen.

Macrocytosis is the commonest abnormality of red cell morphology seen in patients with chronic liver disease. It is identified in up to 90% of patients with alcohol related liver disease, but is not specific for this diagnosis as it occurs in up to 70% of cirrhotic patients of any etiology. The etiology of this macrocytosis is unclear and is often multifactorial. Increases in the mean corpuscular volume (MCV) may be related to reticulocytosis consequent upon anemia, hemolysis and bleeding. It can also be due in part to abnormal (usually increased) cholesterol and phospholipids within the cell membrane or it may be contributed to by deficiency of vitamin B<sub>12</sub> and folic acid seen in both alcoholic cirrhotics and malnourished cirrhotic patients independent of etiology.

Other abnormalities of red cell morphology that can be observed within the blood film of patients with chronic liver disease include the presence of target cells and echinocytes. Target cells result from increased levels of cholesterol and

phospholipids within the cell membrane. This causes alteration of the surface area of the cells and thus the characteristic target appearance on microscopy. Echinocytes are 'spiky' red cells that may be seen in the blood film, although are best appreciated on electron microscopy. Their presence may be related to changes in high density lipoprotein (HDL) within the serum of patients with liver disease. The HDL fraction from affected individuals is able to turn normal red cells into echinocytes. The presence of echinocytes does not seem to carry any pathologic or prognostic significance, as opposed to the more severe red cell abnormality acanthocytosis. Acanthocytes are bizarrely shaped red cell forms that can be appreciated in the blood films of patients with severe advanced (usually) alcoholic liver disease. They can be associated with a hemolytic anemia, this combination being known as spur cell anemia. The presence of acanthocytes implies advanced liver disease and carries a worse prognosis. It has been proposed that the hemolysis arises from the destruction of acanthocytes by the spleen but this is unlikely given that acanthocytes are observed in abetalipoproteinemia, yet, hemolysis does not occur.

Hemolysis has also been described in the setting of alcoholic liver disease in association with jaundice and hypertriglyceridemia. This combination, termed Zieve syndrome, is differentiated from alcoholic hepatitis by the presence of hemolysis. Considerable debate has occurred as to the prevalence and incidence of the syndrome because the components of the syndrome are frequently observed independently in liver disease.

### **Thrombocytopenia and platelet function**

Thrombocytopenia is very common and has been dealt with in the section above relating to coagulation disorders. Prolongation of bleeding time in patients with liver disease, despite adequate circulating platelet numbers, is suggestive of abnormal platelet function. Impaired aggregation to factors such as ADP and arachidonic acid can be demonstrated in patients with liver disease. Local factors related to the vessel wall such as increased production of NO are also important in disease pathogenesis.

### **Neutropenia and neutrophil dysfunction in liver disease**

Patients with cirrhosis are often neutropenic and therefore have a high incidence and prevalence of bacterial infections. Apart from a reduction in absolute neutrophil numbers, abnormalities of neutrophil function have been described in patients with advanced liver disease. A number of investigators have demonstrated increased apoptosis of neutrophils. The exact cause for this is unknown, but patients with neutropenia secondary to chronic schistosomiasis have had reversal of this apoptosis by splenectomy. Neutrophils of patients with cirrhosis also display deficient phagocytosis. Although increased adhesion of neutrophils to endothelium has been demonstrated, there is reduced trans-endothelial migration of neutrophils. Deficiency of the tetrapeptide called tuftsin has been demonstrated in patients with advanced liver disease. Tuftsin is a necessary cofactor for efficient neutrophil function, and the addition of tuftsin to neutrophils from cirrhotic patients reversed the abnormalities in their function. Tuftsin is activated within the spleen, and it has been proposed that the impaired tuftsin activity seen in cirrhosis results from decreased splenic function.



## HEPATOGENOUS DIABETES

The association between diabetes and hepatic cirrhosis was first observed in 1906 when the syndrome hepatogenous diabetes was coined. The prevalence of diabetes amongst cirrhotic patients is reported at between 10 and 70%, with the majority of the remainder displaying some degree of impaired glucose tolerance. The onset of diabetes following a diagnosis of cirrhosis appears to be rapid with up to 20% of patients displaying diabetes within 5 years of diagnosis. Interestingly, the presence of hepatogenous diabetes does not affect the short-term prognosis of patients with cirrhosis but does increase the risk of decompensation and therefore negatively impacts upon the long-term prognosis.

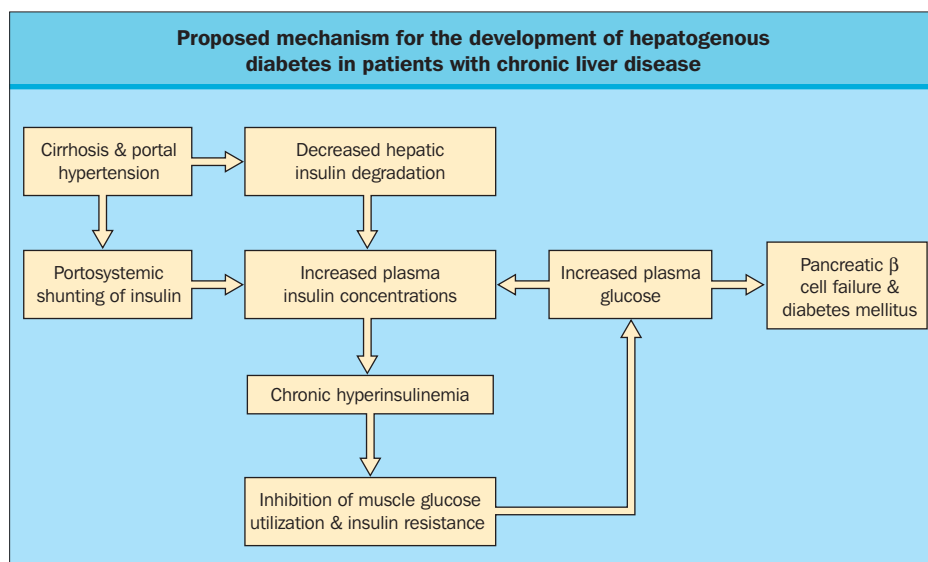
Despite similarities with non-insulin dependent diabetes mellitus (NIDDM), there are clear differences between hepatogenous diabetes and NIDDM. First, there is a family history of diabetes in only 20% of affected individuals and second, obesity has a less important role in pathogenesis. Finally, the incidence of both macro- and microvascular complications appears to occur at a significantly lower frequency than that seen in patients with NIDDM.

The pathogenesis of hepatogenous diabetes is complex and incompletely understood, but it is clear that both insulin resistance and hyperinsulinemia have an important role. Almost all cirrhotics that display insulin resistance have hyperinsulinemia and it is proposed that hyperinsulinemia is the first step in the development of hepatogenous diabetes. The cause of the hyperinsulinemia is unclear although decreased hepatic clearance of insulin and portosystemic shunting are thought to be important. Chronically elevated insulin levels in the serum, as occur in cirrhosis, cause inhibition of muscle glucose utilization primarily due to inhibition of glycogen formation. The molecular mechanism underpinning this effect is unknown, but the end point of this process is peripheral insulin resistance. The point at which this insulin resistance and impaired glucose tolerance develops into diabetes is determined by the capacity of the  $\beta$  cells of the pancreas to respond to the increased need of insulin. **Figure 13.3** summarizes the putative mechanisms in the development of hepatogenous diabetes.

The reversal of hepatogenous diabetes after liver transplantation supports its status as an acquired metabolic syndrome. Although liver transplantation is associated with a deterioration of glucose tolerance and a worsening of diabetes in the initial postoperative period, up to 67% of diabetic cirrhotic patients can be cured by transplantation. This improvement occurs irrespective of the etiology of the liver disease, immunosuppressive regimen, age and body mass index. For patients who continue to have ongoing diabetes post-transplant, a markedly low insulin response during oral glucose tolerance testing pretransplant predicts ongoing diabetes. This is consistent with the persistence of reduced  $\beta$ -cell function post-transplant and suggests that islet cell transplant may be useful for this patient group. Beyond transplantation, the treatment of hepatogenous diabetes mirrors that of NIDDM. Weight loss in the obese patient, regular exercise and compliance with a diabetic diet are cornerstones of management. Where oral hypoglycemic agents are needed, metformin should be avoided. Although the incidence of both macro- and microvascular complications in patients with hepatogenous diabetes is reported to be less than 10%, patients should be screened for retinopathy, renal disease and cardiovascular complications. Current data from natural history studies suggest that just over half of patients die within 10 years of the diagnosis of cirrhosis, mainly of complications of the cirrhosis and end-stage liver disease.

## ACQUIRED HEPATOCEREBRAL DEGENERATION AND HEPATIC MYELOPATHY

Acquired (non-Wilsonian) hepatocerebral degeneration (AHCD) is a syndrome of brain dysfunction associated with a variety of liver diseases. Clinically and pathologically, the condition is similar to Wilson disease. Clinical features of this chronic syndrome are intention tremor, dysarthria, dementia, ataxia of gait, and choreoathetotic movements. The neuropathologic findings include patchy cortical laminar neuronal necrosis, diffuse proliferation of Alzheimer type II glial cells and neuronal dropout in the cerebral cortex, basal ganglia and cerebellum. Multiple bouts of hepatic coma appear to be the only risk factor to



**Figure 13.3 Proposed mechanism for the development of hepatogenous diabetes in patients with chronic liver disease.** The presence of cirrhosis results in both decreased insulin breakdown and portosystemic shunting of insulin which results in increased levels of circulating insulin. Chronic insulinemia results in inhibition of glucose utilization within muscle and ongoing insulin resistance. Increased plasma glucose concentrations result in  $\beta$  cell failure and a vicious cycle of hyperinsulinemia and worsening insulin resistance.

trigger this condition, although Wilson disease must be considered. Liver transplantation has been reported as successfully treating this condition and reduction of lucencies noted within the basal ganglia on magnetic resonance imaging has also been described.

The second syndrome referred to by the term hepatic myelopathy or alternatively, post-shunt myelopathy, is characterized by the presence of progressive spastic paraplegia and hyperreflexia with preservation of normal sensation. Described in patients who have developed spontaneous portosystemic shunts and following surgical shunt creation, the cause of this syndrome is uncertain. Postmortem examinations have revealed demyelination of the lateral column of the spinal cord in some cases.

## FURTHER READING

### **Metabolic bone disease**

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### **Cirrhotic cardiomyopathy**

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### **Hepatogenous diabetes**

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Although shunting of various toxins such as ammonia have been proposed as a cause of this entity, the majority of patients with significant shunting do not develop this syndrome. In addition, the syndrome of myelopathy has been described in patients without evidence of portosystemic shunts suggesting alternative etiologies. Moreover, conventional therapies directed at reducing intestinal uptake of potential toxins fail to improve symptoms. Spastic paraparesis following portosystemic shunting has been reversed by liver transplantation and current thinking suggests that the efficacy of transplant is optimal when spastic paraparesis has been present for less than 1 year, although an exact threshold has yet to be defined.

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- Schenk P, Schoniger-Hekele M, Fuhrmann V, et al. Prognostic significance of the hepatopulmonary syndrome in patients with cirrhosis. *Gastroenterology* 2003; 125:1042–1052. *Original data that demonstrates conclusively the effect of Child Pugh grade on prognosis in patients with hepatopulmonary syndrome.*

## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 14

## Viral Hepatitis A and E

Martin Tagle, Maria de Medina and Eugene R. Schiff

### HEPATITIS A

#### Introduction

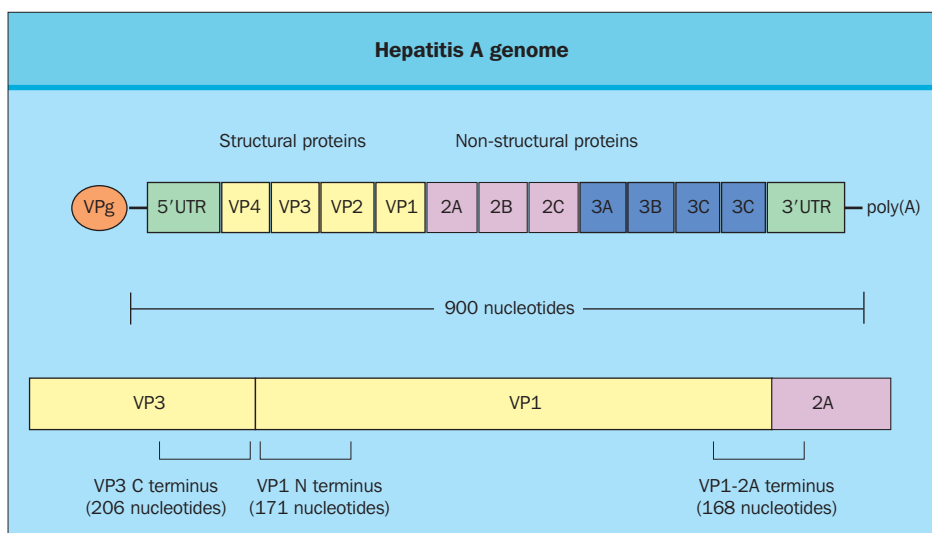
Outbreaks of hepatitis were described in Europe in the 17th and 18th centuries. Epidemiologic analysis that led to the differentiation between hepatitis A ('infectious hepatitis') and hepatitis B ('serum hepatitis') came from studies done among troops during World War II. A diagnostic test became available in 1975, and the hepatitis A virus (HAV) was isolated in cell culture for the first time in 1979. Hepatitis A is the most common defined cause of viral hepatitis worldwide. HAV belongs to the *Picornaviridae* family, which includes the enteroviruses and the rhinoviruses and takes the form of an icosahedral particle 28 nm in diameter, with a composition of 30% RNA and 70% protein. The HAV genome is single stranded, linear, positive sense and contains 7.48 kb RNA (Fig. 14.1). The RNA template has one open reading frame (ORF) that produces a polyprotein that is 2227 amino acid residues long and is cleaved to provide the structural and functional proteins of the virus. It is relatively heat-stable and is readily inactivated by autoclaving, ultraviolet radiation, formalin and iodine. HAV is destroyed by boiling for 5 min, but steaming of shellfish may not be enough to destroy the virus. There is only one serotype of the HAV, but four genotypes have been described in human infections. However, all the human strains are very closely related, and infection with one strain confers immunity to the other strains.

#### Epidemiology and pathogenesis

The virus is found in high titers in stools of infected persons, and the main mode of transmission is fecal-oral. Person to person

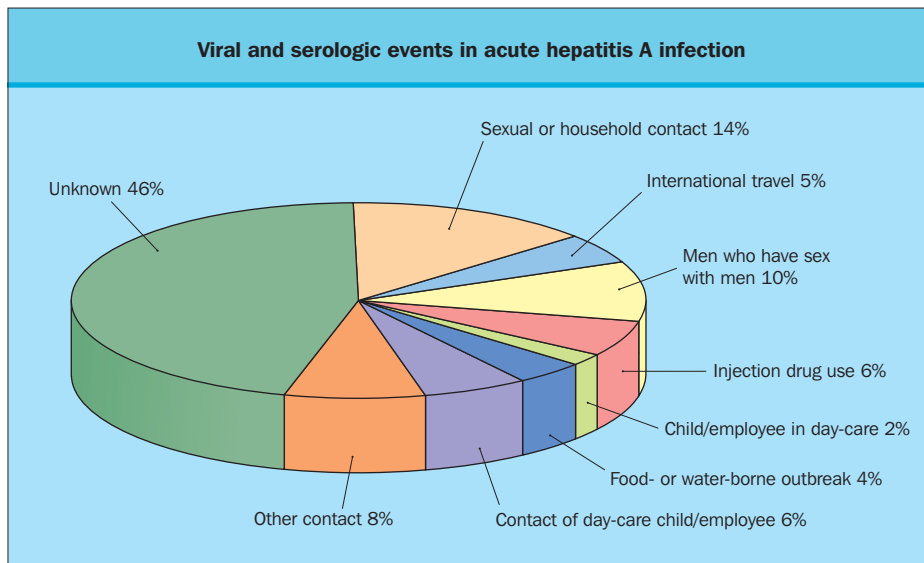
contact with infected family members remains one of the most important mechanisms of HAV transmission. Food-borne or water-borne transmission can occur when food or water is contaminated with HAV and ingested by susceptible persons. Some of the contaminated foods involved in outbreaks of HAV include raw or partially cooked shellfish, clams, oysters, mussels, cockles, milk, salads and hamburgers. In 1997, more than 30 000 cases of hepatitis A were reported to the National Notifiable Diseases Surveillance System (NNDSS) in the USA (Fig. 14.2). There have been cyclic community-wide outbreaks of hepatitis A in the USA every 5 to 10 years. More than 10 000 cases were reported in the USA in 2001. In 2003 an outbreak of hepatitis A was reported among 555 persons, associated with consumption of green onions at a restaurant in Pennsylvania. There were three deaths due to fulminant hepatitis. Green onions and other selected produce items (e.g. strawberries) might be more vulnerable to contamination because plant surfaces are particularly complex or adherent to viral or fecal particles. HAV is highly endemic in less-developed countries where most persons acquire the infection in early childhood. In some parts of the world such as Eastern Europe, the former Soviet Union and parts of Asia, some children escape infection during childhood and reported rates of hepatitis A can be paradoxically higher due to the transmission in older age groups. A gradient from northern to southern Europe exists, in which hepatitis A is almost non-existent in Scandinavian countries but is frequent in Mediterranean countries.

Occasionally, large food-borne outbreaks occur, such as in Shanghai in 1988, associated with raw shellfish. In the early 1980s, an increase in the number of injection-drug users with hepatitis A was observed in the USA, and this increase reached



**Figure 14.1 Hepatitis A genome.** Reproduced from Costa-Mattioli et al (03) by permission of The Society for General Microbiology.





**Figure 14.2 Risk factors associated with acute hepatitis A, 1990-2000, United States.** Reproduced from Center for Disease Control and Prevention (03) by permission of CDC.

a peak in 1986 when 19% of reported cases of hepatitis A were associated with intravenous drug abuse. In 1991, there was a marked increase in the number of cases of hepatitis A reported among homosexual men in USA, Canada and Australia, probably related to unsafe sexual practices. The mechanism of transmission of hepatitis A in this setting is likely due to oral-anal contact. In serologic surveys, anti-HAV-positive persons reported more frequent oral-anal contact, longer duration of homosexual activity and a larger number of sexual partners than persons without serologic evidence of HAV infection.

Outbreaks of hepatitis A have been reported in health care personnel working in a burn unit and in neonatal intensive care units. Transmission of HAV by blood transfusion is extremely rare but donors in the prodromal phase of infection have been shown to transmit the virus by this route. Approximately 50% of patients do not have a source identified for their infection. In the USA, anti-HAV prevalence varies directly with age: among persons 6-11 years of age, the prevalence is 9%; 20-29 years of age, 19%; 40-49 years of age, 33%; and >70 years of age, 75%. Anti-HAV prevalence is inversely related to income and household size. In developing countries, the disease is hyperendemic and most infections occur early in childhood. Different patterns of antibody prevalence may be seen in populations belonging to high and low socioeconomic status from the same developing country.

Communities in developed countries such as the USA can be considered to have high, intermediate, or low rates of hepatitis A on the basis of epidemiologic characteristics such as age-specific rates of infection and temporal patterns of disease incidence. In communities with a high rate, the peak incidence during these epidemics is generally >700 cases/100 000 population, and few cases occur among persons aged >15 years. Around 30-40% of children in these communities acquire infection before 5 years of age and almost all persons become infected before reaching young adulthood. These populations often are relatively well defined, either geographically or culturally and include American Indian, Alaskan Native and selected Hispanic, migrant and religious communities.

In communities with intermediate rates of hepatitis A, most disease occurs over a wider range of ages (i.e. children, ado-

lescents and young adults) than in communities with high rates of hepatitis A. Overall disease rates during epidemic periods typically range from 50 to 200 cases/100 000 population/year. In communities with low rates of hepatitis A, most cases are reported among school-age children, adolescents and young adults; rates reflect little year-to-year variation, and community-wide outbreaks are unusual. Although the most frequently reported source of infection is close contact with a person who has hepatitis A, cases attributed to international travel might account for 10-12% of reported cases because of fewer sources of transmission within the community. Approximately half of reported cases do not have a recognized source. Persons from developed countries who travel to developing countries are at substantial risk for acquiring hepatitis A. Such persons include tourists, military personnel, missionaries and others who work or study in countries that have high or intermediate endemicity of hepatitis A. The decrease in the rates of hepatitis A in the USA during the past several decades probably reflects progress in hygiene, water supplies, better sewage disposal, reduced crowding and, especially, the introduction of the hepatitis A vaccine in 1995 (see section on prevention).

The mean incubation period of hepatitis A is approximately 30 days, with a range of 15-50 days. The incubation period does not seem to be influenced by the route of infection or the magnitude of the inoculum. HAV is transmitted by the fecal-oral route, replicating in the intestine, after which it reaches the liver through the portal circulation. The liver is the major site of replication, which occurs specifically in the hepatocytes. After intrahepatocytic replication, the virus is shed into the sinusoids and biliary canaliculi, passing into the intestine and then being eliminated in feces. Extrahepatic sites of replication such as the oropharynx have been postulated but the pathology is essentially confined to the liver. HAV was originally assumed to be directly cytopathic, but more recent studies suggest that this may not be the case. The initial phase of viral replication in hepatocytes does not appear to damage the host cells and an immunologically-mediated component to the pathogenesis of hepatocyte necrosis has been suggested. This probably involves HAV-specific, human leukocyte antigen (HLA)-restricted cytotoxic T lymphocytes.

### Clinical manifestations

Hepatitis A is very often a subclinical infection of the liver, usually running an anicteric course, particularly in children. The likelihood of having symptoms with HAV infection is related to the person's age. In children <6 years of age, most (70%) infections are asymptomatic; if illness does occur, it is not usually accompanied by jaundice. Among older children and adults, infection is usually symptomatic, with jaundice occurring in more than 70% of patients. Most cases occur 2–6 weeks following exposure to the virus (incubation period). HAV has been documented in stool samples 2–3 weeks prior to the development of symptoms. The disease course is usually benign, starting with a mild prodromal phase appearing 1 to 7 days prior to the onset of dark urine, including flu-like symptoms, fever, malaise, anorexia and nausea induced by fatty foods or tobacco. Most of the patients seek medical attention when they experience dark urine (choloria), which is followed by light-colored stools and jaundice. On physical examination tender hepatomegaly may be present and splenomegaly can be detected in about 15% of cases. In a significant proportion of patients the appearance of jaundice is associated with a rapid resolution of symptoms. The duration of the illness is variable but usually lasts about 3 weeks.

Children and infants can shed HAV for longer periods than adults, up to several months after the onset of clinical illness. Transient skin rashes as well as lymphadenopathy may occur, especially in the cervical area. Prolonged clinical courses have been documented in some patients, secondary to either relapsing or cholestatic hepatitis. Relapsing or biphasic hepatitis has been described in up to 10% of patients and occurs at intervals of 4–15 weeks after the original illness. There is no strong correlation between the severity of the initial and the second phase of the illness. However, relapsing hepatitis seems to be associated with a higher incidence of immune-related clinical and laboratory phenomena. A cholestatic course may develop in a proportion of patients and is characterized clinically by deep and protracted jaundice and the development of acholic (pale) stools and pruritus. Chronic shedding of HAV in feces does not occur; however, shedding can occur in persons who have relapsing illness. HAV infection does not have a chronic phase and does not cause chronic hepatitis. Extrahepatic manifestations of hepatitis A are uncommon and are usually immunologically mediated. Cutaneous vasculitis with immunoglobulin M (IgM) deposits and vasculitis, cryoglobulinemia, cholecystitis has been reported, along with neurologic syndromes such as Guillain-Barré, myelopathy, mononeuritis, meningoencephalitis or exacerbations of pre-existing multiple sclerosis. Hepatitis A infection can also behave as a triggering factor for autoimmune hepatitis, which should be suspected in patients with persistently elevated aminotransferase levels months after the resolution of the icteric phase. Acute pancreatitis and acalculous cholecystitis are also unusual complications of HAV infection that have been described. The age at the time of diagnosis is an important determinant of prognosis. Infection in the very young is associated with an asymptomatic or mild infection, although fulminant cases occur, whereas infection in adults older than 50 years of age is more likely to be associated with a fulminant course. Warning signs of this complication include irritability, changes in sleep pattern and confusion.

### Diagnosis

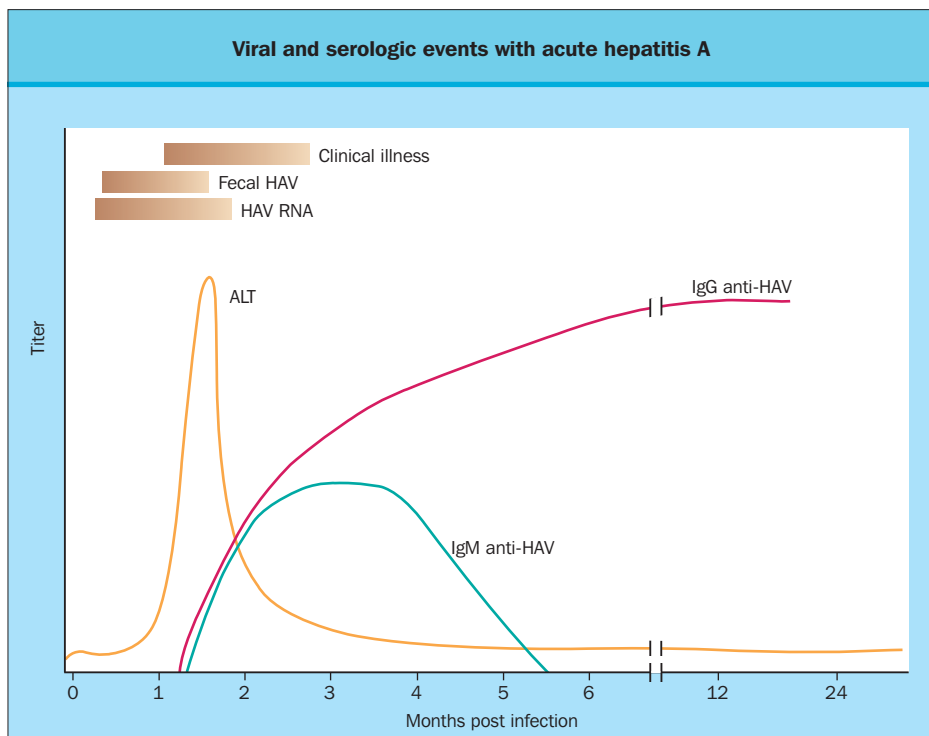
The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are very sensitive markers of hepatocyte necrosis and increase during the prodromal phase of hepatitis A. The peak levels may be measured in thousands of units but rarely exceeding 5000 U/L. However, there is no correlation between clinical severity and degree of transaminasemia. Prothrombin time prolongation is a sign of severity, especially when associated with changes in mental status. High levels of alkaline phosphatase may be seen in cholestatic forms. The aminotransferase levels initially fall rapidly, but may then take many weeks to return to complete normality. The serum aminotransferases reach a peak before the serum bilirubin and return to normal levels in virtually all patients within 6 months. Serum albumin levels usually remain in the normal range.

Serologic testing for IgM antibody to the capsid proteins of HAV (IgM anti-HAV) is a *sine qua non* for the diagnosis of acute HAV infection (**Fig. 14.3**). In most persons, IgM anti-HAV becomes detectable 5–10 days before the onset of symptoms and can persist for up to 6 months after infection. Immunoglobulin G (IgG) anti-HAV, which appears early in the course of infection, reaches high titers during convalescence and remains detectable for the person's lifetime, conferring lifelong protection against the disease. Commonly used commercial assays measure either IgM anti-HAV or total anti-HAV (IgM and IgG). HAV RNA can be detected in the blood and stool of most persons during the acute phase of infection by using nucleic acid amplification methods. Since hepatitis A is a benign, self-limited disease in the great majority of patients, liver biopsy is seldom required. Lobular necrosis of varying degrees has been documented in cases of subfulminant or fulminant liver failure.

### Treatment and prognosis

The great majority of patients who have acute hepatitis A do not require hospitalization. Bed rest is still a traditional component of the management, but there is no evidence to demonstrate that bed rest accelerates the rate of recovery in uncomplicated infections. Patients should be advised to remain active and adjust physical activity according to their general well being. Anorexia, nausea and vomiting may occasionally be severe and require intravenous fluid administration. It is not necessary to modify the dietary habits and the patients should be advised to maintain a balanced diet. Alcohol intake, potentially hepatotoxic medications, sedatives and narcotic drugs should be avoided. Acetaminophen (paracetamol) in doses not exceeding 4 g/day is the safest analgesic even though it is potentially hepatotoxic in larger doses. Cholestyramine is indicated in cases of cholestasis and pruritus, where antihistamines are ineffective. Serum glucose should be monitored regularly in any patient who has an altered mental status, since hypoglycemia may signal the development of encephalopathy in patients at risk for fulminant hepatic failure. Hospitalization is indicated in cases of severe vomiting or in patients with altered mental status. The latter are at risk of developing acute liver failure and they should be managed in coordination with a liver transplantation center. There is no specific drug therapy for uncomplicated hepatitis A infection.

The age at acquisition of hepatitis A virus infection is a major determinant of the severity of the clinical illness. Case fatality rates have been calculated at 0.1% under the age of 15 years, 0.4% in 15–39 year olds, and 1.1% in those older than 40 years.



**Figure 14.3 Viral and serologic events associated with acute hepatitis A virus infection.** ALT, alanine aminotransferase; HAV, hepatitis A virus; IgG, immunoglobulin G; IgM, immunoglobulin M

Among those developing acute liver failure, defined by the presence of encephalopathy, the prognosis is also worse with increasing age and the median age of non-survivors in one study was 47 years compared with 33 years for survivors. Older patients with slowly evolving disease have the worst prognosis.

Concomitant infections or disease may lead to an increase in the severity of acute viral hepatitis A. In general, patients with cirrhosis of any etiology are at high risk of developing a complicated course of acute hepatitis A and should be vaccinated (see section on prevention).

### Prevention

Good hygiene practices and restriction of activities of ill persons who work in the food industry are important. Travelers to endemic areas should be advised against improperly cooked food with particular emphasis on vegetables and shellfish, and encouraged to drink bottled or boiled water. Immune serum globulin (ISG) provides protection against hepatitis A through passive transfer of antibody. Both ISG and immunoglobulin for intravenous administration contain anti-HAV, but ISG is the product used for the prevention of HAV infection. The concentrations of anti-HAV achieved following administration of ISG are below the level of detection of commercially available diagnostic tests. When used for pre-exposure prophylaxis, a dose of 0.02 mL/kg of ISG administered i.m. confers protection for up to 3 months, and a dose of 0.06 mL/kg ISG administered i.m. confers protection for up to 5 months. ISG does not interfere in general with the immune response to inactivated vaccines or to oral poliovirus vaccine or yellow fever vaccine. Persons who have been recently exposed to HAV and who have not previously received hepatitis A vaccine (such as: previously unvaccinated household and sexual contacts of persons who have serologically confirmed acute hepatitis A or persons who have shared intravenous drugs with a person who has serologically confirmed

hepatitis A) should receive a single i.m. dose of ISG (0.02 mL/kg) followed by the first dose of HAV vaccine as soon as possible. If a food handler is diagnosed with hepatitis A, ISG should be administered to other food handlers working at the same place. In the case of a common-source outbreak, ISG should not be administered to exposed persons after cases have begun to occur because the 2-week period during which immunoglobulin is effective will have been exceeded. Additionally, persons who will be traveling to countries with high or intermediate rates of infection within 2 weeks should receive immunoglobulin prophylaxis. No transmission of hepatitis B virus (HBV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), or other viruses has been reported from immunoglobulin for intramuscular administration.

Two inactivated hepatitis A vaccines have been licensed by the Food and Drug Administration in the USA for use in persons aged more than 2 years old to prevent HAV infection (Table 14.1). Hepatitis A vaccine is not approved for use in children younger than 2 years of age because of concern that children who have passively acquired maternal antibodies will have a diminished response to vaccine. Routine vaccination of preschool and school age children living in states where the incidence of hepatitis A is greater than the national average is recommended. Vaccination is also recommended for travelers to endemic areas and other high risk groups. Havrix® is available in two formulations: for persons 2–18 years of age, 720 enzyme-linked immunosorbent assay (ELISA) units (EL U)/dose in a two-dose schedule; and for persons >18 years of age, 1440 EL U/dose in a two-dose schedule. Each mL of the Havrix® vaccine for adults contains 1.440 EL U of viral antigen. The recommended dose for adults is 1 mL i.m. in the deltoid muscle, plus a 1 mL booster dose 6 to 12 months later. Vaqta® is licensed in two formulations: for persons 2–17 years of age, 25 U in a two-dose schedule; for persons >17 years of age, 50 U/dose in a



Recommended schedule for hepatitis A vaccines						
Vaccine	Age group (years)	HAV Ag dose	HBsAg dose (µg)	Volume	No. of doses	Schedule (Months)
Havrix <sup>1</sup>	2–18	720 EL U	N/A	0.5 mL	2	0, 6–12
	19 or older	1440 EL U	N/A	1.0 mL	2	0, 6–12
Vaqta <sup>2</sup>	2–18	25 units	N/A	0.5 mL	2	0, 6–18
	19 or older	50 units	N/A	1.0 mL	2	0, 6–12
Twinrix <sup>3</sup>	1–15	360 EL U	10	0.5 mL	3	0, 1, 6
	16 or older	720 EL U	20	1.0 mL	3	0, 1, 6

HAV Ag, hepatitis A virus antigen; HBsAg, hepatitis B surface antigen  
 EL U=enzyme-linked immunosorbent assay (ELISA) units  
<sup>1</sup>Hepatitis A vaccine, inactivated, GlaxoSmithKline  
<sup>2</sup>Hepatitis A vaccine, inactivated, Merck & Co., Inc.  
<sup>3</sup>Combined hepatitis A and hepatitis B vaccine, GlaxoSmithKline  
 Modified from Lemon in Schiff et al by permission of Lippincott, Williams & Wilkins

**Table 14.1 Recommended schedule for hepatitis A vaccines**

two-dose schedule. Indications for vaccination include: travelers who are 2 years of age or older to areas of intermediate or high prevalence of HAV infection, men who have sex with men, users of illicit drugs, persons with chronic liver disease of any etiology, recipients of liver transplants and laboratory personnel who work with HAV. Protective levels of specific humoral antibodies are detectable in 80–98% of adult recipients 15 days after the first dose and in 96% after 1 month. Estimates of antibody persistence derived from kinetic models of antibody decline indicate that protective levels of anti-HAV could be present for  $\geq 20$  years. On the other hand, in some studies, administration of hepatitis A vaccine to persons with HIV infection resulted in lower seroprotection rates and antibody concentrations, and this phenomenon may be directly related to the degree of CD4<sup>+</sup> cell depletion. The most frequently reported side-effects occurring within 3 days after the 1440 EL U dose were pain at the injection site, headache and malaise. Reviews of data from multiple sources for  $>5$  years regarding adverse events did not identify any serious side-effects among children or adults that could be definitively attributed to hepatitis A vaccine.

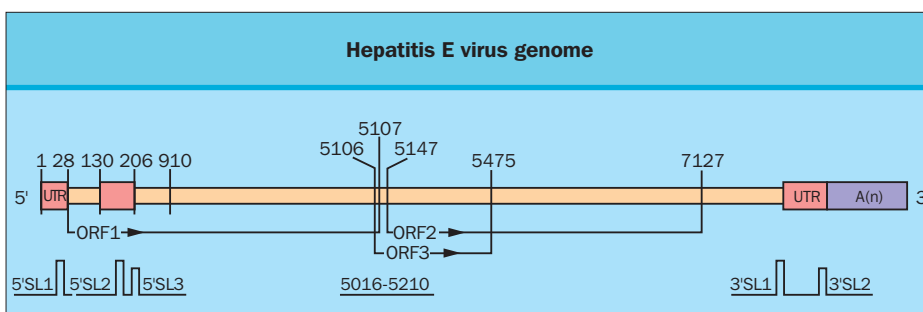
A combined hepatitis A and B vaccine (Twinrix<sup>®</sup>) has been released for use in persons aged  $>18$  years in the USA and

elsewhere. Twinrix is manufactured and distributed by GlaxoSmithKline Biologicals (Rixensart, Belgium) and is made of the antigenic components used in Havrix and Engerix-B (GlaxoSmithKline). The antigenic components in Twinrix have been used routinely in separate single antigen vaccines in the USA since 1995 and 1989 as hepatitis A and B vaccines, respectively. The efficacy of Twinrix is expected to be comparable with existing single antigen hepatitis vaccines. The persistence of anti-HAV and anti-HBV following Twinrix administration is similar to that following single antigen hepatitis A and B vaccine administration at 4-year follow-up (GlaxoSmithKline Biologicals, unpublished data, 2001).

## HEPATITIS E

### Introduction

Hepatitis E is an enterically transmitted form of viral hepatitis that appears to be common in tropical and subtropical areas and epidemics have been reported in Asia, Africa, the Middle East and Central America. An extensive outbreak of water-borne hepatitis was reported in India in 1955. In further retrospective analysis 25 years later, all stored serum samples were found to have IgG anti-HAV but not IgM anti-HAV, evidence against the assumption that the etiologic agent responsible for this outbreak was HAV. This was the first of many documented outbreaks of water-borne non-A, non-B hepatitis. Subsequently, similar outbreaks were reported especially associated with a high fatality rate among pregnant women in South East Asia. In 1990 the genome of the virus was cloned and named hepatitis E virus (HEV), which allowed the development of specific diagnostic tests and expanded the knowledge about this agent. It is a distinct agent which has been associated with fulminant hepatic failure among pregnant women, but, similarly to hepatitis A, has no known chronic sequelae. HEV has not yet been classified taxonomically. Immunoelectron microscopy permitted the identification of HEV particles measuring around 32 nm, spheric shaped, non-enveloped and very similar to those of the *Caliciviridae* family. The virus exhibits spikes and indentations in the particle surface, that give it a feathery aspect. It is disintegrated by exposure to cesium chloride and storage at  $-20^{\circ}\text{C}$ . The genome of HEV consists of 7.5 kb and has three separate, partially overlapping ORFs which encode structural and non-structural proteins (**Fig. 14.4**). Eight different genotypes of HEV have been identified to date: Burma (type 1), Mexican (type 2), North American (type 3), Chinese (type 4), European (types 5, 6 and 7), and the recently discovered Argentinean type (type 8). The existence of many more genotypes is a possibility, as suggested by Austrian investigators who recently isolated the

**Figure 14.4 Hepatitis E virus genome.** ORF, open reading frame. Reproduced from Surgit et al by permission of The American Society for Microbiology Journals Department.

variant HEV-Au1. Despite differences in genotypes, there is only one serotype explaining cross-reactivity for the main viral epitopes, allowing serologic diagnosis with commercially available kits.

### Epidemiology and pathogenesis

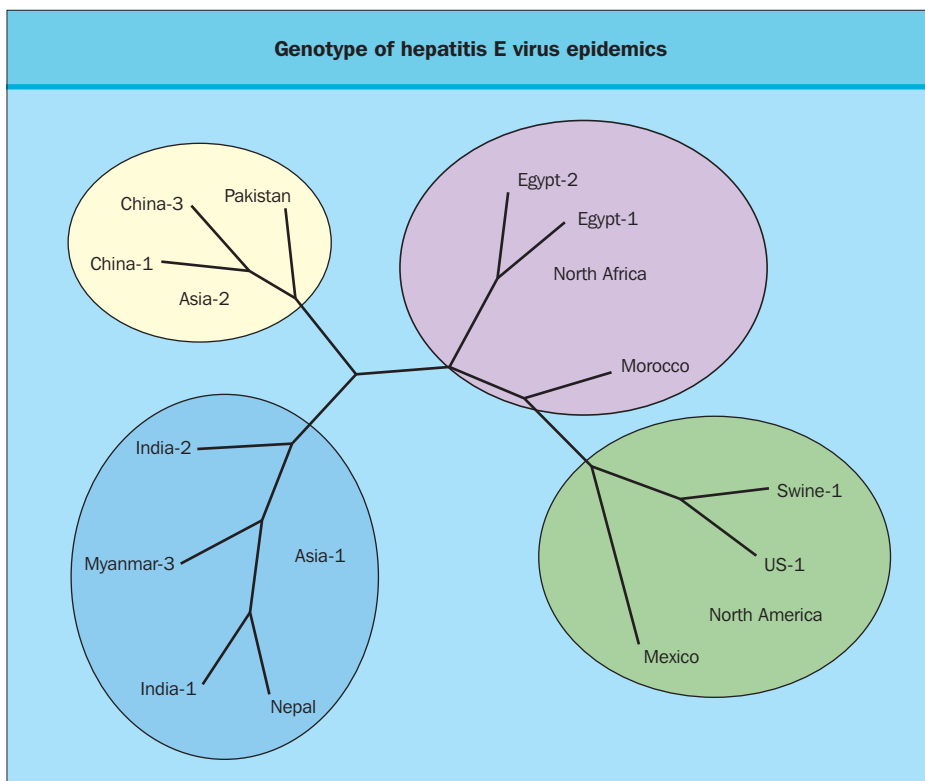
Epidemics of HEV infection have been described throughout Asia, large parts of northeastern and eastern Africa and Mexico (Fig. 14.5). Hepatitis E is implicated in about 50% of sporadic cases of acute viral hepatitis in developing countries. North America and Europe have been considered non-endemic for HEV, although seroprevalence ranges from 1 to 5%. In the last few years some HEV strains associated with sporadic acute hepatitis have been isolated from human serum samples in North America. Spanish investigators analyzed the excreted virus in the urban sewage of Barcelona (Spain) and other countries like USA (Washington DC area), Greece, France and Sweden. Around 84% of sewage samples from Barcelona tested positive for HEV RNA. One of five samples from Washington DC and one of four samples tested from France were positive. HEV RNA was not detected in any of the samples from Greece or Sweden. These data suggest that HEV strains are more widespread than previously thought and endemic HEV infections are likely to be present in Europe and the USA. Recently, seroprevalence studies have demonstrated the endemic nature of HEV in some regions of South America. HEV attacks mostly young adults and is rarely seen in children <15 years of age, in contrast to HAV infection, which is a rare event in adults due to the longstanding immunity against the virus acquired in childhood (see section on hepatitis A in this chapter). In Egypt, the seroprevalence of anti-HEV exceeds 60% in the first decade of life, peaks at 76% in the second decade and remains above

60% until the eighth decade. This is the highest prevalence reported in the world for HEV. Seroconversion to anti-HEV was documented in 4 of 211 travelers from the USA to Thailand, Russia, China and Peru. None of the individuals reported any symptoms of hepatitis before, during or after travel, implying that exposure to HEV resulted in subclinical infections.

It has been proposed that animal reservoirs of HEV may exist in some regions and that human infections may represent a zoonosis. HEV has been isolated in 22% of pooled stool samples from 115 swine farms in the Netherlands. It was found that 90% of wild rats from Hawaii, 77% from Maryland and 44% from Louisiana were seropositive for HEV. In Vietnam, where hepatitis E is endemic, anti-HEV has been detected in 44% of chickens, 36% of pigs, 27% of dogs and 9% of rats. Swine veterinarians in eight different states of the USA were 1.5 times more likely to be anti-HEV positive than healthy volunteer blood donors.

Person-to-person transmission is a potential route of infection, as suggested by the epidemiologic pattern seen in the Mexican outbreak. Pregnant women in the third trimester are at very high risk of developing fulminant hepatitis and this is probably the most prominent epidemiologic feature of this disease. Possible parenteral transmission of HEV can occur as suggested by a report of a hospital outbreak of hepatitis E.

HEV replicates in the liver and is excreted in the feces via the biliary tract. This has been extensively studied in non-human primates, after inoculation with fecal samples obtained from patients with established HEV infection. Pathologic changes in the liver during acute HEV infection can vary from canalicular cholestasis to ballooning degeneration of the hepatocytes and acute portal inflammatory infiltrates. HEV particles appear in



**Figure 14.5 Genotype of hepatitis E virus epidemics.** Reproduced from Jameel, Shabid. Molecular biology and pathogenesis of hepatitis E virus. Expert Reviews in Molecular Medicine, 1999. By permission of Cambridge University Press.

feces during prodromal symptoms of hepatitis E, which can be associated with a viremic phase. Several weeks after exposure, HEV antigen appears in blood and antibody responses start to develop approximately 1 month later, coincident with the ALT peak. The first antibody to be detected is IgM, which declines during the convalescent phase. Simultaneously IgG anti-HEV appears and reaches peak levels later, although the duration of detectable antibody is unknown. The pathogenesis of hepatitis E may be a combination of direct cytotoxicity and immunologic mechanisms.

### Clinical manifestations

The incubation period of hepatitis E is approximately 40 days (2–8 weeks). It is a self-limited disease and cannot be distinguished from other forms of viral hepatitis based on clinical features alone. However, it should be suspected in any person with acute hepatitis with a history of recent travel to underdeveloped areas and negative serologies for hepatitis A, B and C.

Anicteric forms of hepatitis E are very frequent, making the diagnosis in acute phase very puzzling. Fever, arthralgias, general malaise and nausea are common prodromal manifestations. Jaundice and dark urine start shortly after as it occurs in hepatitis A. Anorexia, abdominal discomfort, diarrhea, pale stools, arthralgia, pruritus, rash, hepatomegaly and splenomegaly are described. These symptoms resolve within 6 weeks in most patients. There have been no case reports of chronic hepatitis E infection to date. Severe disease typically occurs in pregnant women during the third trimester with a mortality rate of about 20%. All nine pregnant women in an outbreak in Algeria died of fulminant hepatic failure. This predilection for fulminant forms in pregnant women is not seen in other forms of viral hepatitis, and there is no clear pathophysiologic explanation for this catastrophic occurrence. Sera from 22 patients with fulminant hepatitis in Bangladesh showed anti-HEV IgM in 63.6%, HEV being the most common cause of fulminant viral hepatitis in that series.

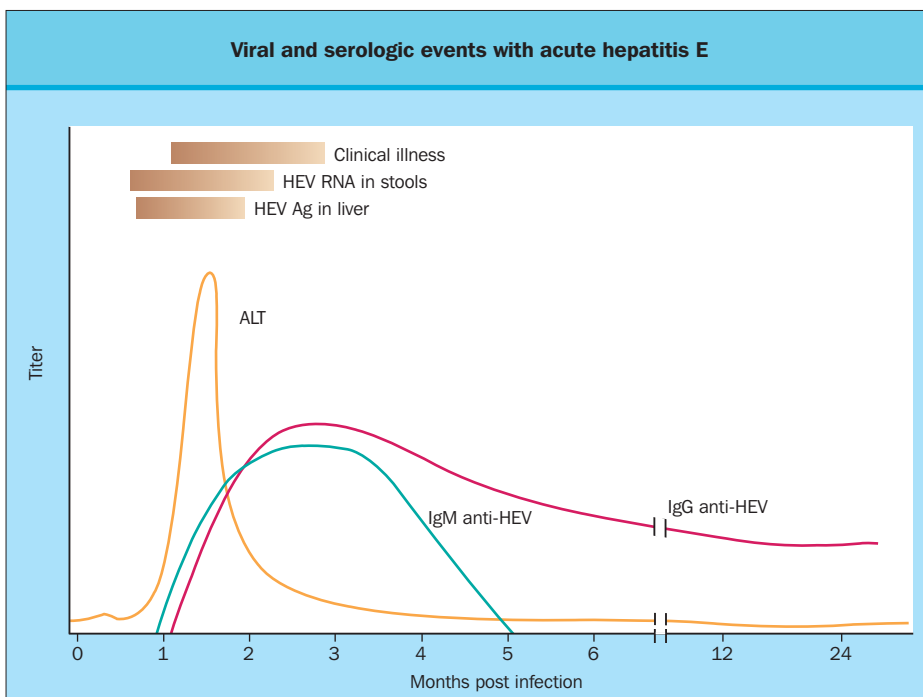
Asymptomatic or subclinical infection may occur but the magnitude of this is not known due to the lack of epidemiologic and serologic studies.

### Diagnosis

The serologic diagnosis of infection with HEV is made by the determination of the anti-HEV antibodies (IgG and IgM) (**Fig. 14.6**). IgM anti-HEV has been identified in most patients and as early as 4 days after the onset of symptoms of the infection. IgM anti-HEV peak titers are coincidental with the serum aminotransferase elevation, and it is usually undetectable within 6 months after the onset of the infection. Essentially IgM anti-HEV is analogous to IgM anti-HAV, and is a reliable marker of recent infection. IgG is a serologic marker of convalescent phase or past exposure to the virus. The serum aminotransferases and serum bilirubin tend to normalize over a 1–6-week period. Due to the possibility of a false-positive reaction, anti-HEV could be confirmed by immunoblot assays. There is controversy regarding the existence of prolonged immunity in cases of infection with HEV. There is some evidence that anti-HEV titers decrease progressively raising the possibility of infection post re-exposure. However, some other authors have documented prolonged immune reaction post acute infection, suggesting the role of immunologic memory. The polymerase chain reaction (PCR) technique has been used to detect HEV in stools, serum and liver of acutely infected patients, but it is still considered experimental. In 1995 HEV infection was identified in a group of United Nations Bangladeshi peace keepers in Haiti by direct fecal HEV RNA determination, allowing infection control within the group and avoiding dissemination of the disease.

### Treatment and prognosis

There is no specific treatment for acute hepatitis E, and the above-mentioned general support measures for hepatitis A apply (see section on treatment for hepatitis A). In acute,



**Figure 14.6 Viral and serologic events associated with acute hepatitis E virus infection.** ALT, alanine aminotransferase; HAV, hepatitis A virus; IgG, immunoglobulin G; IgM, immunoglobulin M.



uncomplicated hepatitis E complete recovery is the rule. The case fatality rate is around 0.5–4.0% and is higher than for hepatitis A. Pregnant women are considered to have the highest mortality, especially during the second and third trimesters. The reported mortality rates range from 20 to 25% in India. No convincing explanation for the pregnancy-related morbidity has been proposed.

A study performed in India among over 400 patients with fulminant hepatitis, where 38% were infected with HEV, revealed that age >40 years, prolongation of prothrombin time >25 s and total bilirubin >15 mg/dL were associated with a poor outcome. Not surprisingly, pregnant women were found to be more prone to develop acute liver failure.

### Prevention

Improvement in the general hygiene conditions and access to clean drinkable water are ideal measures to prevent hepatitis E.

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*This article reviews the main virologic and immunologic features of this relatively recently discovered hepatitis virus.*

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 15

## Hepatitis B and D

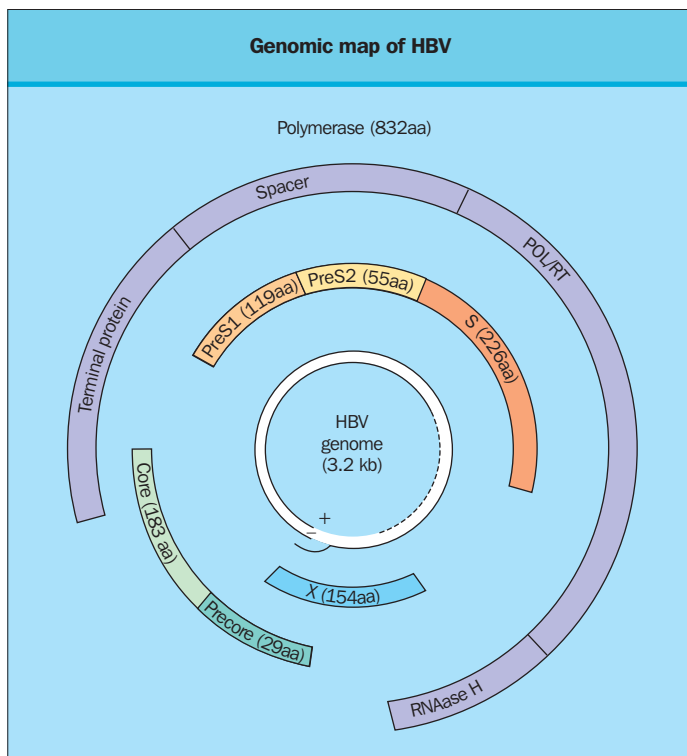
Patrick Marcellin

### HEPATITIS B

#### Introduction

Hepatitis B virus (HBV) belongs to the family of hepadnaviruses. The complete virion or Dane particle is 42 nm in diameter. It consists of an envelope composed of virus-encoded proteins and host-derived lipid components; and a core particle made up of the nucleocapsid protein, the viral genome and the polymerase protein. Hepatitis B virus also produces 22 nm subviral particles in the form of filaments and spheres that contain only envelope proteins. Such particles do not contain the HBV genome and are non-infectious.

The HBV genome is a circular, partially double-stranded DNA of approximately 3200 base pairs in length (Fig. 15.1). There are four open reading frames (ORFs) encoding the envelope (pre-S/S), core (precore/core), polymerase and X proteins. The pre-S/S ORF is divided into pre-S1, pre-S2, and S regions that encode the large (L), middle (M), and small (S) envelope



**Figure 15.1 Genomic map of hepatitis B virus.** Map shows the relaxed circular partially double-stranded DNA with four overlapping open reading frames: envelope (pre-S/S), core (precore/core), polymerase, and X. POL, DNA polymerase; RT, reverse transcriptase; RNAase, ribonuclease.

proteins, respectively. The M and S envelope proteins are found in all forms of viral and subviral particles, whereas the L envelope proteins are predominantly found in complete virions. The precore/core ORF consists of two regions that encode a precore polypeptide, which is post-translationally modified into a soluble protein, the hepatitis B e antigen (HBeAg) and the core protein (HBcAg). The polymerase protein consists of a protein primer, a spacer, a reverse transcriptase/DNA polymerase and an RNAase H. The X protein is not required for the replication of the virus. It is a potent transcriptional transactivator of many promoters including HBV and cellular oncogenes. The HBX protein has been implicated in hepatocarcinogenesis.

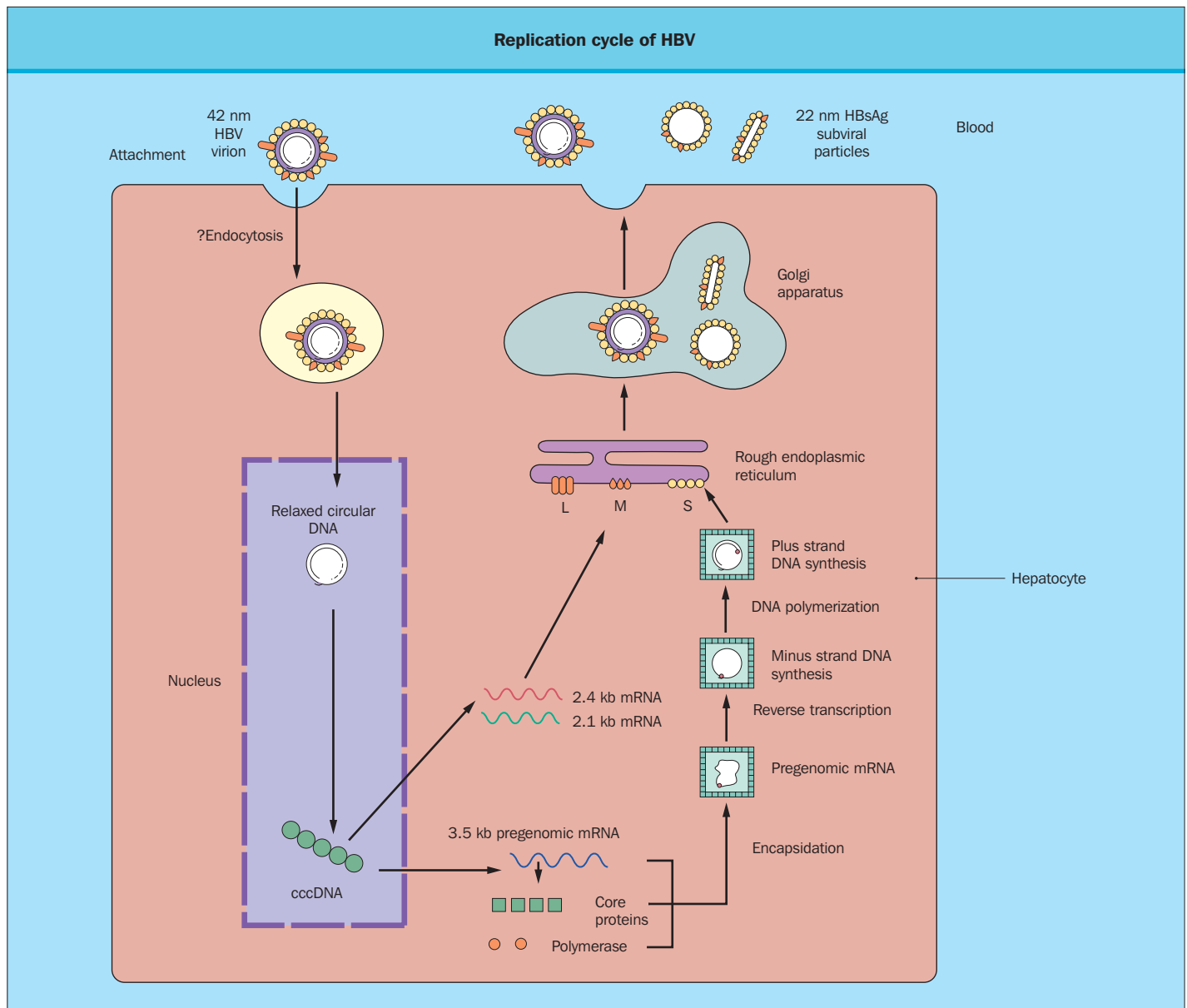
The replication cycle of HBV begins with attachment of the virion onto the hepatocyte membrane (Fig. 15.2). Inside the hepatocyte nucleus, synthesis of the plus strand HBV DNA is completed and the viral genome is converted into a covalently closed circular DNA (cccDNA). The HBV genome replicates by reverse transcription via an RNA intermediate. Transcription of the cccDNA produces a pregenomic RNA that serves both as a template for reverse transcription as well as a messenger RNA for the production of nucleocapsid and polymerase proteins. The pregenomic RNA and nucleocapsid and polymerase proteins are encapsulated in the virus core particle inside which reverse transcription takes place. A new minus strand HBV DNA is produced followed by the synthesis of a new plus strand HBV DNA. Nucleocapsids with the partially double-stranded HBV DNA can re-enter the hepatocyte nucleus to produce more cccDNA or be secreted as complete virions after coating with envelope proteins.

#### Epidemiology

HBV infection is a worldwide health problem. It is estimated there are more than 300 million HBV carriers globally, of whom over 250 000 die from HBV-associated liver disease annually.

#### Prevalence

The prevalence of HBV carriers varies from 0.1–2% in low prevalence areas (USA and Canada, Western Europe, Australia and New Zealand), to 3–5% in intermediate prevalence areas (Mediterranean countries, Japan, Central Asia, Middle East, and Latin and South America), to 10–20% in high prevalence areas (Southeast Asia, China, and sub-Saharan Africa) (Fig. 15.3). The wide range in carrier rate in different parts of the world is largely related to differences in age at infection. The rate of progression from acute to chronic HBV infection is approximately 90% for perinatally acquired infection, 20–50% for infections between the age of 1 and 5 years, and less than 5% for adult acquired infection. Perinatal infection is the predominant mode of transmission in high prevalence areas, probably related



**Figure 15.2 Replication cycle of hepatitis B virus (HBV).** Transcription of covalently closed circular DNA produces 3.5 kb pregenomic RNA which serves as the template for reverse transcription of HBV DNA and translation of core and polymerase proteins; and 2.4 kb and 2.1 kb messenger (m)RNAs which are translated to large (L), middle (M), and small (S) S proteins of the viral envelope.

to the high prevalence (40–50%) of HBeAg in women of reproductive age. Horizontal transmission, particularly in early childhood, accounts for most cases of chronic HBV infection in intermediate prevalence areas, whereas transmission via unprotected sexual intercourse and intravenous drug use are the major routes of spread in low prevalence areas.

#### Modes of transmission and high-risk groups

The incidence of transfusion-related hepatitis B decreased significantly after the exclusion of paid blood donors and the introduction initially of hepatitis B surface antigen (HBsAg) and then anti-HBc screening of donated blood. Currently, the risk of post-transfusion hepatitis B has almost disappeared.

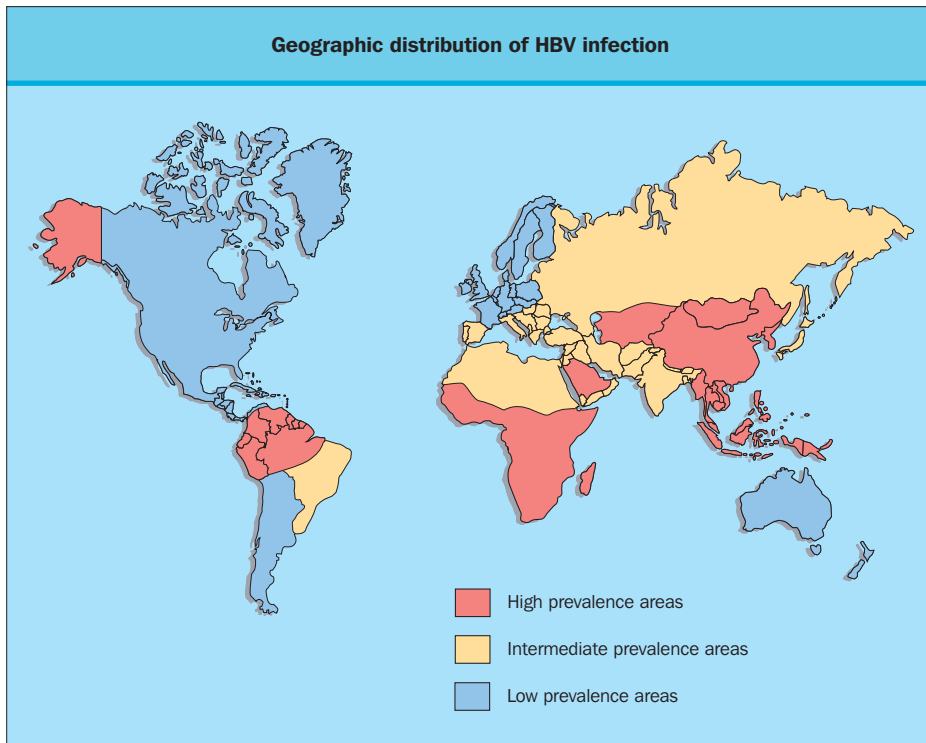
Sexual transmission remains the major mode of spread of HBV in developed countries. The incidence of acute hepatitis B

among male homosexuals has been reduced as a result of decreased high-risk sexual behavior related to the human immunodeficiency virus (HIV) epidemic. Heterosexual transmission of HBV infection still occurs. Sexual transmission accounts for approximately 30% of HBV infections in the USA.

Percutaneous inoculation of blood and body fluid, in particular needle sharing among intravenous drug users, is an important mode of HBV transmission. Other routes include the reuse of contaminated needles in tattoos, acupuncture and ear piercing.

Perinatal transmission of HBV occurs in up to 90% of infants born to HBeAg-positive mothers. Transmission takes place at the time of delivery by maternal–fetal transfusion and exposure to maternal blood in the birth canal, as well as postnatally through close mother–baby contact.





**Figure 15.3** Geographic distribution of hepatitis B virus infection.

Children may acquire HBV infection via minor skin breaks and mucous membranes or close bodily contacts. As HBV can stay outside the human body for a long time, transmission via contaminated household articles such as toothbrushes, razors and even toys may be possible.

Although HBV DNA has been detected in various bodily secretions of HBV carriers, there is no firm evidence of HBV transmission via body fluids other than by sexual contact.

Healthcare workers, particularly surgeons, pathologists and physicians working in hemodialysis and oncology units, have a higher risk of contracting HBV infection via minor skin breaks, cuts and accidental needle puncture. Patient-to-patient transmission via contaminated instruments and needles may also occur in the healthcare setting. Institutionalized persons as well as their attendants and family members also have increased risks of HBV infection.

### Pathogenesis

The pathogenesis of HBV-related liver disease is largely due to immune-mediated mechanisms. However, in some circumstances HBV can cause direct cytotoxic liver injury.

#### Immune-mediated liver injury

Liver injury related to HBV is generally thought to be related to cytotoxic T-lymphocyte (CTL)-mediated lysis of infected hepatocytes. These cells recognize viral antigens presented in association with class I histocompatibility antigens on the hepatocyte membrane causing apoptosis of the infected hepatocytes. Events that enhance immune clearance as in spontaneous or interferon-induced HBeAg seroconversion are often accompanied by exacerbations of liver disease, with flares in serum alanine aminotransferase (ALT) levels and lobular necrosis. Chronic hepatitis B patients who clear HBeAg have been found to have

higher levels of interleukin-12, a cytokine that promotes differentiation of Th1 cells and antigen-specific proliferation of CTLs, than those who remain HBeAg-positive. Patients who develop fulminant hepatitis B frequently have no evidence of HBV replication at presentation; some may even be HBsAg-negative. The fulminant course is believed to be due to rapid and aggressive immune clearance. Immune-mediated viral clearance can also occur through non-cytolytic pathways via the release of cytokines such as tumor necrosis factor- $\alpha$  and interferon  $\gamma$ .

#### Direct cytopathic effects

In general, HBV is not a cytopathic virus. In most patients with chronic hepatitis B, there is no direct correlation between viral load and severity of liver disease. This is particularly true in Asian children and young adults who acquired HBV infection perinatally. These individuals tend to have very high serum HBV DNA levels and abundant intrahepatic virus, but are usually asymptomatic with normal ALT levels and minimal histologic changes. Most patients on hemodialysis also have high serum HBV DNA levels but minimal liver disease. Nevertheless, direct cytopathic liver injury can occur when the viral load is very high, as in some patients with recurrent hepatitis B post-liver transplant [orthotopic liver transplantation (OLT)] who develop fibrosing cholestatic hepatitis (FCH). This is characterized by marked cholestasis, minimal necroinflammation, high levels of intrahepatic HBsAg and HBcAg expression, and rapid progression to liver failure. Another form of direct cytopathic liver injury was initially described in transgenic mice that overexpress pre-S antigens, leading to retention of small S proteins, development of ground glass hepatocytes, liver injury and finally hepatocellular carcinoma (HCC). Similar phenomena have been reported in humans infected with HBV that have mutations in the S gene promoter causing retention of small S protein.

**Role of viral mutants**

Mutations in all regions of the HBV genome have been found in patients with chronic HBV infection. Mutations in the precore region or in the core promoter region (T1762/A1764), hamper the production of HBeAg. These mutations usually appear at a late phase of chronic HBV infection. The patients with these variants have chronic hepatitis B with undetectable HBeAg and detectable anti-HBe despite the presence of moderate or high level HBV replication. HBeAg-negative chronic hepatitis B is generally more severe (higher risk of cirrhosis and HCC). In addition even if the response on therapy is relatively good, the risk of relapse after therapy is very high.

**Hepatocarcinogenesis**

Several lines of evidence support an etiologic association between chronic HBV infection and the development of HCC (see O'Grady 2000, Ch. 25). There is a close correlation between the geographic distribution of HBsAg carriers and the occurrence of HCC. A study of Taiwanese men followed for 9 years found that the relative risk of HCC for HBsAg-positive men was 98. Studies in woodchucks found that chronic infection with hepadnavirus alone is sufficient to cause HCC. Integration of HBV DNA into host chromosomes can be found in the neoplastic liver tissues from most HBsAg-positive patients with HCC. Although HBV is not directly carcinogenic, integration of HBV DNA into the host genome may induce carcinogenesis by activating cellular proto-oncogenes or suppressing growth-regulating genes, or by inducing host chromosomal deletions or translocations. Another mechanism of carcinogenesis may be through chronic liver injury, inflammation and regeneration.

**Clinical features**

Infection with HBV has a wide spectrum of manifestations including subclinical hepatitis, anicteric hepatitis, icteric hepatitis and fulminant hepatitis during the acute phase; and asymptomatic carrier state, chronic hepatitis, cirrhosis and HCC during the chronic phase.

**Acute hepatitis**

Approximately 70% of patients with acute HBV infection have subclinical or anicteric hepatitis, whereas 30% become icteric. Acute liver failure develops in approximately 0.1–0.5% of patients. The incubation period lasts 1–4 months. A serum sickness-like syndrome may develop during the prodromal period. This is followed by constitutional symptoms such as low-grade fever, malaise, anorexia, nausea and vomiting, and right upper quadrant or mid-epigastric pain. Jaundice usually appears as the constitutional symptoms begin to subside. Clinical symptoms and jaundice generally disappear after 1–3 months but some patients may have prolonged fatigue, even after normalization of aminotransferase levels. Elevation of ALT and aspartate aminotransferase (AST) levels, up to 1000–2000 IU/L, is typically seen during the acute phase, with ALT levels higher than AST levels. Prothrombin time, which reflects hepatic synthetic function, is the best indicator of prognosis. In patients who recover, normalization of aminotransferases usually occurs within 1–4 months, followed by normalization of bilirubin levels. Persistent HBsAg lasting more than 6 months indicates progression to chronic infection.

**Chronic hepatitis**

In low or intermediate prevalence areas, only 30–50% of patients with chronic HBV infection have a history of acute hepatitis. The remaining patients in these areas and the majority of patients in high prevalence areas (predominantly perinatal infection) have not experienced acute hepatitis. Many patients are asymptomatic, while others may have non-specific symptoms such as fatigue and mild right upper quadrant discomfort. Some patients experience exacerbations that may be asymptomatic, mimic acute hepatitis or manifest as liver failure. Physical examination may be normal or there may be stigmata of chronic liver disease. In patients with cirrhosis, additional findings such as jaundice, splenomegaly, ascites, peripheral edema and encephalopathy may be present.

Laboratory investigations including liver profile and blood counts may be normal in some patients, but most patients have mild to moderate elevations of AST and ALT levels. During exacerbations, ALT levels may be as high as 50 times the upper limit of normal and  $\alpha$ -fetoprotein (AFP) levels of up to 1000 IU/L may be seen. Progression to cirrhosis is suspected when serum AST levels are superior to ALT levels, when there is evidence of hypersplenism (decreased leukocyte and platelet counts) and impaired hepatic function (hypoalbuminemia, prolonged prothrombin time and hyperbilirubinemia).

**Extrahepatic manifestations**

Extrahepatic manifestations occur in 10–20% of patients with chronic HBV infection. Mediated by circulating immune complexes, acute hepatitis may be heralded by a serum sickness-like syndrome manifested as fever, skin rashes, arthralgia and arthritis, which usually subside with the onset of jaundice.

Approximately 10–50% of patients with polyarteritis nodosa are HBsAg-positive. Vasculitis associated with HBV may affect large-, medium- and small-sized vessels in multiple organs including cardiovascular, gastrointestinal, neurologic and dermatologic systems. The course is highly variable and the mortality rate is high.

Hepatitis B virus-related glomerulonephritis, most commonly membranous glomerulonephritis, is more often found in children. Approximately 30–60% of children with HBV-related membranous glomerulonephritis undergo spontaneous remission. Remission accompanying HBeAg to anti-HBe seroconversion has also been reported. Interferon has been reported to induce remission in small clinical trials. In adults, the course of HBV-related glomerulonephritis may be progressive and response to interferon is poor.

Other extrahepatic manifestations including essential mixed cryoglobulinemia and aplastic anemia have also been reported.

**Special patient groups****Pediatric patients**

Infants infected perinatally are usually asymptomatic but have a high rate (90%) of progression to chronic infection.

Infection with HBV has been estimated to account for 10–25% of all cases of childhood acute hepatitis. Extrahepatic manifestations including Gianotti papular acrodermatitis have been reported in 25% of patients. The acute illness is usually mild. Recovery with HBsAg to anti-HBs seroconversion occurs in >80% of children infected after the age of 1 year.

Serodiagnosis of hepatitis B virus (HBV) infection								
	HBsAg	antiHBs	HBeAg	antiHBe	IgM antiHBc	Total antiHBc	HBV DNA	ALT
Acute hepatitis B	+	-	+	-	+	+	Detectable	Raised
Chronic hepatitis B (wild type)	+	-/+	+	-	-	+	>10 <sup>5</sup>	Raised
Chronic hepatitis B (precore variant)	+	-/+	-	+	-	+	>10 <sup>5</sup>	Raised
Inactive carrier	+	-/+	-	+	-	+	<10 <sup>5</sup>	Normal
Recovered	-	-/+	-	+	-	+	Undetectable	Normal
Vaccinated	-	+	-	-	-	-	Undetectable	Normal
ALT, alanine aminotransferase; HBc, hepatitis B core protein; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IgM, immunoglobulin M.								

**Table 15.1 Serodiagnosis of hepatitis B virus (HBV) infection.**

Children with perinatally acquired chronic HBV infection are usually asymptomatic with normal ALT values, despite high serum HBV DNA levels. In contrast, 15–30% of children with childhood acquired chronic HBV infection are symptomatic with elevated ALT levels and chronic hepatitis on liver biopsies. Progression of chronic hepatitis to cirrhosis is rare in children but cases of childhood HCC have been reported.

#### Immunocompromised patients

Immunocompromised patients may have different clinical manifestations due to increased levels of HBV replication. Abrupt withdrawal of immunosuppressive therapy during cyclic chemotherapy or tapering of steroid treatment may cause a rebound increase in immune clearance and exacerbation of chronic hepatitis B. Although most exacerbations are asymptomatic, fatal hepatic decompensation has been reported.

Chronic infection with HBV is common (about 10%) in patients co-infected with HIV and these patients tend to have higher serum HBV DNA levels, lower rate of spontaneous and interferon-induced HBeAg seroconversion, and an increased risk of cirrhosis. Within the last years, because of the improved survival of HIV-positive patients thanks to HAART, complications due to HBV related cirrhosis (liver failure or HCC) have become a significant cause of mortality.

#### Alcoholics

Alcoholics with HBV infection have been reported to have accelerated liver damage, and an increased risk of progression to cirrhosis and HCC, as well as decreased survival compared with alcoholics who are not HBV-infected.

#### Co-infection with hepatitis C virus

Acute HBV and hepatitis C virus (HCV) co-infection may delay the onset and shorten the duration of HBs antigenemia as well as lower the peak transaminase levels compared with acute HBV infection alone, suggesting a possible interference of HBV replication by HCV. However, dual infection with HBV and HCV has been reported to be associated with increased risks of acute liver failure.

Most patients who are chronically infected with both HCV and HBV have detectable serum HCV RNA but not HBV DNA, indicating that HBV replication is suppressed by HCV. Never-

theless, liver disease is generally more severe and the risk of developing HCC is higher in patients with dual infection than in those infected with either virus alone.

#### Diagnosis

The diagnosis of hepatitis B depends on serologic assays for hepatitis B-associated antigens and antibodies (Table 15.1). Tests for HBV DNA in serum are used to assess level of HBV replication. Liver histology is used to determine the severity and stage of liver disease.

#### Serology assays

Serologic markers of HBV infection are illustrated in Figure 15.4.

#### Hepatitis B surface antigen and antibody

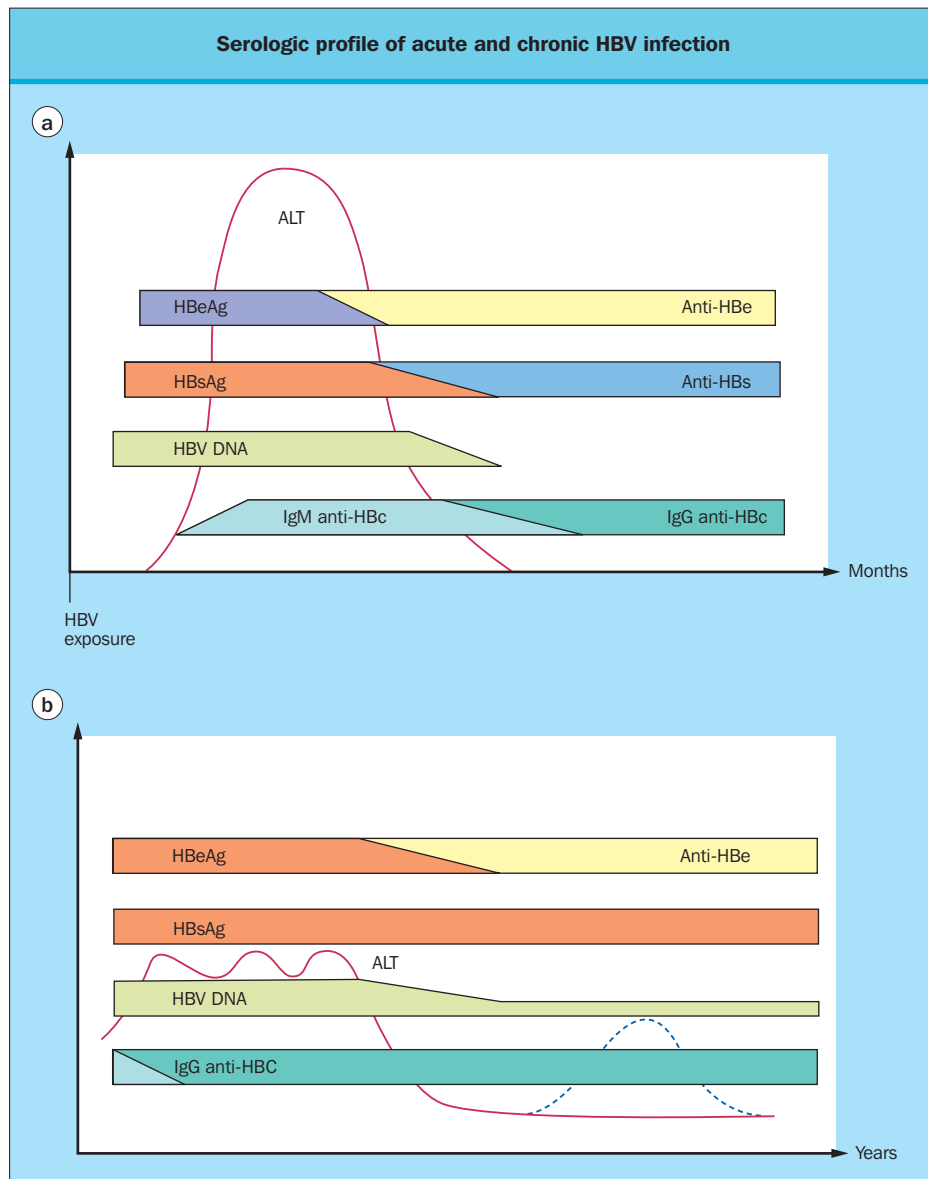
HBsAg is the hallmark of HBV infection. It is usually detectable 1–10 weeks after an acute exposure to HBV, and approximately 2–6 weeks before the onset of clinical symptoms. Most patients who recover from acute hepatitis B clear HBsAg in 4–6 months. Persistence of HBsAg in serum for more than 6 months implies chronic infection.

Anti-HBs is a neutralizing antibody that confers protective immunity to HBV infection. Recovery from acute hepatitis B is indicated by the disappearance of HBsAg and the development of anti-HBs. However, in some patients there may be a window period lasting several weeks to months when neither HBsAg nor anti-HBs can be detected. In addition, in some patients, anti-HBs is transiently detectable then rapidly disappears. Anti-HBs can be detected in individuals who recovered from HBV infection and in those who successfully responded to hepatitis B vaccination. Coexistence of HBsAg and anti-HBs has been found in some patients. These individuals should be considered as carriers because the anti-HBs is usually not capable of neutralizing the circulating virus.

#### Hepatitis B core antigen and antibody

HBcAg is an intracellular antigen and is not detected in serum. Immunoglobulin M (IgM) anti-HBc is the first antibody to develop during acute HBV infection. It is usually detectable within 1 month after the appearance of HBsAg. It is the only marker of HBV infection detectable during the window period between the disappearance of HBsAg and the detection of anti-HBs. The





**Figure 15.4 Serologic profile of acute and chronic hepatitis B virus (HBV) infection.** (a) Acute HBV infection and (b) chronic HBV infection.

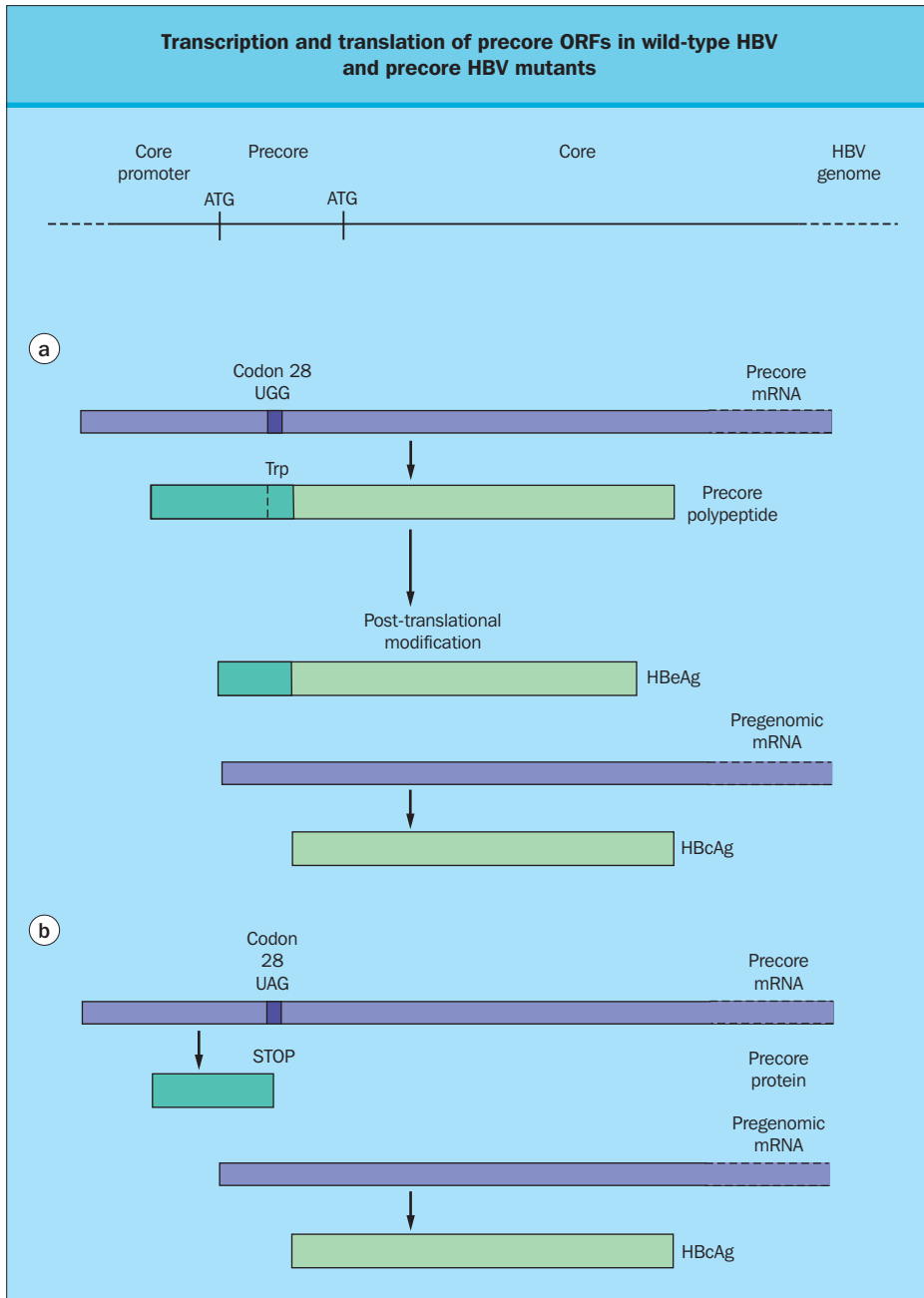
presence of IgM anti-HBc usually indicates recent HBV infection. However, the titer of IgM anti-HBc may increase to detectable levels during exacerbations of chronic hepatitis B, leading to misdiagnosis of acute hepatitis B.

Immunoglobulin G (IgG) anti-HBc (measured as total anti-HBc) is found (alone or in association with anti-HBs) in individuals who recover from hepatitis B and with HBsAg in those who have chronic HBV infection. Isolated presence of anti-HBc in the absence of HBsAg and anti-HBs can be detected in approximately 1% of blood donors in low prevalence areas and in up to 20% of blood donors in endemic areas. This situation usually reflects recovery from acute hepatitis B or after loss of HBsAg at a late stage of chronic HBV infection. Transmission of HBV infection has been reported in blood and organ donors with isolated anti-HBc.

#### Hepatitis B e antigen and antibody

HBeAg is a marker of HBV replication and infectivity. Its presence is usually associated with the detection of HBV DNA

in serum and a high risk of transmission of infection. During acute HBV infection, HBeAg is rapidly cleared, before the disappearance of HBsAg. However, in patients with chronic HBV infection, HBeAg may persist for years to decades. Sero-conversion from HBeAg to anti-HBe is usually associated with the decrease of serum HBV DNA to a low level (below 100 000 copies/mL) and remission of liver disease. However, HBeAg-negative and anti-HBe-positive patients may continue to have active liver disease and moderate levels of HBV DNA in serum. These patients are usually infected with HBV variants with diminished or abolished production of HBeAg. The most common mutation is a G→A change at nucleotide 1896, creating a premature stop codon at codon 28 of the precore region (Fig. 15.5). There is a wide variation in the prevalence of the stop codon mutant in different parts of the world with a high prevalence in the Mediterranean basin, an intermediate prevalence in South East Asia and Japan, and a low prevalence in North America and North Europe. The basis for the variable prevalence is related to the fact that the stop codon mutant is



**Figure 15.5 Transcription and translation of precore and core open reading frames in wild-type hepatitis B virus (HBV) and precore HBV mutants.** (a) Transcription of precore and core regions of wild-type HBV produces the precore polypeptide which undergoes post-translational modification to hepatitis B e antigen (HBeAg). (b) Precore mutant with premature stop codon at codon 28 prevents the production of HBeAg, but the translation of pregenomic RNA is not affected.

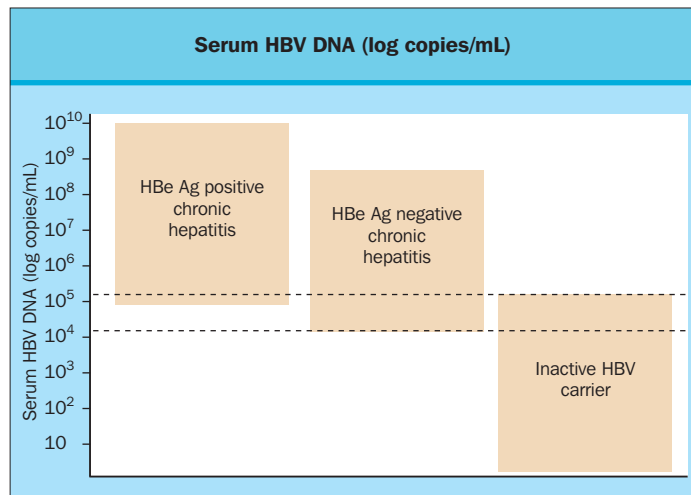
restricted to specific HBV genotypes. Other mutations that can decrease HBeAg production include changes in the core promoter region, most commonly A–T and G–A changes at nucleotides 1762 and 1764, respectively, which downregulates transcription of the precore messenger RNA.

#### Serum HBV DNA assays

Sensitive quantitative assays for HBV DNA in serum have been developed to assess the level of HBV replication. The branched DNA (bDNA) assay can detect HBV DNA levels down to 2000 copies/mL. Polymerase chain reaction (PCR) assays are even more sensitive with detection limits of 50 copies/mL. In patients with acute hepatitis B, serum HBV DNA appears early and may precede the detection of HBsAg. In patients with HBeAg-positive chronic hepatitis, serum HBV DNA levels are

usually much higher than 100 000 copies/mL (median level is  $10^9$ , ranging from  $10^6$  to  $10^{11}$ ) (Fig. 15.6). In patients with HBeAg-negative chronic hepatitis B, serum HBV DNA levels are usually lower (median level is  $10^7$ , ranging from  $10^4$  to  $10^8$ ) and fluctuating with the possibility of periods with very low levels (below  $10^4$ ). In inactive HBsAg carriers, serum HBV DNA levels are typically low (median  $10^3$ , ranging from undetectable to  $10^5$ ). Therefore, it is important to perform serial measurements of serum HBV DNA and ALT levels in order to distinguish true inactive HBsAg carriers from patients with fluctuating HBeAg-negative chronic hepatitis B (Fig. 15.7).

Measurement of the serum HBV DNA level is necessary for the diagnosis [in case of chronic hepatitis with increased ALT levels and persistently low levels of HBV DNA (less than  $10^4$  copies/mL), another cause must be looked for]; for the



**Figure 15.6 Serum hepatitis B virus (HBV) DNA levels.** Range of serum HBV DNA levels in patients with hepatitis B e antigen (HBeAg)-positive and HBeAg-negative chronic hepatitis B and inactive HBV carriers.

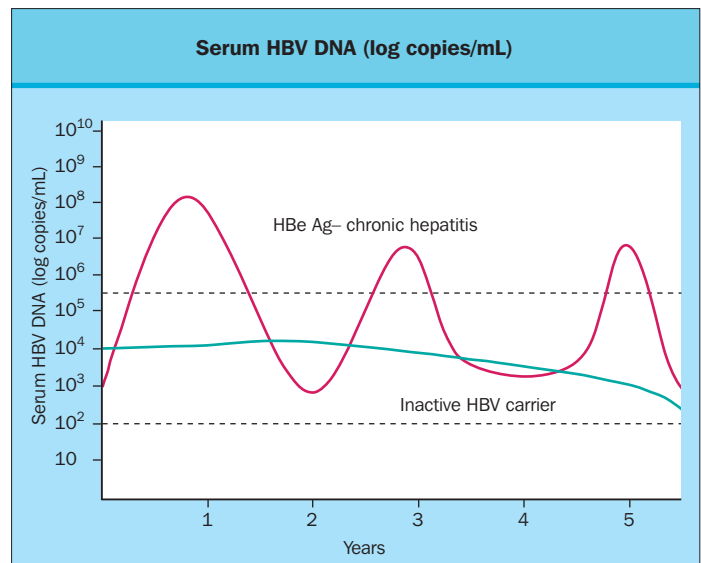
indication for therapy (treatment is indicated if serum HBV DNA level is higher than  $10^4$  in HBeAg chronic hepatitis B and higher than  $10^5$  in HBeAg-positive chronic hepatitis B); and to assess response to antiviral therapy (serum HBV DNA level below  $10^4$  in HBeAg chronic hepatitis B and below  $10^5$  in HBeAg-positive chronic hepatitis B).

### HBV genotypes

Based upon an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into seven unique HBV genotypes, designated A to G. The first reports of classification of HBV genotypes were based on complete genome sequences. However, HBV genotypes can also be determined from a partial sequence of the entire genome such as the pre-S or S gene without determining the entire genomic sequence. Currently, restriction fragment length polymorphisms (RFLP) and the line probe assay (LiPA) are the most commonly used genotyping methods.

Data from multinational clinical trials have confirmed a preponderance of HBV genotypes B and C in Asian and Pacific Islander patients, while genotypes A and D are more common in patients from Western European countries. HBV genotypes B and C are most prevalent in highly endemic areas where vertical transmission is the primary means of transmission. In contrast, HBV genotypes A, D, E, F and G are found in areas where horizontal or sexual transmission of HBV is more common. Data from adefovir clinical trials also demonstrate marked differences in HBV genotypes and subject race with 93% of Asians having genotype B or C and 93% of white patients having either genotype A or D.

All of the cross-sectional studies from endemic areas in Asia demonstrate that genotype C is associated with more advanced liver disease than genotype B. Consistently higher levels of serum HBV DNA and aminotransferase levels have been reported as well as more advanced histology and a greater likelihood of cirrhosis. The prevalence of detectable HBeAg is also higher in patients with genotype C than B. In addition, spontaneous HBeAg seroconversion occurred nearly 10 years earlier in patients



**Figure 15.7 Serum alanine aminotransferase (ALT) time course.** Typical serum ALT time course in a patient with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B and in an inactive hepatitis B virus (HBV) carrier.

with genotype B compared with patients with genotype C. Taken together, these findings suggest that infection with genotype C is associated with more aggressive disease compared with genotype B. The reasons for a more aggressive disease course with genotype C are unclear. Studies comparing the replicative capacity and host immune response to varying HBV genotypes have not been completed. The TA core promoter mutations were significantly more common in genotype C than B patients. Therefore, the higher prevalence of detectable HBeAg and TA core promoter mutations in genotype C patients may, in part, account for the more aggressive disease. However, additional prospective studies are needed to determine which of these virologic factors is most important.

Few studies have evaluated the role of non-B and non-C genotypes on the outcome of infection. Clearly, additional studies comparing HBV genotypes A and D are needed to determine if clinically significant differences in natural history are apparent.

Several retrospective studies performed on trials of conventional or pegylated interferon (peginterferon) showed that the rate of HBeAg seroconversion was higher among patients with genotype A and B than in those with genotype C or D. These data suggest that HBV genotype may be an important predictor of response to interferon, but larger studies are needed in different geographic regions of the world and particularly in patients with HBeAg-negative chronic hepatitis B. In HBeAg-positive chronic HBV patients treated with lamivudine, no significant difference in HBeAg seroconversion was seen in patients with HBV genotype B or C. Finally, a recent study of 694 chronic HBV patients treated with adefovir dipivoxil showed no difference in viral load reduction or HBeAg seroconversion after 48 weeks of treatment among patients with genotype A to G.

### Liver histology

Liver biopsy is seldom indicated in acute hepatitis B. Histologic changes include lobular disarray, acidophilic degeneration of



hepatocytes, focal lobular necrosis, cholestasis and portal inflammation. Bridging necrosis may be present in patients with severe hepatitis.

Liver biopsy is useful in assessing the severity of liver disease, in predicting prognosis, and in discussing the indication for therapy in patients with chronic hepatitis B. The most common features include portal inflammation and periportal necrosis. Periportal necrosis may be severe causing disruption of the limiting plate (piecemeal necrosis or interface hepatitis). As the liver disease progresses, fibrosis, and eventually cirrhosis develop. A third form of liver injury, chronic lobular hepatitis (CLH), which is characterized by spotty necrosis and inflammation within the lobules with mild portal inflammation, is seen during exacerbations of chronic hepatitis B.

Patients are classified according to the stage (degree of fibrosis) and the grade (severity of necroinflammation) of chronic hepatitis. The severity of liver injury (stage and grade) may vary more or less rapidly during the spontaneous course of the disease or according to response to therapy. In recent years, several numeric scoring systems have been developed to provide more objective assessment of liver injury and to permit statistical comparisons of necroinflammation and fibrosis (see O'Grady 2000, Ch. 13). Immunohistochemical staining reveals the presence of HBsAg in hepatocytes. In patients with high level replication, HBeAg can also be detected.

### Natural history

Chronic hepatitis B can induce liver cirrhosis and HCC. Chronic HBV infection is a dynamic process with an early replicative phase with active liver disease and a late low or non-replicative phase with remission of liver disease. Perinatally acquired HBV infection is characterized by a prolonged 'immunotolerant' phase with HBeAg positivity, high levels of serum HBV DNA, low levels of aminotransferases, minimal liver damage and very low rates of spontaneous HBeAg clearance (Fig. 15.8). Patients with childhood or adult acquired infection and chronic hepatitis B usually present in the 'immunoactive' phase with elevated

aminotransferases and liver necroinflammation at histology (HBeAg-positive chronic hepatitis). The rate of spontaneous HBeAg seroconversion may vary in relation to the degree of the activity of the liver disease. Seroconversion from HBeAg to antibody to HBeAg marks the transition from chronic hepatitis to the inactive HBV carrier state with low or undetectable serum HBV DNA and normal aminotransferases, and confers favorable long-term outcome with very low risk of cirrhosis or HCC in the majority of patients. HBsAg clearance occurs at the rate of around 1%/year in chronic HBV infected adults (resolved hepatitis B).

However a proportion of patients continue to have or redevelop high levels of HBV DNA and active hepatitis despite HBeAg seroconversion; these patients usually have HBV variants (with mutations in the precore or the core promoter regions) unable to express HBeAg (HBeAg-negative chronic hepatitis). HBeAg-negative chronic hepatitis represents a late phase in the natural history of chronic HBV infection and is associated with a very low rate of spontaneous disease remission.

### Prognosis

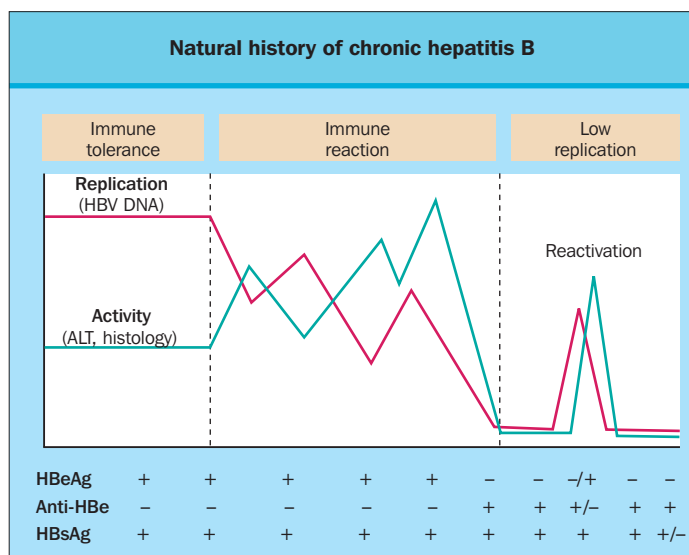
Longitudinal studies of patients with chronic hepatitis B indicate that, after diagnosis, the 5-year cumulative incidence of developing cirrhosis ranges from 8 to 20%. Morbidity and mortality in chronic hepatitis B are linked to evolution to cirrhosis or HCC. The 5-year cumulative incidence of hepatic decompensation is approximately 20% (Fig. 15.9). The 5-year probability of survival being approximately 80–86% in patients with compensated cirrhosis. Patients with decompensated cirrhosis have a poor prognosis (14–35% probability of survival at 5 years).

The incidence of HCC has increased worldwide and nowadays it constitutes the fifth most frequent cancer representing around 5% of all cancers worldwide. The incidence of HCC appears to vary geographically and correlates with the underlying stage of liver disease. The risk of HCC is highest for patients with cirrhosis and usually arises in the clinical setting of compensated cirrhosis which may be silent clinically. The annual incidence in HBV carriers ranges between 0.2% and 0.6%, but it reaches 2% when hepatic cirrhosis is established. The oncogenic mechanism leading to liver cancer involves different pathways that are not fully elucidated. Prevention through universal vaccination has effectively decreased the incidence of liver cancer and new therapeutic agents may delay or avoid the establishment of cirrhosis. The only chance for long-term survival after HCC diagnosis is to achieve early detection through regular surveillance by ultrasound and AFP determination. This will allow the indication of effective therapy such as surgical resection, liver transplantation or percutaneous ablation.

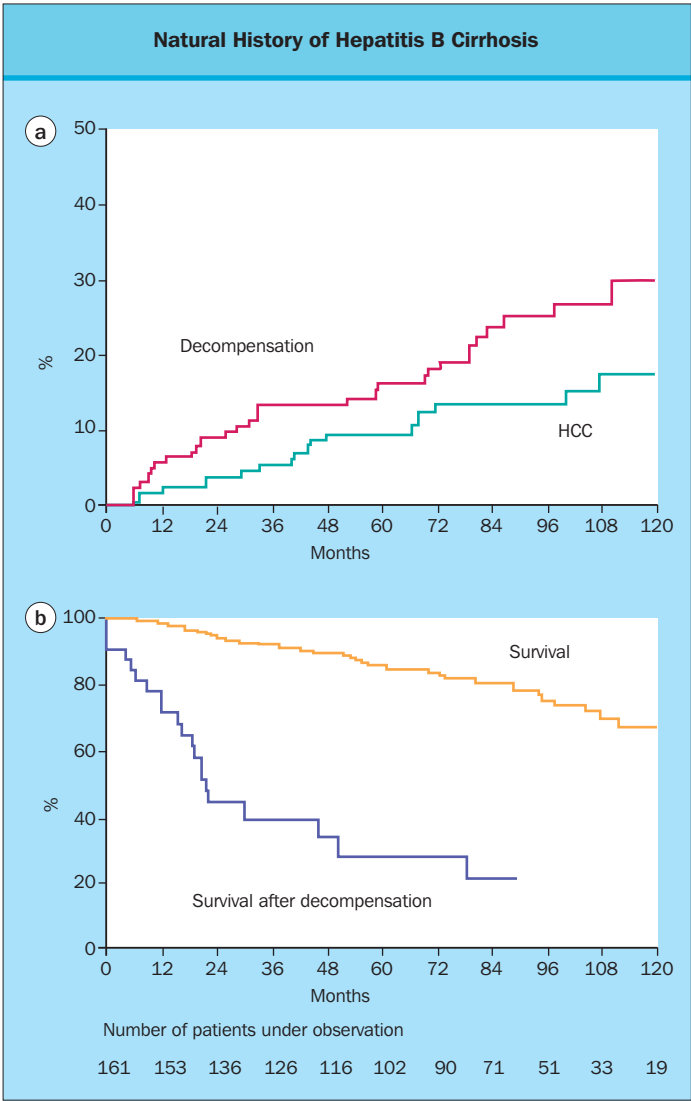
### Management

#### Objectives of treatment

The first objective of treatment is to decrease HBV replication in order to decrease necroinflammation in the liver and therefore prevent the progression of fibrosis. Thus, the interruption of the fibrogenesis process prevents progression to cirrhosis and its complications including HCC and therefore improves survival. The response to treatment can be classified into three phases. The first phase is characterized by the decrease of HBV replication (as reflected by the reduction of serum HBV DNA level); the liver necroinflammation diminishes, fibrosis is stabilized



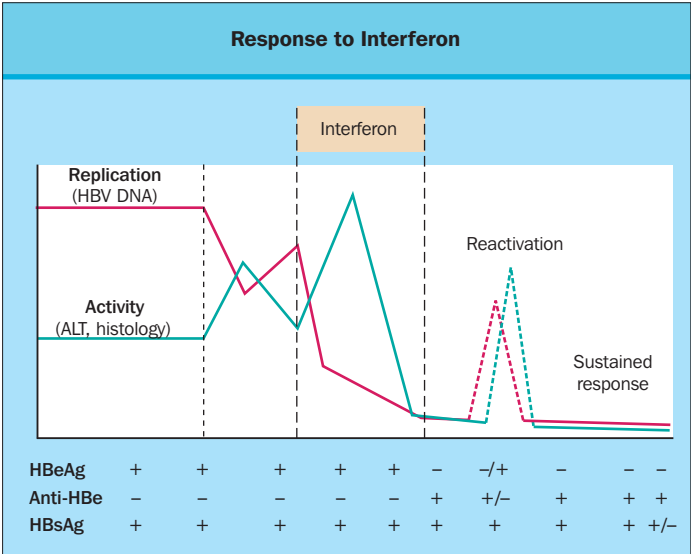
**Figure 15.8 Natural history of chronic hepatitis B.** The natural history has three consecutive phases: immune tolerance, immune reaction, low replication phase.



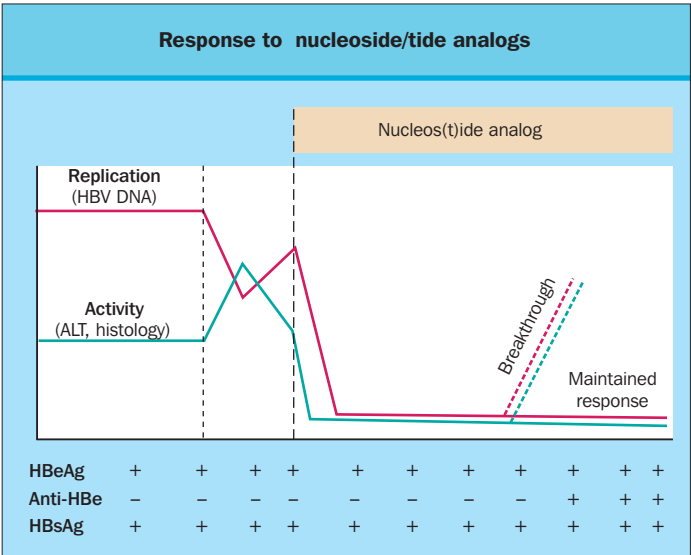
**Figure 15.9 Incidence of decompensation and survival in patients with hepatitis B virus (HBV) related cirrhosis.** (a) Cumulative incidence of decompensation, hepatocellular carcinoma (HCC). (b) Survival and survival after decompensation in untreated Caucasian patients with HBV related cirrhosis. Reproduced from Fattovich by permission of European Association for the Study of the Liver.

or may even regress but the risk of reactivation persists. If the antiviral effect is sufficient (less than 100 000 copies of HBV DNA/mL) and is maintained and accompanied by an effective immune response with clearance of infected hepatocytes, HBe seroconversion may occur; then the risk of reactivation is low. If HBV replication is completely inhibited (as reflected by the absence of detectable HBV DNA in the serum by sensitive assays), with stable HBe seroconversion, loss of detectable HBsAg (with or without HBsAg seroconversion) may occur which is associated with complete disappearance of liver necroinflammation with no risk of reactivation.

With the drugs currently available, two different concepts for the treatment of chronic hepatitis B can be considered: the first concept is that of sustained response obtained after a limited duration of therapy; the second concept is that of maintained response obtained during prolonged administration of therapy.



**Figure 15.10 Profile of sustained response to interferon treatment in a hepatitis B e antigen-positive patient.**



**Figure 15.11 Profile of maintained response to nucleoside or nucleotide analog treatment in a patient with chronic hepatitis B.**

The first strategy is the one usually used with interferon whose duration of therapy is limited by the poor tolerability (Fig. 15.10). Interferon therapy can induce a sustained response after withdrawal of treatment with a non-negligible chance of HBsAg loss or seroconversion; however, the limitation of this strategy is that the rate of sustained response is relatively low and sustained response is mainly obtained in patients with good predictors of response (i.e. patients in the immunoreactive phase with active liver disease and low level replication). The second strategy is the one generally used with nucleoside or nucleotide analogs whose withdrawal is generally accompanied by reactivation. Therefore, therapy needs to be administered indefinitely to obtain a maintained response (Fig. 15.11). Maintenance therapy is possible because of the good tolerability of these drugs. However, the limitation is the occurrence during therapy of

HBV resistant mutants associated with breakthroughs. Furthermore, HBc seroconversion is infrequent and HBsAg loss with or without HBs seroconversion is rarely observed with maintenance therapy with nucleoside or nucleotide analogs.

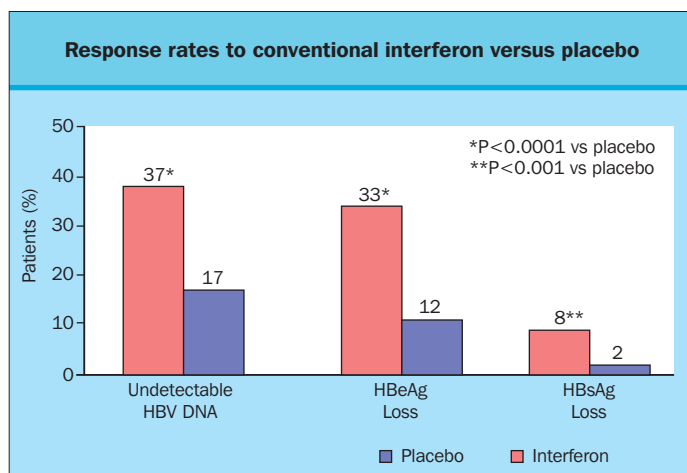
### Treatment with interferon

#### Conventional interferon

Recombinant interferon  $\alpha$  has been used in the treatment of chronic hepatitis B for many years. Interferon  $\alpha$  exerts an antiviral effect on infection with HBV through two mechanisms. First, interferon  $\alpha$  has a direct antiviral effect by inhibiting synthesis of viral DNA and by activating antiviral enzymes. Second, interferon  $\alpha$  exaggerates the cellular immune response against hepatocytes infected with HBV by increasing the expression of class I histocompatibility antigens and by stimulating the activity of helper T lymphocytes and natural killer lymphocytes. Thus, interferon  $\alpha$  induces an early diminution of HBV replication (reflected by a diminution of HBV DNA in serum) and (in patients with HBeAg-positive chronic hepatitis B) a late (about 2 months later) increase in serum ALT levels.

In patients with HBeAg-positive chronic hepatitis B, many controlled studies of interferon  $\alpha$  have been reported. In these studies, with various schedules, the mean virologic response rate was 37% versus 17%, the mean HBeAg loss rate was 33% versus 12% and the HBsAg loss rate was 8% versus 2% in the interferon treated groups versus the placebo groups (Fig. 15.12). A dosage of 5–10 MU thrice a week for 4 to 6 months allows a good efficacy with a satisfactory tolerance.

The discrepancies in the results of the different studies could be due, in part, to the different therapeutic schedules, but are mainly due to the populations of patients included in these trials. A certain number of factors are predictive of a poor response to interferon (Table 15.2). High serum HBV DNA level and low serum ALT levels are predictors of non-response. Also, infection with HBV at birth or early in the patient's life (as it is often the case in countries where HBV infection is hyper-endemic, such as South-East Asia) is a factor of poor response to interferon.



**Figure 15.12 Rates of virologic response.** Rates of virologic response [undetectable hepatitis B virus (HBV) DNA], hepatitis B e antigen (HBeAg) loss and hepatitis B surface antigen (HBsAg) loss in patients with HBeAg-positive chronic hepatitis B treated with interferon  $\alpha$ . Reproduced from a meta-analysis by Wong et al (93) by permission of the American College of Physicians.

In patients with HBeAg-negative chronic hepatitis B, many studies have shown high rates of virologic and biochemical response on-treatment (60–90%). However, the relapse rates observed after withdrawal of therapy were very high (70–90%). Longer duration of treatment (18 or 24 months) was associated with higher sustained response rates. Factors predicting response are not well established.

**Long-term outcome** Most responders have a sustained response. Approximately 10–20% of patients who clear HBeAg after interferon treatment experience reactivation of chronic hepatitis replication, usually within the first year after completing therapy. However, eradication of HBV replication as determined by disappearance of serum HBV DNA by PCR assay seldom occurs in patients who cleared HBeAg only. HBsAg clearance occurs at highly variable rates ranging from 65% in one US study, to 19–24% in European studies, to 9% in a study from Hong Kong. A sustained antiviral response in patients who cleared both HBeAg and HBsAg is invariably followed by a normalization of ALT levels and a decrease in histologic necroinflammation. Long-term studies found that patients with sustained suppression of HBV replication after interferon treatment have improved overall survival and a reduced likelihood of developing hepatic decompensation and HCC.

#### Pegylated interferon

The efficacy of interferon has recently improved with the replacement of conventional interferon by interferon conjugated with polyethylene glycol (peginterferon). This new form of interferon significantly increases the half-life and results in more stable plasma concentrations of interferon. Moreover, pegylation reduces the immunogenicity of the protein (reduction of the production of anti-interferon antibodies). Finally the number of injections has been reduced from three to one weekly, due to improved pharmacokinetics, which is obviously more comfortable for the patient.

#### Predictors of non-response to interferon therapy in chronic hepatitis B

Asian ethnicity
Childhood infection
Male sex
Immunosuppression due to disease (HIV) or therapy
Coexisting HDV infection
Disease caused by core promoter or precore mutant virus (HBeAg-negative)
Low serum ALT levels
High serum HBV DNA levels
Mild grade of liver necroinflammation

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IgM, immunoglobulin M.

**Table 15.2 Predictors of non-response to interferon therapy in chronic hepatitis B.**



Two peginterferons which differ in the quality and quantity of conjugated PEG to interferon have been produced: 12 kDa of linear PEG for interferon- $\alpha$  2b and 40 kDa branched PEG for interferon- $\alpha$  2a. In both cases peginterferons have been shown to be twice as effective overall than the corresponding non-peginterferons in chronic hepatitis C. Therefore, the efficacy of peginterferons has been recently assessed in the treatment of chronic hepatitis B.

A first randomized controlled study of peginterferon  $\alpha$ 2a has been performed in patients with HBeAg-positive chronic hepatitis B. Treatment duration and follow-up were each 24 weeks. At the end of follow-up, treatment response defined by loss of HBeAg with serum HBV DNA level below 500 000 copies/mL with normal ALT was observed in 19–28% of patients receiving peginterferon  $\alpha$ 2a (at a dose of 90  $\mu$ g, 180  $\mu$ g or 270  $\mu$ g per week) versus 12% of patients who received standard interferon 2a. Side-effects associated with peginterferon were comparable to those observed with conventional interferon. The safety profile of peginterferon was comparable to that of conventional interferon with the same frequency of adverse events or laboratory abnormalities.

This study does not prove the superiority of peginterferon  $\alpha$ 2a as compared with standard interferon 2a since the dose of interferon  $\alpha$ 2a used was relatively low (4.5 MU, three times a week) and the differences observed between each of the three peginterferon  $\alpha$ 2a treatment groups and the standard interferon 2a group were not significant. However, the overall rate of response in patients who received peginterferon  $\alpha$ 2a was higher than that observed in the interferon  $\alpha$ 2a group. A retrospective analysis showed that the rates of response were higher with the peginterferon among the most difficult to treat patients (with high HBV DNA levels or low ALT levels). Therefore this study strongly suggests that peginterferon  $\alpha$ 2a is more effective than standard interferon  $\alpha$ 2a for the treatment of chronic hepatitis B.

Three large randomized controlled trials have confirmed the efficacy of peginterferons in HBeAg-positive (one trial with peginterferon  $\alpha$ 2b and one trial with peginterferon  $\alpha$ 2a) and HBeAg-negative chronic hepatitis B (one trial with peginterferon  $\alpha$ 2a). These studies which compared peginterferon monotherapies to the combination of peginterferon and lamivudine (three studies) and lamivudine (two studies) are detailed in O'Grady 2000 (Ch. 13).

#### Adverse reactions

Interferon therapy is associated with a wide spectrum of side-effects. The commonest is flu-like symptoms (fever, headache, malaise and myalgia) during the first 1–2 weeks of treatment. Other adverse effects include fatigue, anorexia, weight loss and mild alopecia. Mild myelosuppression is common but significant neutropenia ( $<1000/\text{mm}^3$ ) or thrombocytopenia ( $<60\,000/\text{mm}^3$ ) requiring dose reduction or premature termination of treatment is uncommon. Psychiatric problems including anxiety, irritability, depression and suicidal tendency may occur even in patients without previous emotional problems. Interferon may also induce the development of autoantibodies, but it seldom leads to overt autoimmune disease except for hyper- and hypothyroidism.

Adverse events observed with pegylated interferons are the same as those observed with conventional interferons. No new and unexpected adverse event was observed.

#### Treatment with nucleoside and nucleotide analogs

One nucleoside analog (lamivudine) and one nucleotide analog (adefovir dipivoxil) are currently approved for the treatment of chronic hepatitis B. Lamivudine and adefovir have the advantages of oral administration and excellent safety profiles. However, they induce a sustained response after withdrawal of therapy in only a minority of patients and therefore the treatment needs to be indefinitely administered in the majority of patients. In addition, the long-term efficacy of lamivudine is limited by the frequent emergence of drug-resistant HBV mutants. Adefovir is associated with a low incidence of resistance but its antiviral effect is not optimal. As a result, more effective therapy with more potent drugs used alone or in combination are under evaluation and the treatment of chronic hepatitis B remains an open issue.

#### Lamivudine

Lamivudine, a nucleoside analog which inhibits HBV DNA synthesis through chain termination, was first developed as a reverse transcriptase inhibitor for use in HIV infection. Lamivudine (2',3'-dideoxy-3'-thiacytidine, 3TC) is a negative enantiomer and it is thought that this may help to explain the very low rates of adverse events noted with this agent. Randomized controlled trials have shown the efficacy of lamivudine in the treatment of HBeAg-positive and HBeAg-negative chronic hepatitis B.

**HBeAg-positive chronic hepatitis B** One randomized placebo controlled trial showed that almost all patients treated with lamivudine (98%) had a reduction of serum HBV DNA levels. Serum HBV DNA levels became undetectable under lamivudine ( $<0.7$  mEq/mL) in 44% versus 16% in the placebo group. At 1 year of treatment, the HBe seroconversion rates were 17% and 6% in the lamivudine and the placebo group, respectively. Normalization of serum aminotransferase (ALT) levels was observed in 41% versus 7% in the placebo group. Histologic improvement defined by a decrease of at least two points of the Knodell score was observed in 52% versus 23% in those on placebo. The rates of virologic, biochemical and histologic response observed in three other randomized controlled trials showed similar results with HBeAg seroconversion rates ranging from 17 to 21%. Lamivudine therapy for 3 years reduces necroinflammatory activity and reverses fibrosis in most patients.

The tolerability and safety of lamivudine are excellent; the incidence of adverse events was similar to that of placebo. Lamivudine therapy seems to be well tolerated for up to 5 years; however data reported on long-term therapy with lamivudine are limited to small series of patients.

Pretreatment factors predictive of response are, as with interferon, high serum ALT levels and a high degree of histologic necroinflammation. Pretreatment ALT levels seem to be the most important: response rates are low and similar to untreated patients in patients with levels below two times the upper limit of normal. The rate of HBeAg loss was highest among patients with pretreatment ALT levels greater than five times the upper limit of normal.

After withdrawal of lamivudine therapy, ALT flare-ups have been reported in 41 individuals treated with lamivudine for a minimum of 3 months and followed for at least 6 months after therapy. Marked flares were observed in 17% and occurred

7–44 weeks after treatment. These flares were all associated with rising levels of HBV DNA unlike the spontaneous flares associated with HBe seroconversion (with falling levels of HBV DNA). Two of these flares were associated with hepatic decompensation.

The major inconvenience of lamivudine is the high rate of occurrence of viral resistance related to mutations in the YMDD motif. Indeed, even if the HBeAg seroconversion rate may be increased by continuing treatment, the frequency of resistance increases with the time from 24% at 1 year, 38% at 2 years, 50% at 3 years and 67% at 4 years. The most important mutation is a substitution of valine or isoleucine for methionine in the YMDD motif of the HBV polymerase gene (rtM204V/I). In many patients this is accompanied by a second mutation substituting methionine for leucine in an upstream region (rtL180M). Lamivudine resistance is more likely to occur in patients with high baseline serum HBV DNA levels.

The emergence of lamivudine-resistant mutants is usually associated with a breakthrough with moderate increase of serum HBV DNA and ALT levels which may remain lower than at baseline for several months. However, severe cases have been reported in patients with cirrhosis. In patients who develop lamivudine-resistant mutants, adefovir is effective and should be initiated rapidly if an increase in ALT is observed, especially in patients with cirrhosis who have a risk of hepatic decompensation. In order to diagnose emergence of resistance earlier (before the appearance of detectable serum HBV DNA by standard assays and before the increase of ALT) monitoring of serum HBV DNA level by a sensitive assay is useful. Indeed, an increase of serum HBV DNA of more than 1 log is generally a sign of the appearance of a resistant mutant and this allows one to switch to adefovir several months before the increase in ALT occurs.

In case of HBe seroconversion during lamivudine therapy, it is usually recommended to prolong treatment for 3 to 6 additional months to decrease the risk of reactivation. In the absence of HBe seroconversion, since withdrawal is almost invariably followed by reactivation, it is usually recommended to continue the treatment as long as HBV replication is suppressed and serum ALT levels remain normal, until the appearance of viral resistance.

**HBeAg-negative chronic hepatitis B** In patients with HBeAg-negative chronic hepatitis B, one randomized controlled study showed an efficacy similar to that observed in patients with HBeAg-positive chronic hepatitis B with the same rate of resistance. HBV DNA was undetectable (non-PCR assays) after 12 months of therapy in 90% of patients (70% with PCR assays). Serum ALT normalized in 75% of patients. A fall in HBV DNA and normalization of ALT occurred only in 5% of patients on placebo. A histologic response was observed in 60% of lamivudine-treated patients. Predictors of response to lamivudine have not been established in this population. In patients with a virologic response at the end of a 12-month course of lamivudine, the sustained response rate 6 months post-treatment was less than 5%.

In studies with prolonged therapy, response rates peak at 12 months and decrease thereafter. At 30 months, rates of virologic and biochemical responses were 30% and 60%, respectively. In a study with more than 600 patients with severe

fibrosis or compensated cirrhosis, lamivudine therapy (median treatment exposure 32 months) was shown to significantly reduce disease progression and the risk of hepatocellular carcinoma.

The rate of resistance observed in patients with HBeAg negative chronic hepatitis was similar to that observed in HBeAg-positive chronic hepatitis. Lamivudine-resistant mutants appeared in 10–40% of patients after 1 year of therapy, and in 50–60% of those treated continuously for 3 years. Likewise in HBeAg-positive patients, the emergence of resistant mutant HBV was accompanied by an increase of HBV DNA and after a few months, by ALT elevation. Flares occurred in 30% of patients and were symptomatic or severe in some cases. Like in HBeAg-positive patients, treatment by adefovir is generally effective in patients who develop lamivudine resistance.

**Special groups** In patients with decompensated cirrhosis, lamivudine has been a major breakthrough. Lamivudine therapy can induce marked improvement in liver disease, impacting favorably on survival. In patients waiting for liver transplantation, time to death or transplantation was significantly longer and transplantation was less frequent (35% versus 74%) in lamivudine-treated compared to a historical untreated cohort. But the clinical effects are slow and impaired by the risk of emergence of resistance which can be associated with flares responsible for liver failure and death. In addition, patients in whom lamivudine resistance develops before transplant have a higher risk of recurrent hepatitis B post-transplant. In this clinical situation, adefovir should be rapidly initiated if lamivudine resistance is suspected in order to prevent the flare.

In case of recurrence of HBV infection after liver transplantation, lamivudine therapy achieves similar results to those in non-transplant patients with respect to inhibition of HBV replication and normalization of ALT. Breakthrough secondary to lamivudine resistance may lead to severe hepatitis flares, rapidly progressive liver failure and graft loss. For these reasons, after transplantation, the optimal prophylactic regimen appears to be the combination of lamivudine with hepatitis B (HBIG).

In HIV-HBV co-infected patients, from the limited data available lamivudine appears to show similar effect in patients on highly active antiretroviral therapy (HAART) in terms of virologic and biochemical response, as in patients not co-infected with HIV. In 40 HIV co-infected patients, 96% had undetectable serum HBV DNA (<5 pg/mL); serum ALT decreased compared to baseline; HBe seroconversion was observed in 11%. Drug-resistant HBV emerged rapidly during monotherapy with lamivudine: the incidence was 50% and 90% at 2 and 4 years, respectively. Note that the recommended dose of lamivudine for HIV is 150 mg twice a day and for HBV is 100 mg once a day. Because of the high rate of resistance to lamivudine and because a large proportion of HIV-HBV-infected patients have already received lamivudine for the treatment of HIV and/or HBV, adefovir or tenofovir (which is registered for the treatment of HIV) are the drugs generally used in the treatment of chronic hepatitis B in this population.

#### **Adefovir dipivoxil**

Adefovir dipivoxil has been recently registered for the treatment of chronic hepatitis B. Adefovir dipivoxil is the oral prodrug of adefovir. Adefovir is a nucleotide analog of adenosine

monophosphate. In vivo, adefovir dipivoxil is converted to the parent compound, adefovir, and through two phosphorylation reactions to adefovir diphosphate, the active intracellular metabolite that interacts with the HBV polymerase. Adefovir diphosphate acts as a competitive inhibitor and chain terminator of HBV replication.

Two large randomized controlled trials have demonstrated that adefovir dipivoxil is effective in patients with HBeAg-positive or HBeAg-negative chronic hepatitis B. Also, adefovir dipivoxil effectively suppresses lamivudine-resistant HBV in chronic hepatitis B patients post-liver transplantation, patients with compensated or decompensated liver disease and patients co-infected with HIV.

**HBeAg-positive chronic hepatitis B** A large, placebo-controlled study enrolled 515 patients randomized to receive adefovir dipivoxil 10 mg daily ( $n = 172$ ), adefovir 30 mg daily ( $n = 173$ ), or placebo ( $n = 170$ ) for 48 weeks. There was a rapid decrease in the median serum HBV DNA level in patients treated with adefovir dipivoxil, with statistically significant differences compared with placebo. At week 48, the median change from baseline in serum HBV DNA was  $-3.5 \log_{10}$  for adefovir (10 mg) compared with  $-0.5 \log_{10}$  copies/mL for placebo. Significantly more patients had undetectable serum HBV DNA levels (below 400 copies/mL) (21% versus 0%,  $P < 0.001$ ) (Fig. 15.13). HBeAg seroconversion was observed in 12% of the patients receiving adefovir 10 mg compared with 6% in the placebo group ( $P < 0.05$ ). ALT normalization was achieved in 48% compared with 16% ( $P < 0.001$ ). A significantly higher proportion of patients receiving adefovir 10 mg showed improvement in liver histology (improvement of at least two points of the Knodell score) at week 48 compared with those receiving placebo (53% versus 25%;  $P < 0.001$ ).

The tolerability and safety profile of adefovir at the dose of 10 mg was similar to that of the placebo. Adefovir at the dose of

30 mg was associated with increase in creatinine levels in some patients. This increase was moderate, occurred after 24 weeks of treatment and resolved in all cases after withdrawal of the drug. However, this study showed the 10 mg dose as the best dose with regard to the benefit/risk ratio and the 10 mg dose is the dose registered.

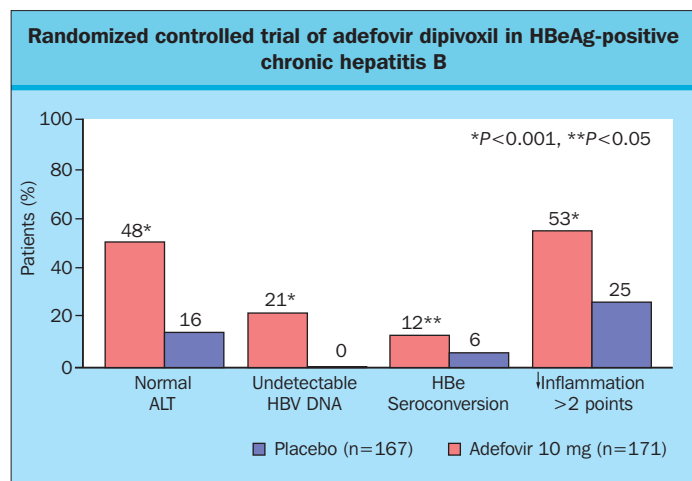
The durability of the response after withdrawal of treatment is not known since the treatment is maintained according to the design of the trial. However, cessation of therapy in some patients without HBe seroconversion was associated with relapse. Therefore, maintenance therapy is recommended. Preliminary results suggest that the antiviral effect is maintained and the rates of virologic response with HBe seroconversion increase with the duration of therapy. However, results of longer follow-up (up to 5 years) are awaited in order to confirm the maintained response and the possible increased efficacy of long-term treatment.

An extensive genotyping study has been carried out in all patients with detectable HBV DNA (by PCR assay). Systematic sequencing did not show any case of emergence of a mutant HBV resistant to adefovir after 48 weeks of treatment with adefovir. Large-scale studies of cohorts of patients under adefovir are in progress to assess the incidence of resistance in the long term. The explanation for the high threshold for resistance to adefovir dipivoxil may reside in its structural features that distinguish it from nucleoside analogs: specifically, its minimal flexible acyclic linker that closely resembles the natural substrate and the fact that it is a nucleotide containing a phosphorus atom, not a nucleoside.

**HBeAg-negative chronic hepatitis B** A large randomized, placebo-controlled study of adefovir dipivoxil has been performed in 185 patients with HBeAg-negative chronic hepatitis B. Patients were randomized in a 2:1 ratio to receive either adefovir dipivoxil 10 mg ( $n = 123$ ) or placebo ( $n = 62$ ) for 48 weeks. Adefovir 10 mg once daily resulted in a significant reduction in serum HBV DNA levels at week 48 compared with placebo, with a median decrease of  $3.9 \log_{10}$  copies/mL versus  $1.3 \log_{10}$  copies/mL ( $P < 0.001$ ). Of the patients treated with adefovir dipivoxil, 51% had no detectable HBV DNA as measured by PCR compared with none in the placebo group ( $P < 0.001$ ) (Fig. 15.14). ALT normalization was achieved in 72% compared with 29% ( $P < 0.001$ ). Significantly more patients in the adefovir group had histologic improvement from baseline to week 48 compared with the placebo group (64% versus 33%,  $P < 0.001$ ).

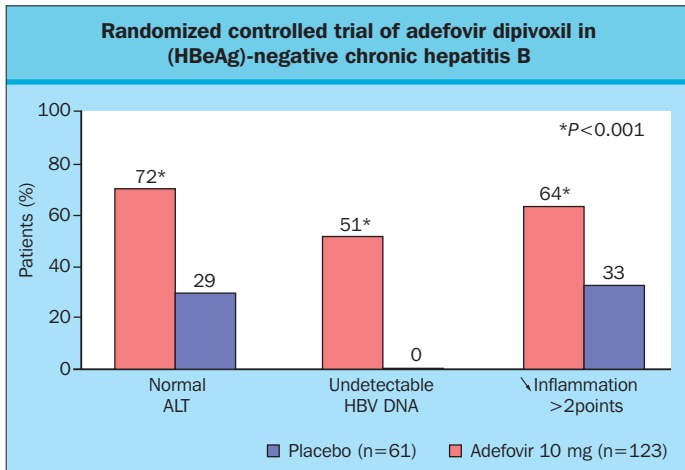
At 4 years of therapy with 10 mg of adefovir daily, the antiviral effect was maintained. In a subgroup of patients which had repeat liver biopsy, a maintained histologic improvement was observed. After 4 years of therapy, tolerability was good without significant side-effects and without the occurrence of significant nephrotoxicity. The incidence of resistance related to the occurrence of specific mutations (rtN236T and rtA181V) was low (3% at 2 years, 11% at 3 years and 18% at 4 years). HBV strains resistant to adefovir are sensitive to lamivudine in vitro and a few case reports seem to confirm this.

**Special groups** Adefovir (at the dose of 10 mg) can be used safely and effectively in patients with decompensated cirrhosis or recurrent hepatitis B after liver transplantation. In a recently reported study, 324 chronic hepatitis B patients pre- and post-



**Figure 15.13 Adefovir dipivoxil in the treatment of hepatitis B e antigen (HBeAg)-positive chronic hepatitis B.** In this randomized controlled trial, at 48 weeks of therapy, the rates of normal alanine aminotransferase (ALT), undetectable hepatitis B virus DNA, HBe seroconversion and histologic improvement were higher in the group of patients receiving adefovir, at the dose of 10 mg daily, as compared with the group receiving a placebo. Data from Marcellin et al (04).





**Figure 15.14 Adefovir dipivoxil in the treatment of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B.** In this randomized controlled trial, at 48 weeks of therapy, the rates of normal alanine aminotransferase, undetectable hepatitis B virus DNA and histologic improvement were higher in the group of patients receiving adefovir, at the dose of 10 mg daily, as compared with the group receiving a placebo. ALT, alanine aminotransferase. Data from Hadziyannis et al (03).

liver transplantation (128 and 196 patients, respectively) with lamivudine-resistant HBV received adefovir dipivoxil for varying lengths of time up to a maximum of 72 weeks (pre-liver transplantation) and 129 weeks (post-liver transplantation), respectively. Serum HBV DNA was reduced by approximately 4 log<sub>10</sub> copies/mL in both groups, and ALT level normalized in 76% and 49% of patients in the two groups, respectively. Patients also had significant improvement of liver function. Kaplan-Meier survival curves estimates by week 48 were 84% and 93% in the pre- and post-transplantation groups, respectively. These 1-year survival rates are significantly better than survival rates observed in historical groups of patients with no treatment. Noteworthy, in a significant number of patients who improved, the liver transplantation was postponed.

Although many of the patients, all of whom had lamivudine-resistant HBV, were medically compromised as a result of advanced liver disease and co-morbidities, there was a low rate of adverse events leading to drug discontinuation in pre- and post-liver transplantation patients (5% and 6%, respectively). A total of 42 deaths (11%) were reported in pre- and post-transplantation patients (24 and 18 patients, respectively). The deaths were considered to be due to complications of progressive liver disease or liver transplantation surgery. There may be some concern about the long-term administration of adefovir in these patients with common concomitant renal dysfunction and therefore dose reduction, according to the package insert, based on creatinine clearance is advised.

In an open-label pilot study conducted in 35 HIV-HBV co-infected patients with lamivudine-resistant HBV and controlled HIV infection, adefovir therapy, at the dose of 10 mg, induced a 4 log decrease in serum HBV DNA levels at 48 weeks. Two patients underwent HBe seroconversion. A transient increase in serum ALT levels was observed in 15 patients without consequence on liver function. The explanation for this observation is unclear. No HBV DNA breakthrough and no viral resistance

were observed through week 48. In addition no significant changes in either HIV RNA or CD4 cell count were observed.

## New agents

### Entecavir

Entecavir, a cyclopentyl guanosine analog, is a potent inhibitor of HBV DNA polymerase, inhibiting both the priming and elongation steps of viral DNA replication. Entecavir is phosphorylated to its triphosphate, the active compound, by cellular kinases. It is a selective inhibitor of HBV DNA and is less effective against lamivudine-resistant mutants than against wild-type HBV.

Two large randomized controlled trials showed that entecavir is effective in patients with HBeAg-positive or -negative chronic hepatitis B. In HBeAg-positive chronic hepatitis B, entecavir induced a marked decrease in HBV DNA levels (7 logs) with undetectable HBV DNA in 91% of patients and histologic improvement in 72%. In HBeAg-negative chronic hepatitis B, entecavir induced also a marked decrease in HBV DNA levels (5 logs) with undetectable HBV DNA in 91% of patients and histologic improvement in 70%. These results were significantly better than those observed in the lamivudine control arms. Interestingly, no resistance was observed at 1 year of therapy in the 432 nucleoside naive patients; however, resistance (mutations rt184, rt202 and rt250) was observed in 5.8% of the patients who had received previous treatment with lamivudine. These results indicate that entecavir is a potent antiviral against HBV.

### Emtricitabine

Emtricitabine (FTC) is a cytosine nucleoside analog with antiviral activity against both HBV and HIV. It is structurally similar to lamivudine: it only differs from by a fluorine at the 5-position of the nucleic acid.

A randomized controlled trial of emtricitabine at the dose of 200 mg versus placebo showed undetectable HBV DNA in 56% of the patients treated and histologic improvement in 62%. However, the rate of HBe seroconversion was low (12%) and no different from that observed in the placebo group. In addition, a relatively high rate of resistance (12.6%) (same YMDD mutations as those associated with lamivudine) was observed. Therefore, the role of emtricitabine as a monotherapy may be limited by its structural similarity to lamivudine with the risk of development of drug resistance. Emtricitabine is available in a combination pill with tenofovir.

### Telbivudine

The natural nucleosides in the β-L-configuration [β-L-thymidine (LdT), β-L-2-deoxycytidine (L-dC) and β-L-2-deoxyadenosine (L-dA)] represent a newly discovered class of compounds with potent, *selective and specific activity against* hepadnavirus. 'In vitro' studies have shown that these compounds have marked effects on HBV replication. Telbivudine (LdT) is at the most developed stage of clinical investigation.

A phase II study including 104 HBeAg-positive patients showed that telbivudine administered at the dose of 600 mg daily, for 1 year induced a mean HBV DNA reduction of 6 logs with undetectable HBV DNA by PCR in 61% and HBeAg loss in 33%. YMDD HBV mutants were found in 4.4% of the patients. The safety profile of telbivudine appeared similar to placebo. Thus, this study confirms the marked antiviral effect of

telbivudine with a safe profile with however a non-negligible 1-year resistance rate (4.4%). On the basis of these data, phase III studies have been initiated.

Another promising  $\beta$ -L-nucleoside compound is val-LdC (valtorcitabine). It is in phase II testing and preliminary results indicate interesting antiviral activity with a good safety profile. A combination of these two compounds which act at different levels of HBV replication could be of interest.

#### Clevudine

Clevudine (1-2-fluoro-5-methyl- $\beta$ -L-arabinosyl uracil, L-FMAU) is a pyrimidine analog with marked 'in vitro' activity against HBV but not HIV. The active triphosphate inhibits HBV DNA polymerase but is not an obligate chain terminator.

Phase II studies showed that clevudine at the dose of 100 mg administered for 4 weeks induced a rapid decrease of HBV DNA (3.5 logs) with interestingly a lasting effect with a slow reincrease of HBV DNA after withdrawal of treatment without rebound. Further studies are in progress to assess the long-term efficacy and safety of this drug.

#### Combinations

##### Combination of pegylated interferon with lamivudine

Previous studies on the combination of interferon and lamivudine suggested that this combination could be more effective than lamivudine monotherapy. However, the results of different studies were discordant which could be due to different treatment regimens which could not be optimal.

**HBeAg-positive chronic hepatitis** In a large randomized controlled study, 307 patients with HBeAg-positive chronic hepatitis B were randomized to receive either the combination of peginterferon  $\alpha$ 2b 100  $\mu$ g per week for 32 weeks then 50  $\mu$ g for 20 weeks and lamivudine 100 mg per day or peginterferon  $\alpha$ 2b at the same dose with placebo. At the end of the 26-week post-treatment follow-up, there was no difference in response rates between the two treatment groups: serum HBV DNA was undetectable by PCR (below 400 copies/mL) in 7% and 9%; HBeAg loss was observed in 36% and 35%; normal ALT was obtained in 32% and 35% in the peginterferon monotherapy and the peginterferon with lamivudine combination therapy groups, respectively. Interestingly, a relatively high rate of HBsAg loss was observed (7% in both groups).

Main predictors of response were HBV genotype and pre-treatment ALT level. Response was 34% for those with ALT levels under three times the upper limit of normal and 50% for those with ALT levels above five times the upper limit of normal. Response was 60% for genotype A versus 42% for genotype B, 32% for genotype C and 28% for genotype D.

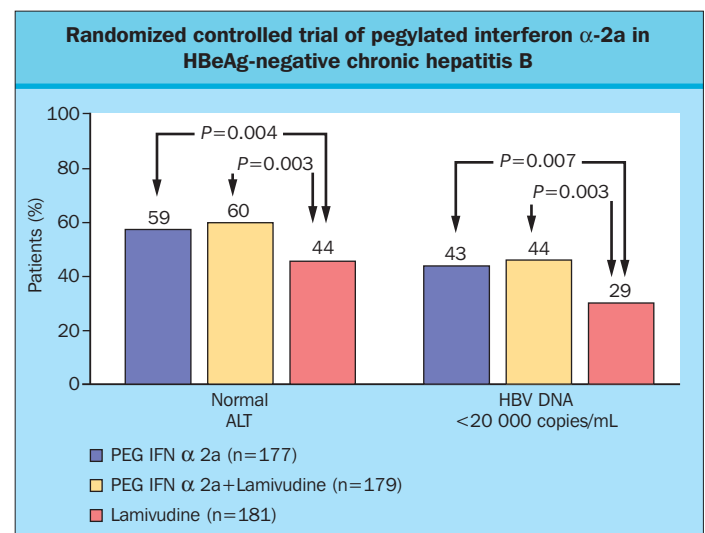
Another large randomized controlled trial compared the efficacy of the combination of peginterferon  $\alpha$ 2a with or without lamivudine versus lamivudine alone. Patients were randomized to one of the following treatments: peginterferon  $\alpha$ 2a, 180  $\mu$ g once weekly plus oral placebo once daily for 48 weeks; peginterferon  $\alpha$ 2a, 180  $\mu$ g once weekly plus lamivudine 100 mg once daily for 48 weeks; lamivudine 100 mg once daily for 48 weeks. At the end of the 24-week post-treatment follow-up, the two peginterferon treatment arms (with or without lamivudine) showed the same efficacy which was superior to that observed in the lamivudine treatment arm: a virologic response (serum

HBV DNA below 100 000 copies/mL by quantitative PCR) in 32%, 34% and 22% of the patients, respectively. HBeAg seroconversion was observed in 32%, 27% and 19%, respectively. Interestingly, a substantial rate of HBsAg loss was observed in this study in patients who received peginterferon  $\alpha$ 2a (3% and 4% versus <1% in the lamivudine group).

These studies show that in patients with HBeAg-positive chronic hepatitis B, 6 months after therapy, the combination of peginterferon with lamivudine (with the simultaneous regimen used) is not superior to peginterferon used in monotherapy.

**HBeAg-negative chronic hepatitis** A phase III, partially double-blinded study has evaluated the efficacy and the safety of peginterferon  $\alpha$ 2a alone or in combination with lamivudine versus lamivudine in patients with HBeAg-negative chronic hepatitis B.

Patients were randomized to one of the following treatments: peginterferon  $\alpha$ 2a, 180  $\mu$ g once weekly plus oral placebo once daily for 48 weeks; peginterferon  $\alpha$ 2a, 180  $\mu$ g once weekly plus lamivudine 100 mg once daily for 48 weeks; lamivudine 100 mg once daily for 48 weeks. In total, 552 patients were enrolled in the study. At the end of the 24-week post treatment follow-up, the two peginterferon treatment arms (with or without lamivudine) showed the same efficacy which was superior to that observed in the lamivudine treatment arm: a biochemical response (normal ALT) was observed in 59%, 60% and 44% of the patients, respectively and a virologic response (serum HBV DNA below 20 000 copies/mL by quantitative PCR) in 43%, 44% and 29% of the patients, respectively (Fig. 15.15). Interestingly, taking into account that HBsAg loss is rarely



**Figure 15.15 Pegylated (PEG)-interferon  $\alpha$ 2a in patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B.** In this randomized controlled trial, 24 weeks after therapy, the rates of response (normal alanine aminotransferase level and serum hepatitis B virus DNA below 20 000 copies/mL) were higher in the two groups which received peginterferon  $\alpha$ 2a (with or without lamivudine) as compared with the group which received lamivudine. There was no difference in response rates between the group which received peginterferon  $\alpha$ 2a alone and the group which received the peginterferon  $\alpha$ 2a plus lamivudine combination. ALT, alanine aminotransferase. Reproduced from Marcellin et al by permission of the Massachusetts Medical Society.

observed in HBeAg-negative patients, a substantial rate of HBsAg loss was observed in this study in patients who received peginterferon  $\alpha$ 2a (4% and 3% versus 0% in the lamivudine group).

Of note, at the end of the 48-week treatment period, there was a higher incidence of lamivudine resistance in the lamivudine monotherapy group when compared with the peginterferon  $\alpha$ 2a plus lamivudine combination group, which confirms previous studies suggesting that interferon decreases the risk of lamivudine resistance.

The adverse events associated with peginterferon  $\alpha$ 2a therapy were similar to those observed in previous trials in patients with chronic hepatitis C. Interestingly, the frequency of the adverse events was lower than that observed in patients with chronic hepatitis C. In particular, the frequency of depression was much lower: 3–4% as compared with 16–20% in patients with chronic hepatitis C.

This study shows that in patients with HBeAg-negative chronic hepatitis B, first, the efficacy, as assessed at 24 weeks post-treatment, of peginterferon  $\alpha$ 2a monotherapy is superior to lamivudine monotherapy and, second, the combination of peginterferon  $\alpha$ 2a with lamivudine (with the simultaneous regimen used) is not superior to peginterferon  $\alpha$ 2a used in monotherapy. However, longer follow-up is needed to confirm this conclusion and to assess the rate of possible late reactivations which are common after treatment in this population of patients.

#### Combination of adefovir with lamivudine

The concept of improving the efficacy by combining two analogs is based on the hypothesis that the combination would maximize the viral suppression and would decrease the occurrence of viral resistance.

One randomized study evaluated the efficacy of the combination of adefovir with lamivudine as compared to lamivudine alone or adefovir alone in 59 patients with HBeAg-positive chronic hepatitis B with lamivudine resistant HBV. There was no significant difference in median serum HBV DNA reduction ( $-3.59$  and  $-4.04$  log copies/mL), rates of ALT normalization (53% and 47%) and HBeAg loss (three patients in each group) between the adefovir–lamivudine combination group and the adefovir monotherapy group. Of note, serum HBV DNA level remained stable and there was no significant biochemical or serologic change during the study in the patients who remained under lamivudine monotherapy. Therefore, the clinical benefit of continuing lamivudine therapy once resistance develops appears to be questionable. However, it seems reasonable, at least in patients with bridging fibrosis or cirrhosis, to continue lamivudine administration after initiation of adefovir until a significant decrease of serum HBV DNA and serum ALT has been obtained. This is based on the observation that a significant number of patients will have an ALT flare after withdrawal of lamivudine which is probably due to rapid emergence of wild type HBV. In one study in which lamivudine-resistant patients received adefovir monotherapy, 37% of patients had an ALT flare soon after discontinuing lamivudine. Such flares have not been observed when patients are maintained on adefovir in combination with ongoing lamivudine maintenance.

Another study compared the efficacy of the combination of adefovir with lamivudine versus lamivudine used in mono-

therapy in 112 treatment-naïve patients (107 HBeAg-positive). There was no significant difference in median serum HBV DNA reduction ( $-5.41$  and  $-4.80$  log copies/mL), rates of undetectable HBV DNA with PCR (39% and 41%) and HBeAg loss (19% and 20%) between the adefovir–lamivudine combination group and the adefovir monotherapy group. Interestingly, there was a lower incidence of lamivudine resistance in the combination group (2%) than in the lamivudine monotherapy group (20%) ( $P < 0.003$ ).

These two studies do not answer the question of the benefit of the long-term treatment with the combination of adefovir and lamivudine as compared to adefovir monotherapy. Large randomized controlled trials with a long follow-up are needed to address this issue.

#### Future treatment strategies

Based on current experience, monotherapy with interferon or an antiviral drug is unlikely to achieve sustained responses in most patients with chronic hepatitis B. Combination therapy as in the case of treatment of HIV infection is the logical next step. However, which agents to combine is unclear. Ideally, the two agents should have synergistic, long-lasting effects on HBV clearance; no added toxicity and potential to decrease resistance. Finally, different therapies may have to be designed for different patient populations. Several practice guidelines on treatment of hepatitis B have recently been published, including those by the American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of the Liver (EASL). The former are summarized in Tables 15.3 and 15.4.

#### Liver transplantation

The early results with OLT for chronic hepatitis B were disappointing, with more than 80% of patients experiencing HBV reinfection. Most importantly, in many patients, reinfection was associated with severe and rapidly progressive liver disease. However the prognosis has been substantially improved with the use of hyperimmune B immunoglobulin and lamivudine or adefovir (see O'Grady 2000, Ch. 37).

**Association for the Study of Liver Disease (AASLD) practice guideline on treatment of chronic hepatitis B**

HBeAg	HBV DNA (copies/mL)	ALT	Strategy
+	$>10^5$	$<2 \times \text{ULN}$	Observe
+	$>10^5$	$>2 \times \text{ULN}$	IFN, lam or adefovir
–	$>10^5$	$>2 \times \text{ULN}$	IFN, or adefovir
–	$<10^5$	$<2 \times \text{ULN}$	Observe

$\times$  ULN, times upper limit of normal; IFN, interferon; lam, lamivudine; adefovir, adefovir dipivoxil; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

**Table 15.3 Association for the Study of Liver Disease (AASLD) practice guideline on treatment of chronic hepatitis B.**



**Association for the Study of Liver Disease (AASLD) practice guidelines for patients with cirrhosis due to hepatitis B**

HBeAg	HBV DNA (copies/mL)	Cirrhosis	Strategy
+/-	>10 <sup>5</sup>	Compensated	Lam or adefovir
+/-	>10 <sup>5</sup>	Decompensated	Lam or adefovir; OLT
+/-	<10 <sup>5</sup>	Compensated	Observe
+/-	<10 <sup>5</sup>	Decompensated	OLT

adefovir, adefovir dipivoxil; IFN, interferon; lam, lamivudine; OLT, orthotopic liver transplantation; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

**Table 15.4 Association for the Study of Liver Disease (AASLD) practice guidelines for patients with cirrhosis due to hepatitis B.**

### Vaccination against HBV

Safe and effective hepatitis B vaccines have been available for the prevention of HBV infection in the past 15 years.

#### Vaccine formulations

Hepatitis B vaccines that are currently available in most countries are genetically engineered and consist of recombinant HBV small S protein (HBsAg) only. Although it has been suggested that incorporation of pre-S antigens into hepatitis B vaccines may increase the immunogenicity, this hypothesis remains to be confirmed.

#### Administration and efficacy

Hepatitis B vaccine is given intramuscularly, usually in three doses at 0, 1 and 6 months. The dose recommended for adults is 10–20 µg and for children 2.5–10 µg. Immune response defined as anti-HBs titers >10 IU/L is as high as 90–95% in immunocompetent persons, but is lower in older individuals (60% above age 60 years) and in immunocompromised patients such as dialysis patients (40%) and organ transplant recipients (80%). An additional one to three doses are recommended for non-responders. Individuals who fail to respond after receiving two complete courses are unlikely to benefit from a third course. Although the anti-HBs titer decreases with time, the duration of protection is probably life-long, because most responders can mount an amnestic anti-HBs response on rechallenge. In addition, clinical infection is rarely observed during long-term follow-up of responders.

The implementation of universal vaccination of all newborns in endemic areas such as Taiwan and Senegal has been shown to dramatically reduce not only the carrier rate among children but also the incidence of childhood HCC.

#### Indications

Vaccination is most important for infants, particularly newborns of HBsAg carrier mothers, because of the high risk of progression to chronic infection after perinatal infection. Universal vaccination of all newborns with incorporation of hepatitis B vaccines into the Expanded Program for Immunization in Children is now recommended in most countries. In infants born to

carrier mothers, an additional dose of HBIG is administered at birth to provide immediate protection. Catch-up vaccination is also recommended for all children and adolescents who have not been previously immunized.

Adults at increased risk of HBV infection such as healthcare workers, spouses of hepatitis B carriers, patients with chronic liver disease, dialysis patients, male homosexuals and intravenous drug abusers should also be vaccinated.

Postexposure prophylaxis should consist of the administration of a single dose of HBIG and the simultaneous initiation of a course of vaccination.

#### Adverse reactions

Adverse reactions to hepatitis B vaccine are uncommon. Approximately 20% of vaccinees may experience mild reactions at the injection site and a minority may have transient flu-like symptoms. Particular concern has been raised by the observation of cases of multiple sclerosis occurring after vaccination. However several large studies did not show a significant difference in the rates of incidence of multiple sclerosis in vaccinated subjects as compared with comparable control populations.

#### Vaccine-escape mutant

Mutations in the HBV S gene have been described in infants born to carrier mothers despite adequate anti-HBs response after vaccination. The commonest mutation is a glycine to arginine substitution at codon 145 (G145R) in the 'a' determinant of HBsAg. This mutation has been shown to decrease the binding of HBsAg to anti-HBs, accounting for the breakthrough infection. The exact incidence of this mutant among vaccinees is not clear but is likely to be <5%. There is no evidence to suggest that the efficacy of current vaccines is diminishing. In addition, there is no evidence to suggest that these mutants are transmitted to other family members or the community.

## HEPATITIS D

### Introduction

Hepatitis D is caused by a defective virus: the hepatitis D virus (HDV). Infection with HDV is closely associated with HBV infection. Although HDV can replicate autonomously, the simultaneous presence of HBV is required for complete virion assembly and secretion. As a result, individuals with hepatitis D are always dually infected with HDV and HBV.

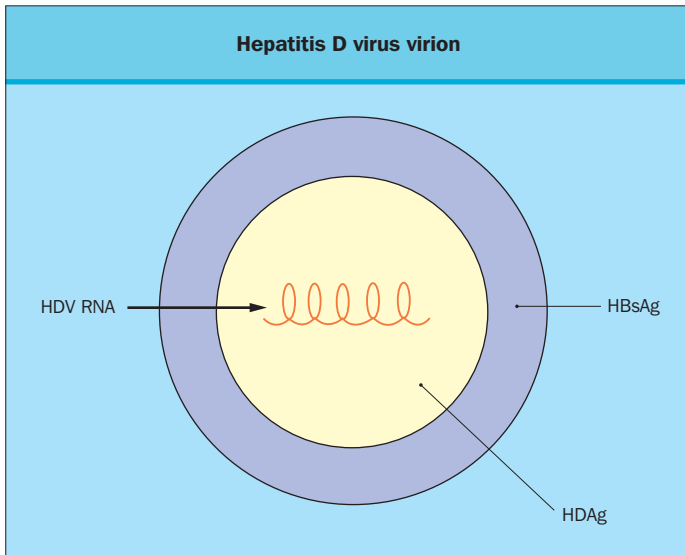
The hepatitis D virion or δ agent comprises a single stranded RNA genome, the hepatitis D antigen (HDAg), and an envelope consisting of HBV envelope proteins (Fig. 15.17). Hepatitis D virus replicates via its complementary or antigenomic RNA.

### Epidemiology

It is estimated that approximately 5% of the HBV carriers worldwide may be infected with HDV, leading to a total of 15 million persons infected with HDV worldwide. However, the geographic distribution of HDV infection does not parallel that of HBV.

#### The Mediterranean Basin

Infection with HDV is endemic in these countries. Infection tends to occur early, affecting mainly children and young adults.



**Figure 15.16** Hepatitis D virion. HDvAg, hepatitis D antigen.

The main route of transmission is inapparent permucosal or percutaneous spread. Intrafamilial transmission has also been reported.

#### **The Far East**

Despite the high prevalence of HBV infection, the prevalence of HDV infection in these countries is, in general, low. Transmission occurs sexually or amongst intravenous drug users.

#### **Western countries**

Infection with HDV is uncommon and predominantly confined to intravenous drug users.

#### **Changes in epidemiology**

Evidence accrued in the last decade, however, is suggestive of a changing trend in the epidemiology of HDV. A decline in HDV prevalence in both acute and chronic hepatitis has been observed in the Mediterranean area, most likely due to universal HBV vaccination, measures to control HIV and socioeconomic improvements, whereas new foci of HDV infection are emerging in other parts of the world. South America, especially the subtropical area, remains an important potential reservoir for new outbreaks of HDV infection.

#### **Natural history**

The natural history of chronic HDV infection is characterized by a wide spectrum of clinical expressions. Since the earliest studies, HDV turned out to be a highly pathogenic virus causing a severe and rapidly progressive disease, with very infrequent spontaneous resolutions. Cirrhosis was shown to develop in up to 70% of the cases. Although HDV cirrhosis, once established, may be a stable disease for many years, co-infection with HDV was shown to significantly increase the risk of HCC and death in patients with compensated HBV cirrhosis. The link between HDV and severe and progressive liver disease has been found at all ages, as suggested by the detection of markers of HDV infection in up to 40% of children with HBsAg-positive cirrhosis.

Most patients with chronic HDV infection are HBeAg-negative. Low levels of HBV DNA replication occur due to the inhibition of HBV replication by HDV.

#### **Clinical features**

Due to its dependence on HBV, HDV infection always occurs in association with HBV infection. The clinical manifestations of HDV infection vary from benign acute hepatitis to fulminant hepatitis (Fig. 15.16), and from an asymptomatic carrier state to rapidly progressive chronic liver disease. In most cases of chronic HDV infection, HBV replication is suppressed to very low levels by HDV. Liver damage in these patients is essentially due to HDV only.

#### **Co-infection**

Co-infection of HBV and HDV in an individual susceptible to HBV infection results in an acute hepatitis. The clinical picture is indistinguishable from that of classic acute hepatitis B and is usually transient and self-limited (Fig. 15.16). However, a high incidence of acute liver failure has been reported among drug addicts. The rate of progression to chronic HDV infection is no different from that observed after classic acute hepatitis B because the persistence of HDV infection is dependent on the persistence of HBV infection.

#### **Hepatitis D virus superinfection**

Hepatitis D virus superinfection of an HBsAg carrier may present as unusually severe acute hepatitis in a previously unrecognized HBV carrier or an exacerbation of pre-existing chronic hepatitis B. Progression to chronic HDV infection is almost invariable.

#### **Latent infection**

This was initially described in the liver transplantation setting. In this situation, the allograft is reinfected with HDV but not HBV. The hepatitis D antigen (HDvAg) can be detected in the liver, but HDV RNA cannot be detected in the serum. During this phase, there is usually no evidence of liver disease. Infection with HDV is abortive unless the graft is later reinfected with HBV.

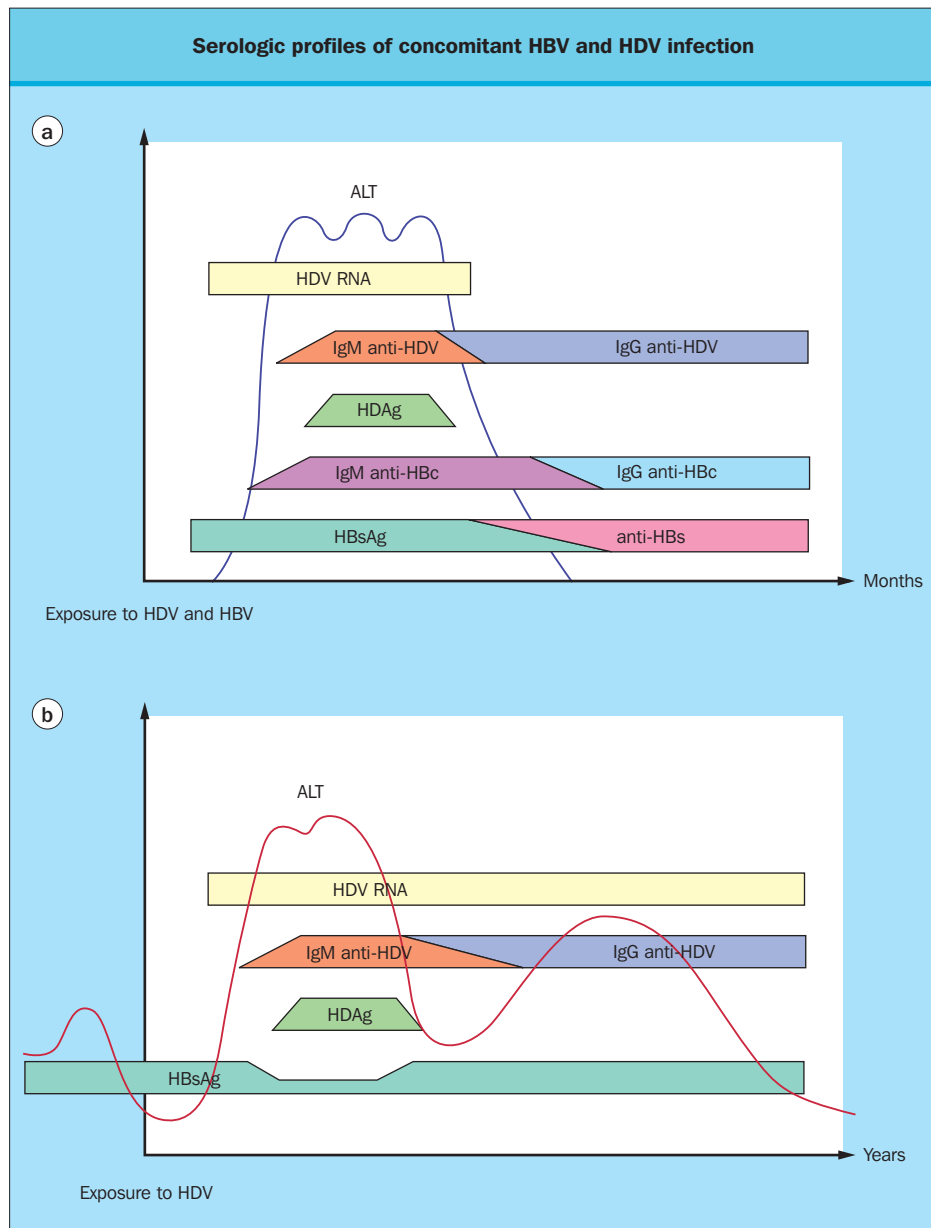
#### **Diagnosis**

The serum biochemical profiles associated with HDV and HBV infections are given in Figure 15.18.

#### **Hepatitis D virus antibody assays**

Total (IgM and IgG) anti-HDV can be detected by enzyme-linked or radioimmune assays. These are the only commercially available tests for HDV infection in many countries. Anti-HDV-IgG appears late in acute hepatitis D. Thus, its clinical value is limited unless repeated testing is performed. Nonetheless, a well-documented anti-HDV seroconversion may be the only way to diagnose acute HDV infection. High-titer IgG anti-HDV is present in chronic HDV infection. It correlates well with ongoing HDV replication.

IgM anti-HDV is transient and delayed if the course of acute hepatitis D is self-limited. In patients who progress to chronic HDV infection, IgM anti-HDV is long-lasting and present in high titer. The IgM anti-HDV titer tends to correlate with the level of HDV replication and severity of liver disease.



**Figure 15.17 Acute hepatitis B virus (HBV) and hepatitis D virus (HDV) co-infection and HDV superinfection.** Serologic profile of (a) acute HBV and HDV co-infection, and (b) HDV superinfection on chronic HBV infection. HDAg, hepatitis D antigen.

#### Detection of serum hepatitis D antigen

In acute HDV infection, serum hepatitis D antigen appears early but is very short-lived and may escape detection if repeated testing is not performed. In chronic HDV infection, hepatitis D antigen is usually undetectable because of the formation of immune complexes with anti-HDV.

#### Detection of serum hepatitis D virus RNA

Serum HDV RNA is an early and sensitive marker of HDV infection in acute hepatitis D. In chronic HDV infection, only 70–80% of patients have detectable serum HDV RNA when tested by hybridization assays but most are positive by PCR assays.

staining of liver tissues. The detection of intrahepatic HDAg has been proposed to be the 'gold' standard for the diagnosis of ongoing HDV infection. Hepatic HDV RNA can be detected by in situ hybridization.

#### Detection of serum HBV markers

Due to the dependence of HDV on HBV, the presence of HBsAg is necessary for the diagnosis of HDV infection. Documentation of the presence of HDV infection relies largely on the detection of anti-HDV. In patients with acute HBV/HDV co-infection, IgM anti-HBc is also present. Tests for serum HDAg and/or HDV RNA should also be performed if available. In patients with HDV superinfection, anti-HDV is usually present in high titers along with serum HDV RNA, while markers of HBV replication may be suppressed and IgM anti-HBc absent.

#### Tissue markers of HDV infection

The antigen HDAg can be detected by immunohistochemical



## Treatment

### Aims

The main goals are to eradicate both HBV and HDV and to ameliorate liver disease. The primary end point is sustained suppression of HDV replication as documented by the undetectability of HDV RNA in serum using PCR assays which is associated with biochemical response and histologic improvement.

### Interferon

Interferon  $\alpha$  is at present the only effective and approved treatment of chronic hepatitis D. Nucleoside and nucleotide analogs active against HBV are not active against HDV. Based on the limited data reported, interferon needs to be used in high doses up to 9 MU thrice weekly for up to 1 year for optimal results. The overall efficacy is uncertain because of conflicting data from two large controlled studies. In the largest multicenter trial, 61 Italian patients with chronic hepatitis D were randomized to receive interferon  $\alpha$  in doses of 5 MU/m<sup>2</sup> thrice weekly for 4 months, followed by 3 MU/m<sup>2</sup> thrice weekly for another 8 months, or placebo. Treatment did not increase the rate of sustained virus clearance or histologic improvement.

A more favorable response was achieved in another Italian study in which 50% of patients who received 9 MU doses of interferon  $\alpha$  for 48 weeks had biochemical and virologic response as well as improvement in liver histology. A long-term follow-up study of patients who participated in this trial showed a benefit in terms of decreased rate of cirrhosis, complications and HCC.

Preliminary results suggest that peginterferon may be at least as effective as conventional interferon in the treatment of chronic hepatitis D. Randomized controlled trials are in progress.

OLT is the only valid therapeutic option for end-stage HDV-related liver disease. The risk of HDV reinfection of the graft has been found to be lower than that of HBV reinfection and can be prevented by the continuous administration of anti-HBs immunoglobulin.

### Prevention

The mainstay of prevention of HDV infection is vaccination against its helper virus, HBV. To date no effective vaccine specific for HDV has been developed, which would represent the only means to eliminate the risk of HDV superinfection for chronic carriers of HBsAg.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 16

## Hepatitis C

Adrian M. Di Bisceglie

### INTRODUCTION

Chronic infection with the hepatitis C virus (HCV) is relatively frequent and widespread through the world. Chronic hepatitis C has become recognized as a significant public health problem both in developed Western countries and around the world. For example, in the USA it is the single biggest indication for liver transplantation and accounts for more than 10 000 deaths each year. There is also growing public awareness of hepatitis C and its complications.

This growing awareness of hepatitis C has led to greater efforts by public health authorities to develop effective strategies to control HCV infection and its complications. In 2002, a National Institutes of Health Consensus Development Conference was held on the management of hepatitis C in Bethesda, Maryland. The proceedings of that meeting were summarized in a Consensus Statement which forms a valuable resource in the management of hepatitis C.

### BACKGROUND

Although it had been recognized for several years that certain forms of hepatitis could be transmitted parenterally, it was only after the discovery of the viral agents responsible for hepatitis B and hepatitis A and serologic tests for these agents that it became apparent some cases of blood-borne hepatitis were due to another agent (referred to as non-A, non-B hepatitis). Many characteristics of the clinical features and natural history of non-A, non-B hepatitis were elucidated by studying cases of post-transfusion hepatitis by monitoring serum aminotransferase activities and liver histology.

The agent responsible for parenteral non-A, non-B hepatitis was discovered using the techniques of molecular biology and cloning by Houghton and co-workers at Chiron Corporation who cloned a fragment of the hepatitis C virus in an expression vector. The cloned fragment was then detected by reaction with antibodies present in human serum from individuals infected with HCV. This landmark discovery led to the development of a diagnostic assay for antibodies to HCV and was followed by full-length cloning of the virus. Polymerase chain reaction (PCR)-based assays to detect the viral genome allowed further clarification of the natural history and epidemiology of hepatitis C.

Even before the discovery of HCV several investigators had tested interferons as therapy for chronic non-A, non-B hepatitis. These early studies indicated that interferon therapy was associated with suppression of serum aminotransferase activities, albeit often transient. Considerable progress has since been made in developing effective therapies against hepatitis C.

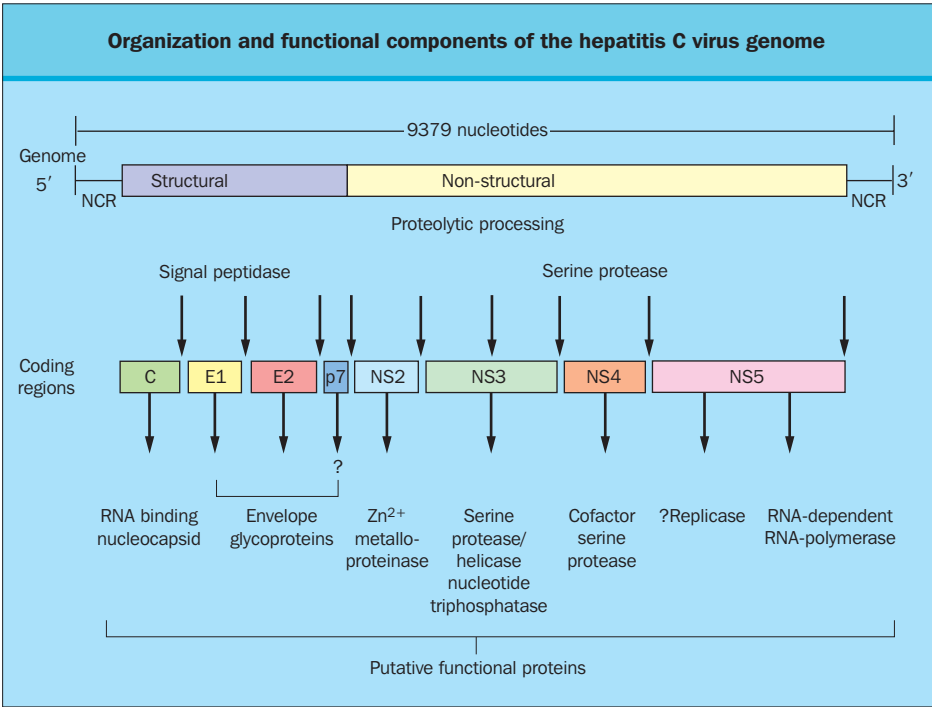
### VIROLOGY OF HCV

HCV is a single-stranded RNA virus belonging to the family Flaviviridae. Its genome is approximately 10 000 nucleotides in length and consists of a single open reading frame (ORF) with non-coding RNA regions at its 5' and 3' ends (Fig. 16.1). The single ORF codes for one large protein which is then cleaved into a series of smaller proteins by viral and host proteases. These smaller proteins constitute the structure of the virus (structural proteins: core, E1 and E2) or form part of the replicative machinery of the virus (the non-structural proteins: NS2, NS3, NS4 and NS5). Both the 5' and 3' non-coding regions of the viral genome appear to be critical to its function. The 5' non-coding region serves as an internal ribosomal entry site (IRES) which binds to cellular ribosomes and facilitates the production of viral proteins. Although the exact function of the 3' end is uncertain, HCV cannot replicate effectively in its absence.

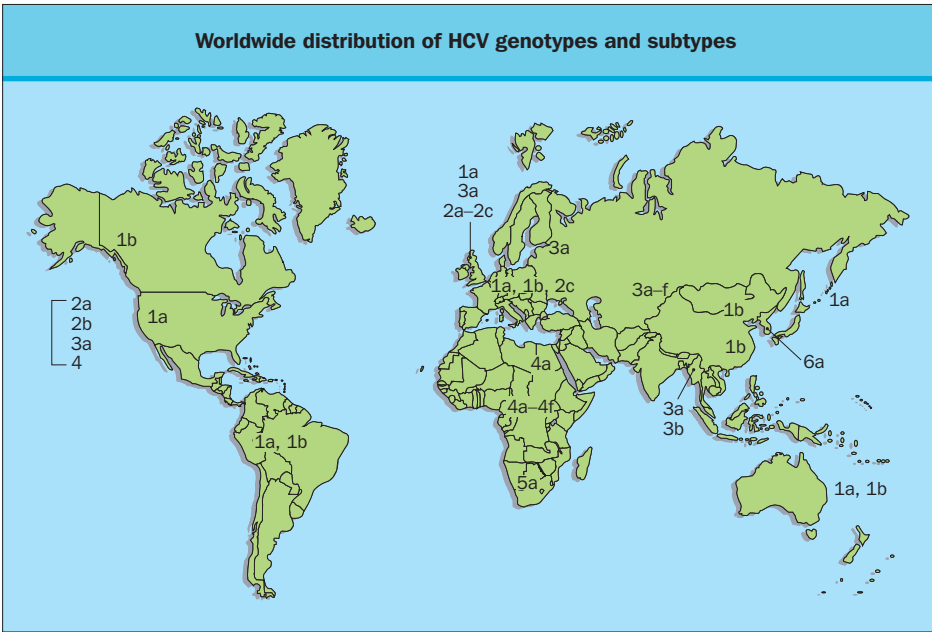
The RNA genome of HCV replicates through an RNA-dependent RNA polymerase (RdRp) coded for by the NS5B gene of HCV. The RNA genome of HCV is very prone to mutation resulting in considerable variability in its RNA sequence. Based on differences in RNA sequence, six major genotypes of HCV have been identified with multiple subtypes. Isolates from individual patients also contain a quasispecies 'swarm' of closely related but distinct viral sequences. HCV genotypes have been of interest from an epidemiologic point of view as they have allowed study of HCV in various populations. Some genotypes are confined to or found largely in certain geographic areas – for example, HCV genotype 5 in southern Africa, genotype 4 in Egypt and the Middle East and genotype 6 in south-east Asia (Fig. 16.2). Genotypes 1, 2 and 3 are found in developed Western countries, Japan and Australia in various proportions. Interestingly, HCV genotype also seems to affect the biologic behavior of the virus with regard to response to antiviral therapy – those patients infected with HCV genotypes 2 or 3 respond much better to interferon-based therapies than those with HCV genotype 1.

HCV is a largely hepatotropic virus that replicates only in humans and certain non-human primates such as chimpanzees and tamarins. These features have made it difficult to grow reliably in cell culture, thus hampering research into its replication and the development of new therapeutic agents. HCV has been reported to grow at low levels in certain lymphocytoid and hepatic cell lines. More recently, researchers have relied on a replicon system which utilizes an artificial construct of non-structural chimeric HCV genes in a cell line derived from hepatocellular carcinoma (HCC). Relatively high levels of HCV replication have been observed in the replicon system and it seems a suitable model for studying the effects of antiviral drugs. Nonetheless this system is limited because only certain





**Figure 16.1** Organization and functional components of the hepatitis C virus genome (NCR, non-coding region).

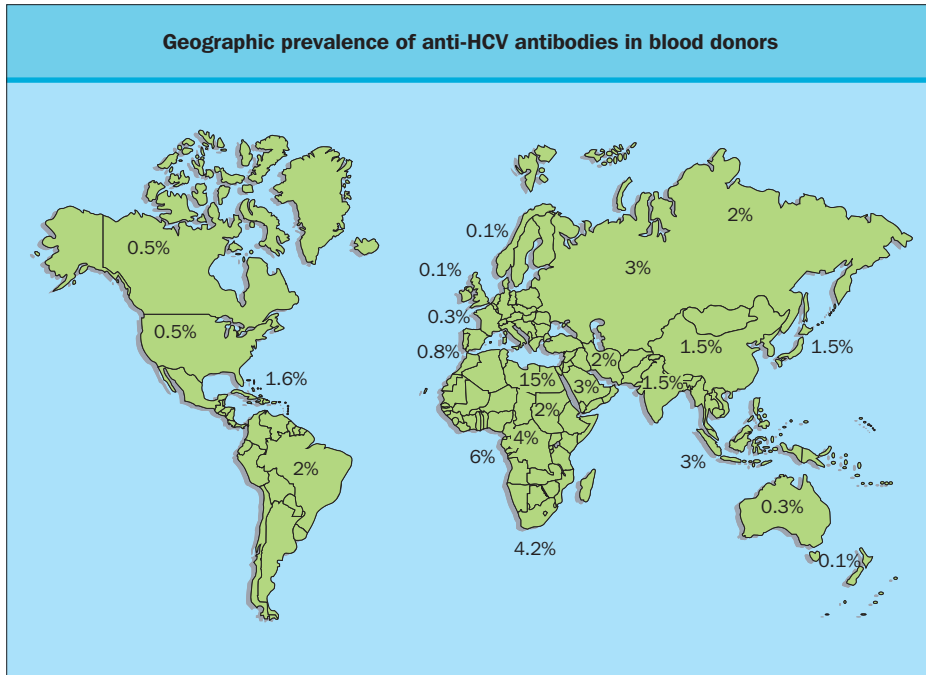


**Figure 16.2** Worldwide distribution of hepatitis C virus genotypes and subtypes.

genotypes of HCV replicate effectively and the structural proteins of HCV are not produced.

A reliable small animal model for HCV propagation has only recently been developed. The chimpanzee has been the best animal model for HCV infection and disease. However, as the use of chimpanzees is extremely expensive, it is not at all practical for most studies of HCV replication to be performed in chimps. Recently HCV replication in transgenic mice chimeric for human livers was described. This system uses SCID/beige mice

crossed with mice carrying a plasminogen activator transgene, urokinase plasminogen activator, under the control of the albumin promoter (Alb-uPA). The transgene leads to accelerated mouse hepatocyte death conferring a replicative advantage for transplanted hepatocytes that lack the uPA transgene. In this application the transplanted hepatocytes could be derived from either humans or chimpanzees. Back-crosses are required to generate mice homozygous for both the SCID trait and the Alb-uPA. Mercer et al (01). demonstrated infection of transplanted



**Figure 16.3 Geographic prevalence of antibodies to hepatitis C virus antibodies in blood donors.**

mice with HCV in the doubly homozygous animals and transient replication of HCV in the hemizygous animals using human hepatocytes.

## **PATHOGENETIC MECHANISMS**

HCV is thought to be a non-cytopathic virus and liver damage is probably immune mediated. The large majority of infected individuals exposed to HCV become chronically infected although the mechanisms underlying this high rate of chronicity are not known. Neutralizing antibodies to HCV can be detected in serum and T-helper (CD4) lymphocytes responsive to both structural and non-structural HCV proteins can often be detected in patients with chronic HCV infection. Cytotoxic T lymphocytes (CTLs) are thought to be particularly important in viral pathogenesis as they recognize viral antigens on cell surfaces in conjunction with major histocompatibility complex (MHC) class I proteins and lyse infected target cells.

Progression of liver disease due to HCV is marked by progressive increases in hepatic fibrosis associated with activation of hepatic stellate cells, presumably through cytokine mediators produced as part of the immune response to HCV.

## **EPIDEMIOLOGY**

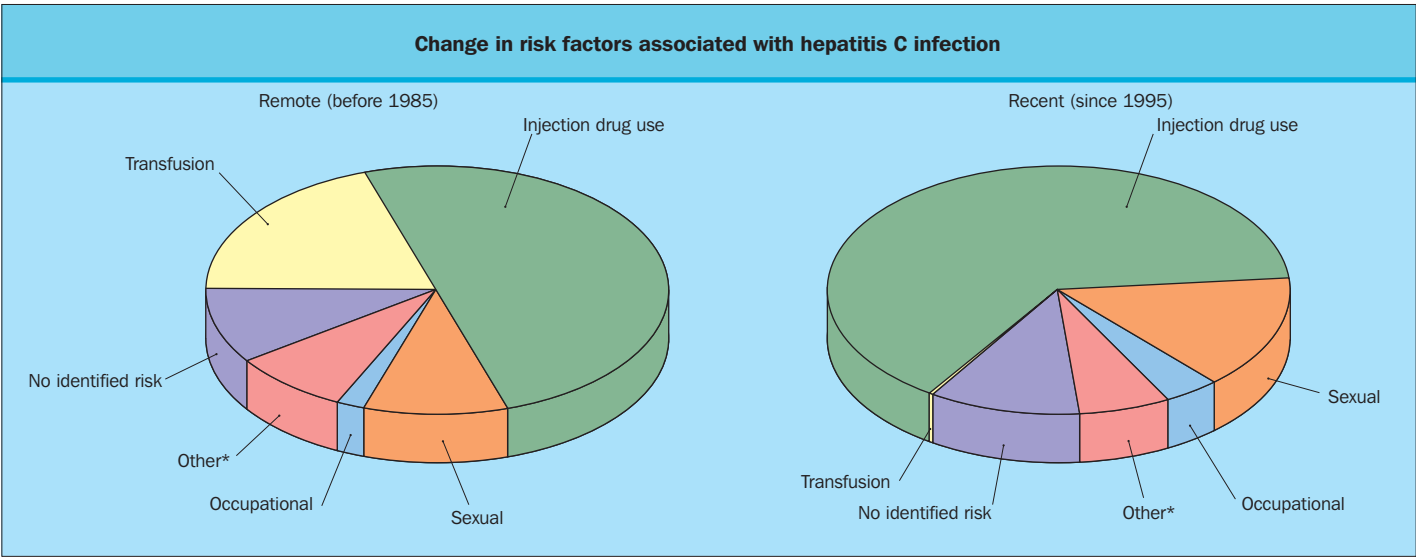
HCV is transmitted by parenteral contact with blood or blood products. Recognized routes of infection include transfusion of blood or blood products, injection drug use, needlestick or other forms of contaminated injury among healthcare workers, maternal–infant transmission and sexual spread. In most developed countries post-transfusion HCV has been virtually eliminated by screening of donated blood, but chronic infection remains prevalent as demonstrated in studies of volunteer blood donors (Fig. 16.3). Injection drug use appears to be the most common remaining risk factor for HCV infection (Fig. 16.4).

Maternal–infant transmission may occur if a mother is seropositive for HCV RNA. This occurs in approximately 5% of infants, although the risk appears to be increased by the presence of co-infection with the human immunodeficiency virus (HIV) in the mother, presumably because the serum levels of HCV RNA are higher in mothers with HIV co-infection.

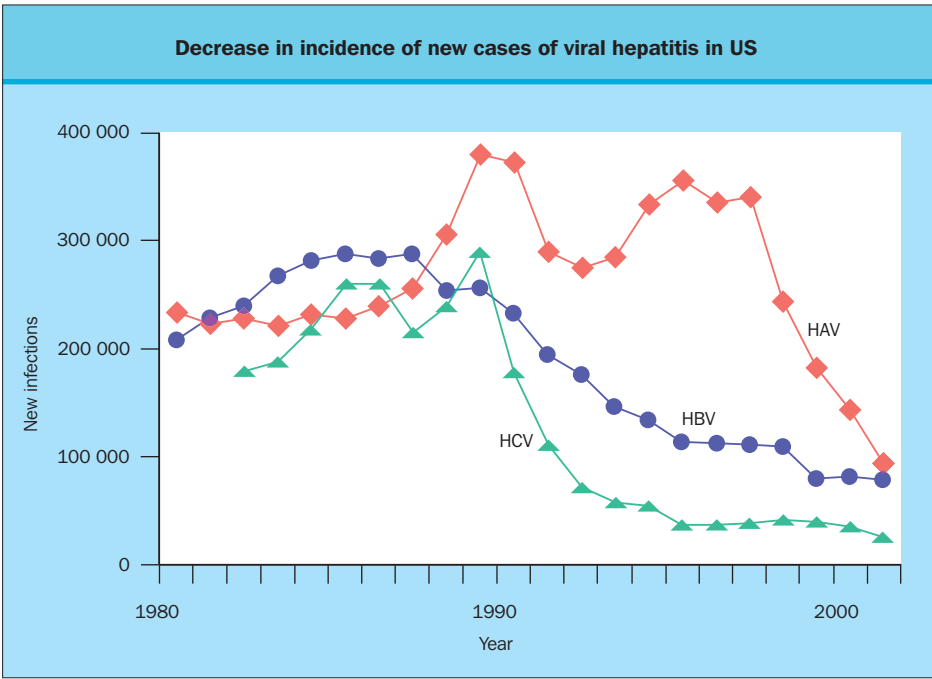
Although occupational exposure to HCV may occur, the risk of acquiring HCV through needlestick injury appears very slight. Sexual transmission of HCV has been well documented to occur but the exact frequency of this occurrence is debated. Studies from the Centers for Disease Control and Prevention (CDC) have shown an increased risk of HCV infection in those individuals with multiple sexual partners or partners known to have HCV infection. However, studies of stable monogamous couples have shown very little evidence of sexual spread of HCV. Furthermore, the risk of HCV infection among men having sex with men is only about 5%, lower than what might be expected if HCV was readily spread by sexual contact.

Recent reports from the CDC have shown a dramatic decrease in the number of new cases of HCV infection in the USA from nearly 300 000 in the early 1990s to only 25 000 in 2001 (Fig. 16.5). Most of this decrease seems to have occurred among injection drug users and can be attributed to various public health measures.

Although the exact incidence and prevalence of HCV infection are not known in the USA accurate estimates of these figures have been possible by the use of sampling methods. Information on HCV incidence is derived from the Sentinel Counties Study while information on the prevalence of HCV is derived from the National Health and Nutrition Evaluation Survey (N-HANES) which is based on a random sample of the adult, non-institutionalized population of the USA. Based on these studies, it is estimated that 1.8% of the population is seropositive for anti-HCV, of whom about 75% have HCV RNA detectable in serum. Thus there are approximately 2.7 million



**Figure 16.4** Change in risk factors associated with hepatitis C viral infection. Modified from CDC ([www.cdc.gov](http://www.cdc.gov)).



**Figure 16.5** Decrease in annual incidence of new cases of viral hepatitis in the USA. Modified from CDC ([www.cdc.gov](http://www.cdc.gov)).

HCV-infected individuals in the USA, a figure that is perhaps underestimated because individuals in institutions, such as prisons and nursing homes, were not sampled.

Given this frequency of HCV in the USA, the impact of this condition is not surprising. In addition to 8000–10 000 deaths each year attributed to HCV, it leads to more than one-third of liver transplants and is associated with expenditures of more than \$1 billion annually.

### DIAGNOSIS OF HEPATITIS C

The diagnosis of hepatitis C is relatively simple and is based on detection of anti-HCV through enzyme-linked immunoassays (EIA) that are both sensitive and specific. The EIA now in use represents a third generation of this assay and consists of recombinant viral protein cores, NS3 and NS5 regions of the viral genome in a solid phase assay. The presence of anti-HCV



<b>Centers for Disease Control and Prevention (CDC) recommendations for HCV testing</b>
<i>Persons who should be tested routinely for HCV infection based on their risk of infection</i>
Persons who ever injected illegal drugs
Persons with selected medical conditions, including:
• Persons who received clotting factor concentrates before 1987
• Persons who were ever on chronic hemodialysis
• Persons with persistently abnormal alanine aminotransferase levels
• Prior recipients of transfusions or organ transplants
Persons notified that they received blood from a donor who later tested positive for HCV
Persons who received a transfusion of blood or blood components before July 1992
Persons who received an organ transplant before 1992
<i>Persons who should be tested routinely for HCV infection based on a recognized exposure</i>
Healthcare, emergency medical and public safety workers after exposure to HCV-positive blood
Children born to HCV-positive women

**Table 16.1 Centers for Disease Control and Prevention (CDC) recommendations for HCV testing.**

can be confirmed using the recombinant immunoblot assay (RIBA), another immunoassay using a different, Western blot-like format. Anti-HCV may persist for years after HCV infection and clearance.

Testing for anti-HCV should be routinely offered to all persons identified as being at risk (Table 16.1).

Individuals who are currently infected with HCV have HCV RNA detectable in their serum. The levels of HCV RNA typically found with human infection are not easily detected using standard hybridization assays and require amplification of either the target (by PCR) or signal. As few as 50 copies of viral RNA per ml of serum can be detected with some modern assays.

The amount of HCV RNA in the circulation is readily quantifiable and is on average 2 to 3 million copies per ml. The level of HCV RNA may however vary considerably from patient to patient but is generally consistent over time in individual patients. The level of HCV RNA in serum does not appear to correlate with the severity of liver disease assessed either by serum aminotransferase activities or liver histopathology. Finally, the serum level of HCV RNA appears to be a good predictor of response to antiviral therapy. Assays to determine HCV genotype are now widely available although they lack standardization. Commonly used assays are based on amplification and sequencing or a line probe hybridization assay.

The duration of infection with HCV is difficult to estimate (Table 16.2). Patient histories are often unreliable and serologic tests do not accurately distinguish acute from chronic HCV infection. Jaundice is uncommon with acute hepatitis C. The

<b>Diagnosis of hepatitis C virus (HCV)</b>				
	<i>Anti-HCV (EIA)</i>	<i>Anti-HCV (RIBA)</i>	<i>HCV RNA</i>	<i>ALT</i>
Acute or chronic hepatitis C	Positive	Positive	Positive	Elevated or normal
Recovered HCV	Positive	Positive	Negative	Normal
False-positive anti-HCV	Positive	Negative	Negative	Normal
ALT, alanine aminotransferase; EIA, enzyme-linked immunoassays; RIBA, recombinant immunoblot assay.				

**Table 16.2 Diagnosis of hepatitis C virus (HCV).**

appearance of anti-HCV may be delayed a few weeks after HCV RNA following acute infection. A liver biopsy would show features of acute rather than chronic hepatitis but is not routinely recommended. The diagnosis of acute hepatitis C cannot be made with certainty unless the patient was known to be HCV negative previously and then developed HCV RNA in serum with or without clinical features of acute hepatitis.

## LIVER HISTOPATHOLOGY

Liver biopsy plays a major role in the management of chronic hepatitis C. The histologic features of chronic hepatitis C commonly include an infiltrate of chronic inflammatory cells within portal areas, interface hepatitis, lobular hepatocellular injury and hepatic steatosis. Less commonly, the portal infiltrates take on the form of lymphoid aggregates or sometimes even lymphoid follicles. Progression of chronic hepatitis C is marked by progressive increases in hepatic fibrosis, consisting largely of collagen. Hepatic fibrosis due to HCV infection begins within the portal triads but may form bridges between portal areas or with terminal hepatic venules (bridging fibrosis). Cirrhosis represents the most advanced form of hepatic fibrosis.

Several systems for scoring the severity of chronic hepatitis C have been developed. They have in common assigning a grade to the degree of necrosis and inflammation and a stage to the degree of fibrosis. These scoring systems have proved to be very useful in clinical trials as a means of measuring improvement in liver histopathology as a result of antiviral therapy, but are also valuable in clinical practice as a means of assessing and following the severity and possible prognosis of liver disease. Commonly used systems include those described by Knodell and colleagues, Scheuer, Ishak and a French collaborative group (METAVIR) (Table 16.3) Brunt (01)).

## CLINICAL FEATURES

The clinical features of acute and chronic hepatitis C are not specific for this form of hepatitis. Many patients with either acute or chronic hepatitis C are asymptomatic. Acute hepatitis C may sometimes result in jaundice, nausea, vomiting and general malaise. Fulminant hepatic failure is an extremely rare complication of acute HCV infection. The symptoms of chronic hepatitis C are non-specific and include fatigue and right upper

The Scheuer scoring systems for liver histopathology in chronic hepatitis		
Grade	Portal/periportal activity	Lobular activity
0	None/minimal	None
1	Portal inflammation	Inflammation but no necrosis
2	Mild piecemeal necrosis	Focal necrosis
3	Moderate piecemeal necrosis	Severe focal cell damage
4	Severe piecemeal necrosis	Damage includes bridging necrosis
Stage	Fibrosis	
0	None	
1	Enlarged, fibrotic portal tracts	
2	Periportal or portal–portal septa but intact architecture	
3	Fibrosis with architectural distortion but no obvious cirrhosis	
4	Probable or definite cirrhosis	

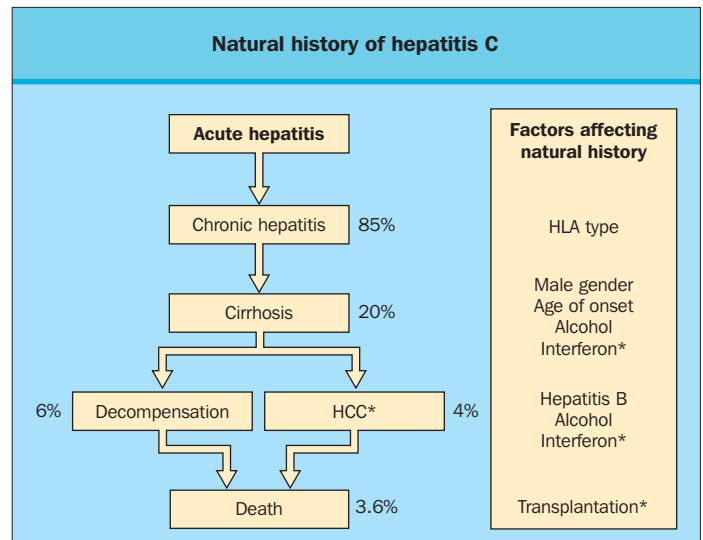
**Table 16.3 The Scheuer scoring system for liver histopathology in chronic hepatitis.** This is a simple scoring system that can be readily adapted to clinical practice.

quadrant pain. Pruritus may sometimes be a presenting symptom for hepatitis C, probably related to cholestatic liver injury. Patients may sometimes present with features of extrahepatic manifestations of chronic hepatitis C such as porphyria cutanea tarda or cryoglobulinemia (see below) or advanced liver disease with cirrhosis and liver failure. Asymptomatic patients with chronic hepatitis C may be detected at the time of routine health evaluations, insurance physicals or at the time of blood donation.

## NATURAL HISTORY AND PROGNOSIS

The natural history of HCV infection is quite variable but is notable for a strong propensity to become chronic. Thus, as many as 85% of those individuals infected with HCV develop persistent infection marked by the ongoing presence of HCV RNA in serum for more than 6 months after the onset, although the rate of chronicity appears to be lower among younger individuals (Fig. 16.6). Among those with chronic infection, there is also considerable variability with regard to rates of progression of hepatic fibrosis. Some patients appear to have very rapidly progressive liver disease and may develop cirrhosis in less than 5 years. On the other hand there are others who do not have significant fibrosis even after 20 years or more of infection.

Some of the factors thought to contribute to severity of chronic hepatitis C include older age at infection, male gender and alcohol consumption. Once cirrhosis has developed, further progression may result in hepatic decompensation or the development of HCC.



**Figure 16.6 Natural history of hepatitis C.** Natural history and factors affecting its outcome. Reproduced from Di Bisceglie (2000) by permission of Wiley-Liss, Inc. \*, these factors improve outcome while others worsen it.

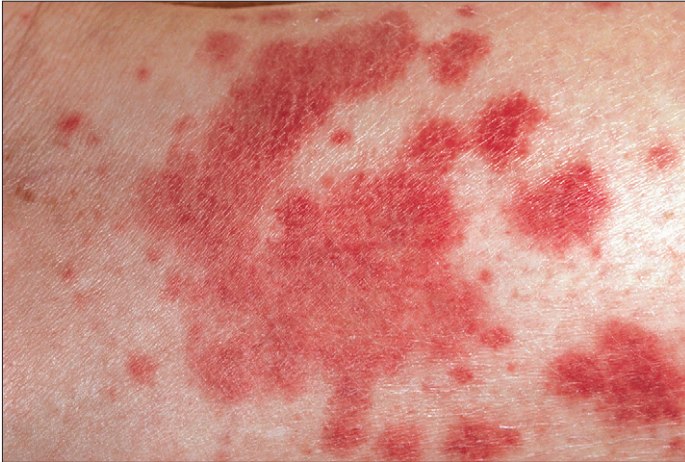
Confirmed extrahepatic manifestations of hepatitis C
Cryoglobulinemia
Membranoproliferative glomerulonephritis (usually with cryoglobulinemia)
Porphyria cutanea tarda
Lichen planus
Sialadenitis (with or without Sjogren syndrome)

**Table 16.4 Confirmed extrahepatic manifestations of hepatitis C.**

## COMPLICATIONS AND EXTRAHEPATIC MANIFESTATIONS

Hepatic decompensation in cirrhosis due to hepatitis C is very similar to liver failure associated with other forms of chronic liver disease. Features of decompensation such as ascites, jaundice and bleeding from esophageal varices occur in about 30% of patients with cirrhosis over a period of 10 years. Once features of hepatic decompensation have occurred, the mortality rate from liver disease increases to approximately 10% per year. For this reason, hepatic decompensation due to hepatitis C represents an indication for liver transplantation.

Some of the well known extrahepatic manifestations of chronic hepatitis C are listed in Table 16.4. Cryoglobulinemia is defined by the presence of cryoglobulins in serum. These are abnormal immunoglobulins that characteristically precipitate out in the cold and can redissolve when warmed. Low levels of cryoglobulins can be detected in as many as 40–50% of patients with chronic hepatitis C but are associated with symptoms only rarely, presumably when high levels are present. Symptoms of cryoglobulinemia include joint aches and skin rashes. The presence of palpable purpura on the lower legs is a characteristic



**Figure 16.7** Palpable purpura on leg of a patient with cryoglobulinemia. Reproduced from Shakil and Di Bisceglie by permission of the Massachusetts Medical Society.



**Figure 16.8** Lesions of porphyria cutanea tarda in a patient with chronic hepatitis C.

feature of cryoglobulinemia (Fig. 16.7). In as many as 70% of cases, cryoglobulinemia is associated with HCV infection, in the form of type II or 'mixed' cryoglobulinemia. This may sometimes be associated with membranoproliferative glomerulonephritis (MPGN) which may result in proteinuria (sometimes severe enough to cause the nephrotic syndrome), hypertension and renal insufficiency. Other complications of cryoglobulinemia include peripheral neuropathy and B-lymphocyte malignancies (rarely). Cryoglobulinemia may be diagnosed by the presence of the typical clinical features with cryoglobulins in serum. The assay for cryoglobulins in serum is not entirely reliable as it depends on the correct handling of blood specimens. The presence of high titers of rheumatoid factor may also indicate the presence of cryoglobulinemia.

Porphyria cutanea tarda (PCT) is a form of porphyria associated with deficiency of the enzyme uroporphyrinogen decarboxylase. The acquired form is by far the most common and is usually associated with some form of liver disease. In as many as 70% of cases, the underlying liver disease is caused by chronic HCV infection. The clinical features of PCT include photosensitivity with blistering lesions on the hands and facial hirsutism (Fig. 16.8). Liver biopsy often shows hepatic fibrosis and iron overload in addition to the usual features of chronic hepatitis C.

Many other conditions have been linked with chronic HCV infection, although a causal relationship may not have been definitively established. Conditions probably associated with hepatitis C include sialadenitis (sometimes with Sjogren syndrome), lichen planus (Fig. 16.9) and glomerulonephritis not associated with cryoglobulinemia.

## TREATMENT OF HEPATITIS C

### General principles of therapy

#### Goals

The goals of therapy for chronic hepatitis C include prevention of progressive liver disease, control of extrahepatic manifestations (where present) and decreasing the infectivity of patients with HCV infection. These end points are difficult to measure and may take many years to evaluate, given the prolonged natural history of this condition. Efforts at therapy have therefore



**Figure 16.9** Characteristic lacy-white appearance of lichen planus in the buccal cavity in a patient with chronic hepatitis C.

focused on elimination of HCV infection which is almost always associated with improvement in the severity of liver disease as assessed by liver biopsy. An algorithm for treatment of chronic hepatitis C is shown in Figure 16.10.

### Patterns of response to therapy

There is considerable variability in the virologic response of patients with chronic hepatitis C to antiviral therapy. These patterns are illustrated in Figure 16.11. Recently, the NIH Consensus Development Panel affirmed that a sustained virologic response (SVR) is the optimal outcome of therapy. SVR is defined as undetectable HCV RNA in serum at the end of therapy and for at least 6 months thereafter. Preliminary studies have shown that once patients achieve an SVR, almost all are 'cured' of their HCV infection as late relapse occurs in fewer than 2 or 3% of patients. SVR is associated with significant improvement on liver biopsy. There is some evidence that SVR also appears to decrease the subsequent risk of HCC.

Other possible responses include loss of detectable HCV RNA from serum during therapy followed by its reappearance when treatment is stopped (relapse). The term non-responder is used for those patients in whom HCV RNA may or may not



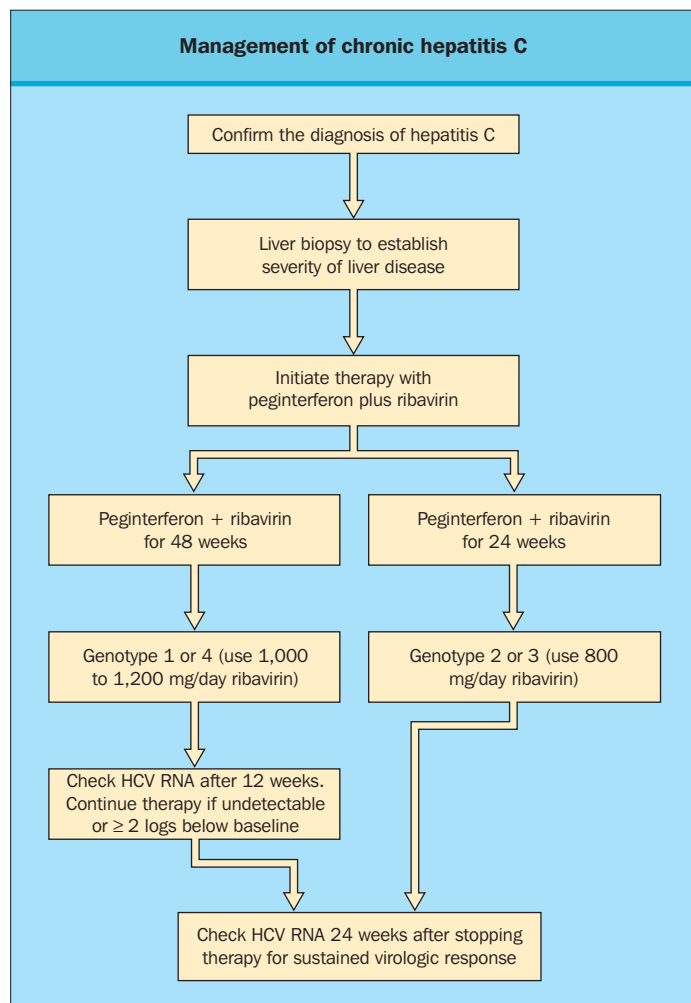


Figure 16.10 Algorithm for management of chronic hepatitis C.

decrease but does not become undetectable during therapy. An uncommon pattern is 'breakthrough' where HCV RNA becomes undetectable on therapy but then reappears even while the patient is still receiving treatment.

In addition to these virologic patterns of response, improvements (reductions) in serum aminotransferases may occur (referred to as a biochemical response) and a decrease in necro-inflammatory activity may be noted on liver biopsy (histologic response). Assessment of antiviral therapy has focused on SVR.

#### Effect of antiviral therapy on hepatic fibrosis

It has long been recognized that interferon therapy of chronic hepatitis C is associated with a significant reduction in the degree of necroinflammatory change within the liver. It has only recently become apparent that antiviral therapy might also have an impact on the degree of hepatic fibrosis. Poynard et al (2002) examined pooled individual data from more than 3000 patients with chronic hepatitis C who have participated in four randomized controlled trials with the same industry sponsor (Schering). Patients enrolled in these four trials were subject to 10 possible different treatment regimens combining standard interferon  $\alpha 2b$ , pegylated (PEG)-interferon  $\alpha 2b$  and ribavirin. They assessed the impact of each regimen by determining the percentage of patients with at least one-grade improvement in necrosis and inflammation using the METAVIR score and with at least one-stage worsening of fibrosis and by the annual fibrosis progression rate. Necrosis and inflammation improved with all treatment regimens, but occurred to the greatest degree with higher doses of peginterferon and ribavirin. The proportion of cases with worsening fibrosis was also lowest in patients treated with this regimen. All treatment regimens were associated with significant reductions in the rate of fibrosis progression assessed in the follow-up liver biopsies. Not only was the rate of progression of fibrosis slowed but reversal of cirrhosis was

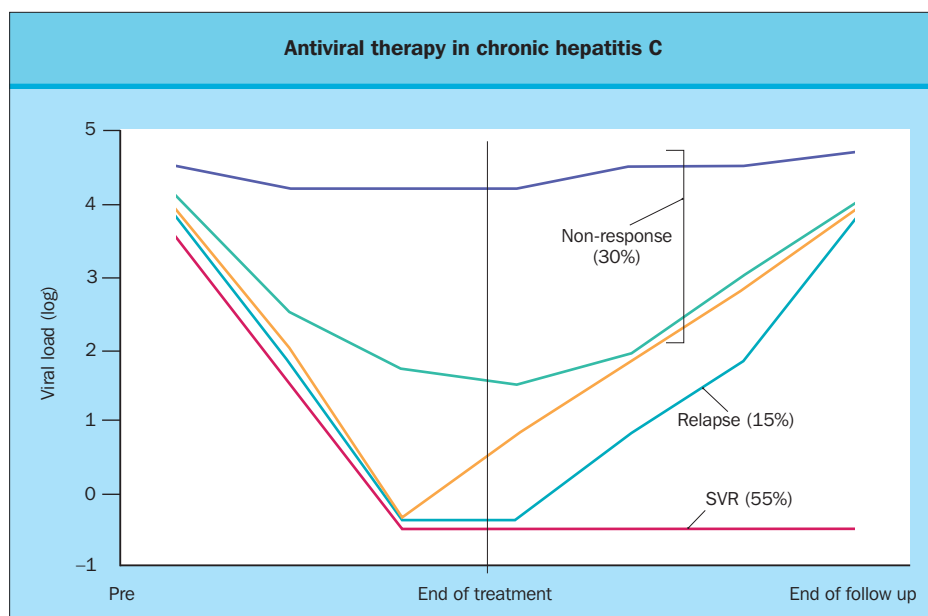


Figure 16.11 Antiviral therapy in chronic hepatitis C.

observed in 75 of 153 (49%) of patients with cirrhosis at baseline.

### **Identification of patients for treatment**

The recent National Institutes of Health (NIH) Consensus Development Conference helped to clarify which patients should be treated. The Consensus Statement began by noting that all patients with chronic hepatitis C were to be considered as potential candidates for antiviral therapy. Treatment was particularly recommended for those patients at increased risk of developing cirrhosis. The Consensus Statement further characterized them as having detectable HCV RNA, a liver biopsy with portal or bridging fibrosis and at least moderate inflammation and necrosis. The majority of these patients would be expected to have elevated serum aminotransferase activities.

For patients with milder forms of liver disease or persistently normal serum alanine aminotransferases (ALTs), it was noted that there were differences of opinion about treatment. Certainly, patients with normal ALTs appear to have similar rates of SVR compared to those with elevated ALTs and they can be treated on an individual basis, taking into account factors such as favorable genotype, presence of hepatic fibrosis, patient motivation, symptoms, age and presence and severity of comorbid illnesses. If patients are not subjected to antiviral therapy, they should be monitored periodically to identify progression of their liver disease promptly. Patients with advanced liver disease in the form of hepatic decompensation or after liver transplantation should not be treated routinely, although therapy could be considered in the context of clinical trials or by experts with experience in these conditions.

Some patients have been previously excluded from therapy because of behavioral problems such as alcohol or drug abuse and comorbid medical and psychiatric conditions. The Consensus Statement recommended that 'efforts should be made to increase the availability of the best current treatments to these patients'.

### **Predictability of response**

Analysis of data from large clinical trials using either standard or peginterferon in combination with ribavirin have identified certain pretreatment host and viral factors as being predictive of achieving SVR. Using multivariate analysis, Manns et al (2001) identified HCV genotype, baseline viral load, age and absence of cirrhosis while Fried et al (2002) found HCV genotype, age and body weight to be predictive of SVR. Of these factors, HCV genotype is clearly the most dominant. Patients infected with HCV genotypes 2 or 3 achieve response rates nearly double those infected with genotype 1.

Other factors that appear to be important in predicting the outcome of therapy but that were not specifically identified in these two pivotal trials include race and the dose of ribavirin. Because very few African-Americans were included in the three pivotal trials described, race could not be separated out as an unfavorable predictive factor. However, other studies have confirmed the very low response rate among African-Americans, even using peginterferon and ribavirin. Substantial research efforts are underway now to clarify the mechanisms responsible for low response rates in this ethnic group. For patients infected with HCV genotype 1, the dose of ribavirin administered appears to be critical. It has been suggested that the use of recombinant human erythropoietin may prevent ribavirin-associated anemia

and allow larger doses of ribavirin to be given or minimize the need for dose reduction.

It has become apparent that the ultimate outcome of antiviral therapy may become apparent early on during treatment. In those patients destined to achieve end of treatment response, levels of HCV RNA fall rapidly and often become undetectable within the first few weeks of therapy. In an analysis of data from two large pivotal trials using peginterferon and ribavirin, Davis (02) found that among 965 treated patients, 778 (80%) achieved an early virologic response (EVR, decrease by at least 2 log<sub>10</sub> units or to undetectable HCV RNA in serum). Among 187 patients without an EVR, only 3 (1.6%) went on to have SVR. These findings suggest that the week 12 HCV RNA level can be used to assess the need to continue therapy, at least in those patients infected with HCV genotype 1. Generally, most clinicians treat genotype 2 or 3 patients for 24 weeks, without assessing EVR.

## **Antiviral therapies**

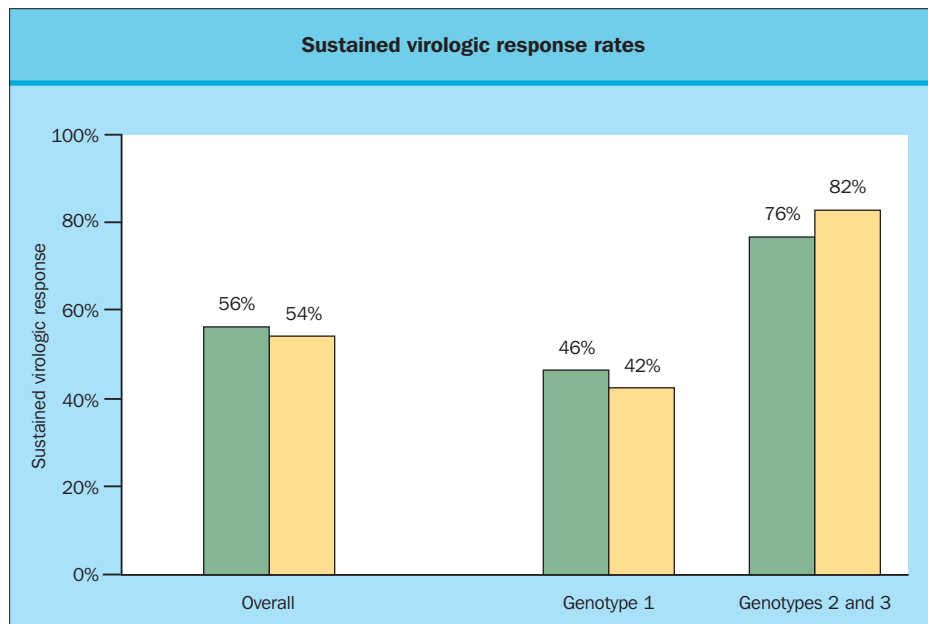
### **Interferons**

Interferons are naturally occurring cytokines produced in response to viral and other infections and were first identified in the 1950s. Recombinant and lymphoblastoid interferons became available in the early 1980s and have been used in the treatment of chronic hepatitis C for nearly 15 years now. Early studies showed that interferon therapy was associated with a decrease in serum aminotransferase activities to normal (a biochemical response) in a substantial proportion of patients, although the proportion having a sustained biochemical response was relatively low.

Interferon was initially used for only 6 months, but then therapy evolved to 12 months in duration for patients infected with HCV genotype 1. The use of peginterferons has largely replaced standard interferons. When used together with ribavirin, SVR rates are now in excess of 50%. Other patterns of response to therapy include relapse and non-response.

Two large studies in interferon-naïve patients followed, one in the USA and the other in Europe and other countries. More than 1700 patients were enrolled in these two studies with a similar design. Patients were randomized to treatment with either interferon  $\alpha$ 2b or ribavirin, for either 24 or 48 weeks (**Fig. 16.12**). The results of these two studies were similar and rates of SVR with combination therapy were more or less double those found with interferon monotherapy. Furthermore, the longer duration of therapy (48 weeks) was also found to be associated with a significantly higher response rate. Thus, interferon monotherapy for only 6 months achieved an SVR of only 6% while combination therapy given for 12 months resulted in SVR rates of 38% and 43% in patients in each of the two studies. These studies found that the addition of ribavirin did not substantially increase the rate of virologic response at end of treatment, but did reduce the relapse rate substantially.

The pegylation of the recombinant interferons appears to be associated with enhanced antiviral efficacy. The process of pegylation involves the addition of a polyethylene glycol (PEG) 'tail' to interferon and is a well-established method for delaying the clearance of proteins and for reducing their immunogenicity. PEG is an inert substance with no direct action against HCV or other viruses. Two forms of peginterferon are available. One is a conjugate of straight-chain PEG with a molecular weight of



**Figure 16.12 Sustained virologic response rates (SVR).** SVR rates in patients treated with pegylated interferon and ribavirin in two pivotal trials, according to genotype (in yellow, data from Manns et al, in green, data from Fried et al). Note that SVR is defined as undetectable hepatitis C virus RNA 24 weeks after completion of a 48-week course of therapy. SVR rates in genotypes 2 and 3 are almost double those for patients infected with genotype 1.

12 000 daltons (12 kDa, PegIntron, Schering Plough) while the other has a branched chain PEG conjugated to interferon with a resultant molecular weight of 40 kDa (Pegasys, Roche). Pegylation has resulted in the interferon persisting in the circulation for approximately 7 days. This is in contrast to the half-life of only several hours for standard forms of interferon. Early studies proved the convenience of once a week injection instead of three times a week injection and the absence of severe adverse events associated with the long plasma half-life of the interferon.

Several large studies were conducted in which the effect of peginterferons was tested without the addition of ribavirin in patients with chronic hepatitis C. Zeuzem (2000) and co-workers randomly assigned 531 patients with chronic hepatitis C to receive either 180 µg of peginterferon α2a subcutaneously once a week for 48 weeks or 6 million units of interferon α2a three times a week for 12 weeks followed by 3 million units three times a week for 36 weeks. In the peginterferon α2a arm, 69% of patients were HCV RNA-negative at the end of therapy compared to only 28% treated with standard interferon. At the end of follow-up (24 weeks after stopping therapy) 39% of those receiving peginterferon α2a remained HCV RNA-negative while only 19% of those treated with standard interferon achieved a sustained virologic response. Rates of sustained biochemical response were 45% with peginterferon α2a and 25% with standard interferon. Interestingly, a discordance was noted between virologic and biochemical responses at the end of treatment (week 48) in more patients receiving peginterferon α2a.

A similar study was conducted in patients with chronic hepatitis C and more advanced liver disease (bridging fibrosis or cirrhosis). In this study, 271 patients were assigned to one of three groups. One group received interferon α2a 3 million units thrice weekly, another received peginterferon α2a 90 µg weekly and the third group was treated with peginterferon α2a 180 µg weekly. End of follow-up response rates were 8% with standard interferon, 15% with 90 µg of peginterferon α2a and 30% with

the higher dose (180 µg per week) of peginterferon α2a. This study illustrates the relatively low response rate seen with standard interferon in patients with cirrhosis. In addition, an obvious dose response can be discerned with the peginterferon. There were concerns that in patients with cirrhosis, the decrease in peripheral blood neutrophil and platelets counts normally observed with standard interferon might be exaggerated. In fact, the rates of dose modification for neutropenia and thrombocytopenia were similar in all three treatment groups (for neutropenia, this was 14% with interferon α2a, 9% for low dose peginterferon α2a and 10% for the higher dose peginterferon α2a and for thrombocytopenia it was 6%, 18% and 18% respectively).

A study involving 1219 patients with chronic hepatitis C was done using peginterferon α2b. In this trial, patients were randomized to one of four treatment groups: standard interferon α2b or peginterferon α2b at doses of 0.5, 1.0 or 1.5 µg/kg of body weight once a week. Again response rates were significantly higher with pegylated than standard interferon, at least at the higher doses. Thus, the sustained virologic response rate was only 12% with standard interferon α2b compared to 17% with peginterferon 0.5 µg/kg/week, 24% with peginterferon 1.0 µg/kg/week and 23% with peginterferon 1.5 µg/kg/week.

### Ribavirin

Ribavirin is a nucleoside analog that was developed in the early 1970s as an antiviral agent with a broad spectrum of activities. Preliminary studies of ribavirin monotherapy in chronic hepatitis C showed a significant decrease in serum amino-transferase activities during therapy with little direct antiviral effect. Subsequently, ribavirin was tested in combination with interferon and was found to substantially increase the rate of SVR, mostly by decreasing relapse of HCV after stopping therapy. Although the long-term use of ribavirin monotherapy for hepatitis C has been found to be associated with significant improvement in liver histopathology, ribavirin is rarely used alone.



The mechanisms of action of ribavirin in hepatitis C are not known. Serum levels of HCV RNA decrease by a very small amount and only transiently when used alone. Ribavirin does however seem to inhibit the action of the HCV RNA dependent RNA polymerase and also induces catastrophic mutations in viral RNA. Ribavirin has significant immunomodulatory action, causing in a shift from Th1 to Th2 profile of circulating T lymphocytes.

When ribavirin was combined with interferon in the therapy of chronic hepatitis C it was found to substantially improve SVR rates. The first large study using the combination evaluated the use of interferon  $\alpha 2b$  with ribavirin in the retreatment of patients experiencing a relapse of HCV after initial viral response to interferon monotherapy. It was found that the addition of ribavirin increased the rate of SVR approximately 10-fold compared to retreatment with interferon alone. It was on the basis of this study that the combination of interferon and ribavirin was first Food and Drug Administration (FDA)-approved in the USA.

### Combination therapy

Given the increased response rates to therapy observed with peginterferons, it was natural to test them in combination with ribavirin. At least three large pivotal trials of this therapy have been conducted so far. These studies have many patient inclusion and exclusion criteria in common including raised serum aminotransferases, detectable serum HCV RNA, absence of other liver diseases or coexisting HIV infection.

The results of the first studies were reported by Manns et al in 2001. In this study, 1530 patients with chronic hepatitis C were randomized to one of three treatment groups: standard interferon (3 million units thrice weekly) with ribavirin for 48 weeks (1000–1200 mg daily based on body weight), peginterferon  $\alpha 2b$  1.5  $\mu\text{g}/\text{kg}/\text{week}$  with ribavirin 800 mg daily for 48 weeks or peginterferon  $\alpha 2b$  1.5  $\mu\text{g}/\text{kg}/\text{week}$  for 4 weeks and then 0.5  $\mu\text{g}/\text{kg}/\text{week}$  for 44 weeks. The rates of sustained virologic response were greatest in the group receiving higher dose peginterferon  $\alpha 2b$  with ribavirin (54%), compared to 47% with both the lower dose peginterferon and standard interferon combined with ribavirin. Pretreatment variables influencing the outcome of therapy included HCV genotype, viral load, body weight, age and degree of hepatic fibrosis. Patients infected with HCV genotype 1 and having a high viral load appear to be a particularly difficult group to treat with only 30% experiencing a sustained virologic response, even with optimal therapy.

Because peginterferon dosing is based on weight and because two different doses of ribavirin were evaluated, an extensive post-hoc analysis was conducted to try and evaluate the impact of these variables on treatment outcome. This analysis revealed that the likelihood of SVR increased as the dose of ribavirin increased (expressed as mg/kg of body weight). A threshold value of 10.6 mg/kg could be established, below which rates of SVR decreased significantly. Also, when the dose of ribavirin was controlled on a mg/kg basis, the estimated effect of the higher dose of peginterferon  $\alpha 2b$  compared with the lower dose was larger implying that weight-based dosing of both interferon and ribavirin might be appropriate.

A large international trial using peginterferon  $\alpha 2a$  in combination with ribavirin was completed in 2002. In this trial,

patients with chronic hepatitis C were randomized into one of three groups. The first group received standard interferon  $\alpha 2b$  with ribavirin at standard doses; the second group received peginterferon  $\alpha 2a$  at a dose of 180  $\mu\text{g}/\text{week}$  with daily oral placebo to control for the effect of ribavirin; the third group was treated with peginterferon 180  $\mu\text{g}/\text{week}$  with ribavirin 1000–1200 mg based on body weight. Treatment was for 48 weeks and follow-up for 24 weeks after the end of treatment. SVR rates were 45% with standard interferon and ribavirin, 30% with peginterferon alone and 56% with peginterferon in combination with ribavirin. Approximately 7% of patients experienced a relapse of hepatitis C after achieving an end of treatment response in the two groups receiving ribavirin while in the group receiving peginterferon alone, the rate of relapse was substantially higher (29%). There was a marked difference in rates of SVR in patients with different genotypes. Thus, 46% of those with genotype 1 achieved a sustained response with peginterferon and ribavirin, compared to 76% for those infected with non-1 genotypes (mostly genotypes 2 and 3). Again, the group with both genotype 1 and high viral load proved the most difficult to treat and only 41% of them had an SVR.

The last of the three pivotal trials was designed to assess the effect of duration of therapy and the dose of ribavirin. Thus patients were randomized to receive peginterferon  $\alpha 2a$  for either 24 or 48 weeks, with either 800 or 1000–1200 mg/day of ribavirin. Within each of these treatment groups, patients were stratified at entry according to HCV genotype (1 or non-1) and viral load (greater than or less than 2 million copies/mL). SVR was assessed at the end of 24 weeks of follow-up in all groups. In patients infected with HCV genotype 1, the highest rates of response were noted in patients treated for 48 weeks with the higher dose of ribavirin (51%). Interestingly however, rates of SVR were virtually identical in all four treatment groups in patients infected with genotypes 2 and 3 (73–78%). These data clearly indicate that patients infected with HCV genotype 2 or 3 can successfully be treated with only 24 weeks of therapy and less ribavirin. This represents substantial savings in terms of both cost and side-effects (see Figs 16.10 and 16.12).

### Side-effects of interferon and ribavirin

The side-effect burden of therapy with interferon and ribavirin is considerable and accounts for the relatively high proportion of patients requiring dose reduction or premature discontinuation of therapy. The most troublesome side-effects of interferon include a 'flu'-like illness, fatigue, achiness and neuropsychiatric effects which include depression, irritability and emotional lability. Leukopenia and thrombocytopenia occur as a result of bone marrow suppression by interferon.

The side-effects of standard and peginterferons appear to be similar. Thus, the proportion of patients requiring dose reduction or discontinuation of therapy was similar in the groups receiving either peginterferon or standard interferon plus ribavirin in two of the pivotal studies described above. Only neutropenia and injection site reactions occurred more frequently with peginterferon. The incidence of bacterial infections was, however, no greater with pegylated than with standard interferon.

The use of interferon may uncommonly be associated with adverse events which may be serious or even life-threatening. Some of these include acute psychosis or severe depression

associated with suicidal ideation or attempt, systemic bacterial infection, interstitial pneumonitis, cardiac events such as congestive heart failure, arrhythmias or myocardial infarction. In general these severe side-effects resolve when interferon is stopped. The induction of autoimmune thyroid disease (usually hypothyroidism) is more common, occurring in about 4% of treated patients; while this is rarely serious it may not resolve when the use of interferon is discontinued.

The most frequent side-effect of ribavirin is dose-dependent hemolysis which may require dose reduction for anemia. This anemia has sometimes been associated with myocardial ischemia in the form of angina or even myocardial infarction. Ribavirin is a potent teratogen and has been associated with spontaneous abortions and birth defects in experimental animals. Every effort must be made to avoid pregnancy during and for 6 months after taking ribavirin, both in women and men, because of the risks of teratogenicity.

### **Treatment of special populations**

#### ***Treatment of acute hepatitis C***

It has been known for some time that interferon therapy of acute hepatitis C is associated with greater rates of SVR than those seen in chronic hepatitis C. However this phenomenon has been difficult to study because of the relative paucity of patients diagnosed with acute hepatitis C, now that blood transfusion has been eliminated as a source of infection. A recent report showed that treatment with standard interferon alone for a period of only 6 months was able to eliminate HCV infection in nearly 100% of patients with acute hepatitis C. The NIH Consensus Panel felt, however, that further study was needed, perhaps because the optimal timing to begin therapy is not known, as some patients will clear HCV infection by themselves. In addition, the optimal duration of therapy is not known. The use of ribavirin in combination with interferon has not received extensive evaluation, just as peginterferon has not been adequately tested. However, it may prove very difficult to ever get these optimal studies done. Given the remarkable effectiveness of monotherapy for 6 months, this may simply become the standard of care. Clearly peginterferon with or without ribavirin, while not adequately evaluated, may also be used to treat acute hepatitis C.

#### ***Treatment of non-responders***

Retreatment of patients with interferon alone who did not respond initially to monotherapy appears futile, as rates of SVR are very low. However, sustained response rates as high as 30% have been observed when they are retreated using ribavirin in combination with interferon. There are few data available on the use of peginterferon with ribavirin in the retreatment of previous non-responders. Preliminary data from the HALT-C trial shows that although the rate of end of treatment response may be quite high in previous non-responders retreated with peginterferon and ribavirin (26%), relapse is frequent and only 11% achieved SVR. Selected patients with good predictors of response or particularly severe liver disease could be targeted for retreatment. Long-term maintenance therapy, in which patients are given continuous treatment with interferon over a period of several years, is still under study but has the potential of reducing the risk of progression of liver disease to cirrhosis, hepatic decompensation or HCC.

### ***Treatment of relapse***

There are no clear guidelines for the management of patients experiencing an end of treatment response to combination antiviral therapy who then go on to have a relapse of their hepatitis C. Certainly those patients who relapsed after interferon monotherapy should be considered for retreatment using combination therapy with ribavirin. It is possible that retreatment using combination therapy for longer time periods may be successful in patients who relapse after combination therapy, but this has not been tested in clinical trials. Furthermore, patients who relapsed but who did not receive the full dose of ribavirin (perhaps because of anemia) could be retreated with full dose ribavirin, perhaps supplemented with recombinant erythropoietin. Again this approach has not been validated in clinical trials.

### ***Co-infected patients***

Because of the common modes of transmission, patients with hepatitis C are sometimes co-infected with other blood-borne viral agents, including hepatitis B virus (HBV) and HIV. There are no clear guidelines for the management of patients co-infected with HBV (as marked by the presence of hepatitis B surface antigen in serum) and HCV. Interferon is effective against HBV, although higher doses are usually used for HBV and peginterferons have not been tested extensively in this situation. Other antiviral agents, such as lamivudine and adefovir, are effective against HBV and may be used in combination with interferon in co-infected persons.

Thomas (2002) has estimated that as many as 200 000 patients are co-infected with both HIV and HCV. Morbidity and even death due to liver disease has emerged as significant problems in the management of co-infected patients as better therapies have been implemented against HIV and patients live longer. HIV infection appears to worsen HCV infection by increasing the rate of chronicity, by accelerating the progression of liver disease and by decreasing the rate of response to antiviral therapy. Medications used to control HIV infection are associated with risks of hepatotoxicity which seem to be even worse in patients with HCV co-infection.

Definitive trials of antiviral therapy directed against HCV using the combination of peginterferon and ribavirin in co-infected patients suggest that although lower than in the general population, rates of SVR are reasonable. There has been some concern raised about the risk of lactic acidosis in patients with HIV taking nucleoside analogs (particularly old I) and other antiviral drugs for HIV when ribavirin is added to the regimen.

### ***Decompensated cirrhosis and post-transplant hepatitis C***

Treatment of patients with advanced liver disease using interferon is potentially risky because these patients usually already have thrombocytopenia and leukopenia which are exaggerated by interferon. The occurrence of severe bacterial infections, such as spontaneous bacterial peritonitis, has also been documented to occur in this setting. Nonetheless, small numbers of patients with decompensated cirrhosis have been treated. The proportion achieving an SVR appears to be smaller than in those with milder liver disease. Interestingly however, if patients with decompensated cirrhosis achieve a sustained virologic response and then go on to liver transplantation, it seems that the risk of recurrent hepatitis C is virtually eliminated, a major benefit of

therapy. The dosing of interferon is not well established in patients with very advanced liver disease, but in general should be lower than in otherwise healthy individuals.

Recurrence of HCV infection is almost universal after liver transplantation and may result in recurrence of severe liver disease and cirrhosis in a proportion of patients within a few years. There is growing evidence of benefit from antiviral therapy in these patients with recurrent hepatitis C. In this setting, ribavirin should be used with extreme caution because of the typical renal insufficiency associated with use of calcineurin inhibitors to prevent rejection. Furthermore, the rate of SVR is substantially lower than in pretransplant patients with mild liver disease (no more than 10% using current treatment regimens). However it appears that the use of interferon after transplant may be associated with some histologic benefit.

## PREVENTION OF HCV INFECTION

Substantial progress has already been made in preventing the spread of HCV infection. HCV has been virtually eliminated from the blood supply and the number of new cases is decreasing. Further decreases in the incidence of hepatitis C would require a reduction in cases associated with injection drug use. This would require either the primary prevention of illegal drug injecting or, possibly, secondary prevention among drug users by methods such as needle exchange programs.

Another approach to preventing the spread of HCV involves development and use of vaccines against HCV. There is currently no vaccine in clinical use or even undergoing clinical trial in large numbers of patients. Development of a vaccine has been hampered by the lack of a clear understanding of what constitutes a sterilizing immune response to HCV and by the considerable variability of the HCV genome. Vaccines based on recombinant forms of HCV envelope proteins have been tested in chimpanzees with at least partial success.

## FURTHER READING

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## FUTURE OUTLOOK

Further studies are still needed to clarify the use of existing therapies. Thus, while the optimal dose of ribavirin has been established by these recent studies, the optimal dosing of peginterferon deserves further study. It is not clear if lower doses of peginterferon can perhaps be used for some patients – perhaps those infected with non-1 genotypes, or those with low levels of HCV RNA in serum. Because body weight has proven to be a factor influencing the response to antiviral therapy, further study of weight-based dosing may be appropriate, particularly for those patients at the extremes of high or low body weight. Studies such as the HALT-C trial and follow-up of patients achieving SVR in other clinical trials will hopefully clarify whether antiviral therapy against hepatitis C is effective in achieving the goals of slowing or preventing the progression of liver disease.

Considerable efforts have gone into developing new approaches to therapy of hepatitis C, largely based on the design of specific antiviral agents aimed at inhibiting various viral enzymes or functions, such as the HCV polymerase, HCV protease or the ribosomal docking function of the IRES. Several antiviral agents are in the process of development based on this progress. Preliminary data were recently reported on the antiviral effect of a novel protease inhibitor, showing very potent antiviral effect in the short term. Issues that are likely to arise with the use of specific antiviral agents include the risks of unexpected toxicity, development of viral resistance due to mutations and possibly the need for continuous or long-term therapy. It is likely that peginterferons will continue to play a major role in therapy of HCV for some time to come, at least until the widespread availability of other antivirals. Given that peginterferon and ribavirin are effective in eliminating HCV infection in nearly 80% of patients infected with genotypes 2 or 3 when given for a relatively short period of time, this combination of drugs may remain the mainstay of therapy in this subgroup of patients.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 17

## Miscellaneous Infections of the Liver

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### INTRODUCTION

Infections frequently involve the liver, either as part of a disseminated infection or due to a specific tropism of the infectious agent for the liver and biliary tree. The liver is at particular risk of infection due to the large vascular supply and in particular the close relationship to the venous drainage system of the gut, by which many infectious agents gain access to the body. Given that many infections will present non-specifically, with elevations in serum aminotransferases or alkaline phosphatase as the only clue to involvement of the liver, the clinician needs to be aware of what infections to which a particular host may be susceptible, as well as the best means to achieve diagnosis of a particular pathogen. In this chapter, we will describe infections other than classic viral hepatitis (A, B, C, D and E) with respect to their presentations as hepatic diseases.

### HEPATIC MANIFESTATIONS OF SYSTEMIC DISEASE

#### Hepatosplenic candidiasis

##### Epidemiology

Hepatosplenic candidiasis (HSC; otherwise known as chronic disseminated candidiasis) is a form of invasive fungal infection that primarily affects the liver, kidneys and spleen. Most often the disease is caused by *Candida albicans*, but rare cases of *Candida tropicalis*, and *Candida glabrata* as well as other species have been described. It most often occurs in the setting of hematologic malignancies. It has been recognized as a distinct form of invasive fungal disease in this setting for the last two to three decades, although the incidence of HSC is rising because of more widespread use of cytotoxic agents. One large retrospective study in Taiwan reported an incidence of up to 8% in 500 patients with acute leukemia receiving chemotherapy. Patients with these conditions are at risk secondary to a combination of mucotoxic chemotherapeutic regimens and periods of neutropenia. Other potential risk factors are intravascular catheters and use of broad-spectrum antibiotics. However, hepatosplenic candidiasis in non-leukemic patients is rare. The most likely pathophysiology is that the mucosal damage caused by chemotherapeutic agents causes invasion of the bloodstream and hematologic spread of *Candida*, which is a part of the normal gastrointestinal flora. Given that the portal system would then be the first site of dissemination, its location puts the liver and spleen at particular risk of infection. Coupled with periods of neutropenia, this places patients with hematologic malignancies at extreme risk of hepatosplenic candidiasis.

#### Clinical signs, symptoms and diagnosis

The presentation of hepatosplenic candidiasis is usually subacute, lasting weeks to months. The most common presenting sign of hepatosplenic candidiasis may only be a persistent fever despite appropriate broad-spectrum antimicrobial therapy. Patients may also present with abdominal pain, nausea, vomiting and an elevated serum alkaline phosphatase. Blood cultures are positive in less than 50% of patients. Hepatic lesions can usually be identified on magnetic resonance imaging (MRI), computed tomography (CT) scan and ultrasound. However, these lesions may disappear in periods of neutropenia or only become visible after neutropenia has resolved. One series described serial sonography as a means of increasing sensitivity. Typically, the lesions associated with hepatosplenic candidiasis appear irregularly-shaped and hypoechoic on ultrasound. A definitive diagnosis usually requires a biopsy of these lesions demonstrating budding yeast or pseudohyphae. Given the focality of these lesions, ultrasound-guided or laparoscopic biopsies are recommended. Histologically, early lesions consist of a mixture of polymorphonuclear inflammatory cells and mononuclear cells that surround a center of viable yeast. After treatment, these lesions then become a center of necrotic fungal elements surrounded by lymphocytes, macrophages and giant cells. The periphery is made up of fibrotic tissue.

#### Treatment and prophylaxis

There is wide variation in treatment protocols for hepatosplenic candidiasis, and to date no definitive regimen is agreed upon. Amphotericin is the drug of choice, and most sources recommend amphotericin B in combination with 5-flucytosine. Amphotericin can cause significant renal toxicity and electrolyte abnormalities. If the patient has underlying renal impairment, liposomal formulations of amphotericin can be used. Once stable, the patient can be switched to fluconazole. Most sources recommend continuing therapy until radiographic evidence of disease has disappeared or a total of 4 g of amphotericin has been given. Fluconazole has also been used successfully in non-neutropenic patients as primary treatment, providing the *Candida* isolate is susceptible to fluconazole; some species, such as *Candida krusei*, are intrinsically resistant to fluconazole. Other azole formulations and caspofungin have activity against *Candida* species, but there is too little information about their use in this setting to recommend them as initial treatment.

The overall incidence of disease had declined significantly in the last decade, attributed largely to institution of fluconazole prophylaxis as standard of care for patients with prolonged neutropenia in most large cancer centers. This has been confirmed in several studies, including one large autopsy study in which there were fewer cases of candidal liver infections in patients on fluconazole prophylaxis.

## Histoplasmosis

### Epidemiology

Histoplasmosis is endemic in certain areas in North, Central and South America. In the USA it is the most common cause of fungal infection in the Ohio and Mississippi River valleys, although it is present in most of the USA. Cases reported in residents of areas that are not endemic usually represent reactivation of disease acquired during previous travel to endemic zones. Disseminated histoplasmosis occurs in patients with impaired cellular immunity as in patients with acquired immunodeficiency syndrome (AIDS), advanced age, malignancy and diabetes or even in immunocompetent patients if the yeast burden is high enough. Disseminated disease occurs in approximately 1/2000 exposed individuals. While disease normally starts in the lungs, as it occurs when infectious microconidia are inhaled and then transform into pathogenic yeast-phase organisms, liver involvement is extremely common in disseminated histoplasmosis. Rarely, histoplasmosis may occur in the liver without any evidence of disseminated disease.

### Clinical signs and symptoms

Most people who are infected with histoplasmosis remain asymptomatic or experience a self-limited course that consists of fever, cough and fatigue. The symptoms of disseminated disease uniformly include fever, weight loss and malaise. Patients may also present with hepatomegaly and lymphadenopathy. Alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are usually elevated to twice the upper limit of normal. Patients can also have a markedly elevated lactate dehydrogenase (LDH). Patients may present with shock, multiorgan failure and disseminated intravascular coagulation (DIC). In cases that are not disseminated and only hepatic involvement is reported, patients usually present with right upper quadrant pain and a hepatic mass.

### Diagnosis

Diagnosis of disseminated disease can be made by biopsy of the lung, lymph node or liver. Oval, budding yeast are seen on methenamine silver stain. On hepatic biopsy, granulomatous hepatitis will be present with or without necrosis. Blood cultures and smears may also be useful. Urinary histoplasmosis antigen can be obtained if disseminated disease is suspected. It has a sensitivity of 90% in patients with disseminated disease.

### Treatment

In patients with severe pulmonary, hepatic or disseminated disease, treatment should be initiated with amphotericin B. In patients who are to be treated as outpatients or in whom amphotericin B is initially used with good clinical response, itraconazole can be used as it has good activity against histoplasmosis. Itraconazole can also be used in AIDS patients as secondary prophylaxis.

## SYSTEMIC DISEASE – BACTERIAL

### Listeriosis

#### Background

Hepatic involvement in the course of any serious bacterial infection is common. Like many other bacteria, *Listeria* has a

propensity for invading the liver. This has been well documented in both human and animal studies. In fact, before *Listeria* was given the name *Listeria monocytogenes* it was first called *Listeria hepatolytica* because of the common finding of focal necrosis in the liver during animal studies.

### Epidemiology

*Listeria* is a motile, Gram-positive organism. Infection is commonly associated with pregnancy, extremes of age, chronic disease states and immunosuppression. Transmission of *Listeria* is usually through contaminated food. Outbreaks have been associated particularly with meat or dairy products, such as undercooked hotdogs, which incur 12 times the risk, or undercooked chicken, which is associated with a 20-fold increase in risk. Recent outbreaks of listeriosis have also been associated with chocolate milk, delicatessen meats, unpasteurized dairy products, soft cheeses, blue-veined cheeses and Mexican-style cheese products.

### Clinical presentation

The most common presentation of listeriosis, especially in the elderly, pregnant and newborn populations, is meningitis or a meningoencephalitis. However, there are three distinct entities comprising the hepatic involvement of *Listeria*: hepatitis, single liver abscess and multiple liver abscesses. In the case of hepatitis, the diagnosis is often mistaken initially for acute viral hepatitis. It is often not until other more common signs of listeriosis, such as meningeal signs, develop that *Listeria* is considered in the differential diagnosis of acute hepatitis. Patients may initially present with fever and right upper quadrant tenderness. Physical examination may reveal hypotension and hepatomegaly. Laboratory tests will usually demonstrate markedly elevated AST and ALT, with peak aminotransferase levels as high as in the 5000–6000 U/L range. Patients may also initially present with a febrile gastroenteritis. Diarrhea is likely to be non-bloody and last for a median of 40 h. Median duration of fever with this presentation is approximately 1 day.

Solitary abscesses have mostly been described in patients with diabetes. They rarely have associated meningitis or bacteremia. Patients may present with fever and right upper quadrant pain. They have an excellent prognosis with proper treatment. Multiple liver abscesses caused by *Listeria* carry a worse prognosis. In one study, only 13% of patients survived. Given the likelihood of many extrahepatic sites of *Listeria*, it is likely that multiple abscesses are a sign of disseminated disease.

### Diagnosis

The diagnosis is usually confirmed when *L. monocytogenes* is isolated from blood cultures. In culture, the organism possesses flagellae and exhibits a tumbling motility at 25°C. It is commonly misidentified as a diphtheroid, and reports of diphtheroids in blood in a patient with a compatible clinical syndrome should prompt the clinician to query the laboratory about the possibility of *Listeria* and consider empiric antibiotic treatment. A diagnostic clue that might help distinguish *Listeria* from acute viral hepatitis is the presence of high temperatures ( $\geq 39^\circ\text{C}$ ) and shaking chills, which is atypical for viral hepatitis but common in *Listeria* infection.



**Treatment**

Intravenous ampicillin is usually effective in the treatment of hepatitis. Single abscesses usually require drainage and may require addition of an aminoglycoside. However, in the case of multiple abscesses and disseminated disease, high-dose ampicillin in combination with gentamicin is recommended.

**Melioidosis****Background**

Melioidosis is caused by a Gram-negative, motile bacillus called *Burkholderia pseudomallei*. Until 1992, the organism was known as *Pseudomonas pseudomallei*. Before the return of troops from endemic areas during the Vietnam War, melioidosis was virtually unheard of in the USA and Western Europe, but with increasing global travel and appreciation of the disease more cases have been identified.

**Epidemiology**

The organism is an environmental saprophyte in soil and fresh water in endemic regions. It is endemic to Southeast Asia, northern Australia, southern Asia and China, with most cases being reported from northern Thailand and northern Australia. In endemic areas, melioidosis is a disease of the rainy season. The primary mode of acquisition is now thought to be percutaneous, with subsequent dissemination of organisms to distant sites such as the lungs, skin, joints and liver. It normally affects individuals with diabetes, chronic renal failure, alcoholic liver disease or any degree of immunosuppression. Up to 50% of patients with melioidosis have diabetes associated with poor glucose control. The peak incidence is in the fourth and fifth decades of life.

**Presentation**

Disease usually occurs by inoculation or inhalation rather than ingestion. The incubation period following inoculation ranges from 1 to 21 days (mean 9 days) in those with symptomatic disease, with shorter periods seen in those with heavy exposure to the organism. Most infections are asymptomatic, but when symptoms are present they may be either acute or chronic in nature. Disease can present many years after initial exposure, with a reactivation syndrome similar to what occurs with tuberculosis, although such cases appear to be rare. The presenting signs and symptoms depend on the organ(s) involved, and range from a full-blown sepsis syndrome to non-specific fever. In the liver, the disease is characterized by abscess formation, which may be accompanied by other abscesses, particularly in the spleen, or radiologic evidence of pulmonary involvement.

**Diagnosis**

Abdominal ultrasound is helpful in identifying the hepatic abscess of melioidosis, although no ultrasonographic features of melioidosis are specific for this infection. If there are also splenic abscesses this can help in establishing the diagnosis in endemic areas, as other causes of liver abscess in these areas are less likely to cause a concomitant splenic lesion. While serologies may be helpful, definitive diagnosis is made by culturing the organism from the abscess site or blood. If the diagnosis is suspected based on residence or travel in an endemic area, the microbiology laboratory should be notified to enhance recovery of the organism, although *B. pseudomallei* grows on a wide range of commercially available media. On examination,

the Gram-negative organisms have a characteristic 'safety pin' appearance. Direct immunofluorescence microscopy from sputum, urine or purulent drainage is 98% specific. It is only 70% sensitive, but can make the diagnosis in 30 minutes or less.

**Treatment**

Melioidosis can be a difficult disease to treat. *B. pseudomallei* is inherently resistant to many antibiotics, including first and second generation penicillins and cephalosporins, as well as the aminoglycosides. It should be treated with either ceftazidime or a carbapenem such as meropenem, although sulfa agents may be used as adjunctive therapy in severe infections. If hepatic abscesses are large, they need to be drained. Patients may be slow to respond to antibiotics, but resistance to either ceftazidime or carbapenems is rare so physicians should keep these drugs patiently on board as the first-line regimen. Patients are usually treated with at least 2 weeks of intravenous therapy, followed by a prolonged period of oral therapy for a minimum of 3 months, usually with high dose trimethoprim-sulfamethoxazole (TMP-SMX)

**Brucellosis****Background and epidemiology**

*Brucella* is a small, non-motile, Gram-negative coccobacillus. Worldwide, it is a significant cause of disease in domesticated animals with humans as an incidental host. Brucellosis can be an occupational hazard for livestock workers and anyone in the meatpacking industry. There are several possible routes of exposure, including direct inoculation through breaks in the skin, via the conjunctiva, inhalation and ingestion of contaminated food. It can be transmitted to humans through ingestion of unpasteurized dairy products or by handling meat or blood samples from infected animals. There are four species that are pathogenic to humans (*Brucella melitensis*, *Brucella abortus*, *Brucella suis* and *Brucella canis*). Hepatic involvement has been widely reported, primarily from *abortus*, which is found in cattle and *B. melitensis*, which is found in goats.

**Clinical presentation**

Brucellosis is a well-documented cause of fever of unknown origin with varied and often non-specific symptoms, the most prominent of which is fever, thus earning the sobriquets of undulant, Mediterranean or Malta fever. Patients will present with fatigue, fever, night sweats, weight loss and headache, which may be either acute or subacute in onset. Patients may develop these symptoms acutely, or the disease may also lay dormant for many years in which case patients will present with a more indolent course that is only episodically symptomatic. Physical findings, if present, will be limited to minimal lymphadenopathy or hepatosplenomegaly. In the acute phase, up to 10% of patients will have hepatitis. Laboratory values will reveal an elevation in AST, ALT and alkaline phosphatase. Patients may also present in either the acute or the chronic phases of the disease with hepatic abscess formation, but this is much less common than a clinical hepatitis. Other common manifestations of localized infection are osteoarticular involvement, epididymo-orchitis, meningitis and endocarditis.

**Diagnosis**

Risk factors for this disease, such as contact with animal tissues or ingestion of unpasteurized milk or cheeses, should prompt

specific microbiologic testing. The organism can be isolated from blood cultures, which are relatively insensitive and may need to be held for prolonged periods (7–21 days) to reveal growth of the organism. Routine techniques used for culturing of blood are not optimal for recovery of the organisms, and so the microbiology laboratory should be alerted to perform special techniques to enhance recovery. Given the difficulty involved in culturing the organism, serologic testing is often used to establish the diagnosis. Biopsy of the liver usually demonstrates non-caseating granuloma formation.

### Treatment

In adults and children over 8 years old, treatment is doxycycline and rifampin (rifampicin) for 6 weeks, or doxycycline for 6 weeks plus streptomycin intramuscularly daily for 14–21 days. Other agents, such as fluoroquinolones, have some activity against *Brucella* but should not be used as monotherapy. In children less than 8 years of age, treatment consists of TMP-SMX for 45 days plus rifampin (rifampicin) for 6 weeks; alternatively, gentamicin may be used.

## SYSTEMIC DISEASE – VIRAL

### Cytomegalovirus

#### Background

Cytomegalovirus, CMV, is a member of the Herpesviridae family. Infection is common early in life, but is usually asymptomatic. It can be acquired transplacentally, through saliva, blood and urine; and may be associated with congenital abnormalities. In the adult population, disease can represent primary infection or reactivation. Symptomatic infection in immunocompetent patients is rare, but can occur. In all states that compromise T-cell immunity symptomatic infection is frequent, putting patients who are post-transplant and patients with HIV infection at particular risk. In these patients, infection can be severe and life threatening.

#### Clinical presentation

The clinical spectrum associated with active CMV disease is vast. It can cause pneumonia, gastrointestinal findings, hepatitis, central nervous system involvement, retinitis and nephritis. In one review analyzing the presentation in the immunocompetent population, abnormal liver specific tests were the most common clinical finding along with the less common fever, malaise and night sweats. In this 'mononucleosis-like syndrome', patients may also present with lymphadenopathy and lymphocytosis with greater than 10% atypical lymphocytes. In primary hepatitis, hepatomegaly is a common presenting feature. In the transplant population, CMV infection is most likely to occur within the first 18 months post-transplant. Patients who are seronegative prior to transplant but receive a graft from a seropositive donor are particularly at risk. However, reactivation disease in a previously seropositive patient can also occur and be quite severe. In transplant patients, the allograft is a particular target for CMV disease thus making CMV infection a risk for graft failure. Additionally, CMV has a number of host proteins that interact with the host immune response, and thus may independently increase the risk of other opportunistic infections.

In patients with HIV infection, CMV disease typically occurs when the CD4 count is less than 100. The most common

presentations in this population are retinitis, esophagitis and colitis. Liver enzyme abnormalities are common in these patients and active replication in the liver can be documented in 30–50% of patients with evidence of disease elsewhere. Active replication in the liver can also cause an acalculous cholecystitis and even a sclerosing cholangitis in these patients. A markedly disproportionately elevated alkaline phosphatase may be a sign of biliary disease in patients with CMV.

### Diagnosis

CMV infection is defined as the isolation of CMV virus or the detection of viral proteins or nucleic acid in any body fluid or tissue specimen. The diagnosis can be established using viral culture detection of viral antigens, or CMV DNA by polymerase chain reaction (PCR)-based techniques. PCR can be used in both a qualitative and quantitative modality.

### Treatment and prophylaxis

CMV in the immunocompetent host is usually self-limiting and generally no antiviral therapy is warranted. The recommendations for treatment of CMV in the immunocompromised host are varied. In AIDS patients and in transplant recipients with active CMV, the consensus is that an induction period with intravenous ganciclovir for at least 2–3 weeks is considered gold standard for treatment. However, for the maintenance therapy that follows the induction period, there is no general agreement about optimal therapy. Oral ganciclovir is often implemented in the maintenance period. In HIV patients, it is usually continued lifelong or until immune reconstitution has occurred. Oral valganciclovir, which is a pro-drug of ganciclovir, has been approved by the Food and Drug Administration (FDA) for both the induction and maintenance periods.

Prophylaxis in the transplant population is a complicated topic. The recommendations vary based on the organ allograft. However, the general principle is that patients who are seronegative for CMV and receive a transplant from a seropositive donor are at highest risk of CMV disease. Aggressive monitoring for CMV antigenemia is often implemented post-transplantation, including use of leukocyte depleted red cells. Typically, high-risk patients undergo an induction period of IV ganciclovir for the first 2 weeks post-transplant. Immunoglobulin is also often administered post-transplant as additional prophylaxis in donor CMV positive/recipient CMV negative cases. A recent study demonstrated that after this initial 2 weeks of therapy, the patient could then be switched to oral ganciclovir. However, there is also some data to support use of oral agents for the entire period of prophylaxis. There is no consensus on duration of therapy, but patients usually remain on prophylaxis in the first 1–3 months post-transplant. The issue of prophylaxis should be addressed on an individual patient basis taking into account the immunosuppressive medical regimen and risk of renal toxicity.

### Epstein-Barr virus

#### Background

Epstein-Barr virus (EBV) is a widely disseminated herpesvirus which is spread by intimate contact between susceptible persons and those shedding EBV. It is a member of the  $\gamma$  herpesvirus family and infects B lymphocytes. This typically results in a latent infection in which there is persistence of the viral genome

throughout the life of the infected person. The virus is highly adapted to persistence, such that only a few gene products are expressed during this period of latency, thus facilitating avoidance of host immune defenses. By the time they are adults, 80% of people will have been infected by EBV. EBV is spread via aerosolized particles or mouth-to-mouth contact. It is usually transmitted through close contact in young children or through oral contact in teenagers and young adults. Once EBV infects an individual, its DNA is incorporated into the host genome. This integration results in immortalization of peripheral blood B cells to form lymphoblastoid cell lines.

### **Clinical manifestations and disease spectrum**

Primary infection of EBV in most individuals results in a clinical syndrome known as infectious mononucleosis. Patients will present with exudative pharyngitis, fever and cervical lymphadenopathy. In most patients the pharyngeal symptoms are prominent, but in others the degree of lymphadenopathy is much greater than the symptoms of pharyngitis. For some patients, hepatitis is a prominent presenting sign. Other signs and symptoms include petechiae, periorbital or palpebral edema, and maculopapular rash. While splenomegaly is common, hepatomegaly is not. Liver enzymes may be up to five times normal. Jaundice is usually only present in approximately 5% of individuals and is caused by a rise in direct bilirubin. Liver biopsy, though rarely performed, would demonstrate a mild increase in portal inflammation and may have an inflammatory infiltrate of lymphocytes. Hepatic parenchyma may show some mild hepatocellular ballooning. Mild-moderate cholestasis has also been reported. Treatment of infectious mononucleosis is usually supportive, as most immunocompetent children and adults recover uneventfully. The disease is self-limiting, but may last several weeks.

Sporadic fatal mononucleosis is a separate clinical entity. It occurs in 1/300 cases of infectious mononucleosis. Patients will present with similar symptoms as compared to infectious mononucleosis; however, the symptoms, especially the organomegaly, are usually more pronounced. Laboratory values will reveal progressive pancytopenia, DIC and hepatic failure. Histology reveals fulminant hepatic necrosis. Median survival time is 4 weeks. Patients often die of fulminant hepatic failure or splenic rupture. In other cases, hemophagocytic lymphohistiocytosis can be seen, which is characterized by diffuse histiocytic proliferation and hemophagocytosis. Patients may present with fever, generalized lymphadenopathy, hepatosplenomegaly, hepatitis, pancytopenia and coagulopathy.

EBV has also been associated with a variety of lymphoproliferative disorders including X-linked lymphoproliferative disease (XLP) and post-transplant lymphoproliferative disorders (PTLD). Males with XLP have a selective immunodeficiency against EBV due to mutation in the gene *SAP* (signaling lymphocyte activation molecule associated protein), which appears to be involved in regulation of CD8 T-cell responses. Primary infection of males with this disorder results in an overwhelming immune response, and death can result from extensive liver necrosis. In addition to PTLD, there is also a strong association with Hodgkin and non-Hodgkin lymphomas. The liver can be involved in all of the above clinical entities with varying pathology.

### **Diagnosis**

The diagnosis of EBV infection is usually made by demonstrating a positive heterophile antibody, otherwise known as the Monospot test, in the serum. If present, the presence of an atypical lymphocytosis is also supportive of the diagnosis, although atypical lymphocytes can be found in a number of other viral infections, such as CMV, viral hepatitis, mumps, human immunodeficiency virus (HIV), as well as acute drug reactions. The heterophile test is both sensitive and specific, so it is rare to need to perform EBV-specific antibody testing. Serologies can also be performed to look for a variety of viral antigens including the viral capsid antigen (VCA) and the Epstein-Barr nuclear associated antigen (EBNA). The presence of IgM antibodies against VCA suggests acute disease, whereas IgG against EBNA virtually excludes acute infection, unless the person was documented by be IgM VCA positive and anti-EBNA negative recently.

## **SYSTEMIC DISEASE – SPIROCHETES**

### **Leptospirosis**

#### **Background and epidemiology**

Leptospirosis is a zoonosis that is endemic in the tropics and infects a variety of wild and domestic animals. Human infection, which is often an acute febrile illness, has been described under many different names including swineherd disease, mud fever, Fort Bragg fever, 7-day fever, autumn fever and in its most severe form, Weil disease. Leptospirosis is caused by the spirochete *Leptospira interrogans*. It is a tiny, tightly coiled organism that is usually 0.1  $\mu\text{m}$  by 6  $\mu\text{m}$  in size. There is only one species, *L. interrogans*, in the genus *Leptospira* although there are many antigenic variations and 240 serovars have been identified. These serotypes are arranged under 23 serovars. At least 27 serovars of leptospira occur naturally in the USA. The three clinically most important serovars are *Leptospira icterohemorrhagiae*, *Leptospira canicola*, and *Leptospira pomona*. The serovars *L. icterohemorrhagiae*, initially found in rats but later found in dogs, cattle, and swine, is responsible for about 50% of the cases of Weil disease. The other two serovars that are also found in dogs, cattle, and swine usually cause non-icteric illness. Leptospirosis is a worldwide disease. The incidence is higher in countries with more temperate climates as these environments are more hospitable to the organism. In certain tropical regions, up to 30% of the population has reported to be seropositive for leptospirosis. In the USA the highest incidence of leptospirosis is in Hawaii, but it has been reported in a variety of states.

The disease occurs in a wide range of domestic and wild animals. Rats are the most common host for *L. icterohemorrhagiae*. The serovars *L. canicola* is principally found in dogs, whereas *L. pomona* is found in livestock. Interspecies spread of specific serovars among animal hosts is frequent. Asymptomatic host animals carry high numbers of leptospires in their kidneys. The organism is shed in the urine of these animals. Human infection can occur either by direct contact with urine, contaminated water or soil.

The usual portal of entry in humans is through tiny cuts and abrasions or through contact with the conjunctiva. Infection has also been reported after prolonged exposure of intact skin to contaminated water, and drinking contaminated water supplies.



The many routes of transmission put anyone who has potential contact with animals or contaminated water supplies at risk. Outbreaks have been reported in many different vocational groups, everything from sewer workers to fish farmers to cattle workers. There is also a significant risk associated with any recreational water sport, such as water skiing or canoeing.

### **Clinical presentation**

Weil syndrome, which was first described over 100 years ago by Adolf Weil in Heidelberg, Germany, is the name of the severe syndrome associated with leptospirosis. However, as with many diseases, there is indeed a spectrum of clinical manifestations. The majority of cases have subclinical or mildly symptomatic courses called 'anicteric leptospirosis'. In this presentation, individuals may present with fever, which may be episodic and occur every 3 days, myalgias, headaches and a transient rash. A minority of these patients will also have an aseptic meningitis. Symptoms are usually mild and usually remit after 1 week.

About 5–10% of patients will present with the more serious syndrome, otherwise known as Weil syndrome or 'icteric leptospirosis'. The disease course is biphasic. After an initial incubation period of 7–14 days, patients present in the 'septicemic phase'. As the name suggests, patients are jaundiced, with fever, severe headache, myalgias and a change in mental status. They will often have hepatomegaly and right upper quadrant pain as well. Though hyperbilirubinemia is present, the aminotransferases may only be minimally elevated. Other complications of multiorgan failure may ensue, including acute renal failure, pulmonary hemorrhage, thrombocytopenia and myocarditis. The overall mortality rate with this presentation is estimated to be between 5 and 15%. The next phase of the illness, if the patient recovers from the first phase, is the 'immune phase'. Complications in this phase of the illness occur from immune-mediated damage. It has been postulated that symptoms and pathology during this period are related to immune-complex formation and subsequent inflammation. Patients typically have central and peripheral nervous system involvement.

### **Diagnosis**

Even though the clinical diagnosis of leptospirosis is almost always a challenge, the laboratory diagnosis is usually simple. During the initial phase of the disease, leptospires can be cultured from the blood or cerebrospinal fluid (CSF). The culture may take as long as 30 days to show any growth and therefore prolonged incubation is essential. A positive culture is diagnostic. During the second phase the organism can be seen in the urine, by dark field microscopy, which may remain positive for months. Serologic diagnosis is more commonly employed. The microagglutination test (MAT) is the generally recognized basis for serodiagnosis. This test is available only from the US Centers for Disease Control and Prevention, and can recognize specific serotypes.

### **Treatment**

The evidence for effective antimicrobial therapy is not conclusive. There is evidence that doxycycline administered at a dose of 100 mg twice a day may be helpful in reducing duration and severity of symptoms in the anicteric presentation. There is also some evidence that doxycycline at a dose of 200 mg once per week may be effective as short-term prophylaxis for

travelers to endemic areas. However, the evidence for antibiotic treatment in more serious cases is not conclusive. While penicillin may offer some benefit in reducing symptoms and disease duration if introduced very early in the course of symptoms, it has not been shown to reduce mortality if administered after 4 days of symptoms. After this duration of disease, antibiotics have little effect on disease outcome and mortality. One recent study did explore the use of ceftriaxone compared to penicillin and found them to be equally effective in time to fever resolution in severe leptospirosis. There was no difference in organ dysfunction or mortality between these two groups.

### **Syphilis**

#### **Incidence**

Syphilitic hepatitis is a rare presentation of an extremely common and treatable disease. It has been identified for over 400 years, yet still causes diagnostic confusion; and thus, the incidence of syphilitic hepatitis is difficult to estimate. Jaundice is reported to occur in approximately 1–12% of syphilis cases. However, when random patients were evaluated for liver test abnormalities in the setting of their diagnosis of syphilis, over 50% of patients had evidence of hepatitis.

#### **Presentation**

Hepatitis may be part of primary, secondary or tertiary syphilis, but is most commonly seen in the setting of the rash of secondary syphilis. Thus, this diagnosis should be considered in any patient with risk factors for syphilis who presents with a maculopapular eruption, particularly of the palms and soles, fever, arthralgias and diffuse adenopathy. The history of a primary lesion (chancre) may or may not be obtained, as these are painless and may be unreported by the patient unless in a site visible to the patient. There may be slight elevations in AST and ALT and usually there is disproportionately elevated alkaline phosphatase.

#### **Diagnosis**

Given the rareness of this entity, other possible causes for hepatitis should be explored and excluded such as viral or autoimmune hepatitis. Laboratory values will confirm syphilis with the presence of a positive Venereal Disease Research Laboratories (VDRL) test, rapid plasma regain (RPR) test and fluorescent treponemal antibody absorption (FTA-ABS) test. Both false positive and false negative serologic tests are possible. False positive tests may occur as a result of autoimmune disease, hepatitis C and HIV, whereas false negative tests may be a result of early testing (during primary disease) or the prozone reaction, which is caused by the presence of high antibody titers in vast excess of the antigen used for the diagnostic test. If syphilis is strongly suspected, the laboratory should be asked to repeat the testing with diluted serum, which will reveal reactivity. If performed, liver biopsies often demonstrate active inflammation. There have been reports of focal necrosis and granuloma formation in these patients. However, hepatic architecture is usually well preserved. Treponemia may or may not be present on biopsy. Histological changes are thought to vary depending on virulence and duration of spirochetemia.

**Treatment**

Syphilitic hepatitis responds to the appropriate antimicrobial treatment, which should be determined based on the expected stage of syphilis (primary, secondary, latent or neurosyphilis). Penicillin is used for the treatment of syphilis, the dose of which is determined by the stage of disease. Patients with HIV, in particular, should be carefully examined for the presence of neurologic involvement and treated accordingly. Patients with hepatitis as a manifestation of their syphilis seem to have a high incidence of a Jarisch-Herxheimer reaction upon initiation of treatment. This reaction is a systemic process involving myalgias, fever, chills and hypotension upon initiation of antibiotics. It is thought to be caused from the release of a pyogen from the dying spirochetes and may last up to 1 day. Some authors have pretreated patients with steroids, or administered non-steroidal anti-inflammatory drugs (NSAID) if the reaction occurs. The reaction is usually self-limited.

**GRANULOMATOUS DISEASES OF THE LIVER**

A common clinical conundrum is the finding of granulomas on liver biopsy. While there is a vast list of conditions that can cause granulomas, in this section we will focus on the infectious pathogens that most commonly present as granulomas of the liver. Some less common conditions are presented in **Table 17.1**.

Less common causes of granulomatous hepatitis		
Infection	Clues from clinical history	Diagnostic test
Histoplasmosis	History of exposure in Ohio or Mississippi river valley	Urinary histoplasma antigen
Coccidiomycosis	History of exposure in southwest USA	Serologies
Brucellosis	Exposure to animal tissues, ingestion of unpasteurized dairy products	Serologies
Syphilis	History of chancre Unprotected sexual activity	RPR
Q fever	Direct contact with infected animals (ex. an abattoir worker) or exposure to unpasteurized dairy products	Serologies
CMV	Mononucleosis-like syndrome if immunocompetent Transplant patient HIV positive patient	CMV pp65 Monospot-negative CMV PCR
EBV	Mononucleosis syndrome	Monospot
Schistosomiasis	Exposure to endemic area	Detection of eggs in stool or urine
<i>Bartonella</i>	Exposure to cats	Culture or serologies
CMV, cytomegalovirus; EBV, Epstein-Barr virus; PCR, polymerase chain reaction; RPR, rapid plasma reagin.		

**Table 17.1** Less common causes of granulomatous hepatitis

**Mycobacterium tuberculosis****Background**

Primary infection of the liver with *Mycobacterium tuberculosis* (MTB) is rare. However, with the increase in frequency of MTB, especially in immunosuppressed and chronically ill patients, MTB of the liver has been reported. Few primary cases have been identified though they do exist. Most cases identified are in patients who also have pulmonary or gastrointestinal manifestations of disease with MTB, and thus represent a constellation of findings. Hepatic MTB is found in 50–80% of patients dying of pulmonary MTB and in 91% of these patients at autopsy.

**Clinical presentation**

Hepatic MTB should be considered in any patient with fever, right upper quadrant pain and abnormal abdominal imaging, particularly in patients with radiographic findings compatible with pulmonary tuberculosis. Hepatic MTB usually presents as one of three entities:

- miliary MTB of the liver associated with generalized miliary MTB,
- primary hepatic miliary MTB without other organ involvement, or
- primary nodular lesion called a tuberculoma or a frank abscess.

The nodular form is often mistaken for a malignancy. Patients will often have normal AST and ALT on presentation with hepatic MTB. The most common laboratory abnormalities are elevated alkaline phosphatase, hypoproteinemia and hyperglobulinemia.

**Diagnosis**

A tissue sample should be obtained by either CT-guided or ultrasound-guided biopsy or by laparoscopy. If the biopsy reveals caseating granulomas, it is considered diagnostic. However, often non-caseating granulomas will be present in MTB. If this occurs, diagnosis can be established by acid-fast stain, culture or PCR. Acid-fast stain and culture are more likely to be positive in cases of miliary TB.

**Treatment**

Patients should be treated with four-drug MTB therapy typically consisting of isoniazid, rifampin (rifampicin), ethambutol and pyrazinamide. Large abscesses usually require percutaneous drainage. In abscesses that do not respond to medical therapy and percutaneous drainage, surgical removal may be indicated.

**Mycobacterium avium intracellulare (MAI)****Background**

MAI is an ubiquitous saprophytic organism. While exposure is common, disease from this organism is rare. Disease is thought to occur either through inhalation or ingestion. Although disease in normal hosts has been reported, individuals with compromised T-cell immunity, particularly those with HIV, are particularly susceptible to infection with MAI, which typically presents as disseminated disease. MAI is the most commonly reported hepatic opportunistic infection in patients with AIDS.

**Clinical signs and symptoms**

Patients usually develop MAI late in the course of their HIV infection, when CD4 counts are less than 100/mm<sup>3</sup>. They often present with fever, weight loss, anorexia and diarrhea from

disseminated disease. With liver involvement they will have hepatomegaly, elevated alkaline phosphatase and a mild elevation in liver enzymes. Alkaline phosphatase elevation is likely from obstruction of the terminal branches of the biliary tree by granulomatous inflammation.

### Diagnosis

Lysis centrifugation of blood cultures may yield the organisms, although recovery may take several weeks. If performed, liver biopsy specimens should be stained for acid-fast bacilli. Granulomatous hepatitis may be present, but granuloma formation can be altered in patients with late-stage AIDS; because of their immune dysfunction, they are less likely to generate the response necessary to form granulomas.

### Treatment and prophylaxis

Treatment of disseminated disease should always include at least two agents that are active against MAI. Every regimen should contain either azithromycin or clarithromycin; commonly, ethambutol is implemented as the second drug. Many clinicians have added one or more of the following as second, third or fourth agents: clofazimine, rifabutin, rifampin (rifampicin), ciprofloxacin, and in some situations amikacin. Patients with HIV infection and CD4 counts less than 50 mm<sup>3</sup> are at particular risk for MAI infection and should be placed on prophylactic treatment. Either clarithromycin or azithromycin is the agent of choice for chemoprophylaxis.

## Leprosy

### Background

Leprosy, or Hansen disease, was first identified in 1874 by the Norwegian physician, Gerhard Henrik Armauer Hansen. It is a chronic infectious disease caused by the organism, *Mycobacterium leprae*. It is a systemic disease that normally affects the skin and peripheral nerves. Leprosy has been documented since ancient times and is still endemic in some tropical and subtropical, developing countries. It has long been feared as an incurable disease and its victims have been subject to discrimination and ostracism. In the Old Testament, it was considered divine punishment for sin. Its name originates from the Latin word *lepros*, which means defilement. In the early 1990s, the World Health Assembly started a campaign to eliminate leprosy by 2000. At that time the incidence worldwide was estimated to be 12/10 000 people. After implementation of multidrug therapy throughout the world, the current prevalence globally is now decreased to 1/10 000 people with 83% concentrated in India, Brazil, Burma, Indonesia, Madagascar and Nepal.

*M. leprae* cannot be cultured in vitro which has made studying the organism in culture very difficult. It can grow in the footpads of mice and in the footpads of nine-banded armadillos. Armadillos remain a reservoir for the disease and contribute to the incidence in humans in the southwest USA. The main route of transmission is probably through aerosol spread. The organism then infects the nasal and respiratory mucosa. It cannot be transmitted through intact skin.

### Clinical presentation

Based on the Ridley-Jopling classification, Leprosy has two distinct phenotypic presentations: tuberculoid and lepromatous with borderline categories in between the two. Tuberculoid

leprosy generally causes relatively few large macular hypopigmented lesions or erythematous anesthetic lesions which are well defined with raised margins. Lepromatous leprosy, on the other hand, causes more widespread disease. Visceral involvement is common in these cases. The World Health Organization (WHO) has a simpler classification which takes into account the number of lesions, but bases the classification mostly on the number of acid-fast bacilli (AFB) seen on the smear. These categories fall into multibacillary and paucibacillary and largely correlate with the lepromatous and tuberculoid categories respectively. These classification systems are used equally to describe clinical presentations, so clinicians should be familiar with both.

In multibacillary leprosy, the incidence of liver involvement is between 48 and 100%. It is considered rare in cases of tuberculoid or paucibacillary leprosy. Most patients with visceral organ involvement, however, remain asymptomatic with normal liver tests. Abnormal liver tests, however, do not correlate to number of granulomas or AFB on biopsy. Abnormal liver tests in leprosy are so rare that other causes of concomitant liver disease should be excluded.

### Diagnosis

The diagnosis of leprosy is based on the following clinical criteria:

- hypopigmented or reddish patches with definite loss of sensation,
- thickened peripheral nerves, and
- AFB on skin smears or biopsy material.

On liver biopsy, granulomas will be seen. The histology of the granuloma varies. The term 'lepromatous hepatitis' is reserved for limited portal and centrilobular granulomas, with or without lymphocytes, minimal fibrosis and multiple AFB. PCR is available, but is not used in clinical practice.

### Treatment

The first-line drugs for treatment of leprosy are rifampin (rifampicin), clofazimine and dapsone. All patients should receive a multidrug regimen with monthly supervision. Length of treatment and combination of drugs remain controversial. In the USA, the standard treatment regimens seem to differ slightly from the WHO recommendations. In the USA, multibacillary disease is treated with dapsone, rifampin (rifampicin) and clofazimine for 2 years. Paucibacillary disease is treated with dapsone and rifampin (rifampicin) alone for 1 year. The WHO recommends rifampin (rifampicin) only monthly instead of daily, and the treatment courses are much shorter. Adjunctive therapy, like corticosteroids and thalidomide, may be needed for neuropathies and erythema nodosum.

## LIVER ABSCESES

The most common cause of liver abscess formation is bacterial or pyogenic abscesses. For the purpose of this discussion, however, we will focus on the other causes of liver abscesses including echinococcus and amebiasis.

## Echinococcosis

### Life cycle

Echinococcosis is caused by the larval stages of various tapeworms (cestodes) from the genus *Echinococcus*. Two of these



species are well-known causes of liver disease in humans worldwide. *Echinococcus granulosus* causes cystic echinococcosis which is otherwise known as cystic hydatid disease. *Echinococcus multilocularis* causes alveolar echinococcosis or alveolar hydatid disease.

*E. granulosus* is a small tapeworm that rarely exceeds 7 mm in length. The adult worm lives firmly attached to the mucosa of the small intestine of its definitive host, most commonly the dog. The definitive host for *E. multilocularis* is usually the red or arctic fox although dogs and cats have also been implicated. Ungulates are usually the intermediate host for both species, but humans can be accidentally infected by ingesting feces containing gravid proglottids, each of which contain several hundred eggs. Once these eggs infect humans, a larva is released. The larva then penetrates the intestinal epithelium and is transported through the blood stream to target organs such as the liver and lungs. Once the target organs have been penetrated, a fluid-filled cyst begins to develop. Once this cyst differentiates into multiple layers, the hydatid cyst is formed. The adult forms can then develop within the cyst. These adult forms, known as protoscolices can develop into secondary cysts or into mature worms.

### Epidemiology

*E. granulosus* infection can occur worldwide while *E. multilocularis* seems to only occur in the northern hemisphere. Cystic hydatidosis is a significant public health problem in South and Central America, the Middle East, some sub-Saharan countries, China and the former Soviet Union. In the last several years, *E. multilocularis* has become an increasingly serious public health issue. In some parts of northern China and Alaska, for instance, the prevalence has been reported as high as 5%. In endemic areas of southern Germany, eastern France and northern Switzerland, the estimated prevalence is 1–10/100 000. The recent increased prevalence in these regions has been attributed to a sharp increase in the wild fox population.

### Pathology and clinical signs

*E. granulosus* causes well-demarcated cysts most commonly in the liver, but also in the lungs and other organs such as the kidney, spleen, heart and brain. Clinical signs and symptoms can be highly variable depending on the location of the cysts in the liver. The fully developed hydatid cyst is usually filled with fluid. These cysts may contain thousands of organisms and liters of fluid. They usually cause damage by the process of displacing host tissue and vessels. Rarely, the cysts can rupture. When this occurs, the massive release of cystic fluid into the peritoneum can cause an anaphylactic reaction and then potentially disseminated secondary echinococcosis.

*E. multilocularis* lesions are almost exclusively found in the liver though rarely they too can form in the lungs, brains and other organs. The hepatic lesions are usually made up of scattered areas of small cysts and vesicles. These cysts are typically slow-growing, with an estimated incubation period of 5–15 years. Unlike *E. granulosus*, the lesions are not usually well delineated from surrounding uninfected tissue. In advanced disease, a central area of necrosis will form. Scattered areas of calcification may also be seen. Similar to disease caused by *E. granulosus*, *E. multilocularis* can cause symptoms from the mass effect of the enlarging cysts. These cysts upon rupture can cause biliary

obstruction, pancreatitis and cholangitis. Their mass effect has also been implicated in cases of Budd-Chiari. Secondary bacterial infection of the cysts can result in liver abscesses.

### Diagnosis

Diagnosis of echinococcosis can be extremely challenging. At first glance, the signs and symptoms may not differ much from other forms of liver disease. Laboratory values may reveal a peripheral eosinophilia and abnormal liver tests. Non-invasive imaging studies are usually first applied and then combined with immunologic studies. Sonography can be used consistently to identify cysts in the liver (**Fig. 17.1**). Parasitic hepatic cysts can be distinguished from non-parasitic hepatic cysts, for which the prevalence has been reported as high as 2.5%, by identifying a defined wall surrounding the cyst. Occasionally, actual parts of the parasite can be identified within the cyst by sonography. Sonography, though highly specific for identifying echinococcal cysts can have somewhat limited sensitivity early in infection, particularly in the first year of infection. Unless the lesion is calcified, the limit of detection of ultrasound is a 1.5 cm lesion. Echinococcal cysts have been reported to grow at a rate of 0.5–1.5 cm/year. Recently, more advanced modalities such as contrast-enhanced wide-band Doppler ultrasonography and positron emission tomography (PET) scans have been shown to be helpful in increasing sensitivity of imaging techniques in aiding diagnosis.

Serology, though frequently tested either by enzyme-linked immunosorbent assay (ELISA) or latex agglutination, has a low sensitivity, in one study ranging from 28 to 48%. The specificity of these tests is generally good, however positive serology may not differentiate between active and past infection. Differentiating *E. granulosus* from *E. multilocularis* as well as establishing diagnosis in more challenging cases in which serologies may be negative can be accomplished by performing PCR on the DNA in the cyst fluid.

### Treatment

The standard of care for echinococcal cysts is still evolving. In the past, the approach has been surgical removal of the cysts.



**Figure 17.1** Ultrasound of multiple hydatid cysts. (Courtesy of Dr Sanaa Kamal.)

The procedure has long been associated with high morbidity and mortality. Now with the advent of effective chemotherapeutic agents, namely the benzimidazole carbamate derived agents, mebendazole and albendazole, achieving cure without removing the cysts surgically has been more promising. Most providers advocate a combined approach of percutaneous treatment by PAIR (puncture, aspiration, injection and reaspiration) combined with pre- and post-procedure chemotherapy.

## Amebiasis

### Epidemiology

It is estimated that amebiasis affects 500 million people each year. 100 000 people are thought to die each year from this disease. Amebiasis is caused by the protozoan *Entamoeba histolytica*. Although this disease can be a cause of morbidity and mortality worldwide, it is more likely to affect individuals living in, or immigrating from, developing countries. For the purpose of this chapter, we will focus on amebic liver abscesses, which is the most common manifestation of extraintestinal amebiasis. Amebic liver abscess can occur even after short-term travel to endemic areas. Men are 10 times more likely than women to have amebic liver abscesses, and immunosuppression also increases the risk of liver abscess formation.

### Life cycle and pathogenesis

Infection in the human host normally begins after ingestion of fecally-contaminated water or food containing *E. histolytica* cysts. The cysts then can safely travel through the stomach and small intestine. In the large intestines, motile and potentially invasive trophozoites emerge from the cysts. In most cases the trophozoites remain in the intestinal mucin layer forming new cysts that result in a self-limited and asymptomatic infection. The cysts can then be excreted in the stool and cause further infection through fecal-oral transmission. However, when the trophozoites invade the mucosal layer and intestinal epithelium, colitis, further infection of the liver and other organs via the portal system and hematogenous spread may occur.

Interestingly, *E. histolytica* has several mechanisms by which it may evade the immune system and thereby establish chronic infection. On its surface, the trophozoite has a lectin molecule that allows it to attach to the colonic mucosa. This lectin molecule has antigenic cross-reactivity with the human leukocyte antigen, CD59. Also, it seems that the amebic cysteine proteinases rapidly degrade C3a and C5a in the complement pathway. These proteinases also have shown the ability to degrade secretory immunoglobulin A (IgA) and serum immunoglobulin G (IgG). Lastly, these organisms have the ability to suppress the macrophage respiratory burst and down-regulate the presentation of antigens by major histocompatible complex (MHC) class II molecules.

### Clinical signs and symptoms

Infection with *E. histolytica* is asymptomatic in 90% of individuals. In the 10% who do experience symptoms, more than 90% of these individuals will have intestinal amebiasis. This may manifest itself as colitis with or without necrosis, toxic megacolon, or even as a perianal fistula. Patients with amebic liver abscesses normally present with fever, right upper quadrant pain or a dull generalized abdominal pain 8–20 weeks after exposure. Diarrhea is present in less than one-third of

infected individuals, although sometimes there is a more chronic presentation. Involvement of the diaphragmatic surface of the liver may result in cough, pleural pain or referred shoulder pain. Less commonly, patients may have vague gastrointestinal symptoms such as nausea and vomiting, or change in their stool habits. On physical examination, the most common finding is hepatomegaly. Laboratory findings may consist solely of leukocytosis and anemia. If the infection is acute, liver tests may be within normal limits except for an elevated ALT. However, if the infection is chronic, ALT is likely to be normal, but alkaline phosphatase may be elevated.

### Diagnosis

Amebic abscesses can typically be identified either by ultrasound, CT or MRI. Once an abscess had been identified it is important to consider risk factors for amebiasis. Amebic abscesses are usually solitary and located in the right lobe of the liver, although multiple abscesses are often seen (Fig. 17.2). Since the radiographic features are indistinguishable from pyogenic abscess, specific serologic testing may be performed. Serologic tests will be negative in very early infection (>1 week) but will ultimately be positive in nearly all individuals with amebiasis; the problem is that such tests remain positive for life and will not distinguish past infection from current disease in residents of endemic areas. Aspiration of the lesion is usually not necessary, unless it is needed to eliminate other possibilities, such as a pyogenic abscess. If aspiration is done, the fluid is typically described as 'anchovy paste', and consists mostly of necrotic hepatocytes. Trophozoites are seen in less than 20% of cases and usually just in the peripheral part of the lesion.

There is actually another species that looks identical to and can be mistaken for *E. histolytica*, *Entamoeba dispar*. However, *E. dispar* is considered non-pathogenic. Differentiating between the two species is thus of extreme clinical importance, but relying solely on appearance is difficult. *E. histolytica* trophozoites are more likely to contain erythrocytes that have been ingested, but otherwise the two species can appear identical. Microscopy of stool is also only about 60% sensitive. The most reliable tests are



**Figure 17.2 Amebic abscess.** (Courtesy of Dr Sanaa Kamal.)

antigen detection in the stool, *E. histolytica*-specific DNA by PCR, or isoenzyme analysis of stool culture. Stool culture, though more sensitive than microscopy, can take up to 1 week; so antigen testing, which usually takes 2–3 h, and PCR, which may take 2–3 days, may prove to be more useful in establishing a diagnosis of *E. histolytica*.

### Treatment

Commonly, treatment for amebic liver abscess is metronidazole (500–750 mg orally three times daily for 7–10 days). This therapy is effective in about 90% of patients. If patients are slow to respond or relapse following therapy, aspiration and/or a prolonged course of metronidazole should be considered. Intraluminal infection should also be treated even if stool studies are negative. Either Paromomycin or Iodoquinol can be used to treat intraluminal disease.

## PARASITIC INFECTIONS OF THE LIVER AND BILIARY TREE

### Schistosomiasis

#### Background

In the mid-19th century Theodor Bilharz first described a parasitic infection called bilharzia that would later become known as schistosomiasis. It is caused by a trematode blood fluke from the genus *Schistosoma*. The species that infect humans are *Schistosoma hematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma intercalatum* and *Schistosoma mekongi*. *S. mansoni* and *S. japonicum* are the two species that most commonly infect the liver and biliary tree, while *S. hematobium* infects the bladder and urinary tract. There are over 70 countries in tropical and subtropical areas of Asia, South America, Africa and the Caribbean that are endemic for schistosomiasis. It is estimated that over 200 million people have this infection, and that 120 million are symptomatic.

#### Life cycle

Infection is caused by direct contact with water that contains free-swimming larvae. These larvae penetrate the skin of humans or other hosts. The larvae shed their tails and the resulting schistosomula penetrate the capillaries and lymphatic vessels and enter the lungs mostly through the venous system. They then enter the circulation in the left heart and travel to the liver through the portal system. The worms mature in the portal system. From the systemic circulation they may then travel to either the liver or the urinary tract where they produce eggs. Eggs are shed in the urine or feces. If the eggs are retained in tissue, chronic schistosomiasis occurs. The eggs can hatch in freshwater and infect snails where the life cycle commences again.

#### Clinical presentation

At the site of inoculation with the larvae, a local response may occur. In primary infection, it usually consists of a maculopapular eruption. In sensitized individuals, 'swimmer's itch', a more severe dermatitis may occur at the site of infection. Acute schistosomiasis, otherwise known as Katayama fever, usually occurs within 2–3 weeks of freshwater exposure in endemic areas. Symptoms include fever, myalgias, right upper quadrant

pain and bloody diarrhea. Respiratory symptoms are also reported. Tender hepatomegaly is present as is splenomegaly in certain cases. Patients will usually have a peripheral eosinophilia.

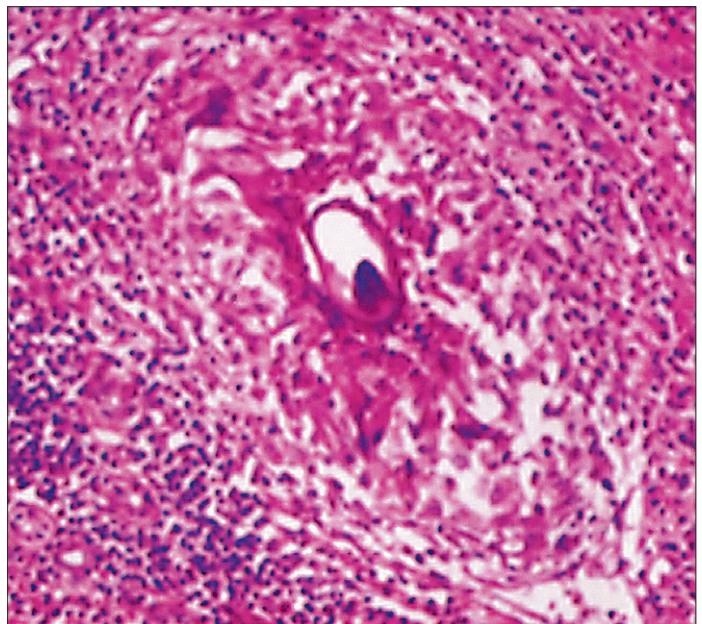
In chronic liver involvement, patients develop granulomatous disease. The granuloma formation and inflammation from the retained eggs cause presinusoidal inflammation and periportal fibrosis (Fig. 17.3). This condition is referred to as clay-pipe-stem fibrosis. It is a major cause of portal hypertension in endemic areas. In certain areas, such as Egypt, this problem is compounded by co-infection with hepatitis C; the two infections together result in a very rapid progression to advanced fibrosis compared to either infection alone.

### Diagnosis

Definitive diagnosis can be made by detecting the eggs in the feces or urine. Antibody testing can be helpful in ruling out infection, but it does not distinguish between past or present disease. Recently, an antigen test has been developed. Two parasite proteins, circulating anodic antigen (CAA) and circulating cathodic antigen (CCA), are present in the blood during infection. These proteins may be detected by ELISA in active infection.

### Treatment and prophylaxis

Treatment of schistosomiasis consists of praziquantel with a single dose of 40 mg/kg. If infection with *S. japonicum* is confirmed or even suspected a higher dose of 60 mg/kg should be implemented. Feces or urine should be re-examined after 1 month to confirm cure. If praziquantel is ineffective, oxamniquine can be used as an alternative. In acute infection, if there is cerebral involvement, corticosteroids may prove to be helpful in reducing cerebral edema. Artemisinin in combination with praziquantel can be used for chemoprophylaxis. However, the best form of prevention is avoidance of contaminated water sources. Travelers should avoid swimming in fresh-water in endemic



**Figure 17.3** Liver biopsy schistosomiasis egg causing granulomatous hepatitis. (Courtesy of Dr Sanaa Kamal.)



areas. In these areas, bathing water should be heated for 5 min prior to use and pretreated with chlorine or iodine. Some sources also suggest allowing bath water to stand for 3 days prior to use as the larvae only survive for 48 h.

## Ascariasis

### Background

Ascariasis is a helminthic infection caused by the nematode *Ascaris lumbricoides*. *A. lumbricoides* is both the most prevalent and largest of human helminths. Adult worms can be up to 50 cm in length and 6 mm in width. Ascariasis is most commonly seen in crowded rural areas. It is related to the density of population, dietary habits, sanitation and agricultural customs. It is estimated that 1.4 billion people are infected with *Ascaris*. Ascariasis is a global problem as it is endemic in parts of Southeast Asia, China, India, Africa and Latin America. In the USA, it is estimated that over 4 million people are infected. A large percentage of those infected in the USA are thought to be immigrants from endemic areas.

### Life cycle

Infection with *Ascaris* usually occurs after ingestion of embryonated eggs that exist in the hospitable environment of moist soil. These eggs can commonly infect water and food supplies. After ingestion, the outer layer of the egg is digested by gastric juices. The larvae emerge in the duodenum. In the small intestine, the larvae can penetrate the intestinal mucosa and enter the portal system thereby entering the liver and then freely move either through the bloodstream or the lymphatic system throughout the body. Once the larvae reach the bronchial tree and upper respiratory tract, they are then swallowed and re-enter the digestive system. At this point, they undergo a sexual maturation in the small intestine that takes approximately 3 months. The adult worm then is able to exist in the lumen of the human intestine through the use of its muscles. Its lifespan may be anywhere from 6 months to up to 2 years during which time the female adult will release millions of egg into the feces.

### Clinical signs and symptoms

The clinical presentation of ascariasis is very much related to the organism's life cycle. In most hosts, the infection is asymptomatic. However, if the organism burden is high enough, clinical disease will be present. Hepatobiliary ascariasis is one of the most common manifestations of this disease. Not only can the larvae infect the liver, but the worm itself can enter the hepatic ductal system from the ampulla of Vater in the small intestines. Therefore, the spectrum of disease ranges from hepatic abscess to biliary disease. In the liver, live larvae do not cause an inflammatory response. However, if the organism dies in the liver, or in any organ for that matter, a granulomatous reaction occurs. In some areas ascariasis is as common as gallstones as a cause of biliary disease. Given the range of pathophysiology, the symptoms can be anything from simple biliary colic to vague weight loss and epigastric discomfort to fever and intense right upper quadrant pain. Protein malnutrition, especially in children, is a common manifestation of ascariasis. Depending on the degree of liver involvement and/or biliary

disease accounting for any obstruction, liver enzyme tests may be abnormal. While the worm is present in the bloodstream of the patient a peripheral eosinophilia will also be present.

### Diagnosis

Ascariasis can be diagnosed by finding the worms, larvae or eggs in the feces. If the infection is only with male worms, no eggs will be detected in the stool. Diagnosis of hepatobiliary ascariasis is usually made with ultrasound and/or endoscopic retrograde cholangiopancreatography (ERCP). Ultrasound is useful in detecting worms in the biliary tree and hepatic abscesses. On ERCP, ductal ascariasis will present as smooth linear filling defects. ERCP allows the added benefit of then removing the worms from the ductal system and, if present, in the duodenum. Liver abscesses caused by *Ascaris* have no distinct clinical features and percutaneous needle aspiration is often needed for both therapeutic and diagnostic purposes. Examination of the fluid will reveal broken or collapsed eggs. The necrotic focus in the liver is usually surrounded by granulomatous hepatitis.

### Treatment

Treatment of hepatobiliary ascariasis usually consists of supportive care for the cholangitis/pancreatitis that may be present as well as oral administration of antihelminthic agents to paralyze the worms in the intestine. Antihelminthic agents that are commonly used include pyrantel pamoate, mebendazole and albendazole, all administered as single doses. Parasite paralysis and death with clearance from the intestine can take up to 3 days. Endoscopic intervention with worm extraction is performed if patients do not respond to treatment within a few days or if after 3 weeks the worms in the ducts have not migrated out into the intestine.

## Liver flukes

### Life cycle and epidemiology

The groups of parasitic trematodes commonly referred to as the liver flukes include the *Opisthorchis* and *Clonorchis* species as well as *Fasciola hepatica*. These organisms asexually reproduce in their intermediate host, which is usually the snail. They then multiply into thousands of free-swimming cercariae which penetrate freshwater fish and are then infective to humans when eaten raw, as in the case of *Opisthorchis* and *Clonorchis*, or encyst on plants like watercress, as is the case with *Fasciola*. In the human, they undergo sexual reproduction. As adults, they are flat, hermaphroditic worms that range in size from a few millimeters to several centimeters. They pass directly into the bile ducts through the ampulla of Vater. *F. hepatica* can actually penetrate the intestinal mucosa into the peritoneum and then enter the liver and the biliary tree from there. They then excrete their eggs into feces. The adult worm can survive for decades in the bile ducts.

Epidemiologically, *Opisthorchis* and *Clonorchis* are very similar. They are found primarily in Eastern Europe and Asia. *Clonorchis* is endemic in China, Hong Kong, Japan, Taiwan and Vietnam, where dogs and cats can serve as reservoirs. *Fasciola*, however, is quite distinct epidemiologically from *Clonorchis* and *Opisthorchis*. It primarily affects sheep and cattle and can be found where they are raised worldwide.

**Clinical course**

Most people infected with liver flukes are asymptomatic. However, in acute infection, if the worm burden is high enough, patients will be symptomatic. Patients will present with fever, right upper quadrant pain, anorexia, and diarrhea 10–26 days after ingestion of the larvae that may last a few days to weeks during the migrating stage of the larvae. Liver tests and imaging are likely to be normal, but patients will have a peripheral eosinophilia. Over time the flukes can cause mechanical injury to the bile ducts. They are also thought to secrete substances that are toxic and immunogenic in the biliary tree. This inflammation results in periductal fibrosis, bile duct dilatation and epithelial hyperplasia. This pathology places patients at risk for cholangitis, cholangiohepatitis and even cholangiocarcinoma.

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**Diagnosis and treatment**

Diagnosis of liver fluke infection can be accomplished by demonstrating eggs in the stool, bile or ascitic fluid. However, stool studies may be negative as eggs may only be produced intermittently. An ELISA test for antiparasitic antibodies and antigens can then be performed. *Opisthorchis* and *Clonorchis* can be successfully treated with one single oral dose of praziquantel. *Fasciola*, however, is best treated with bithionol which can be obtained in the USA from the Centers for Disease Control and Prevention (CDC). *Fasciola* is relatively resistant to praziquantel, and it has not been shown to be effective in eradicating infection.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 18

## Autoimmune Hepatitis

Christian P. Strassburg, Arndt Vogel and Michael P. Manns

### INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory disease of the liver, characterized by a loss of tolerance against hepatic tissue. Historically, AIH was first defined by Waldenström in 1950 when he described a form of chronic hepatitis in young women showing jaundice, elevated  $\gamma$ -globulins and amenorrhea, which eventually led to liver cirrhosis. This form of chronic hepatitis was later also noticed in combination with other extrahepatic autoimmune syndromes. Mainly due to similarities with systemic lupus erythematosus and in particular the presence of antinuclear antibodies (ANA) it was initially termed lupoid hepatitis by Mackay (56). Systematic evaluation of the cellular

and molecular immunopathology, of the clinical symptoms and of laboratory features has subsequently led to the establishment of autoimmune hepatitis as a separate clinical entity which has been subclassified based on serology, and is treated by a specific therapeutic strategy (Table 18.1). A recently established and meanwhile revised scoring system allows for a reproducible and standardized approach to diagnosing and consequently treating AIH in a scientific context (Table 18.2). The use and interpretation of seroimmunological and molecular biological tests together with the exclusion of other etiologies and the scoring system permits a precise discrimination of autoimmune hepatitis from other etiologies of chronic hepatitis, in particular from chronic viral infection as the most common cause of chronic hepatitis worldwide.

#### Differential diagnosis of autoimmune hepatitis and diagnostic tests

<i>Suspected differential diagnosis</i>	<i>Test performed to exclude</i>
Hepatitis C infection (HCV)	Anti-HCV (HCV RNA)
Hepatitis B and D (HBV, HDV)	HBsAg, anti-HBc (HBV DNA) Anti-HDV, HDV DNA only when HBsAg positive
Hepatitis A virus	Antibodies, serology: IgG, IgM
Hepatitis E virus	Only if suspected
Epstein-Barr virus	Only if suspected
Herpes simplex virus	Only if suspected
Cytomegalovirus	Only if suspected
Varicella zoster virus	Only if suspected
Drug induced hepatitis	History, if applicable withdrawal of drug. LKM-2, LM autoantibody in selected cases
Primary biliary cirrhosis	Antimitochondrial antibodies specificity of reactivity: PDH-E2, BCKD-E2 Liver histology: copper deposition in bile ducts Unresponsive to steroids
Primary sclerosing cholangitis	Cholangiography
Wilson disease	Ceruloplasmin, urine copper, eye examination, quantitative copper in liver biopsy
Hemochromatosis	Serum ferritin, serum iron, transferrin saturation, liver histology: iron staining, quantitative iron in biopsy Genetic testing: C282Y, H63D mutation of HFE gene in Caucasoids
$\alpha_1$ -Antitrypsin deficiency	Phenotype testing: PiZZ/PiSS/PiMZ/PiSZ
HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; IgG, immunoglobulin G; IgM, immunoglobulin M; LKM, liver–kidney microsomal autoantibodies; LM, liver microsomal.	

Table 18.1 Differential diagnosis of autoimmune hepatitis and diagnostic tests.



International diagnostic criteria for the diagnosis of autoimmune hepatitis	
Parameter	Score
Gender	
Female	+ 2
Male	0
Serum biochemistry	
Ratio of elevation of serum alkaline phosphatase versus aminotransferase	
>3.0	- 2
1.5-3	0
<1.5	+ 2
Total serum globulin, $\gamma$ -globulin or IgG	
Times upper normal limit	
>2.0	+ 3
1.5-2.0	+ 2
1.0-1.5	+ 1
<1.0	0
Autoantibodies (titers by immunofluorescence on rodent tissues)	
Adults	
ANA, SMA or LKM-1	+ 3
>1:80	+ 2
1:80	+ 1
1:40	0
<1:40	
Antimitochondrial antibody	
Positive	- 4
Negative	0
Hepatitis viral markers	
Negative	+ 3
Positive	- 3
Other etiological factors	
History of drug usage	
Yes	- 4
No	+ 1
Alcohol (average consumption)	
<25 g/day	+ 2
>60 g/day	- 2
Genetic factors: HLA DR3 or DR4	+ 1
Other autoimmune diseases	+ 2
Response to therapy	
Complete	+ 2
Relapse	+ 3
Liver histology	
Interface hepatitis	+ 3
Predominant lymphoplasmacytic infiltrate	+ 1
Rosetting of liver cells	+ 1
None of the above	- 5
Biliary changes	- 3
Other changes	- 3
Seropositivity for other defined autoantibodies	+ 2
ANA, antinuclear antibodies; IgG, immunoglobulin G, LKM-1, liver-kidney microsomal autoantibodies; SMA, smooth muscle antibodies. Interpretation of aggregate scores: definite autoimmune hepatitis (AIH), >15 before treatment and >17 after treatment; probable AIH, 10-15 before treatment and 12-17 after treatment. Reproduced from Alvarez et al (99) by permission of Wiley-Liss, Inc.	

Table 18.2 International diagnostic criteria for the diagnosis of autoimmune hepatitis.

## DEFINITION AND DIAGNOSIS OF AUTOIMMUNE HEPATITIS

AIH is a chronic inflammatory disease of the liver that has usually been present for more than 6 months at the time of diagnosis. In 1992, an international panel met in Brighton, UK, to establish diagnostic criteria for AIH because it was recognized that several features including histologic changes and clinical presentation are also prevalent in other chronic liver disorders. In this and in a revised report the group noted that there is no single specific test for the diagnosis of AIH. In contrast, a set of diagnostic criteria was suggested in form of a diagnostic scoring system designed to classify patients as having probable or definite AIH. According to this approach the diagnosis relies on a combination of indicative features of AIH and the exclusion of other causes of chronic liver diseases (**Table 18.2**). AIH predominantly affects women in any age group, and is characterized by marked elevation of serum globulins, in particular  $\gamma$ -globulins, and circulating autoantibodies. The clinical appearance ranges from an absence of symptoms to a severe or fulminant presentation and responds to immunosuppressive treatment in most cases. An association with extrahepatic autoimmune diseases such as rheumatoid arthritis, autoimmune thyroiditis, ulcerative colitis and diabetes mellitus and a family history of autoimmune or allergic disorders has been reported.

Autoantibodies are one of the distinguishing features of AIH. The discovery of autoantibodies directed against different cellular targets, including endoplasmatic reticulum membrane proteins, nuclear antigens and cytosolic antigens has led to a suggested subclassification of AIH based upon the presence of three specific autoantibody profiles. According to this approach, AIH type 1 is characterized by the presence of ANA and/or anti-smooth muscle antibodies (SMA) directed predominantly against smooth muscle actin. AIH type 2 is characterized by anti-liver–kidney microsomal autoantibodies (LKM-1) directed against cytochrome P450 (CYP) 2D6 and with lower frequency against uridine diphosphate (UDP)-glucuronosyltransferases (UGT). AIH type 3 is characterized by autoantibodies against a soluble liver antigen (SLA/LP) that has been identified as a UGA suppressor serine tRNA-protein complex.

Although the histologic appearance of AIH is characteristic, there is no specific histologic feature that can be used to prove the diagnosis. Percutaneous liver biopsy should be performed for grading and staging, as well as for therapeutic monitoring. Histologic features usually include periportal hepatitis with lymphocytic infiltrates, plasma cells and piecemeal necrosis. With advancing disease, bridging necrosis, panlobular and multilobular necrosis may occur and ultimately lead to cirrhosis. A lobular hepatitis can be present, but is only indicative of AIH in the absence of copper deposits or biliary inflammation. In addition, granulomas and iron deposits argue against AIH.

Viral hepatitis should be excluded by the use of reliable, commercially available tests. The exclusion of ongoing hepatitis A, B and C virus infection is sufficient in most cases. The exclusion of other hepatotropic viruses such as cytomegalovirus, Epstein-Barr virus (EBV) and herpes group viruses may only be required in cases where there is suspicion of such infections or if the diagnosis of AIH based on the above-mentioned criteria remains inconclusive.

The probability of AIH decreases whenever signs of bile duct involvement are present, such as elevation of alkaline phosphatase, histologic signs of cholangiopathy and detection of antimitochondrial antibodies (AMA). If one or more components of the scoring system are not evaluated, a score pointing to a probable diagnosis can be compiled.

## EPIDEMIOLOGY AND PATHOGENESIS

AIH is a rare disorder. Based on limited epidemiological data, the prevalence is estimated to range between 50 and 200 cases per 1 million in Western Europe and North America among the Caucasian population. The prevalence of AIH is similar to that of systemic lupus erythematosus, primary biliary cirrhosis (PBC) and myasthenia gravis, which also have an autoimmune etiology. Among the North American and Western European Caucasian population AIH accounts for up to 20% of cases with chronic hepatitis. However, chronic viral hepatitis remains the major cause of chronic hepatitis in most Western societies. In countries in which viral hepatitis B and C are endemic, such as in Asia and Africa, the incidence of AIH appears to be significantly lower. Additional epidemiological analyses are required to comprehensively elucidate the prevalence and geographical distribution of AIH.

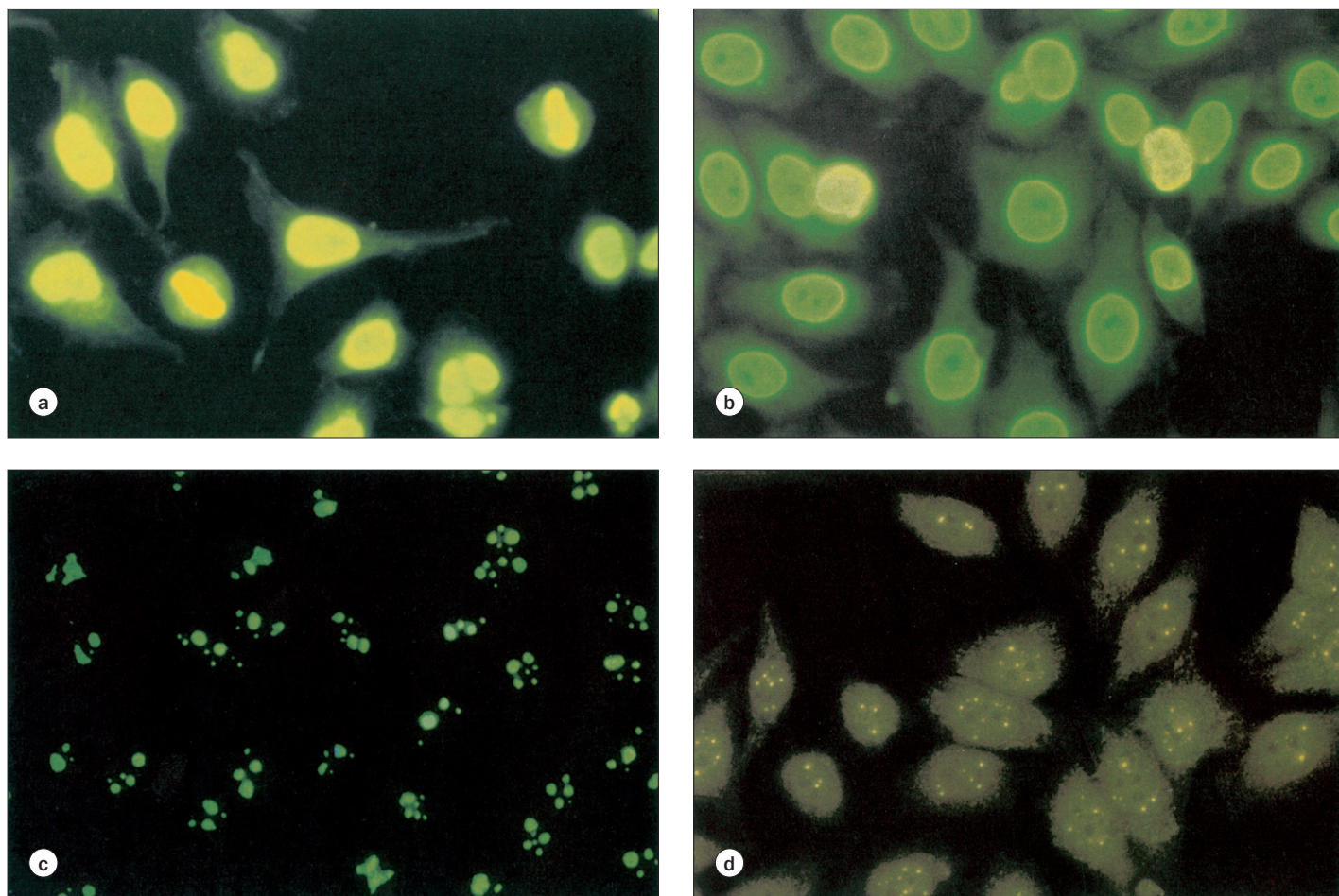
There is no doubt that a loss of self tolerance is the pathophysiological process driving AIH. However, the cause of this disease remains elusive. A number of concepts have been pursued to elucidate the causative agents or mechanisms leading to AIH. The prominent feature of serum autoantibodies has led to the determination, characterization and evaluation of humoral autoimmunity in AIH. In rare cases, evidence has pointed to a viral etiology of AIH, potentially resulting in either a virus triggered, sustained autoaggressive immunological reaction, or an autoimmune reaction directly induced by hepatotropic viruses. Additionally, genetic factors appear to represent a mandatory component of the pathogenesis of AIH, which has been demonstrated through the prevalence of selected HLA alleles in patients suffering from AIH. The determination of genetic markers is at present not part of the routine work-up of AIH patients or the determination of their treatment strategy.

## AUTOANTIBODIES IN AIH

Circulating autoantibodies are a hallmark of AIH. Autoantibodies are the single most important finding determining diagnosis, treatment and discrimination of autoimmune disease from chronic viral infections. The identification, molecular cloning and recombinant expression of hepatocellular autoantigens have enabled the implementation of precise testing systems and the scientific evaluation of humoral autoimmunity associated with AIH. Autoantibodies with significance for AIH are: antinuclear antibodies (ANA), smooth muscle antibodies (SMA), liver–kidney microsomal antibodies (LKM), soluble liver antigen/liver pancreas antibodies (SLA/LP), liver cytosolic (LC-1) and asialoglycoprotein receptor antibodies (ASGPR).

### Antinuclear antibodies

ANA are directed against functional and structural components of the cell nucleus, against nuclear membranes or DNA. The target antigens are a heterogeneous and incompletely defined



**Figure 18.1 Indirect immunofluorescence micrographs of ANA found in AIH and other autoimmune diseases, and detected on immobilized Hep-2 cells.** (a) Typical aspect of homogeneous nuclear staining found in a patient with autoimmune hepatitis (AIH) type 1 with titers exceeding 1:160. These autoantibodies are frequently directed against double-stranded DNA and histones and are a typical finding in type 1 AIH. (b) Aspect of the nuclear membranous (rim) immunofluorescence pattern found in a patient with AIH type 1 at titers exceeding 1:160. In this pattern autoantibodies are directed against lamins (lamin B, but also lamin A and C). Membranous immunofluorescence is not a frequent finding and can indicate the existence of mixed immune syndromes including vasculitis and other features of systemic lupus erythematosus. (c) Indirect immunofluorescence study showing a nucleolar ANA fluorescence pattern. This pattern is rarely seen in AIH, but is common in rheumatological diseases such as scleroderma and polymyositis. If present in AIH type 1, it can be indicative of overlap syndromes with rheumatological disorders. (d) Indirect immunofluorescence study showing multiple nuclear dots. This pattern is not typical for AIH and is mainly found in about 20% of patients with primary biliary cirrhosis (PBC). Usually AMA are present at the same time but can also be missing in cases of antinuclear antibody (ANA)-positive, antimitochondrial antibody (AMA)-negative PBC. These autoantibodies are directed against the sp100 nuclear antigen (100 kDa).

group of cellular proteins. To date, subtyping of the various ANA antigens offers no diagnostic or prognostic advantage. ANA are also detected in PBC, primary sclerosing cholangitis (PSC), viral hepatitis, drug-related hepatitis and alcoholic liver disease, and investigations have been aimed at identifying target antigens that are specific for AIH. ANA are determined by indirect immunofluorescence on cryostat sections of rat liver and on Hep-2 cell slides. Most commonly, a homogeneous or speckled immunofluorescence pattern is encountered. ANA have been found to be reactive with centromeres, ribonucleoproteins and cyclin A. They represent the most common autoantibody in AIH and occur in high titers usually exceeding 1:160 (Fig. 18.1 a–d).

#### Anti-smooth muscle antibodies

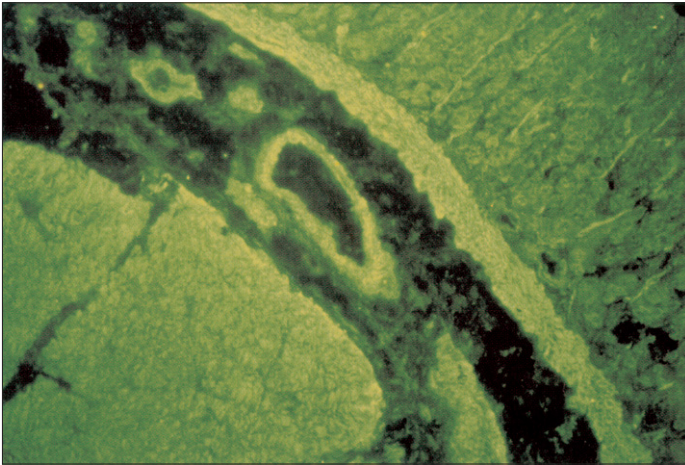
Anti-SMA are directed against components of the cytoskeleton such as actin, troponin and tropomyosin. They frequently occur in high titers in association with ANA. However, SMA auto-

antibodies also occur in advanced diseases of the liver of other etiologies, in infectious diseases and rheumatic disorders. In these cases titers are often lower than 1:80. SMA autoantibodies are also determined by indirect immunofluorescence on cryostat sections of rat stomach (Fig. 18.2). SMA are associated with the HLA A1-B8-DR3 haplotype and, probably more as a reflection of this status, affected patients are reported to be younger at disease onset and have a poorer prognosis.

#### Liver–kidney microsomal antibodies

LKM are directed against proteins of the endoplasmic reticulum (microsomal protein). In 1973, Rizzetto discovered autoantibodies reactive with the proximal renal tubulus and the hepatocellular cytoplasm by indirect immunofluorescence (Fig. 18.3). These autoantibodies termed LKM-1 were associated with a second form of ANA-negative AIH. Between 1988 and 1991 the 50 kDa antigen of LKM-1 autoantibodies was identified as cytochrome

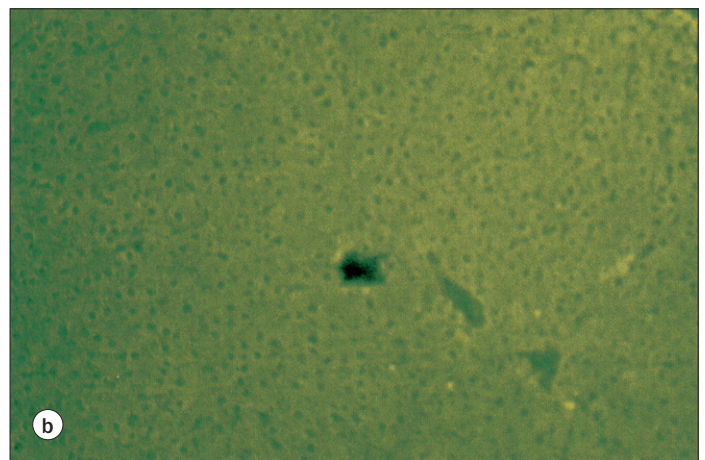
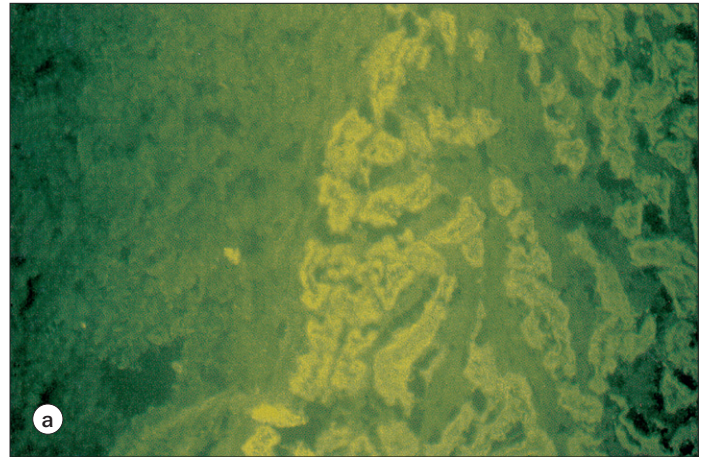




**Figure 18.2 Typical immunofluorescence pattern of SMA autoantibodies detected on rat stomach cryostat sections.** This serum shows immunoreactivity with the muscularis mucosae and muscularis propria layers of rat stomach. Note that the mucosa is excluded from reactivity. This autoantibody is often detected in conjunction with antinuclear antibodies in autoimmune hepatitis type 1.

P4502D6 (CYP2D6). LKM-1 autoantibodies recognize a major linear epitope between amino acids 263 and 270 of the CYP2D6 protein. These autoantibodies inhibit CYP2D6 activity in vitro and are capable of activating liver infiltrating T lymphocytes. This indicates a combined humoral and cellular immune mechanism leading to the development of LKM autoantibodies. In addition to linear epitopes, LKM-1 autoantibodies have also been shown to recognize conformation dependent epitopes. However, the recognition of epitopes located between amino acid 257 and 269 appears to be a specific autoimmune reaction of autoimmune hepatitis and somewhat discriminatory against LKM-1 autoantibodies associated with chronic hepatitis C virus infection. The endoplasmic reticulum based CYP2D6 has been found to be detectable on the hepatocellular surface and its expression appears to be regulated by cytokines.

Antibodies against microsomal proteins form a heterogeneous group spanning several immune-mediated diseases including AIH, drug-induced hepatitis, autoimmune polyendocrine syndrome type 1 (APECED), and chronic hepatitis C (HCV) and D (HDV) (Table 18.3). LKM autoantibodies against CYP1A2 as well as 2A6 are found in patients with APECED and hepatic involvement. Anti-CYP2A6 autoantibodies also occur in HCV infection. LKM autoantibodies, which are characterized by an immunofluorescence pattern selectively staining the hepatocellular but not renal cell cytoplasm, have been found to be directed against CYP1A2. These autoantibodies are also found in APECED syndrome with hepatic involvement and additionally occur in dihydralazine-induced hepatitis. A second type of LKM autoantibodies, LKM-2, are directed against CYP2C9 and are detectable in ticrynafen-associated hepatitis. A third group of LKM autoantibodies, LKM-3, were identified in 6–10% of patients with chronic hepatitis D virus infection (HDV). These autoantibodies are directed against family 1 UDP-glucuronosyltransferases (UGT1A), which are also a superfamily of drug metabolizing proteins located in the endoplasmic reticulum membrane. LKM-3 autoantibodies have been identified in HDV infection but also in AIH type 2 patients. They can also occur in



**Figure 18.3 Indirect immunofluorescence showing LKM-1 autoantibodies on rat kidney and liver cryostat sections.** Serum of a patient with autoimmune hepatitis type 2. (a) Typical indirect immunofluorescence pattern of liver-kidney microsomal (LKM-1) autoantibodies detecting the proximal (cortical) renal tubules (left of micrograph) but excluding the distal tubules (right of micrograph) located in the renal medulla, which corresponds to the tissue expression pattern of the autoantigen CYP2D6. (b) Using rat hepatic cryostat sections a homogeneous cellular immunofluorescence staining is visualized excluding the hepatocellular nuclei (LKM-1).

LKM-1-negative and ANA-negative AIH. In addition, LKM-positive sera display reactivity with a number of to-date undefined antigens with molecular weights of 35 kDa, 57 kDa, 59 kDa and 70 kDa. These autoantibodies are predominantly found in AIH, HCV infection and halothane hepatitis. LKM autoantibodies are visualized by indirect immunofluorescence on rodent cryostat sections. Subclassification is achieved by enzyme-linked immunosorbent assay (ELISA) and Western blot, preferably using recombinant antigens.

#### Antibodies against soluble liver antigen

Antibodies against SLA were detected in a patient with ANA-negative AIH. It is now clear that the liver pancreas (LP) antibodies recognize the same target protein structure leading to the designation SLA/LP autoantibodies. Anti-SLA/LP were found to be highly specific for AIH and are detectable in about 10–30% of all patients with AIH. In 1992, Gelpi et al identified specific autoantibodies present in patients with a severe form of

Molecular definitions of the most important autoantigens relevant to the serological diagnosis of autoimmune hepatitis			
Antibody	kDa	Target antigen	Disease
<b>Autoantigens of the endoplasmic reticulum (microsomal autoantigens)</b>			
LKM-1	50	Cytochrome P4502D6	Autoimmune hepatitis type 2 Hepatitis C
LKM-2	50	Cytochrome P4502C9	Ticrynafen-induced hepatitis
LKM-3	55	UGT1A	Hepatitis D-associated autoimmunity/Autoimmune hepatitis type 2
LKM	50	Cytochrome P4502A6	Autoimmune hepatitis type 2 Autoimmune polyendocrine syndrome type 1 (APS-1) Hepatitis C
LM	52	Cytochrome P4501A2	Dihydralazine-induced hepatitis Hepatitis with autoimmune polyendocrine syndrome type 1 (APS-1)
	57	Disulfidomerase	Halothane hepatitis
	59	Carboxyl esterase	Halothane hepatitis
	35	?	Autoimmune hepatitis
	59	?	Chronic hepatitis C
	64	?	Autoimmune hepatitis
	70	?	Chronic hepatitis C
<b>Autoantigens of the cytosol (soluble liver proteins)</b>			
LC1	58–62	Formiminotransferase cyclodeaminase	Autoimmune hepatitis type 2 Autoimmune hepatitis Hepatitis C?
SLA/LP	50	UGA repressor tRNA-associated protein	Autoimmune hepatitis (type 3)
LC-1 liver cytosolic antigen; LKM, liver–kidney microsomal autoantibodies; LM, liver microsomal. LP, liver–pancreas antigen; SLA, soluble liver antigens.			

**Table 18.3** Molecular definitions of the most important autoantigens relevant to the serological diagnosis of autoimmune hepatitis.

autoimmune chronic hepatitis. These antibodies precipitated a UGA suppressor serine tRNA–protein complex, which is probably involved in cotranslational selenocysteine incorporation in human cells. Subsequently, SLA/LP antibodies have been identified as being directed against a UGA suppressor serine tRNA–protein complex and not against cytokeratins 8 and/or 18 or glutathione S transferases as previously suggested. The exact function and role of this autoantigen in autoimmunity are so far unclear. Regarding the disease specificity, anti-SLA/LP may be linked to the pathogenesis of the autoimmune process.

### Other autoantibodies

Antibodies against liver cytosol type 1 (LC-1) were found in up to 50% of patients with AIH type 2. Less frequently, anti-LC-1 may be associated with SMA and ANA in sera from patients with AIH type 1 and chronic hepatitis C infection. In addition anti-LC-1 proved to be the only serological marker in 10% of patients with AIH. Anti-LC-1 are visualized by indirect immunofluorescence, however their characteristic staining may be masked by the more diffuse pattern of LKM-1 antibodies. The antigen recognized by anti-LC-1 was identified as formiminotransferase cyclodeaminase (FTCD). FTCD is a metabolic enzyme involved in the conversion of histidine to glutamic acid, and is most highly expressed in the liver. It is bifunctional and composed of

distinct FT and CD domains connected by a short linker. Anti-LC-1 sera recognize distinct epitopes on FTCD preferentially localized to the FT domain of FTCD. Contrary to most other autoantibodies in AIH, anti-LC-1 seems to correlate with disease activity and may be useful as a marker of residual hepatocellular inflammation in AIH.

Antibodies against the ASGPR were observed in up to 90% of all patients with AIH and can coexist with ANA, SMA and anti-LKM-1. However, they are not disease specific and can also be found in viral hepatitis, drug-induced hepatitis and PBC. Levels of anti-asialoglycoprotein antibodies correlate with inflammatory disease activity and might be used as an additional marker to monitor treatment efficacy.

Antibodies to neutrophil cytoplasmic antigens (ANCA) were detected in 65–95% of sera from patients with AIH type 1, and additionally in sera from patients with PSC. ANCA are detected by immunofluorescence, which distinguishes two patterns: cANCA with a diffuse cytoplasmic staining of neutrophils and pANCA which exhibit a rim-like staining of the perinuclear cytoplasm. In AIH, atypical pANCA (also termed xANCA) are usually found that display a pANCA immunofluorescence pattern but do not show reactivity with myeloperoxidase, one of the major autoantigens of classical ANCA. The discrimination of ANCA is difficult, because ANA frequently also stain ethanol-



fixed neutrophils. The target antigen in AIH is unknown, but, apart from myeloperoxidase, proteinase 3 and elastase have been ruled out as candidates. The role of ANCA in AIH is unclear, but routine determination might be useful to identify patients formerly classified as having cryptogenic hepatitis.

## ETIOLOGY OF AIH

Conclusive evidence of a single etiology of AIH has not yet been presented. Many findings have pointed towards a viral etiology which has been investigated by numerous studies. However, a viral etiology of AIH remains a matter of controversy. In anecdotal reports the relationship of the hepatitis A virus, hepatitis B virus, EBV and herpes simplex virus (HSV) with autoimmune hepatitis has been implicated. As a potential mechanism molecular mimicry between viral and body proteins has been suggested. In this respect, it was shown that the B-cell epitope of CYP2D6, which is targeted by LKM-1 autoantibodies, displays homology with the immediate early antigen IE175 of HSV. A case has been reported in which the only difference in HLA identical twins with discordant manifestation of AIH was HSV exposure.

HCV infection is associated with a broad array of serological markers of autoimmunity and immune-mediated syndromes. LKM autoantibodies are present in 3–5%. However, this serological autoimmunity differs with respect to recognition of antigen targets (CYP2D6 and CYP2A6), recognition of epitopes (AIH mainly 257–269, HCV more diverse and also more conformation dependent epitopes) and the clinical presentation. From these considerations it is unlikely that HCV is etiologically responsible for AIH.

Apart from viral agents a genetic predisposition must be regarded as a mandatory prerequisite of AIH. However, the genetic background of AIH does not follow a Mendelian pattern and a conclusive role of a single genetic locus capable of explaining the etiology of AIH has not yet been identified. AIH is therefore considered as a complex trait like most other human diseases, which means that there are numerous genes acting alone or in concert to reduce or increase the risk of that trait. The inheritable component of AIH is currently regarded as small. However, the absence of evidence does not mean evidence of absence and these data have yet to be established.

The most conclusive association with AIH is related to the major histocompatibility complex (MHC) alleles. The significance of HLA polymorphisms in terms of disease susceptibility and severity lies in the observation that most autoimmune reactions are T-cell dependent and all T-cell mediated responses are MHC restricted. In European patients several studies identified a strong genetic association with the HLA haplotype A1-B8-DRB1\*0301 and DRB\*0401 (Table 18.4). Patients with the HLA A1-B8-DRB1\*0301 haplotype were found to be younger at disease onset, relapse more frequently under immunosuppressive treatment and more frequently require liver transplantation. Subsequent investigations of the encoding HLA alleles identified that genetic susceptibility to AIH is related to the six amino acid sequence LLEQKR at position 67–72 of the DRB1 polypeptide. Within these six amino acids the critical amino acid appears to be that found on position 71 – namely, lysine or arginine on susceptibility alleles and alanine on resistance alleles. Polymorphisms within this region may affect the predisposition to autoimmune diseases by several mechanisms. This

Classification of autoimmune hepatitis based on genetic markers		
HLA	DR3	DR4
Genotype	DR B1*0301	DR B1*0401 (DR B1*0405 in Japanese)
Age at onset	<30	>40
Disease activity	+++	+
Treatment response	++	++++
Relapse after treatment	+++	+
Liver transplantation	+++	+
DR $\beta$ chain amino acid as risk factor	Lysine at AA71	?

**Table 18.4** Classification of autoimmune hepatitis based on genetic markers.

includes shaping of the T-cell repertoire, peptide selection and presentation as well as peptide transport. However, exactly how particular HLA alleles contribute to autoimmunity is still poorly understood. Analysis of MHC genes in different populations of AIH patients revealed that the immunogenetic associations with AIH vary regionally. Along this line an alternative model based on valine/glycine dimorphism at position –86 of the DR  $\beta$ -polypeptide has been proposed, which better represents the key HLA associations in patients from Argentina and Brazil. These findings are likely to be influenced by genetic heterogeneity that exists in evolutionary divergent populations. On the other hand they may reflect different underlying etiologies in terms of initiating events such as different viral triggers. Regardless of the mechanisms, it is apparent that the MHC locus per se is insufficient for the development of autoimmune diseases, as shown by the decrease of the autoimmune disease concordance rate of siblings in comparison to that of monozygotic twins. Therefore several studies have focused on other non-MHC genes and variations in a number of genes that are important in the regulation of immune responses have been associated with the development of AIH.

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) has diverse effects on immune responses and inflammatory cells. In some contexts, TNF- $\alpha$  promotes self-tolerance and T-cell deletion, whereas in others TNF- $\alpha$  promotes T-cell activation and autoimmune diseases. Two polymorphisms in the TNF promoter have been identified: one at position 308 (TNF2) and another at position 238 (TNFA). Some studies have shown that TNF2 and TNFA lead to increased constitutive and inducible expression compared with the wild type TNF, although this remains a matter of debate. TNF2 has been associated with an increased risk of developing AIH of a type with an unfavorable clinical course. However, TNF- $\alpha$  exhibits a strong linkage disequilibrium with the HLA A1-B8-DR3 haplotype and stratification analysis indicates that the association with the TNF2 promoter polymorphism is interdependent with DRB1\*0301. This is further



confirmed by findings in a population where the disease is not primarily associated with HLA-DRB1\*03. AIH in Brazilian patients is obviously not linked to TNF- $\alpha$  polymorphisms at position -308. Polymorphisms of other candidate genes encoding proinflammatory and immunoregulatory cytokines such the IL-1B, IL-1RN and IL-10 genes are not associated with AIH.

Cytotoxic T-lymphocyte antigen 4 (CTLA4) and CD28 are important regulators of T-lymphocyte activation and polymorphisms of *CTLA4* have been linked to a variety of autoimmune diseases. A single nucleotide polymorphism at position -318 of the *CTLA4* gene is located in the promoter region. Another polymorphism, an A/G variation at position +49 in the first exon of the gene leads to a threonine to alanine change in the leader peptide and was found to correlate with a reduced inhibitory function of CTLA4. A similar effect on CTLA4 function has been suggested for the third polymorphism, a microsatellite AT repeat, which is located in the 3' untranslated region of the last exon and has at least 20 alleles. All three polymorphisms are in strong linkage disequilibrium, which makes it difficult to distinguish which variations actually cause the functional difference. In addition, an increased transmission of the 49A alleles and *CTLA4* (AT)<sub>n</sub> has been reported in children with type 1 AIH, but interestingly no deviation in transmission was found in AIH type 2. This genetic heterogeneity might indicate genetic differences between type 1 and type 2 AIH.

In a recent analysis a lack of genetic association between idiopathic autoimmune liver diseases and hepatitis as part of APECED has been demonstrated. APECED is a rare autosomal recessive disorder which is characterized by an immune-mediated destruction of endocrine tissues, chronic candidiasis and additional ectodermal disorders. Typically, the disease presentation includes candidiasis, hypoparathyroidism and adrenocortical failure. The spectrum of associated clinical disease components includes insulin-dependent diabetes mellitus, autoimmune thyroid disease, pernicious anemia and chronic active hepatitis. While the phenotype of the syndrome is largely heterogeneous, APECED is viewed as a genetically homogenous syndrome caused by mutations in a recently identified gene, the autoimmune regulator, *AIRE*. The expression pattern and the predicted protein function suggest that *AIRE* plays a role in the induction and maintenance of immune tolerance. Interestingly, the recently created *AIRE* knock out mice present like human APECED patients with a variable combination of endocrine and non-endocrine autoimmune components with a variable age of onset. The variety of autoimmune diseases seen in APECED patients suggested that *AIRE* might contribute to the etiology of other autoimmune disorders. However, recent findings indicate that common mutations in the *AIRE* gene do not play a major role in sporadic autoimmune diseases such as autoimmune thyroid disease, diabetes mellitus type 1, Addison disease and autoimmune liver diseases, and are therefore a unique feature of APECED. Similar to AIH type 2, hepatitis in APECED is associated with autoantibodies directed against cytochrome P450 proteins. In patients with APECED, autoantibodies to CYP1A2 and CYP2A6 have been described as markers of an autoimmune liver disease. However, recent data indicate that only antibodies to CYP1A2 are specific for hepatitis in APECED, albeit with a low sensitivity. Furthermore, it has been demonstrated that there is no overlap between the different molecular targets of microsomal autoantibodies in AIH and APECED patients.

CLINICAL FEATURES AND SUBCLASSIFICATION

Clinical features

Systematically, AIH is part of the syndrome of chronic hepatitis, which is characterized by sustained hepatocellular inflammation of at least 6 months duration and elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of 1.5 times the upper normal limit. In about half of AIH patients an acute onset of AIH is observed and rare cases of fulminant AIH have been reported. In the remainder, the clinical presentation is not spectacular and characterized by fatigue, right upper quadrant pain, jaundice and occasionally also by palmar erythema and spider nevi. In later stages, the consequences of portal hypertension dominate, including ascites, bleeding esophageal varices and encephalopathy. A specific feature of AIH is the association of extrahepatic immune-mediated syndromes including autoimmune thyroiditis, vitiligo, alopecia, nail dystrophy, ulcerative colitis, rheumatoid arthritis, diabetes mellitus and glomerulonephritis (Table 18.5).

Subclassification

Immunoserological parameters assume a central role in the subclassification of AIH and allow the discrimination of clinically differing groups of patients. The International Autoimmune Hepatitis Group (IAIHG) has not recommended these subdivisions for other than research purposes, because autoantibodies do not define distinct therapeutic groups. However, they noted that the distinction between AIH type 1 and type 2 has already been widely adopted in clinical practice.

AIH type 1 is characterized by ANA and in most cases also SMA autoantibodies. In 97% of patients hypergammaglobulinemia with elevated immunoglobulin G (IgG) is present. Representing 80% of the cases of AIH, this form is the most prevalent subclass, which was historically described as lupoid, classical or idiopathic AIH. Of these patients, 70% are female with a peak incidence between the ages of 16 and 30 years. However, 50% are older than 30 years. An association with other immune syndromes is observed in 48%, with autoimmune thyroid disease, synovitis and ulcerative colitis being the most prevalent. The

Extrahepatic associations of autoimmune hepatitis are present in 10–50% of patients	
Frequent	Autoimmune thyroid disease Ulcerative colitis Synovitis
Rare or individual reports	Rheumatoid arthritis Lichen planus Diabetes mellitus CREST syndrome Autoimmune thrombocytopenic purpura Vitiligo Nail dystrophy Alopecia
CREST, calcinosis cutis, Raynaud phenomenon, esophageal hypomobility, sclerodactyly, telangiectasia.	

Table 18.5 Extrahepatic associations of autoimmune hepatitis are present in 10–50% of patients.

clinical course is often unspectacular and acute onset is rare. About 25% have cirrhosis at the time of diagnosis.

Autoimmune hepatitis type 2 is characterized by the presence of LKM-1 autoantibodies. In 10% LKM-3 autoantibodies are also present. In contrast to AIH type 1, additional organ-specific autoantibodies are present such as antithyroid, antiparietal cell and anti-Langerhans cell autoantibodies. Extrahepatic immune syndromes such as diabetes, vitiligo and autoimmune thyroid disease are also more prevalent. Serum immunoglobulin levels are moderately elevated with a reduction of immunoglobulin A (IgA). AIH type 2 affects 20% of AIH patients in Europe but only 4% in the USA. There is again a female predominance. The peak age at presentation is around 10 years but AIH type 2 is also observed in adults, especially in Europe. AIH type 2 carries a higher risk of acute presentation and progression to cirrhosis.

Autoimmune hepatitis type 3 is characterized by SLA/LP autoantibodies, but 74% also have other serological markers of autoimmunity, including SMA and AMA. AIH type 3 has a lower prevalence than AIH type 2. 90% of affected patients are female with a peak in age between 20 and 40 years. AIH type 3 is a matter of debate and further evaluations will have to determine whether it represents an entity in itself or is a variation of AIH type 1. However, it is important to diagnose anti-SLA/LP-positive AIH, which occurs in 10% of AIH cases as the only serological markers. This may therefore decrease the likelihood of misclassification.

### Differentiation of cryptogenic hepatitis and overlap syndromes

Cryptogenic hepatitis is an etiologically undefined chronic hepatitis. It is unclear how many of these patients in fact suffer from AIH without the presence of serum autoantibodies detectable with the available state-of-the-art techniques. In about 13% of these patients, who had initially been tested by indirect immunofluorescence for ANA, SMA and LKM, it was possible to detect SLA autoantibodies and contribute to their diagnostic clarification. Clinically this group of cryptogenic hepatitis resembles AIH type 1 with respect to age and sex distribution, HLA antigen types, inflammatory activity and response to therapy.

Overlap syndromes are conditions in which there are leading symptoms of AIH, but with additional markers or symptoms pointing to other diseases within the differential diagnosis of AIH. Among these are PBC in 8% with serum AMA and histologic signs of cholangitis, PSC in 6% with typical changes of the cholangiography, and autoimmune cholangitis in 10% with ANA, SMA and histologic inflammation of the biliary system. However, a concise and universally accepted definition of an overlap syndrome is currently lacking. In addition the frequency of this condition is a matter of controversy.

A clinically significant association is virus-associated autoimmunity, which describes the coexistence of autoantibodies and virus infection. The most important associations are HCV infection and HDV infection in which LKM autoantibodies can be detected in 2–5% and 6–12%, respectively. AIH type 2 and HCV infection with LKM autoantibodies are clinically distinct entities (**Table 18.6**). LKM autoantibodies in virus infection are present at lower titers and recognize more conformational and diverse epitopes than in genuine AIH. This discrimination is relevant since it forms the basis for mutually exclusive thera-

Comparison between genuine and virus-induced autoimmunity		
	<i>Autoimmune hepatitis</i>	<i>Viral hepatitis</i>
Autoantibody titer	↑ ↑ ↑	↑
Linear autoepitopes	+ + +	+
Conformational epitopes	+	+ + + +
Inhibitory antibodies	+ +	+ +
Autoimmune response	Homogenous	Heterogeneous
Treatment	Immunosuppression	(Antiviral)

**Table 18.6** Comparison between genuine and virus-induced autoimmunity.

peutic strategies: immunosuppression in AIH and interferon in chronic virus hepatitis.

### NATURAL HISTORY AND PROGNOSIS

Data describing the natural history of AIH are scarce. The last placebo-controlled immunosuppressive treatment trial was published in 1980. The value of these studies is limited considering that these patients were only screened for epidemiological risk factors for viral hepatitis and had not been characterized by standardized diagnostic criteria. Nevertheless these studies revealed that untreated AIH had a very poor prognosis and 5- and 10-year survival rates of 50% and 10% have been reported. They furthermore demonstrated that immunosuppressive treatment significantly improved survival.

Recent data revealed that up to 30% of adult patients had histologic features of cirrhosis at diagnosis. In 17% of patients with periportal hepatitis cirrhosis developed within 5 years, but cirrhosis develops in 82% when bridging necrosis or necrosis of multiple lobules is present. The frequency of remission (86%) and treatment failure (14%) are comparable in patients with and without cirrhosis at presentation. Importantly, the presence of cirrhosis does not influence the 10-year survival rate (90%) and these patients require a similarly aggressive treatment strategy.

Almost half of the children with AIH already have cirrhosis at the time of diagnosis. Long-term follow-up revealed that only a few children can completely stop all treatment and about 70% of children receive long-term treatment. Most of these patients relapse when treatment is discontinued, or if the dose of immunosuppressive drug is reduced. About 15% of patients develop chronic liver failure and are transplanted before the age of 18 years.

In elderly patients, a more severe initial histologic grade has been reported, but the frequency of definite cirrhosis seems not to differ from that of younger patients. At follow-up, about 30% of patients develop cirrhosis. Response to immunosuppression is similar in old and young patients and up to 90% of the older patients reach complete remission. However, in a study from the UK, 41% of the elderly patients with AIH received no immunosuppressive therapy and the prognosis did not appear to be worse than that for younger patients who usually receive

Treatment regimen* and follow-up examinations of autoimmune hepatitis regardless of autoantibody type						
	Monotherapy			Combination therapy		
Prednis(ol)one	60 mg			30 mg		
	Reduce within 4 weeks to maintenance dose 20 mg or lower			Reduce within 4 weeks to maintenance dose 10 mg or lower		
Azathioprine	n.a. (consider maintenance with azathioprine: monotherapy: 2 mg/kg body weight)			50–150 mg		
After remission is reached (treatment length 12–24 months) monitor, consider repeat biopsy, avoid premature treatment reduction						
Prednis(ol)one	Reduction of daily dose by 2.5 mg/week			Reduction of daily dose by 2.5 mg/week		
Azathioprine	n.a.			Reduction: 25 mg every 3 weeks to 50 mg per day		
Examination	Before therapy	During therapy before remission – 4 weeks	Remission under therapy – 3–6 months	Cessation of therapy – 3 weeks	Remission post-therapy – 3–6 months (x 4)	Evaluation of relapse
Physical	+		+	+	+	+
Liver biopsy	+		(+/-)			+
Blood count	+	+	+	+	+	
Aminotransferases	+	+	+	+	+	+
γglutamyltransferase	+	+	+			
γglobulin	+	+	+	+	+	+
Bilirubin	+	+	+	+	+	+
Coagulation studies	+	+	+	+	+	
Autoantibodies	+	+/-				+
Thyroid function tests	+	+/-				+
n.a. – not applicable. In the elderly patient with low inflammatory activity the indication to treat must be weighed against side-effects and some of these patients may best remain untreated. *The table reflects the Mayo Clinic approach. In our own experience, monotherapy with prednisolone beginning with 50 mg and tapered by 10 mg every 10 days to a maintenance dose of 15–20 mg is equally effective as combination therapy with 1 mg/kg body weight of azathioprine for 3 weeks and tapering to 50 mg daily combined with prednisolone therapy tapered to 10 mg daily. There is no published evidence of an advantage of an individual tapering regimen and different tapering and dosing regimens are employed by different centers. In the individual young patient without severe symptoms and with low inflammatory activity [inactive biopsy, alanine aminotransferase (ALT) <5 × upper limit of normal] treatment can be initiated with maintenance doses.						

Table 18.7 Treatment regimen\* and follow-up examinations of autoimmune hepatitis regardless of autoantibody type.

treatment. Hepatocellular carcinoma can complicate AIH cirrhosis, although with a lower frequency than in other etiologies of cirrhosis.

## TREATMENT

### Medical treatment

The indication for treatment of AIH is based on inflammatory activity and not so much on the presence of cirrhosis. In the absence of inflammatory activity immunosuppressive treatment has only limited effects. Independent of AIH type, treatment is initiated with prednis(ol)one alone or in combination with azathioprine. Both strategies are equally effective. The use of prednisone or its metabolite prednisolone is equally effective since chronic liver disease does not seem to have an effect on the synthesis of prednisolone from prednisone. It is important

to differentiate between virus infection and autoimmune hepatitis prior to starting therapy as corticosteroids induce viral replication while interferon can lead to dramatic disease exacerbations in AIH.

An indication for treatment is present when aminotransferases are elevated twofold, γ-globulin levels are elevated and histology shows moderate to severe periportal hepatitis. Symptoms of severe fatigue are also an indication for treatment. An absolute indication exists in cases with a 10-fold or higher elevation of aminotransferase levels, histologic signs of severe inflammation and necrosis, and with disease progression.

The treatment regimen and suggested follow-up examinations are summarized in Table 18.7. Therapy is usually administered over the course of 2–3 years. The decision between monotherapy and combination therapy is guided by the balance between efficacy and toxicity. Long-term corticosteroid therapy leads to



Side-effects of prednis(ol)one and azathioprine	
Side-effects of prednis(ol)one	Acne Moon-shaped face Striae rubrae Dorsal hump Obesity Weight gain Osteoporosis Aseptic bone necrosis Psychiatric symptoms (euphoria, psychosis, depression) Diabetes mellitus Cataract Hypertension
Side-effects of azathioprine	Nausea Vomiting Abdominal discomfort Hepatotoxicity Rash Leucocytopenia Teratogenicity? Oncogenicity?

**Table 18.8** Side-effects of prednis(ol)one and azathioprine.

cushingoid side-effects that may reduce patient compliance considerably (Table 18.8). Serious complications such as diabetes, osteopenia, aseptic bone necrosis, psychiatric symptoms, hypertension and cataract formation also have to be anticipated in long-term treatment. Side-effects are present in 44% of patients after 12 months and in 80% of patients after 24 months of treatment. Prednis(ol)one monotherapy is possible in pregnant patients. Azathioprine reduces reliance on corticosteroids, especially in maintenance therapy. It bears a theoretical risk of teratogenicity but this fear has not been borne out in clinical experience. In addition, abdominal discomfort, nausea, cholestatic hepatitis, rashes and leucopenia can be encountered. These side-effects are seen in 10% of patients receiving a dose of 50 mg/day. From a general point of view, a postmenopausal woman with osteoporosis, hypertension and elevated blood glucose would be a candidate for combination therapy. In young women, pregnant women or patients with hematological abnormalities, prednis(ol)one monotherapy may be the treatment of choice.

Treatment is initiated according to the regimen in Table 18.8. Continuous administration is essential since most cases of relapse are the result of erratic changes of medication and/or dosage. Dose reduction is aimed at finding the optimal maintenance regimen for each individual patient. Since histology lags 3–6 months behind the normalization of serum parameters, therapy has to be continued beyond the normalization of aminotransferase levels. Usually, maintenance doses of prednis(ol)one range between 2.5 and 10 mg. After 12–24 months of therapy prednis(ol)one can be tapered over the course of 4–6 weeks to test whether a sustained remission has been achieved. Tapering regimens should be attempted with great caution and only after obtaining a liver biopsy that demonstrates a complete resolution of inflammatory activity. Relapse of AIH, and the risk of progression of fibrosis,

is almost universal when immunosuppression is tapered in the presence of residual histologic inflammation.

Outcomes can be classified into four categories: remission, relapse, stabilization and treatment failure.

- Remission is a complete normalization of all inflammatory parameters including histology. This is achieved in 65% of patients after 24 months of treatment. Remission can be sustained with azathioprine monotherapy with doses up to 2 mg/kg body weight. This prevents cushingoid side-effects. However, side-effects such as arthralgia (53%), myalgia (14%), lymphopenia (57%) and myelosuppression (6%) have been observed.
  - Relapse is characterized by a threefold increase of aminotransferase levels and the reoccurrence of clinical symptoms. Relapse is present in 50% of patients within 6 months of treatment withdrawal and in 80% after 3 years. Relapse is associated with progression to cirrhosis in 38% and liver failure in 14%. Occurrence of a relapse calls for re-initiation of standard therapy and perhaps long-term maintenance with prednis(ol)one or azathioprine monotherapy.
  - Stabilization is the achievement of a partial remission not fulfilling the definition of remission. Since 90% of patients are expected to attain remission within 3 years, the therapeutic strategy should be re-evaluated in this subgroup of patients, particularly with regard to the use of the newer immunosuppressive agents. Ultimately, liver transplantation may be required.
  - Treatment failure characterizes a progression of clinical, serologic and histologic parameters during standard therapy. This is seen in about 10% of patients. In these cases the diagnosis of AIH has to be carefully reconsidered to exclude other etiologies of chronic hepatitis. In these patients, new or experimental regimens can be administered but, again, liver transplantation may become necessary.
- When standard treatment fails or drug intolerance occurs, alternative therapies such as cyclosporin, tacrolimus, cyclophosphamide, mycophenolate mofetil, rapamycin, ursodeoxycholic acid (UDCA) and budesonide can be considered. The efficacy of these options has not yet been definitively established.
- Budesonide is a synthetic steroid with high first-pass metabolism in the liver, which should limit systemic side-effects compared to conventional steroids. A small study showed that the drug was well tolerated and aminotransferase levels were normalized, but a contradictory study found that budesonide therapy was associated with a low frequency of remission and a high occurrence of side-effects. It is not clear whether budesonide offers an advantage over conventional corticosteroids when cirrhosis and portosystemic shunts are present. The main advantage of budesonide for the future treatment of AIH may be to replace prednisone in long-term maintenance therapy to reduce corticosteroid side-effects. The long-term role of second generation corticosteroids, to sustain remission in AIH patients with reduced treatment related side-effects, requires further controlled studies.
- Cyclosporin is a lipophilic cyclic peptide of 11 residues produced by *Tolypocladium inflatum* that acts on calcium dependent signaling and inhibits T-cell function via the interleukin 2 gene. Of all the alternative agents, the greatest experience to date has been with cyclosporin and it is undoubtedly effective and well tolerated in the short to medium term. However, the principal

difficulty in advocating widespread use of the drug as first-line therapy relates to its toxicity profile, particularly with long-term use (increased risk of hypertension, renal insufficiency, hyperlipidemia, hirsutism, infection and malignancy).

Tacrolimus is a macrolide lactone compound with immunosuppressive capabilities exceeding those of cyclosporin. The mechanism of action is similar to that of cyclosporin but it binds to a different immunophilin. The application of tacrolimus in a small study led to an improvement of aminotransferase and bilirubin levels with a minor increase in serum blood urea nitrogen (BUN) and creatinine levels over a 1-year period. Although tacrolimus represents a promising immunosuppressive candidate drug, larger randomized trials are required to assess its role in the therapy of AIH.

Mycophenolate is a non-competitive inhibitor of inosine monophosphate dehydrogenase, which blocks the rate-limiting enzymatic step in de novo purine synthesis. Mycophenolate has a selective action on lymphocyte activation, with marked reduction of both T- and B-lymphocyte proliferation. A pilot study of patients with AIH type 1 who were unresponsive to standard therapy demonstrated normalization of aminotransferase levels within 3 months in the majority of patients. These preliminary data suggest that mycophenolate may represent another promising treatment strategy for AIH.

Cyclophosphamide can induce remission with 1–1.5 mg/kg/day in combination with corticosteroids. However, cyclophosphamide with its potentially severe hematologic side-effects should be considered an experimental treatment option.

UDCA is a hydrophilic bile acid with putative immunomodulatory capabilities. It is presumed to alter HLA class I antigen expression on cellular surfaces and to suppress immunoglobulin production. Uncontrolled trials have shown a reduction in histologic abnormalities, as well as clinical and biochemical improvement, but not a reduction in fibrosis AIH type 1. However, its role in AIH therapy or in combination with immunosuppressive therapy remains uncertain.

## LIVER TRANSPLANTATION

In approximately 10% of AIH patients liver transplantation remains the only life-saving option. The indication for liver transplantation in AIH is similar to that in other chronic liver diseases and includes clinical deterioration, development of cirrhosis, bleeding esophageal varices and coagulation abnormalities despite adequate immunosuppressive therapy. There is no single indicator or predictor for the necessity of liver transplantation. Candidates for liver transplantation are usually patients who do not reach remission within 4 years of continuous therapy. Indicators of a high mortality associated with liver failure are histologic evidence of multilobular necrosis and progressive hyperbilirubinemia. The long-term results of liver transplantation for

AIH are excellent and well within the range of other indications for liver transplantation.

The potential for AIH to recur after liver transplantation has been a matter of controversial debate. The first case of recurrent AIH after liver transplantation was reported in 1984, and was based upon serum biochemistry, biopsy findings and steroid reduction. Studies published during the past years indicate that the rate of recurrence of AIH ranges between 10 and 35%, and that the risk of AIH recurrence is perhaps as high as 68% after 5 years of follow-up. It is important to consider the criteria upon which the diagnosis of recurrent AIH is based. When transaminitis is chosen as a practical selection parameter many patients with mild histologic evidence of recurrent AIH may be missed. It is therefore suggested that all patients with suspected recurrence of AIH receive a liver biopsy, biochemical analyses of aminotransferases as well as a determination of immunoglobulins and autoantibody titers. Significant risk factors for the recurrence of AIH have not yet been identified although it appears that presentation with acute liver failure before transplantation protects against the development of recurrent disease. A risk factor for the development of recurrent AIH is the presence of specific HLA antigens that may predispose toward a more severe immunoreactivity. In two studies recurrence of AIH appeared to occur more frequently in HLA DR3-positive patients receiving HLA DR3-negative grafts. However, this association was not confirmed in all studies. Interestingly, there have been no conclusive data to support the hypothesis that a specific immunosuppressive regimen represents a risk factor for the development of recurrent AIH. However, data indicate that patients transplanted for AIH require continued steroid treatment in 64% versus 17% of patients receiving liver transplants for other conditions. Based on these results and other studies it would appear that maintenance of steroid medication in AIH patients is indicated to prevent not only cellular rejection but also graft-threatening recurrence of AIH. Corticosteroid withdrawal should therefore be performed with great caution in this setting.

Autoantibody prevalence and titers have been studied in patients receiving liver transplantations for AIH and PBC. In general, autoantibody types persist in the majority of patients after transplantation. In PBC AMA persisted, albeit at lower titers in almost 100% of the patients. In AIH, autoantibodies of the specific subtype present before transplantation were detected at lower titers in 77% of post-transplant patients in one study and were found in 82% of those patients who did not develop recurrence of AIH. A recent study has suggested that an increase in titer exceeding levels detected prior to transplantation may be indicative of AIH recurrence. The majority of published data presently do not support a prognostic role for autoantibodies in AIH and liver transplantation.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 19

## Primary Biliary Cirrhosis

Raoul Poupon and Renée Eugénie Poupon

### INTRODUCTION

Primary biliary cirrhosis is a chronic cholestatic disease of unknown cause, most often diagnosed in middle-aged women. Morphologically, primary biliary cirrhosis is characterized by portal inflammation and necrosis of cholangiocytes in the small and medium-sized bile ducts. The disease is characterized biochemically by a cholestatic pattern, and immunologically by the almost constant presence of mitochondrial antibodies and less often by that of nuclear antibodies. Although primary biliary cirrhosis is a progressive disease, the rate of progression varies greatly from one patient to another. The terminal phase is characterized by hyperbilirubinemia and morphologically by cirrhosis. It is now well established that orthotopic liver transplantation (OLT) is the treatment of choice for patients entering the terminal phase of the disease. A variety of therapeutic agents have been proposed for the treatment of patients with primary biliary cirrhosis. However, most have been found ineffective or too toxic to be widely used. There is accumulating evidence that long-term administration of ursodeoxycholic acid (UDCA) is safe and prolongs survival free of OLT. UDCA has also been combined with other drugs, in particular corticosteroids, in an attempt to enhance favorable effects on the outcome of patients, or to provide beneficial effects in the subset of patients who have a suboptimal response to UDCA therapy alone.

### EPIDEMIOLOGY

According to epidemiologic studies, estimates of the prevalence range from 100 to 200 per million, with a strong female preponderance (10:1) and a median age of between 50 and 55 years at diagnosis.

The prevalence of primary biliary cirrhosis in families with one affected member is about 4% which is much higher than in the general population. Similarly, the occurrence of other immune-related disorders is common in first-degree relatives of patients with primary biliary cirrhosis. There is a weak association between the disease and the human lymphocyte antigen (HLA) DR8 antigen. An excess frequency of haplotype C4A/Q0 has also been reported.

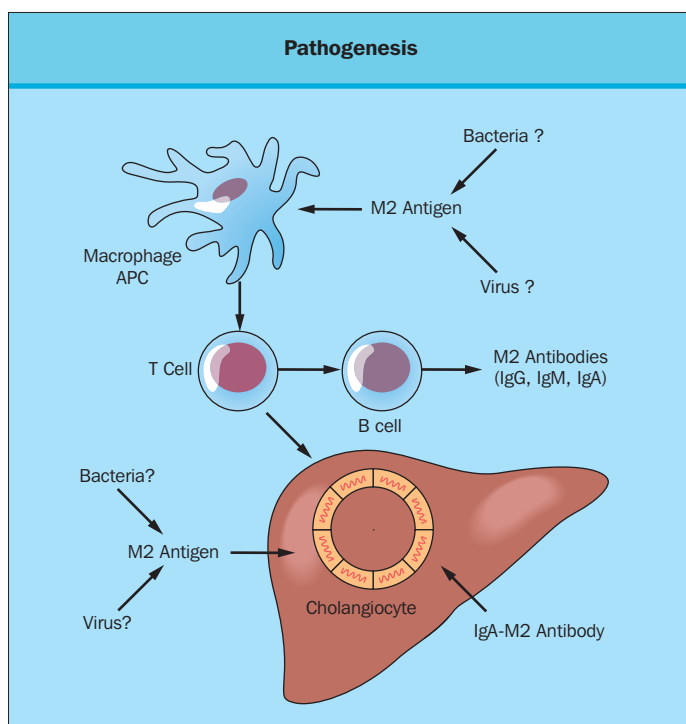
### PATHOGENESIS AND IMMUNOLOGIC ABNORMALITIES

The initial lesion is a focal and segmental destruction of interlobular bile ducts. This results in chronic cholestasis which progresses towards 'biliary' cirrhosis. Numerous immunologic abnormalities are found in primary biliary cirrhosis. The most

characteristics are an increase in serum immunoglobulin M (IgM) levels, the presence of circulating autoantibodies (in particular antimitochondrial and antinuclear antibodies), a defective suppressor T-cell activity, and abnormalities of macrophage function (Fig. 19.1).

#### Antimitochondrial antibodies and other antibodies

Antimitochondrial antibodies (AMAs) are present in about 90% of patients with primary biliary cirrhosis. They are non-organ and non-species specific antibodies. The AMAs in primary biliary cirrhosis were shown initially to react with a trypsin-sensitive antigen (M2) of the internal mitochondrial membrane. Using Western immunoblotting, it was then shown that M2 contains several antigenic determinants with molecular masses of about 75–70, 56–50, 48–43 and 36 kDa. The identity of this non-organ and non-species-specific autoantigen was unknown until 1988, when two groups independently found that the M2 antigen of 70–75 kDa (which was cloned by Gershwin and coworkers in 1987) was the E2 component of the pyruvate



**Figure 19.1 Pathogenesis of primary biliary cirrhosis.** Macrophages present the M2 antigen or the cross-reactive peptide to CD4 lymphocytes by means of class II antigen. CD4 T cells help CD8 T cells to become cytotoxic effector cells for cholangiocytes expressing the M2 antigen. CD4 cells also activate B cells to produce M2 antibodies.

Antinuclear antibodies and the diseases in which they are found	
Antibody specificity	Disease (% prevalence)
Anticentromere	Scleroderma, CREST, primary biliary cirrhosis (10%)
Antilamins	SLE, scleroderma, autoimmune hepatitis, occasionally primary biliary cirrhosis (<5%)
Anti-gp210	Primary biliary cirrhosis (20–30%)
Anti-Sp100	Primary biliary cirrhosis (20–30%)
CREST, calcinosis cutis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, telangiectasia; SLE, systemic lupus erythematosus.	

**Table 19.1 Antinuclear antibodies and the liver diseases in which they are found**

dehydrogenase complex. It later became clear that the antigenic determinants of M2 identified by Western blotting were all components of pyruvate dehydrogenase or the 2-oxoacid dehydrogenase complex located on the mammalian inner mitochondrial membrane. Anti-M2 mainly reacts with the 74 kDa antigen of pyruvate dehydrogenase, because about 95% of sera are positive in immunoblotting.

Other non-organ and non-species-specific antibodies are frequently detected in AMA-positive and AMA-negative patients with primary biliary cirrhosis. These include antinuclear, anti-smooth muscle, rheumatoid factor and antithyroid antibodies.

Antinuclear antibodies are observed in up to 70% of patients with primary biliary cirrhosis (Table 19.1). In 10–15% of cases they are anticentromere antibodies and, when present, are generally associated with the CREST syndrome (calcinosis, Raynaud phenomenon, esophageal motility disorders, sclerodactyly and telangiectasia). Approximately 30% of patients with primary biliary cirrhosis have autoantibodies which give a nuclear rim pattern in immunofluorescence studies. In a few patients, these antibodies are directed against the receptor for lamin B, a constitutive protein of the inner nuclear membrane. These antibodies are more frequent in autoimmune hepatitis and lupus erythematosus. In a larger proportion of patients with primary biliary cirrhosis (20–30%), these autoantibodies are directed against a nuclear pore protein of 210 kDa.

A third type of antinuclear antibody observed in biliary cirrhosis gives a speckled appearance by immunofluorescence, but nucleoli and condensed chromosomes are not stained. These autoantibodies react with a nuclear protein of 100 kDa which is thus known as Sp100. These antibodies are present in about 20–30% of patients. Some studies suggested that anti-gp210- and Sp100-associated primary biliary cirrhosis may have a more severe course.

### Immune effectors involved in cholangiocyte necrosis

The main targets of the immune reaction are cholangiocytes. Several arguments suggest that mitochondrial antibodies are not cytotoxic and are not responsible for this phenomenon. In effect, they are not always present in primary biliary cirrhosis. The severity of the disease is unrelated to the antibody titer.

After liver transplantation, the antibodies persist, even in the absence of evidence of disease recurrence. The mitochondrial antibodies may be present in the absence of any lesion suggestive of biliary necrosis. Bile duct destruction is directly linked to the cytotoxicity of T lymphocytes or to cytokines produced by CD4 and CD8 cells in contact with biliary lesions – in particular, a predominance of TH1 over TH2 cytokines.

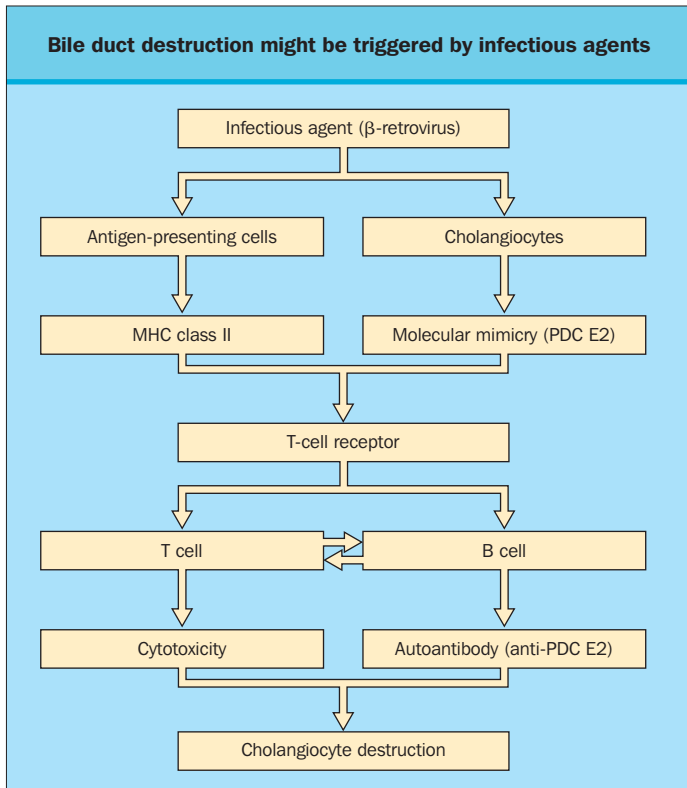
### Epitopes involved in the immune reaction leading to cholangiocyte destruction

Several lines of evidence suggest that the antigen(s) recognized by the AMAs could be the target for T lymphocytes on cholangiocytes. In effect, the pyruvate dehydrogenase complex E2, or a cross-reactive molecule, is overexpressed on cholangiocytes at the luminal domain, independently of the presence or not of circulating AMAs. The T lymphocytes of patients with primary biliary cirrhosis have cytotoxic activity against autologous cholangiocytes. CD4 T cells, with specific reactivity against pyruvate dehydrogenase, are present in the portal inflammatory infiltrate. Two explanations have been offered to account for the overexpression of the M2 epitope at the luminal domain of epithelial cells. First, normal cholangiocytes cocultivated with supernatants containing murine mammary tumor virus, the related retrovirus thought to be involved in the pathogenesis of primary biliary cirrhosis, show the aberrant expression of pyruvate dehydrogenase complex subunit E2 (PDC-E2). Second, immunoglobulin A (IgA) PDC-E2 antibodies could trap PDC complex proteins during their continuous transcytosis towards the bile duct lumen. The epitope recognized by autoreactive CD8+ T cells is similar to that recognized by the autoantibody. B-cell, helper and cytotoxic T-cell immunodominant epitopes contain a shared peptide sequence within the inner lipoyl domain of PDC-E2.

### Hypotheses explaining the immune response

As in other autoimmune diseases, environmental factors have been incriminated in genetically susceptible hosts. One such factor is bacterial infection, the main argument being cross-reactivity between AMAs and antigens of *Escherichia coli* and other bacteria. Recent data suggest that IgA PDC-E2 antibodies may be involved in the cholangiocyte destructive process. The data indicate that these bacteria express an epitope in common with pyruvate dehydrogenase. In rabbits, injection of a lysate of *Salmonella minnesota* R-mutants elicits antibodies against mitochondrial antigens of 50–70 kDa. As in primary biliary cirrhosis, these antibodies recognize the lipoyl domain of the E2 component of pyruvate dehydrogenase. In patients with primary biliary cirrhosis, mutants of *Escherichia coli* represent a large proportion of bacteria found in the urine and the intestine. One characteristic of these mutants is a liposaccharide defect in the bacterial membrane, leading to an increased immunogenicity. Some experimental data suggest that the liposaccharide of the R-mutant forms, and lipid A associated with it, might escape the reticuloendothelial system and be excreted in bile. In other words, these compounds could be the target of the immune reaction and explain the production of AMAs through molecular mimicry, by a mechanism somewhat similar to that observed in HLA B27 positive ankylosing spondylarthritis.

An agent closely related to the murine mammary tumor virus or the murine mammary tumor virus itself, at least in a subset



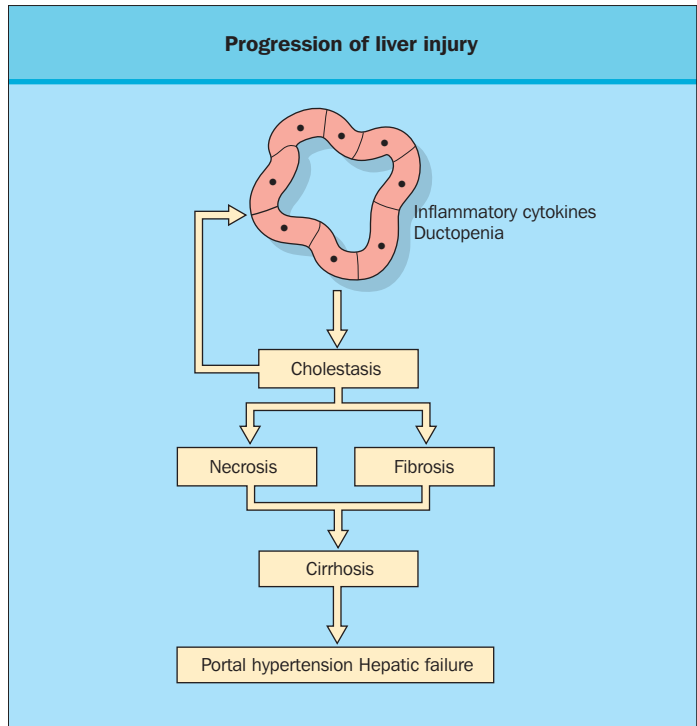
**Figure 19.2 Bile-duct destruction might be triggered by infectious agents.** Viruses are thought to evoke autoimmune response by several mechanisms, including expression of ‘hidden’ autoantigens, abnormal expression of major histocompatibility complex (MHC) molecules, or direct and indirect cross-reactivity between exogenous and endogenous structures.  $\beta$ -retrovirus might be in antigen-presenting cells or hilar lymph nodes and cholangiocytes of patients with primary biliary cirrhosis, a disease characterized by B-cell and T-cell activation leading to antimitochondrial (pyruvate dehydrogenase complex E2 subunit; PDC-E2) antibody and cholangiocyte destruction.

of patients with the phenotype of primary biliary cirrhosis, has been incriminated as a trigger of the bile duct destruction. Virus DNA sequences were found in perihepatic lymph nodes. Biliary cells in culture could be infected and show aberrant expression of PDC-E2-like antigen (**Fig. 19.2**).

More than 80% of patients with primary biliary cirrhosis show abnormal sulfoxidation of xenobiotics, compared to only 20–25% of healthy controls. Sulfoxidation is one step in the metabolism of certain drugs, especially chlorpromazine. Patients who develop cholestatic hepatitis on chlorpromazine have defective sulfoxidation. It is also one of the metabolic pathways leading to the production of endogenous sulfate. It has been postulated that this deficit could make patients with primary biliary cirrhosis more susceptible to the toxicity of certain endogenous compounds or some drugs.

## MECHANISMS OF PROGRESSION OF THE DISEASE

Disease progression is characterized by periportal lesions, ductular proliferation, fibrous and necroinflammatory septa which progress between portal triads leading gradually to nodule formation, then to cirrhosis (**Fig. 19.3**). The degree of severity of bile duct inflammatory lesions and of ductopenia and the



**Figure 19.3 Progression of liver injury.**

degree of severity of periportal lesions are highly related to the degree of severity of fibrosis. By contrast, the degree of severity of biliary lesions and the degree of severity of periportal or lobular lesions are poorly correlated. Periportal lesions are either the consequence of an immune aggression or the consequence of the cholestasis resulting from bile duct destruction.

## DIAGNOSIS AND CLINICAL COURSE

### Symptoms and physical examination

Before 1970, the diagnosis of primary biliary cirrhosis was often made relatively late. Patients presented in the terminal phase, with cholestasis, signs of portal hypertension and hepatocellular insufficiency. Now, most patients are diagnosed before the terminal phase; many are asymptomatic, while others have symptoms such as pruritus or fatigue.

Pruritus is seen in approximately 50% of patients at the time of diagnosis. It is generally intermittent, occurring mainly in the evening and night. In 5–10% of patients, pruritus starts during the last 3 months of pregnancy, and this can lead to the diagnosis of cholestasis of pregnancy. The arguments against this diagnosis are the persistence of pruritus and laboratory test abnormalities after delivery, features that are never observed in cholestasis of pregnancy. Jaundice rarely precedes pruritus. A precise cause for the rise in serum bilirubin level should be identified in such a setting (e.g. drug-induced hepatitis, hyperthyroidism, migration of gallstones, and intercurrent diseases). The jaundice of primary biliary cirrhosis is usually a sign of poor prognosis, because it reflects the severity of the disease.

Currently, primary biliary cirrhosis is often diagnosed in the absence of symptoms, but in the presence of abnormal liver test results, in particular increased serum activity of aminotransferases,  $\gamma$ -glutamyltranspeptidase (GGT) or alkaline phosphatase. In



some patients, the diagnosis of primary biliary cirrhosis is made during the work-up for an associated autoimmune disease such as sicca syndrome, Raynaud phenomenon or thyroid disease.

The physical examination is very important in primary biliary cirrhosis, because certain signs have prognostic value and thus reflect the severity of the disease. Jaundice, hepatomegaly, splenomegaly, ascites or edema point to advanced or progressive disease, while hyperpigmentation, cutaneous exfoliation, lichenification, xanthelasmata, and xanthomata have little or no prognostic value. Hyperpigmentation is most evident on the trunk and arms and areas of exfoliation and lichenification related to pruritus are seen. Xanthelasmata are frequent in female patients with marked hypercholesterolemia. Bone pain resulting from osteopenia is now rarely observed at the time of diagnosis.

### Laboratory tests

Most patients with primary biliary cirrhosis have abnormal liver tests characterized by mild elevation of aminotransferases [alanine aminotransferase (ALT) or aspartate aminotransferase (AST)] activity, frank elevation of GGT and alkaline phosphatase activities, and increased levels of immunoglobulins (mainly IgM). Increased erythrocyte sedimentation rate is constant while C reactive protein level remains low. The changes in biochemical tests are in part related to the stage of the disease and to histologic lesions. The degree of elevation of alkaline phosphatase and GGT may be related to the severity of ductopenia. The increase in serum AST activity and immunoglobulin G (IgG) levels reflect the degree of periportal and lobular necrosis and inflammation. Hyperbilirubinemia in most cases reflects the existence of cirrhosis. Serum bilirubin level is the most powerful prognostic factor in primary biliary cirrhosis. A serum bilirubin level higher than  $17 \mu\text{mol/L}$  ( $>1.0 \text{ mg/dL}$ ) is predictive of development of cirrhosis in patients with early histologic stages of the disease. Thrombocytopenia is usually indicative of portal hypertension.

As in other cholestatic diseases, serum cholesterol levels – with a frank increase in high-density lipoprotein (HDL) cholesterol – are often elevated. In patients with severe disease, an increase in low-density lipoprotein (LDL) cholesterol, associated with the presence of lipoprotein X, is usually found.

Measurement of serum markers of fibrosis may be helpful. Hyaluronic acid level has been shown to be a marker of extensive fibrosis. Although serum bile acid levels are not routinely determined, they are correlated with the degree of severity of histological lesions, in particular with the degree of ductopenia and fibrosis. The main interest of repeated measurements of serum bile acids is the evaluation of the compliance to UDCA treatment, and thus its efficacy.

### HISTOPATHOLOGY

Primary biliary cirrhosis is characterized by chronic, non-suppurative destructive cholangitis mainly affecting interlobular and septal bile ducts. The inflammatory infiltrate is essentially composed of lymphocytes and mononuclear cells in close contact with the basal membrane of cholangiocytes undergoing necrosis. In some cases, the infiltrate consists of plasma cells, macrophages and polymorphonuclear cells, especially eosinophils. The inflammation can take the form of epithelioid granulomas. These are present more often in early disease. There are few (if

any) arterial lesions. In contrast, portal venules are often compressed and occluded by the inflammatory reaction. Bile duct destruction and paucity are best assessed by determining the presence (or absence) of a bile duct relative to the presence of a branch of the hepatic artery. In normal liver, 70–80% of the portal arteries are accompanied by bile ducts. Bile duct paucity is usually defined by a percentage less than 50.

Histologic lesions are classically divided in four stages (Fig. 19.4 a–d). Stage I is characterized by portal inflammation and florid bile duct lesions. In this stage, inflammation remains confined to the portal triads. Disease progression is characterized by the gradual increase of periportal lesions, ductular proliferation and periportal hepatitis (stage II). Periportal regions become focally irregular and the lesion is characterized by cellular necrosis, separation of hepatocytes by inflammatory cells and macrophages. Piecemeal necrosis is usually moderate, but, in some cases, may be as severe as that observed in autoimmune hepatitis. Ductular proliferation is characteristic of stage II disease. It seems to be caused by metaplasia of periportal hepatocytes and their gradual acquisition of phenotypic characteristics of cholangiocytes. Stage III is characterized by a distortion of the hepatic architecture with numerous fibrous septa and periportal cholestasis. Cirrhosis – i.e. the existence of regenerative nodules – defines stage IV.

It is often difficult to distinguish these stages from each other. In fact, the lesions develop progressively at different rates in the different parts of the liver, such that an overlap between stages is usual.

### Diagnosis

Diagnosis of primary biliary cirrhosis is usually very easy to make (Fig. 19.5). To establish the diagnosis of primary biliary cirrhosis the following criteria are mandatory:

- biochemical evidence of cholestasis based usually on the elevation of both serum alkaline phosphatase and GGT activities;
- presence of M2 antibodies at a titer of 1/80 or higher when using immunofluorescence techniques;
- histopathologic evidence of non-suppurative cholangitis and destruction of small- or medium-sized bile ducts.

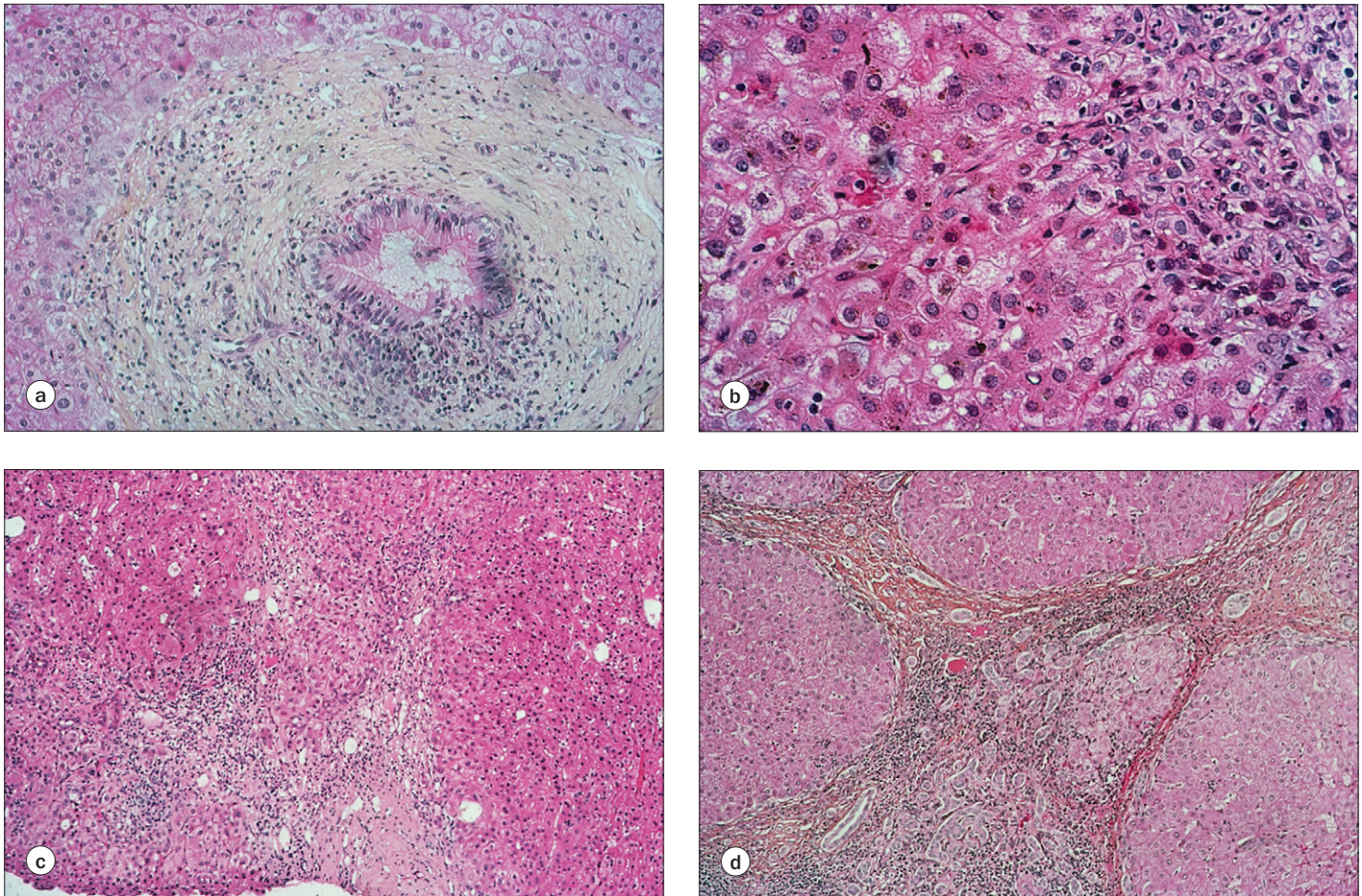
The size of the liver biopsy specimen is critical. The probability of observing cholangitis and bile duct destruction increases with the number of portal tracts because of the patchy distribution of the lesions. At least 15 portal tracts should be present, and multiple sections should be reviewed to adequately appreciate or rule out cholangitis and ductopenia.

The diagnosis of AMA-negative primary biliary cirrhosis should lie on the following criteria:

- presence of both biochemical cholestasis and typical destructive cholangitis, and
- no evidence or signs suggestive of sclerosing cholangitis [normal cholangiogram by endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance imaging (MRI)], sarcoidosis and other etiologies of chronic cholestasis in adults.

It is our policy to consider liver biopsy mandatory in the management of patients with primary biliary cirrhosis except in older patients (age over 70 years) with no or minimal disturbance of liver function tests and positive AMAs.





**Figure 19.4 Histological staging.** (a) Stage I. Non-suppurative destructive cholangitis affecting a medium-sized interlobular bile duct. Biliary epithelium is partly destroyed. Original magnification  $\times 50$ . (b) Stage II. Proliferating ductules with rupture of limiting plate. Original magnification  $\times 100$ . (c) Stage III. Inflammatory fibrous septa with aggregates of lymphoid cells. Original magnification  $\times 25$ . (d) Stage IV. Cirrhosis. Several regenerative nodules are seen. Original magnification  $\times 25$ .

In addition to aiding in diagnosis, histologic stage and some individual liver histologic features, such as the degree of severity of the lymphocytic piecemeal necrosis and of ductopenia, are valuable prognostic indicators in addition to serum bilirubin.

The diagnosis of primary biliary cirrhosis is sometimes very difficult for the following reasons: the typical histological lesions of primary biliary cirrhosis or primary sclerosing cholangitis are

not present in the liver specimen, the classic stricturing lesions are not obvious particularly in the intrahepatic form of primary sclerosing cholangitis especially when MRI is used to visualize the biliary tree.

In approximately 5–10% of the patients, M2 antibodies are absent or present at a low titer, when immunofluorescent techniques are used. In some patients, antinuclear antibodies, anti-gp210 and/or anti-Sp100 are present; in some of them antibodies against the major M2 components (PDC-E2) are present using enzyme-linked immunosorbent assay (ELISA) or Western blotting techniques and finally elevated IgM levels are usually observed. Thus, in the absence of M2 antibodies, the diagnosis of primary biliary cirrhosis can still be made if gp210 or Sp100 antibodies or frank elevation of IgM coexists with biochemical cholestasis and histopathologic evidence of bile duct lesions.

### Differential diagnosis

Up to 10% of patients have characteristics of both primary biliary cirrhosis and autoimmune hepatitis. In those patients, the following features are found: strikingly elevated aminotransferase activity, moderate to severe piecemeal necrosis lesions, marked

Diagnosis of primary biliary cirrhosis	
Criteria mandatory for diagnosis	
<ul style="list-style-type: none"> <li>• Cholestasis</li> <li>• M2 antibodies and/or Anti Sp100-Gp210</li> <li>• Destructive cholangitis</li> </ul>	

**Figure 19.5 Diagnosis of primary biliary cirrhosis.**

increase in IgG levels, and sometimes antiactin or antinuclear antibodies with homogeneous pattern by immunofluorescence. Patients who present the characteristics of both diseases have been diagnosed with so-called 'overlap syndrome' or 'autoimmune cholangiopathy'. In some patients, features of autoimmune hepatitis occur in those who initially had typical primary biliary cirrhosis. This mixed form must be identified, because in addition to UDCA treatment, they have to be treated with corticosteroids or even with cyclosporine.

Several drugs have been reported to induce cholestasis, cholangitis and ductopenia. The most frequently incriminated drugs are phenothiazines, haloperidol, imipramine, amoxicillin and clavulanic acid, and Cox-2 inhibitors. Acute onset of cholestasis (sometimes associated with pruritus) is usually observed and resolves completely after several weeks or months after the medication is discontinued. AMAs are absent. Ductopenia, fibrosis or even cirrhosis may occur.

Primary sclerosing cholangitis is characterized by chronic inflammation and fibrosis of intra- or extrabiliary ducts. In this setting, AMAs are absent. Cholangiography shows the classic stricturing and beading of the extra- and intrahepatic bile ducts. Liver biopsy shows the concentric periductular fibrosis that leads to progressive atrophy or even to the disappearance of the bile ducts.

Sarcoidosis can cause considerable diagnostic difficulty. Characteristics of these two diseases are compared in Table 19.2. Sarcoidosis and primary biliary cirrhosis usually have typical characteristics that allow clear distinction between the entities. However, sarcoidosis may be complicated by severe chronic cholestasis and portal hypertension, a clinical form that can mimic primary biliary cirrhosis. Finally, sarcoidosis and primary biliary cirrhosis may coexist in a single patient.

Cholestatic sarcoidosis compared with primary biliary cirrhosis		
	<i>Sarcoidosis</i>	<i>Primary biliary cirrhosis</i>
Gender	Equal	80% female
Age of onset	Young	Middle age
Pruritus	Yes (rare)	Yes (frequent)
Erythema nodosum	Yes	No
Respiratory symptoms	Yes (frequent)	Yes (rare)
Hepatosplenomegaly	Yes	Yes
Hilar adenopathy	Usual	Rare
Pulmonary infiltrate	Yes	Rare
Positive mitochondrial antibody	No	90%
Elevation of serum angiotensin converting enzyme	50%	10%
Positive Kveim-Siltzbach test	70%	No

**Table 19.2 Cholestatic sarcoidosis compared with primary biliary cirrhosis**

Causes of chronic cholestasis in adulthood
• Primary biliary cirrhosis
• Autoimmune hepatitis – overlap syndrome
• Primary sclerosing cholangitis <ul style="list-style-type: none"> <li>– intrahepatic</li> <li>– intra- and extrahepatic</li> </ul>
• Drug-induced cholestasis
• Low phospholipid associated cholelithiasis (LPAC) syndrome ( <i>ABCB4/MDR3</i> gene defect)
• Sarcoidosis
• Idiopathic ductopenia
• Lymphoma
• Stenosis, strictures and developmental abnormalities of the biliary tree (Caroli disease, congenital hepatic fibrosis)
• Allograft rejection, graft versus host disease
• Parenteral nutrition

**Table 19.3 Causes of chronic cholestasis in adulthood**

*MDR3* or *ABCB4* gene mutations may induce type 3 familial intrahepatic cholestasis in infancy. In young adults, the *MDR3* gene defect is associated with a peculiar form of cholestasis associated with symptomatic intrahepatic and gallbladder cholelithiasis.

Idiopathic adulthood ductopenia is a term proposed to describe a form of chronic cholestasis of unknown origin with clinical onset in adulthood, associated with loss of intrahepatic bile ducts. In these patients, AMAs are absent; there is no drug history; and cholangiographic findings show a normal biliary tree.

Other causes of chronic cholestasis are listed in Table 19.3.

## ASSOCIATED DISORDERS

Scleroderma, especially minor forms such as the CREST syndrome, may be observed in approximately 10% of patients. Systemic forms of scleroderma are often limited to a minor esophageal motor disorder or to a reduction in carbon dioxide diffusion capacity. Raynaud phenomenon is usually observed in 10–20% of the patients. Anticentromere antibodies, characteristic of CREST syndrome, are found in 10–20% of the patients. Sjögren syndrome, most often in its minor form, is frequent in primary biliary cirrhosis. Arthropathies and arthralgias are noted in 5–50% of patients with primary biliary cirrhosis. The clinical significance varies greatly. Thyroid abnormalities and anti-thyroid antibodies are observed in approximately 15% of patients with primary biliary cirrhosis. The most frequent forms are hyperthyroidism and Hashimoto disease. Hyperthyroidism may be associated with a rise in serum bilirubin and aggravation of cholestasis.

Several cases of villous atrophy associated with gluten intolerance have been reported in patients with primary biliary cirrhosis. The number of cases reported indicates more than a



random association. Primary biliary cirrhosis should be suspected in a patient in whom celiac disease is discovered and liver test results are abnormal. A similar association has been reported in patients with sclerosing cholangitis. In this setting, in contrast to primary biliary cirrhosis, a gluten-free diet led to an improvement in liver test results. Villous atrophy is sometimes asymptomatic.

The frequency of gallstones can reach 30–50% according to the duration and severity of the disease. It is higher in patients with cirrhosis. Migration of stones through the bile ducts can aggravate cholestasis. Treatment of hypercholesterolemia with fibrates can lead to the formation of gallstones, which disappear when the drug is withdrawn. Supersaturation of the bile by calcium has been reported during long-term treatment with cholestyramine.

Pancreatic abnormalities, particularly involving the tail and detected by pancreatography have been reported in primary biliary cirrhosis. Severe pancreatic insufficiency is however rare and does not generally explain steatorrhea observed in patients with marked cholestasis.

Lung involvement is frequent in primary biliary cirrhosis but is generally subclinical. Pulmonary fibrosis occurs most frequently in patients with CREST syndrome or Sjögren syndrome. The hepatopulmonary syndrome, characterized by hypoxemia, and intrapulmonary vascular dilatation can also be observed in patients with advanced primary biliary cirrhosis. Pulmonary hypertension can occur in primary biliary cirrhosis, as in other forms of cirrhosis.

Renal tubular acidosis is frequent but usually subclinical. An increased susceptibility to urinary tract infections has been reported but has not been confirmed in other studies. Neuropsychiatric problems (e.g. anxiety, fatigue, depression) are frequent in women with primary biliary cirrhosis, especially those with Sjögren syndrome.

The incidence of breast cancer appears to be higher in women with primary biliary cirrhosis. In contrast, the incidence of other cancers is not different from expected rates.

The onset of pregnancy in patients with primary biliary cirrhosis is rare because the disease usually occurs after menopause or causes amenorrhea itself. Pregnancy, especially in the last few weeks, induces subclinical cholestasis. Pregnancy in patients with primary biliary cirrhosis generally leads to an aggravation in cholestasis. In contrast, in UDCA-treated women with primary biliary cirrhosis, pregnancy produces further improvement of liver biochemistries but after delivery, frank relapse usually occurs.

## COMPLICATIONS OF PRIMARY BILIARY CIRRHOSIS

Pruritus is present in approximately 70–80% of patients with primary biliary cirrhosis. It is usually moderate, but can be severe in approximately 5–10% of cases. The pathogenesis of pruritus is unknown. Opiate antagonists (naloxone and nalmefene) modulate the perception of pruritus and induces an opiate withdrawal syndrome, suggesting a possible role of endorphins.

Fatigue is reported by patients as a symptom that often dramatically impairs their quality of life. Three studies focused on the impact of fatigue score using specific questionnaires. The prevalence of fatigue varied greatly between these studies

(8–80%) and might be related to patient selection criteria. Scores of fatigue were augmented in patients with primary biliary cirrhosis but no relationships were found between these scores and any parameter of disease severity. As the pathogenesis of fatigue in primary biliary cirrhosis still remains to be defined, no specific treatments are available.

In primary biliary cirrhosis, osteopenia is mainly caused by osteoporosis, whereas osteomalacia is rare. Osteopenia is present in 10–35% of the patients at the time of diagnosis. This frequency does not differ from that observed in other chronic liver diseases, such as sclerosing cholangitis, hemochromatosis or chronic hepatitis.

Deficiencies of fat-soluble vitamins (A, D, E and K) can occur in primary biliary cirrhosis, especially in advanced or severe forms. This deficiency is mainly due to malabsorption but rarely results in clinical manifestations. Vitamin A deficiency must be corrected with care, because overdosage can lead to liver damage, particularly fibrosis. Vitamin D deficiency should be corrected, given the risk of osteopenia.

Portal hypertension is frequent in the course of primary biliary cirrhosis, generally developing after the onset of cirrhosis. In fewer than 5% of cases, portal hypertension or gastrointestinal bleeding may be the first manifestation of the disease. Portal hypertension can occur in the absence of cirrhosis and may be caused by portal inflammation, compression of small portal venules, and regenerative nodular hyperplasia. Patients with primary biliary cirrhosis and portal hypertension complicated by gastrointestinal bleeding should first be treated with  $\beta$  blockers and, in case of failure, with sclerosis or ligation of varices. If these treatments fail, liver transplantation should be preferred to portacaval shunt surgery.

Hepatocellular carcinoma appears to be a rare complication of primary biliary cirrhosis. When hepatocellular carcinoma occurs, the liver is cirrhotic. The low incidence of hepatocellular carcinoma may be explained by the relatively late formation of regenerative nodules in the course of the disease.

## PROGNOSIS AND TREATMENT

Most of the patients in whom primary biliary cirrhosis was diagnosed when asymptomatic will develop symptoms in the following years (**Fig. 19.6**). In a 40-month period, 10–30% of asymptomatic patients become symptomatic. The mean duration of the asymptomatic phase is extremely variable but is on the order of 6 years. The 5-year survival rate of asymptomatic patients is approximately 90%. After 5 years of progression, the proportion of survivors is significantly lower than in a paired control population. The duration of the symptomatic phase varies greatly but can last for 10 years. The mean 5-year survival rate among symptomatic patients is 50%, with a range of 30–70%. In terms of histologic progression, the median time to progress from histologic stage II to stage IV, i.e. cirrhosis, is 4 years. The terminal phase of the disease is defined when serum bilirubin level is higher than 100  $\mu\text{mol/L}$  (6 mg/dL), with or without signs of portal hypertension (gastrointestinal bleeding, ascites or encephalopathy). The disease progression is summarized in **Table 19.4**.

Shapiro et al (1979) showed the importance of serum bilirubin as a factor prognostic for survival in primary biliary cirrhosis. They noted that, after a relatively stable phase, serum

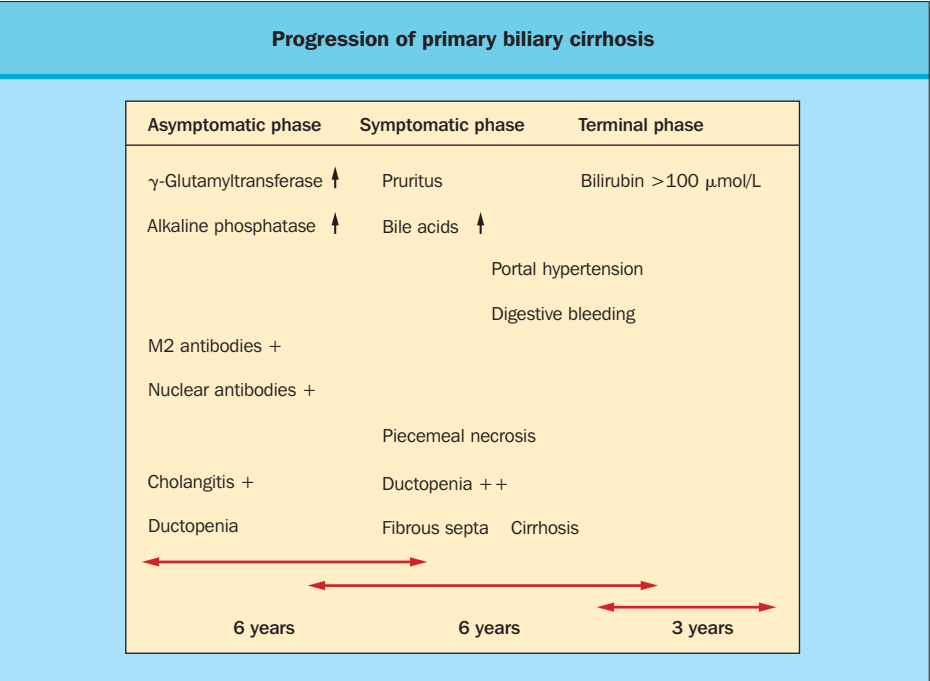


Figure 19.6 Progression of primary biliary cirrhosis.

Progression of primary biliary cirrhosis with time	
Feature	%
Hepatomegaly	+30
Splenomegaly	+28
Ascites	+25
Gastrointestinal bleeding	+22
Cirrhosis	+50
Bilirubin >105 μmol/L (>6.2 mg/dL)	+25
Albumin <29 g/L	+35
Reproduced from Christense 1980 et al (80) by permission of The American Gastroenterological Association.	

Table 19.4 Progression of primary biliary cirrhosis with time. Cumulative percentage of patients with each feature after 4 years of follow-up

bilirubin increased sharply in the months preceding death. In patients with serum bilirubin levels above 34 μmol/L (2 mg/dL), the mean survival was 4 years; in those with values above 102 μmol/L (60 mg/dL) 2 years, and in those with values above 170 μmol/L (10 mg/dL) 1.4 years. The extraordinary importance of bilirubin for short-term and long-term survival was confirmed in all later studies. Several prognostic models have been proposed to improve the prognostic value of serum bilirubin. These models are complex or are not highly superior to serum bilirubin alone. The Mayo risk score has been used as a prognostic index in patients with primary biliary cirrhosis (Table 19.5).

It is important to emphasize that the prognostic value of serum bilirubin level for survival free of liver transplantation is similar in UDCA-treated patients as in non-treated patients.

Calculation and interpretation of the Mayo risk score for primary biliary cirrhosis.	
<b>Step 1:</b> Calculate R	
$R = 0.871 \times \log(\text{bilirubin in mg/dL}) + (-2.53 \log_e [\text{albumin in g/dL}]) + 0.039 \text{ age in years} + 2.38 \log_e \text{ prothrombin time in s} = 0.859$ (if edema is present).	
<b>Step 2:</b> To obtain the probability of survival for at least t more years, one reads $S_0(t)$ from the table below and computes $S(t) = [S_0(t)]^{\exp(R-0.57)}$	
t (years)	1      2      3      4      5      6      7
$S_0(t)$	0.970   0.941   0.883   0.833   0.774   0.721   0.651

Table 19.5 Calculation and interpretation of the Mayo risk score for primary biliary cirrhosis

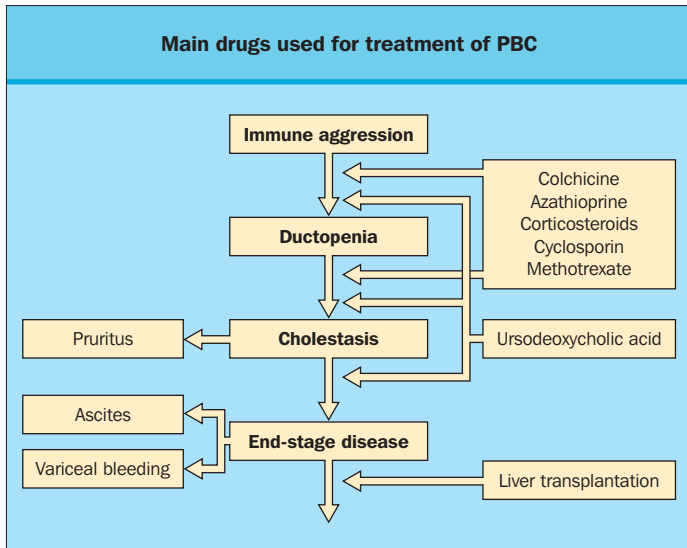
Medical treatments

As with all chronic diseases, the most difficult choice for assessing efficacy is the endpoint(s). Survival is clearly a valid criterion of efficacy, but creates a number of problems; for example:

- the need for long-term follow-up of a large number of patients; and
- ethical considerations if the treatment is ineffective or carries a high risk of side-effects.

One way of shortening the study period is to use surrogate markers. Given the lack of validated surrogate markers in primary biliary cirrhosis, survival free of liver transplantation appears to be one of the best endpoints. Finally, with long-term treatment, the balance between benefit and adverse effects is of crucial importance.

As primary biliary cirrhosis is known to be an autoimmune disease, most drugs used for its treatment have been selected on



**Figure 19.7** Main drugs for the treatment of primary biliary cirrhosis and their therapeutic targets.

the basis of their immunosuppressive or anti-inflammatory properties. **Figure 19.7** lists the main drugs and their therapeutic targets.

Azathioprine, an immunosuppressive drug that is frequently used to prevent allograft rejection, was evaluated in two trials. Azathioprine had little clinical value and a slight benefit in terms of survival. Azathioprine is no longer used in the treatment of primary biliary cirrhosis.

D-Penicillamine was selected for its anticupruritic properties because copper accumulates in the liver of patients with primary biliary cirrhosis as much as in those with Wilson disease, and its immunosuppressive and antifibrotic properties. D-Penicillamine (tested in a total of 748 patients) had no therapeutic benefit on primary biliary cirrhosis but was associated with a high incidence of serious side-effects (up to 30%). It has now been abandoned for the treatment of primary biliary cirrhosis.

### Corticosteroids

In a 1-year trial, 36 symptomatic patients who had no complications of cirrhosis were randomized to receive either prednisolone (30 mg/day, progressively reduced over 8 weeks to 10 mg/day maintenance dose) or a placebo. Prednisolone treatment improved transaminase and alkaline phosphatase activities, procollagen III peptide and IgG but not serum bilirubin, pruritus, and fatigue in the first weeks; there was a trend to an improvement in liver histology. These beneficial effects were offset (in addition to the usual adverse effects of corticoids) by increased bone loss.

### Cyclosporin

In a randomized clinical trial, 19 precirrhotic patients received cyclosporin (4 mg/kg/day) and 10 patients received a placebo. After 1 year, cyclosporine improved pruritus, bilirubin levels, ALT and alkaline phosphatase activities, gammaglobulin and AMA titer. In a subgroup of 20 patients who have completed the 2-year study period, cyclosporine therapy apparently had a beneficial effect on liver histology; however, changes in liver morphology in such a small group of patients should be

interpreted cautiously. Most patients had renal toxic effects, and hypertension. In a large European trial (346 patients, median follow-up 2.5 years), cyclosporin therapy had similar effects on laboratory parameters, no significant effect on histologic progression, and a slight increase in survival rate. Cyclosporin has a very modest therapeutic effect and causes severe adverse effects.

### Colchicine

Colchicine has been used for its antifibrotic and anti-inflammatory properties. Colchicine (0.6 mg/day) improves serum levels of bilirubin, albumin, alkaline phosphatase, cholesterol and aminotransferase but not the severity of symptoms or histologic features. No change in mortality has been demonstrated.

Although colchicine treatment was associated with an improvement in a number of liver tests, a beneficial long-term effect on disease progression remains to be established. Drug toxicity was minimal.

The efficacy of colchicine combined with UDCA versus UDCA alone was evaluated in the treatment of patients with non-advanced primary biliary cirrhosis in a 2-year controlled study. This study suggests that colchicine appears to provide a slight benefit relative to UDCA alone in these patients, a result confirmed by a recent meta-analysis of five clinical trials.

### Methotrexate

Following pilot studies showing that hepatic inflammation and bile duct injury improved in selected patients treated with methotrexate, an initial randomized trial reported no benefit from methotrexate.

More recently, an observational cohort study described the course of 110 patients from the time they began therapy with methotrexate until the time the drug was discontinued or until at least 5 years of continuous use had elapsed. UDCA was added to methotrexate for most of the patients as the benefit of UDCA became increasingly clear through the years. The relative risk of failure (death, transplantation) associated with methotrexate use did not approach statistical significance while the risk of failure of patients on UDCA was significantly lower than that of patients not on UDCA. Because methotrexate was not well tolerated and because there was no evidence of efficacy, the authors concluded that methotrexate use could not recommend routinely.

Kaplan et al (2004) recently reported the results of a small trial of colchicine plus UDCA versus methotrexate plus UDCA including 60 patients enrolled in 1988 and followed for up to 10 years. Approximately one-half of the patients had cirrhosis and serum bilirubin levels ranged from less than 10  $\mu\text{mol/L}$  up to 160  $\mu\text{mol/L}$ , making the sample very heterogeneous. The results suggest a beneficial effect of methotrexate in a subset of selected patients having early stage disease.

A third study published in abstract form, called 'PBC ursodiol and methotrexate or placebo study' (PUMPS) has randomized 226 patients to UDCA and methotrexate or to UDCA plus placebo. At entry into the trial, most of the patients had a serum bilirubin of 17  $\mu\text{mol/L}$  or less and about half of them had early histologic stage disease. After 7-year follow-up the hazard ratio for death with or without OLT was such that the trial was stopped early due to futility.



Therefore, methotrexate cannot be recommended routinely as monotherapy or as adjuvant therapy of UDCA in primary biliary cirrhosis.

### **UDCA as the first-line therapy**

The only drug currently approved specifically for the treatment of primary biliary cirrhosis is UDCA. Although the mechanisms of action of UDCA in cholestasis have not been clearly elucidated so far, increasing experimental evidence supports the use of UDCA in cholestatic diseases. Accumulation of the hydrophobic bile acids in the cholestatic liver may induce liver cell injury and may lead to fibrosis and cirrhosis. UDCA stimulates biliary secretion of endogenous bile acids. UDCA reduces aberrant expression of HLA class I molecules of cholestatic hepatocytes. Experimental findings suggest that UDCA exerts its anticholestatic effects by protection of cell membranes at the level of the biliary tree and by stimulation of impaired hepatobiliary secretion. UDCA exerts antiapoptotic effects not only in cholestatic hepatocytes but also in a number of other experimental conditions of cell injury. Mitochondrial perturbations induced by hydrophobic bile acids in cholestasis are prevented by UDCA in hepatocytes.

The increasing use of UDCA as the first-line therapy is based on a body of evidence showing that this bile acid slows the progression of the disease towards its irreversible terminal phase.

### **Effect of UDCA therapy on survival**

Long-term UDCA treatment improves survival free of OLT. This has been demonstrated by the analysis of the combined raw data of three clinical trials involving 548 patients, followed for up to 4 years and treated with the optimal dose regimen of UDCA (13–15 mg/kg/day) (see below).

Other evidence comes from long-term observational studies. Survival of UDCA-treated patients is better than that predicted by the Mayo model for primary biliary cirrhosis. However, it should be emphasized that 10-year survival of UDCA-treated patients with primary biliary cirrhosis remains slightly lower than that of an age- and sex-matched general population, the difference being mainly explained by the mortality among patients with cirrhosis at the outset of treatment (Fig.19.8).

### **Effect on histology**

UDCA treatment slows the histological progression and delays the onset of cirrhosis. No significant changes in histological stages of the disease have been found in any trial of up to 2 years in duration. In contrast, a long-term clinical trial (median follow-up 3.4 years) has provided evidence that the rate of progression to cirrhosis is lower in the UDCA group than in the placebo group (10 versus 42%), a finding confirmed by a combined analysis of major trials.

Using a multistage continuous time Markov model to analyze the histological progression rate, UDCA therapy was associated with lower yearly transition rates from early stages to late stages (extensive fibrosis or cirrhosis). The median times of remaining free of late stages were 75 and 25% at 5 years of follow-up in UDCA- and placebo-treated patients, respectively.

### **Other effects**

UDCA therapy consistently ameliorates serum liver tests including serum bilirubin that keeps its potent prognostic value

in patients receiving UDCA treatment. UDCA therapy delays the onset and severity of portal hypertension. The risk of newly developing endoscopically confirmed varices was 16% for the UDCA-treated patients compared with 58% for the placebo-treated patients after 4 years of follow-up.

### **Optimal dose of UDCA**

Among all the published clinical trials only three used the initially proposed dose, namely 13–15 mg/kg/day. Two recent clinical trials were conducted to address the issue of the optimal daily dose. These two studies show that doses ranging from 13 to 20 mg/kg/day afford the optimal enrichment in biliary UDCA as well as the most significant changes of liver tests. Doses equal to 10 mg/kg/day or less should be considered as suboptimal for treating primary biliary cirrhosis.

However, it should be emphasized that the dose regimen must be adapted to the patient status. In particular, in patients having pruritus, hyperbilirubinemia, severe ductopenia or cirrhosis, the following is recommended:

- starting with a low dose, i.e. 200 mg/day, to avoid side-effects;
- checking serum bile acid and bilirubin levels every month. The optimal daily dose may only be reached 6 to 8 months after the outset of treatment.

Serum UDCA and endogenous bile acid levels can be assessed to determine the optimal dose, which in fact may vary from one patient to another because of differences in absorption, metabolism of bile acids and liver metabolic activity.

### **Resistance to UDCA therapy**

Resistance to UDCA therapy may be defined by the development of cirrhosis or progression to the terminal phase in patients receiving UDCA.

The biochemical response and the degree of severity of lymphocytic piecemeal necrosis assessed under UDCA therapy are the two independent predictors of development of cirrhosis. In patients already having cirrhosis at the outset of UDCA treatment, normalization of serum biochemistries is significantly less frequent than in patients diagnosed at an early stage of the disease. In cirrhotic patients, the degree of elevation of serum bilirubin measured under UDCA therapy predicts the 5-year occurrence of decompensation of cirrhosis or death.

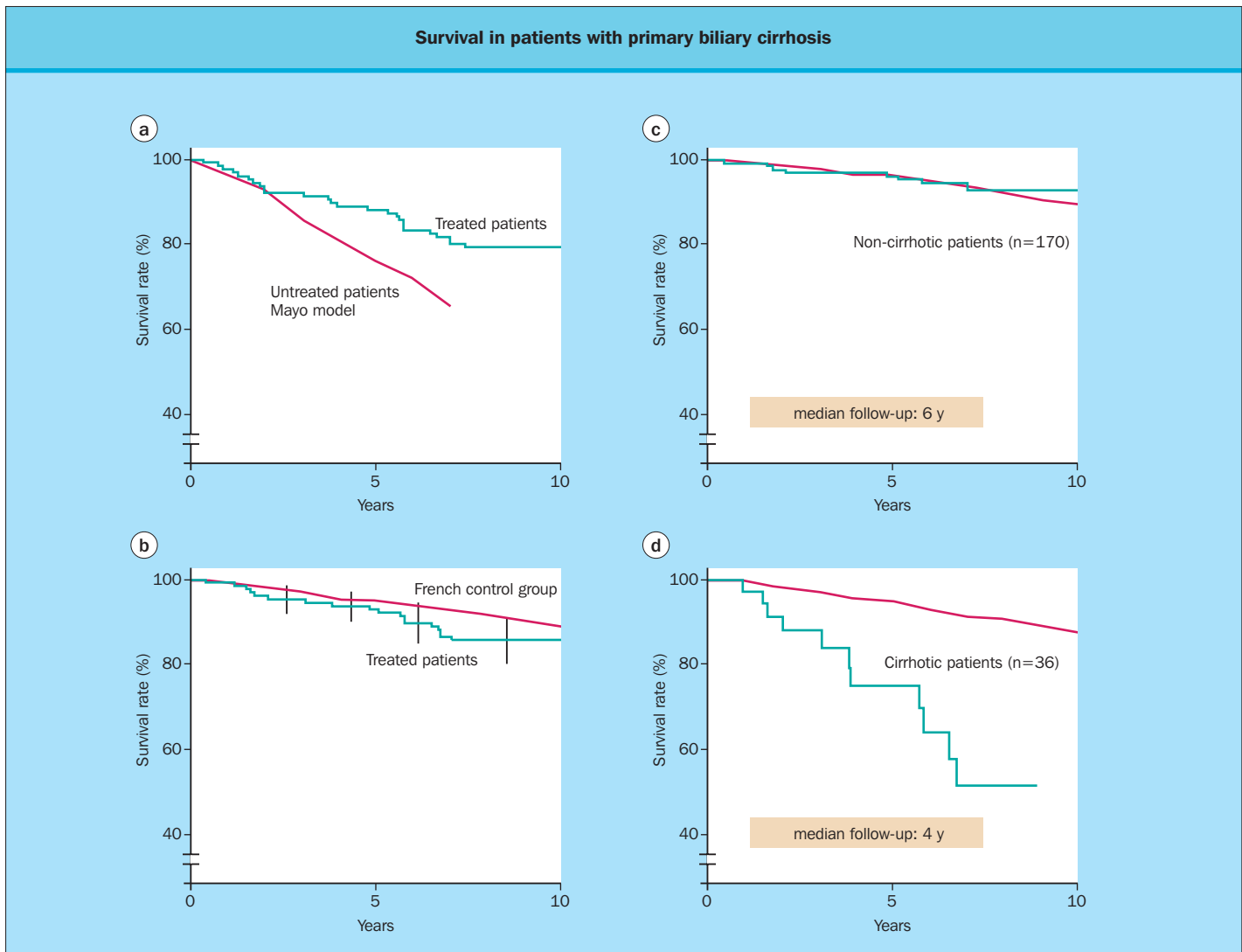
In a patient with a suboptimal response to UDCA, the following possibilities should first be checked:

- non-compliance and inappropriate dosage regimen (both necessitate the determination of individual serum bile acids);
- coexisting autoimmune thyroid dysfunction or celiac disease;
- postbiopsy arteriportal fistula.

The most common and unrecognized cause of resistance is the PBC-autoimmune hepatitis overlap syndrome. The diagnosis of the typical form of this syndrome (coexisting features of both diseases at presentation) is easy. In contrast, the occult form of the syndrome manifested by moderate to severe interface hepatitis and intermittent flares of ALT is often missed.

### **Treatment of primary biliary cirrhosis resistant to UDCA therapy**

The benefit from the combination of corticosteroids and UDCA in the overlap syndrome is obvious.



**Figure 19.8 Survival in patients with primary biliary cirrhosis** (a) Comparison of observed survival free of orthotopic liver transplantation (OLT) of ursodeoxycholic acid (UDCA)-treated patients with primary biliary cirrhosis (solid line) with survival predicted by the updated Mayo model (dotted line). Observed survival was significantly higher than survival predicted by the Mayo model ( $P < 0.04$ , one sample log rank test). (b) Comparison of the observed survival in the UDCA-treated cohort (solid line) with survival of a standardized French population (dotted line). Observed survival of the treated primary biliary cirrhosis patients was significantly lower than that of the control population ( $P < 0.01$ , one sample log rank test). (c) Observed survival of non-cirrhotic patients (solid line) was not different from that of the French control population (dotted line). (d) Survival of cirrhotic patients (solid line) was significantly lower than that of the control population (dotted line) ( $P < 0.0001$ ). Reproduced from Poupon et al (99) by permission of Wiley-Liss, Inc.

So far, three classes of drugs have been tested in combination with UDCA: colchicine, methotrexate and corticosteroids. The combination of colchicine with UDCA failed to show major additional benefits of colchicine. However, it cannot be excluded as the treatment period in randomized clinical trials was too short. Another explanation could be that the dose of colchicine, usually 1 mg/day, was too low. However, because colchicine is mainly excreted into bile, cholestasis may lead to overdose and side-effects. It could thus be hazardous to increase the daily dose of colchicine.

The combination of methotrexate with UDCA has been tested in several studies. Methotrexate adds little or no benefit in combination with UDCA in most of the patients.

The combination of corticosteroids with UDCA has been tested in several short-term studies. These studies show further

improvement in biochemistries with or without improvement in liver histology. These results are encouraging, but again, we have to take into account the risk/benefit ratio in the long term.

## LIVER TRANSPLANTATION

OLT is the only therapeutic option for end-stage primary biliary cirrhosis. Most centers consider patients with primary biliary cirrhosis to be excellent candidates for liver transplantation, because they are usually young to middle-aged women without other major organ disease.

The efficacy of liver transplantation in patients with primary biliary cirrhosis was demonstrated definitively in 1989. The observed survival of 161 primary biliary cirrhosis patients who had undergone liver transplantation was compared with the

predicted survival (Mayo model) of these same patients if they had been treated conservatively. The survival probabilities in the liver transplant recipients 6 months after liver transplantation were significantly higher than the Mayo predictions. These findings have been confirmed in additional studies.

The most difficult question is when to transplant. Liver transplantation should be performed while the patient is well enough to tolerate the operation, yet sufficiently late in the course of the disease to make it cost effective. The two current criteria for liver transplantation in primary biliary cirrhosis are progressive hyperbilirubinemia ( $>100\text{--}150\text{ }\mu\text{mol/L}$ ,  $5.9\text{--}8.8\text{ mg/dL}$ ) and/or ascites. A less common criterion is uncontrolled recurrent gastrointestinal bleeding. Intolerable pruritus and intractable fatigue are highly subjective criteria and must remain exceptional indications for liver transplantation.

Survival for 1 year following liver transplantation for primary biliary cirrhosis now exceeds 75% in most centers. An excellent 5-year survival rate of 71% was reported in the largest series (194 patients). Most deaths occur in the first 3 months and are caused by primary non-function, rejection or infection.

Rehabilitation is usually excellent and most survivors return to work (at least part time). After liver transplantation, patients with primary biliary cirrhosis may expect prompt resolution of the symptoms and complications related to liver disease although

osteodystrophy tends to worsen in the first 3-4 months after transplantation, and then bone loss stops and bone mass is restored almost to normal within 2-3 years.

The effect of liver transplantation on extrahepatic manifestations of the autoimmune phenomena is more controversial. After liver transplantation, 90% of patients show some improvement in keratoconjunctivitis sicca and approximately 50% show complete remission of such symptoms. Improvement in arthropathy and Raynaud phenomenon is experienced by 80-90%. These effects may be caused by immunosuppression rather than liver grafting itself.

There is significant controversy as to whether primary biliary cirrhosis recurs after transplantation. Serologic abnormalities in the form of raised IgM levels and AMAs persist in most patients receiving transplant for primary biliary cirrhosis, but levels tend to decrease. Liver test abnormalities following transplant are non-specific and cannot reliably differentiate the many causes of cholestasis in the liver allograft. Thus, considerable emphasis has been placed on histologic findings. However, even with biopsy the risk of recurrence of primary biliary cirrhosis following transplant remains unclear. On the basis of combined features 'compatible with primary biliary cirrhosis', the rate of recurrence varies from 30 to 50% during the 5-10 years after OLT but is rarely severe enough to result in graft failure.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 20

## Primary Sclerosing Cholangitis

K. V. Narayanan Menon and Russell H. Wiesner

### INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic cholestatic syndrome of unknown etiology that is characterized by chronic cholestasis and diffuse inflammation and fibrosis that involve the entire biliary tree. The pathologic process obliterates intrahepatic and extrahepatic bile ducts, which ultimately leads to biliary cirrhosis, portal hypertension and liver failure.

The syndrome, PSC, was first described by Delbet in 1924, and as of 1980 only 100 cases were documented in the English literature. It was therefore considered a rare disease before the widespread application of endoscopic retrograde and percutaneous transhepatic cholangiography in the late 1970s, which allowed the diagnosis of PSC to be made without surgery (**Fig. 20.1**). The recognition of the association between PSC and inflammatory bowel disease coupled with the use of screening biochemical liver tests as part of routine general examination has further increased the frequency with which PSC is diagnosed. Today, the detection of abnormal liver tests in patients with inflammatory bowel disease leads to an earlier diagnosis of PSC, frequently when the patient has minimal or no symptoms related to liver disease. These advances in the diagnosis of PSC have led to a better understanding of this previously seldom diagnosed syndrome; in particular, its clinical features and natural history.



**Figure 20.1 Cholangiogram.** Classic cholangiographic findings in the setting of PSC are noted including strictures, beading and irregularities of the intrahepatic and extrahepatic biliary system.

Despite these advances, the etiology remains unknown and no curative therapy has been identified. Immunopathogenic mechanisms seem likely to play a major etiologic role based on the strong association of PSC with certain human leukocyte antigens (HLA haplotype), the presence of autoantibodies, a close relationship with other autoimmune diseases and the presence of multiple immunologic abnormalities. However, to date, target antigens have not been identified and, importantly, immunosuppressive therapy has had minimal impact on disease progression.

### EPIDEMIOLOGY

Although the prevalence of PSC in the USA is unknown, it can be estimated because of its frequent association with chronic ulcerative colitis (CUC). Studies have shown a 2.4–7.5% prevalence of PSC in patients with CUC. Because the incidence of CUC ranges from 40 to 225 cases/100 000 population, the incidence of PSC in the USA is estimated to be between two and seven cases/100 000 population. The prevalence of PSC in one midwestern community in the USA was 20.9/100 000 men and 6.3/100 000 women. These estimates are supported by the results of epidemiologic studies from Sweden and Norway, where the incidence of CUC and PSC was noted to be 171 and 6.3 cases/100 000 population, respectively. These results, however, probably underestimate the actual incidence of PSC because the disease can occur in patients with normal serum levels of alkaline phosphatase, and 20–30% of patients with PSC do not have associated inflammatory bowel disease. Therefore PSC seems to be more common than previously suspected, and may have a frequency similar to that reported for primary biliary cirrhosis (PBC).

### PATHOPHYSIOLOGY

The cause of PSC remains unknown despite extensive investigation into various mechanisms related to bacteria, viral infections, toxins, genetic predisposition and immunologic mechanisms, all of which have been postulated to contribute to the pathogenesis and progression of this syndrome (**Table 20.1**).

#### Bacteria toxin theory

The close association of inflammatory bowel diseases, especially CUC, with PSC has been intriguing and led several investigators to postulate that portal bacteremia or absorption of toxins or toxic bile acids from the inflamed colon may play a major role in the pathogenesis of PSC. It was postulated that the primary event in PSC was a chronic, low-grade portal vein bacteremia that caused chronic biliary tract inflammation and fibrosis. However,

Pathophysiology of PSC		
<i>Causal factor</i>	<i>Supportive features</i>	<i>Contradicting aspects</i>
Colonic bacteria or toxins	Association with UC Bacterial peptides induce changes seen in PSC	Portal bacteremia not a feature of severe PSC Clinical association with quiescent PSC
Viral	Some viruses (e.g. CMV) can cause bile duct damage	No direct link between any virus and PSC has been established
Genetic	Strong HLA associations reported	Associations are not totally consistent
Immunologic	Hypergammaglobulinemia Autoantibodies Circulating immune complexes Complement activation Abnormal cell mediated immunity	No clinical response to immunosuppressive therapy

**Table 20.1 Pathophysiology of primary sclerosing cholangitis (PSC).**

The pathophysiology is probably multifactorial involving some or all of these proposed causative factors. UC, ulcerative colitis; CMV, cytomegalovirus; HLA, human leukocyte antigen.

investigators were unable to demonstrate a significant incidence of portal vein bacteremia in patients undergoing surgery for severe, uncontrolled CUC. Furthermore, detailed hepatic histologic analysis revealed that portal phlebitis, the hallmark of portal vein bacteremia, was mild or absent in most patients with PSC. Thus, little evidence supports the hypothesis that portal bacteremia plays a major pathogenic role in this syndrome.

It has been postulated that PSC is caused by a reaction to toxic bile acids, such as lithocholic acid, formed by the bacterial action in the diseased colon that allows absorption of the toxin directly into the portal blood. This hypothesis has also been refuted as multiple studies have been unable to demonstrate major abnormalities in bile acid metabolism in patients with PSC or CUC.

Proinflammatory bacterial peptides synthesized by colonic bacteria in a rat model caused portal inflammation and neutrophilic cholangitis similar to the histopathologic lesion seen in early PSC. Animals that had experimentally induced colitis experienced an eightfold increase in biliary excretion of these proinflammatory bacterial peptides. In another study, investigators demonstrated that intestinal bacterial overgrowth in rats was associated with hepatic inflammation similar to that seen in PSC. Thus, further investigation into the potential pathogenic role that proinflammatory bacterial peptides may play in PSC seems warranted. However, other evidence suggests that increased absorption of toxins by way of an inflamed colon does not play a major part in the pathophysiology of PSC. For example, the severity of CUC does not appear to predict the likelihood of PSC developing, since most patients with PSC have mild or quiescent inflammatory bowel disease. Moreover, PSC has been known to develop years before the onset of CUC and may develop years after proctocolectomy for CUC. In addition, PSC

is diagnosed in a substantial number of patients who have no past or present clinical or histologic evidence of CUC. These findings suggest that colonic disease leading to increased absorption of bacterial products or toxins is not a major factor in the pathogenesis of this syndrome.

The remaining 'toxin' worth mentioning in relation to the pathophysiology of PSC is copper. It has been shown that PSC leads to an accumulation of hepatic copper. This probably represents an epiphenomenon related to cholestasis since it has now been well demonstrated that increased hepatic copper levels are found in patients with all causes of chronic cholestasis. Furthermore, D-penicillamine therapy, which is known to reduce in hepatic copper levels, was not shown to be beneficial in PSC patients in a controlled clinical trial. Therefore, there is little evidence to support hepatic copper toxicity being a major factor in the pathophysiology or disease progression of PSC.

### Viral infection

Infection of biliary epithelial cells by viral agents has been postulated by some to be involved in the pathogenesis of PSC. Previous studies have excluded hepatitis A, B and C viruses as causative factors in PSC. Cytomegalovirus has been implicated in the pathophysiology of PSC. It can cause intralobular bile duct destruction and has been implicated in causing a decrease in the number of intralobular bile ducts. However, fibrosis and the destruction of large ducts have never been shown to be associated with cytomegalovirus infection. More importantly, inclusion bodies, typical of cytomegalovirus infection, have never been demonstrated histologically in bile duct epithelial cells or hepatocytes from patients with PSC.

Other reports suggesting that reovirus type 3 can induce cholangitis and biliary atresia in weanling mice and primates raised the possibility that this virus may also play a causative role in PSC. However, more recent data have shown that neither the prevalence nor the titers of antibodies to reovirus type 3 differ between patients with PSC and normal adult controls. Thus, there is no evidence to support the hypothesis that the pathophysiology of PSC is related to a viral infection.

### Genetic predisposition

Genetic factors have been proposed to contribute to the pathogenesis of PSC. Familial clustering of PSC has been reported. In addition multiple studies have shown a strong association between PSC and certain HLA haplotypes suggesting genetic susceptibility. The first HLA haplotypes shown to be associated with PSC were B8, DR3, DR2, and A1, B8, DR3. In 1990, investigators reported a 100% association of PSC with the HLA-DRw52a (or DRB3\*0101). Subsequent studies failed to confirm this observation but demonstrated that only 52–55% of PSC patients are positive for the HLA-DRw52a haplotype. Later, HLA haplotypes DR3 and DR6 were also found to be highly associated with PSC. Finally, a strong association of PSC with the DRB1\*1301, DQA1\*1301, and DQB1\*0603 alleles was demonstrated.

Meanwhile, investigators began to examine the relationship between HLA genotype and prognosis. One study revealed that HLA-DRw52a-positive PSC patients had a significantly diminished estimated median survival compared with the HLA-DRw52a-negative group. Other studies also reported that PSC patients with HLA-DR4 haplotype and DR3, DQ2 heterozygosity experienced relatively rapid disease progression. In contrast,

another report concluded that HLA-DR and HLA-DQ haplotypes do not represent markers for a more rapid disease progression. Detailed analysis of the characteristics of each study population and additional studies are needed to clarify this issue.

### Immunologic cause

To date, the most attractive hypothesis for the pathogenesis of PSC is immune-mediated damage of bile ducts. For example, the HLA haplotypes associated with PSC (i.e. HLA-B8, HLA-DR3) are also associated with known autoimmune disorders. In addition, abnormal humoral and cellular immune profiles have been described in PSC patients, which although inconclusive, support the hypothesis. Finally, a shared and specific epitope has been detected on human colonic and biliary epithelium. Circulating autoantibodies against this specific epitope were detected in the serum of two-thirds of PSC patients.

The immunohumoral abnormalities reported in PSC patients include

- hypergammaglobulinemia, often with a disproportionate elevation of serum IgM levels;
- increased titers of antismooth muscle, antinuclear, anticolonic, antiportal tract, perinuclear antineutrophilic cytoplasmic (pANCA) and antiendothelial cell antibodies;
- increased levels of circulating immune complexes in serum and bile; and
- activation of the complement system and abnormal clearance of immune complexes from the circulation.

Recent interest has focused on the high prevalence of pANCA in PSC patients with or without CUC. Currently, pANCA is considered a marker of PSC despite the variation in its specificity and sensitivity reported in studies due to methodologic differences. Nevertheless, pANCA is not suggested for screening of PSC since it is not pathognomonic and its titer widely fluctuates during the course of the disease. The pattern of pANCA staining in PSC has been characterized as atypical perinuclear and is distinct from that seen in Wegener granulomatosis, possibly reflecting a difference in the target antigen. The antigen(s) that reacts with pANCA in PSC remain unknown. Proposed antigens include cathepsin G, a chymotrypsin-like protease, and the bactericidal/permeability increasing protein, an endotoxin-binding protein of polymorphonuclear granulocytes. A number of studies have investigated the prognostic significance of pANCA in PSC. These reports demonstrated that the titer of pANCA in PSC patients does not correlate with liver histology, clinical activity and biochemical profiles, but has been associated with more extensive biliary disease. Of interest, pANCA titers can persist after liver transplantation and proctocolectomy. Further studies are needed to identify the antigenic determinants of pANCA in PSC and to better understand the role of these antibodies in PSC.

In an early study, the inhibition of leukocyte migration by biliary antigens in PSC patients suggested the presence of a cellular-mediated immune mechanism in its pathogenesis. The cellular immune abnormalities described in PSC patients include the following:

- a significant decline in the total number of circulating T cells due to a disproportionate decrease in CD8 (suppressor/cytotoxic) cells leading to an increase in the ratio of CD4+ to CD8+ cells;

- a significantly increased ratio of CD4+ to CD8+ T cells in the blood of those with cirrhosis compared with non-cirrhotics;
- an increase in the absolute number and percentage of peripheral B cells;
- an increase in the absolute number and percent of  $\gamma\delta$ -T cells in the peripheral blood and portal areas; and
- a dominant usage of the V  $\beta$  3 gene segment of the T-cell receptor in the T lymphocytes infiltrating the liver.

These findings suggest altered immunoregulation in PSC. Further studies to characterize the activated T lymphocytes infiltrating the liver are necessary to elucidate their role in the pathogenesis of PSC.

The enhanced expression of HLA class II antigens on the biliary epithelial cells of patients during the early stages of PSC and after extrahepatic biliary obstruction has been reported. Although this finding may suggest an autoimmune activation of host lymphocytes by the bile duct epithelia that become capable of presenting self or foreign antigens, it most likely reflects an epiphenomenon of the disease since it is not specific for PSC.

Adhesion molecules such as the intercellular adhesion molecule (ICAM)-1, a ligand for the leukocyte adhesion receptor lymphocyte function-associated antigen (LFA)-1, are involved in the communication between T lymphocytes and antigen-presenting cells (APCs). ICAM-1 has been detected on bile duct epithelia in cirrhotic-stage PSC; increased serum levels of circulating ICAM-1 have also been found in PSC. The fact that the expression of HLA-DR predates the expression of ICAM-1 in bile ducts of PSC patients suggests minimal participation of the latter in the initiation of PSC. Nevertheless, LFA-1 is over-expressed in intrahepatic lymphocytes, implying that additional unidentified adhesion molecules may exist in the bile ducts of patients with PSC. Proinflammatory cytokines induce the expression of ICAM-1, and HLA class I and II molecules on human bile duct epithelia. However, the importance of cytokines in the pathogenesis of PSC remains unclear and further studies are needed.

Finally it has been proposed that PSC is the consequence of an environmental insult that leads to the autoimmune destruction of biliary epithelia in a genetically predisposed individual. Understanding the molecular basis of the genetic and autoimmune milieu involved in PSC is a prerequisite to clarifying the pathogenesis of PSC and devising rational and effective medical therapies.

### CLINICAL FEATURES

The syndrome of PSC can affect any age group and any race, but it appears to occur predominantly in young Caucasian males. In the Mayo Clinic series of 174 adult PSC patients, two-thirds were male and the mean age at the time of diagnosis was 39 years. The clinical presentation of PSC can range from the finding of an increased serum alkaline phosphatase level in an asymptomatic patient to a complication of portal hypertension, such as the onset of ascites, variceal bleeding or portosystemic encephalopathy. With increasing frequency, PSC is being diagnosed in patients with established inflammatory bowel disease who develop abnormal biochemical liver function tests. However, even patients with advanced-stage PSC can have normal liver tests.



Frequency of symptoms and signs of PSC	
Symptom/sign	Frequency (%)
Fatigue	66
Pruritis	59
Jaundice	59
Hepatomegaly	48
Weight loss	34
Splenomegaly	34
Cholangitis	28
Hyperpigmentation	14
Ascites	7
Variceal bleed	6
Xanthoma	4
Hepatic encephalopathy	2

**Table 20.2 Frequency of symptoms and signs of primary sclerosing cholangitis (PSC).** Profile of the signs and symptoms present at the time of diagnosis.

### Symptoms and signs

Many patients are asymptomatic at the time of presentation and diagnosis; however, most of them have a cholestatic biochemical profile that is characterized by an increased serum alkaline phosphatase level. Of the 75% of patients who are symptomatic, presentation can be with a variety of symptoms including fatigue, pruritus or jaundice. Ascending cholangitis secondary to bacterial infection of the biliary tree, characterized by pain, fever and jaundice, is an unusual presenting feature unless the biliary tree has a stricture or has been previously surgically or endoscopically manipulated. Other signs and symptoms are similar to those that occur in patients with other chronic liver diseases such as weight loss, ascites variceal bleeding from an esophageal or stomal source, and hepatic encephalopathy (Table 20.2). On physical examination, nearly 50% of PSC patients will have hepatomegaly or jaundice at the time of diagnosis. Other common signs include splenomegaly, hyperpigmentation and xanthomas.

### DIAGNOSIS

Criteria used to diagnose PSC have evolved with time. Originally, the diagnosis of PSC was established only at laparotomy, by palpation of a fibrotic common bile duct, and a biopsy of the common bile duct which excluded bile duct cancer. Later, operative cholangiography revealed beading and irregularity of the extrahepatic and intrahepatic bile ducts, which today are the characteristic radiologic findings and are diagnostic of PSC in the appropriate clinical setting. The development of endoscopic retrograde cholangiopancreatography (ERCP) and transhepatic cholangiography (THC), both of which became technically feasible in the late 1970s, have had the greatest impact on the diagnosis of PSC. With ERCP, the spectrum of PSC rapidly expanded to include patients with isolated intrahepatic disease alone, and the number of cases of PSC diagnosed per year

increased two to threefold at most centers. In addition, the important relationship between PSC and the development of cholangiocarcinoma was confirmed using serial cholangiograms in patients with PSC. Therefore, cholangiocarcinoma, once considered an exclusionary criterion for the diagnosis of PSC, was shown to be within the spectrum of complications that developed during the course of PSC. More recently, non-invasive techniques such as magnetic resonance imaging (MRI) and magnetic resonance cholangiography (MRC) may allow for the diagnosis of PSC, and also can be useful in the diagnosis of cholangiocarcinoma.

Currently, the diagnostic criteria for PSC are centered on characteristic cholangiographic findings, which are by no means entirely specific for PSC; however, in the appropriate clinical setting these findings are useful in establishing the diagnosis of PSC. Cholangiographic changes similar to those found in PSC can be seen in other conditions and can cause difficulty in making the correct diagnosis. In general, these conditions also can be readily diagnosed by obtaining a detailed history and by carefully evaluating the cholangiographic and histologic findings, which are frequently helpful in excluding causes of secondary sclerosing cholangitis.

The diagnostic inclusion and exclusion criteria for PSC are outlined in Table 20.3.

These include:

- a cholestatic biochemical profile (alkaline phosphatase level of more than 1.5 times the upper limits of normal for 6 months or more). PSC can be diagnosed with a normal alkaline phosphatase level;
- a cholangiogram showing strictures in the intrahepatic, or extrahepatic biliary tree, or both (Fig. 20.1);
- a liver biopsy showing inflammatory fibrosis of interlobular and septal bile ducts, as well as early evidence of obliteration in the absence of evidence of other causes of chronic liver disease; and
- exclusion of causes of secondary sclerosing cholangitis (see Table 20.3).

### Biochemical and immunologic testing

Biochemical abnormalities are not specific for the diagnosis of PSC. However, a cholestatic profile of 6 or more months' duration is the hallmark of PSC, and most patients have at least a twofold increase in serum levels of alkaline phosphatase activity. Nevertheless, patients with cholangiographically documented PSC in whom alkaline phosphatase levels were normal have been described and a number of these patients had advanced histologic-stage disease on liver biopsy.

Serum aminotransferase levels are increased in 92% of patients, but are usually less than five times the upper limits of normal, except in children, and the mean increase is about three times the upper limits of normal. In patients with the highest aminotransferase levels, histologic features of autoimmune hepatitis have been noted, and an overlap syndrome including histologic and clinical features of both PSC and autoimmune hepatitis has been described.

While serum bilirubin levels are typically elevated in patients with advanced PSC, they tend to fluctuate widely during the course of the disease. At the time of diagnosis, serum albumin levels are decreased and prothrombin times prolonged in 17 and 16% of patients, respectively. Hypergammaglobulinemia is present

<b>Diagnostic criteria for PSC</b>	
<b>Inclusion criteria</b>	
Chronic cholestatic biochemical profile (serum alkaline phosphatase level >2 times upper limits of normal for >6 months)	
History of inflammatory bowel disease	
Typical cholangiographic abnormalities; beading and stricturing of the extrahepatic bile ducts, or both	
Liver histology compatible with PSC:	
Fibro-obliterative duct lesion	
Periductal fibrosis	
Ductopenia	
Biliary cirrhosis	
Presence of pANCA (non-specific)	
<b>Exclusion criteria</b>	
Biliary calculi (unless secondary to bile stasis)	
History of biliary tract surgery other than cholecystectomy	
Congenital abnormality of the biliary tree (Caroli disease)	
Cholangiopathy associated with acquired immune deficiency	
Ischemic stricturing secondary to trauma or surgery	
Bile duct neoplasm	
Exposure to biliary toxin (floxuridine, formalin, thiabendazole)	
Evidence of concurrent liver disease (PBC, autoimmune chronic active hepatitis, drug-induced cholestatic hepatitis)	

**Table 20.3 Diagnostic criteria for primary sclerosing cholangitis (PSC).** These include a range of inclusion and exclusion criteria. pANCA, antineutrophil cytoplasmic antibody binding in a peri-nuclear pattern; PBC, primary biliary cirrhosis.

in approximately 30% of patients, and serum IgM levels were increased in 45% of patients. Antinuclear antibodies and anti-smooth muscle antibodies were found in 55 and 35% of adult patients, respectively, in a single study. Other studies have found a much lower prevalence of these antibodies, and the titers are often lower than those observed in patients with typical type I autoimmune hepatitis. Antimitochondrial antibodies (AMA) occurred in less than 5% of PSC patients. However, the AMA recognized a different antigenic epitope from those recognized by AMA in patients with PBC.

The pANCA is associated with both CUC and PSC. Approximately 65–85% of patients with PSC with and without inflammatory bowel disease have pANCA. Furthermore, pANCA was found in 25% of relatives of patients with PSC, in one study. However, other studies from the UK have been unable to confirm the presence of antineutrophilic cytoplasmic antibodies in relatives of PSC patients. Recently, investigators reported that 92% of patients with type I autoimmune hepatitis had a high titer pANCA. However, the pANCA in PSC were composed of both IgG<sub>1</sub> and IgG<sub>3</sub> subclasses while the pANCA in the autoimmune hepatitis patients had an IgG<sub>1</sub> predominance. More

importantly, the antigens recognized by the pANCA in PSC and autoimmune hepatitis appear to be different. For example, following treatment of neutrophils with DNase-1, the pANCA staining pattern from PSC sera is diffusely cytoplasmic, while that from patients with autoimmune hepatitis sera is granular. Further studies are obviously needed to evaluate the role of antineutrophilic cytoplasmic antibodies, but to date they appear to be non-specific as a diagnostic marker for PSC and there is little evidence that they play a major etiologic role.

### Radiology

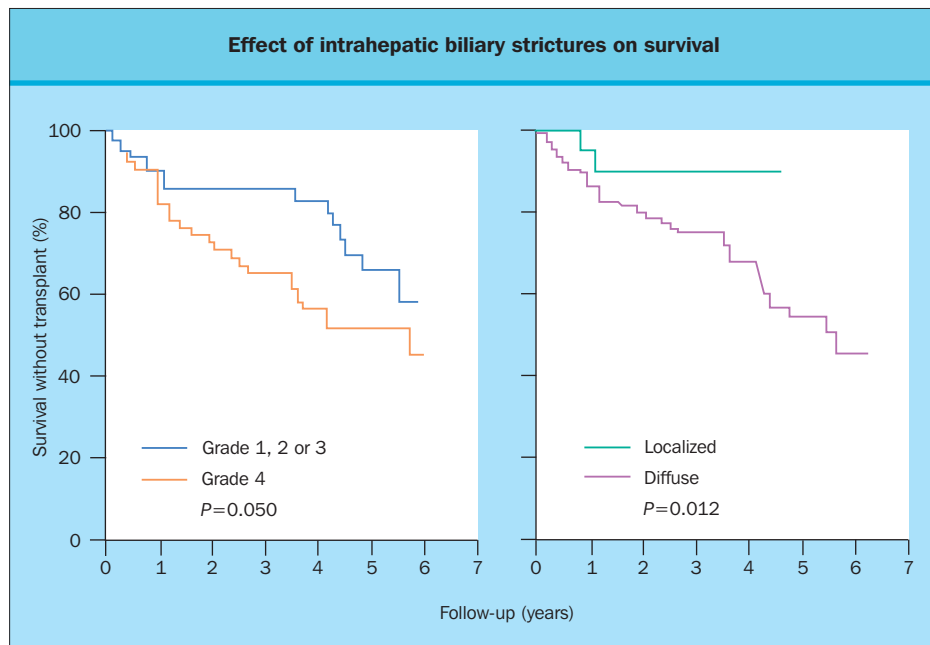
Imaging of the biliary tract is essential to make the diagnosis of PSC. The method of choice is ERCP, but adequate cholangiographic studies can be obtained non-invasively by MRC or using the percutaneous transhepatic approach in those patients in whom ERCP is unsuccessful.

The typical cholangiographic findings in PSC reveal multifocal stricturing and beading involving both the intrahepatic and extrahepatic biliary tree (see Fig. 20.1). Involvement may be of the intrahepatic bile ducts alone, extrahepatic bile ducts alone, or both. These strictures typically are short and annular with intervening segments of normal or slightly dilated bile ducts which are diffusely distributed, producing the classic beaded appearance. Pathologically, this corresponds to the intrahepatic cholangiectasis and bile duct obliteration typically seen in PSC.

The gallbladder and cystic duct can be involved in up to 15% of cases. In a large series of patients with PSC, 81% had involvement of both the intrahepatic and extrahepatic bile ducts, 11% had involvement of the intrahepatic bile ducts alone, and 8% had involvement of only the extrahepatic bile ducts without involvement of the intrahepatic bile ducts. A variation of PSC termed 'small duct PSC' affects bile ducts that are too small to be seen on cholangiography; thus, cholangiograms are normal and the diagnosis is made on the basis of liver histology.

Cholangiographic findings have also been noted to be of prognostic importance in PSC. Patients having high-grade and diffuse stricturing of the intrahepatic bile ducts were found to have an overall poor prognosis (Fig. 20.2). Therefore, therapeutic intervention aimed at relieving extrahepatic bile duct obstruction with balloon dilatation or surgical choledochojejunostomy may not have a significant impact on survival if the patient has severe intrahepatic disease and should be avoided in such patients.

Markedly dilated bile duct segments, a polypoid mass within the bile duct, or progressive stricture formation on serial cholangiograms were features highly suggestive of the development of a cholangiocarcinoma. In such cases, multiple biopsies and brushings from the bile duct should be obtained at the time the cholangiogram is performed in an attempt to make the diagnosis of cholangiocarcinoma. However, such techniques are usually diagnostically insensitive and positive in only up to 40–60% of cases in which bile duct cancer is present. A recent report suggested that cholangiocarcinoma in PSC patients may be detected using positron emission tomography. Use of newer techniques such as digital image analysis (DIA) and fluorescent in-situ hybridization (FISH) may be useful in making the diagnosis of cholangiocarcinoma. MRI may also help in demonstrating hilar masses or marked thickening of the bile ducts suggestive of cholangiocarcinoma.



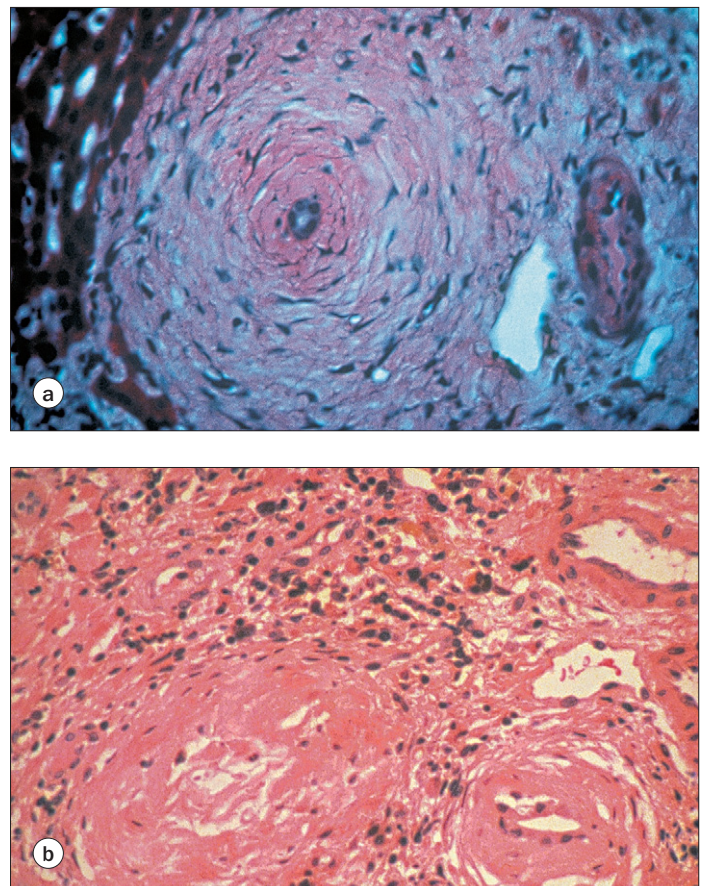
**Figure 20.2 Effect of intrahepatic biliary strictures on survival.** Effects of (a) intrahepatic stricture grade, and (b) extent of intrahepatic strictures on survival curves for patients with PSC. End point was either death or liver transplantation. Reproduced from Craig et al (91) by permission of the American Journal of Roentgenology.

## Histology

The advent of liver transplantation has allowed the morphologic findings of both the extrahepatic and intrahepatic bile duct pathology to be more thoroughly studied. Biopsy specimens from the extrahepatic bile ducts show a thickened fibrous wall, often with mixed inflammatory infiltrates, which tend to cluster around biliary glands. These changes are non-specific and do not allow the pathologist to distinguish between a benign localized postoperative biliary stricture and a stricture that is associated with PSC. The changes in the cholangiographically demonstrable large intrahepatic bile ducts resemble the findings in the extrahepatic bile ducts in PSC. Fibrosed bile duct segments may alternate with areas of cholangiectasis to produce the beaded appearance seen on cholangiograms.

In the early stages of PSC, histologic changes typically consist of bile duct proliferation, edema in some portal tracts and ductopenia in others, often associated with fibrous cholangitis (pericholangitis). Therefore, if a liver biopsy specimen displays histologic features of extrahepatic biliary obstruction in some portal tracts and periductal fibrosis in others, the diagnosis of PSC should be entertained when other clinical features are present. Although helpful in establishing the diagnosis of PSC, these histologic findings are in themselves not diagnostic of PSC and clinical correlation is essential in establishing the diagnosis of PSC. Fibrous obliterative cholangitis is occasionally present in histologic specimens. It represents transformation of bile ducts to a solid fibrous cord and is nearly diagnostic of PSC (Fig. 20.3). This histologic finding is present in fewer than 7% of percutaneous liver biopsies obtained from PSC patients.

Although hepatic parenchymal manifestations of PSC are often not diagnostic, we have found them important for staging the disease and in determining the prognosis. Similar to the histologic staging established for PBC, four histologic stages



**Figure 20.3 Liver biopsy.** (a) Periductal fibrosis and early obliteration typically seen in patients with PSC and (b) complete bile duct obliteration. Reproduced from Wiesner et al (94) by permission of the Mayo Foundation for Medical Education and Research.



have been defined for PSC (Table 20.4). Survival based on histologic stage is shown in Figure 20.4. Patients with cirrhotic-stage disease clearly have a diminished survival probability when compared with patients with non-cirrhotic-stage disease.

### Differential diagnosis

The differential diagnosis of liver disorders that can present with cholestasis and the presence of ductopenia on liver histology include PBC, sarcoidosis, chronic graft versus host disease, idiopathic adulthood ductopenia, drug-induced ductopenia and liver allograft rejection. In addition, other disorders that can present with cholestasis alone include alcoholic hepatitis, drug-induced hepatitis, viral hepatitis and autoimmune hepatitis. The latter disorders generally can be differentiated on the basis of clinical history, liver biochemistry, viral serology, histology and cholangiography.

Histology in PSC	
Portal stage (stage I)	Portal edema, inflammation, and ductal proliferation; abnormalities do not extend beyond limiting plate
Periportal stage (stage II)	Periportal fibrosis, inflammation with or without ductular proliferation; piecemeal necrosis may be present
Septal stage (stage III)	Septal fibrosis or bridging necrosis can be identified
Cirrhotic stage (stage IV)	Biliary cirrhosis

**Table 20.4 Histology in primary sclerosing cholangitis (PSC).** The histologic staging of PSC.

The most useful test in distinguishing PBC from PSC is a cholangiogram. The extrahepatic bile ducts are not involved in PBC and radiographic abnormalities present in the intrahepatic ducts in patients with cirrhotic PBC are usually distinguishable from those seen in classic PSC. The AMA is also positive in PBC but not PSC. Other causes of chronic cholestasis include hepatic involvement with histiocytosis and drug-induced ductopenia associated with drugs such as phenothiazine. Ductopenia in adults is labeled as idiopathic when serum studies are negative for AMA, a cholangiogram is normal, inflammatory bowel disease is absent and viral injury can be excluded.

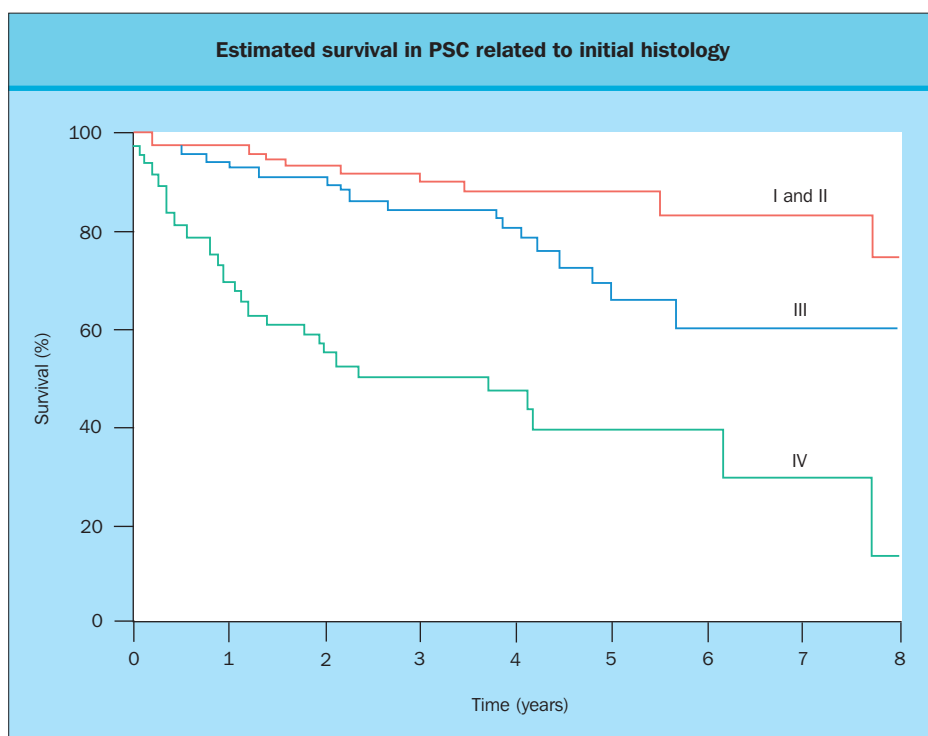
Investigation of HIV-infected patients has led to the recognition of a condition termed 'AIDS-related sclerosing cholangitis'. Typically, these patients have right upper quadrant pain and a cholestatic biochemical profile. An ultrasound examination may show thickened bile ducts and less often bile duct stricturing and/or dilatation. Features on cholangiography are essentially indistinguishable from those of PSC. In a study examining the natural history of AIDS-related sclerosing cholangitis, the median CD4+ lymphocyte count was low in all patients. Cryptosporidiosis with or without cytomegalovirus infection has been identified as a potential infective cause of 'AIDS-related sclerosing cholangitis' in 75% of cases.

Other diseases that can mimic PSC include histiocytosis-X, cystic fibrosis, amyloidosis and sarcoidosis. All these entities can present with intrahepatic bile duct stricturing, typical of that seen in PSC.

### Complications of PSC

The complications of PSC can be broadly divided into the following:

- complications of chronic cholestasis;
- specific complications of primary sclerosing cholangitis; and
- complications of portal hypertension.



**Figure 20.4 Estimated survival in PSC related to initial histology.** Kaplan-Meier estimated survival by histologic stage on liver biopsy at study entry. Reproduced from Wiesner et al (89) by permission of Wiley-Liss, Inc.

Complications associated with chronic cholestasis and PSC
Complications associated with chronic cholestasis
Fatigue
Pruritis
Steatorrhea
Fat malabsorption
Fat-soluble vitamin deficiency
Complications characteristic of PSC
Bacterial cholangitis
Gallbladder and biliary stenosis
Dominant bile duct strictures
Cholangiocarcinoma
Hepatocellular carcinoma

**Table 20.5 Complications associated with chronic cholestasis and primary sclerosing cholangitis (PSC).** These are a combination of the complications common to all causes of chronic cholestasis and those characteristic of PSC.

#### Complications of chronic cholestatic liver disease

These include fatigue, pruritus, steatorrhea, and fat-soluble vitamin deficiencies and associated consequences (Table 20.5). Fatigue, although a non-specific symptom, is a frequent complaint of PSC patients. Fatigue of notable severity can manifest early during the course of PSC and generally parallels progression of the liver disease. While little is known about the specific cause of fatigue, a recent study has suggested that fatigue associated with cholestasis may be attributable to altered serotonergic neurotransmission in the brain. However, at this time, no specific therapy is available for this symptom.

Pruritus, another common symptom of PSC, can be debilitating and can lead to severe excoriations and diminished quality of life. Although the pathogenesis of pruritus remains unknown, several hypotheses have been suggested, the most recent of which implies that pruritus may be related to increased availability of endogenous opiate ligands at the central opiate receptors. The theory that endogenous opiates are involved in the pathogenesis of pruritus associated with cholestasis is supported by the fact that exogenous opiates induce pruritus, cholestasis is associated with increased opiate neurotransmission, and pruritus, in the setting of cholestasis, can be ameliorated by using opiate antagonists.

In general, the severity of pruritus tends not to parallel disease progression in PSC; in fact, during the end stages of PSC, pruritus tends to diminish. Various therapies have been used to treat pruritus (Table 20.6). In most patients, pruritus can be readily controlled; however, in a small number of patients, pruritus is associated with a marked diminished quality of life and, in rare cases, can be an indication for liver transplantation.

Steatorrhea associated with fat-soluble vitamin deficiency has also been described in patients with PSC. Fat malabsorption in PSC, like that seen in PBC, is most often related to diminished intestinal concentration of bile acids. In PSC, however,

Therapies used to treat pruritis
Cholestyramine
Phenobarbital
Antihistamine
Rifampin (rifampicin)
Activated charcoal
Plasmapheresis
Phototherapy
Testosterone
Opiate antagonists
Ursodiol
Propofol

**Table 20.6 Therapies used to treat pruritus.** Treatment options for pruritus, including non-pharmacologic approaches.

steatorrhea also can be caused by chronic pancreatitis, bacterial overgrowth or celiac disease, all of which can be associated with PSC. These causes of steatorrhea should be considered in the differential diagnosis if steatorrhea occurs in a PSC patient who is not jaundiced and does not have cirrhotic-stage disease on liver biopsy. Patients who have cirrhosis, jaundice, or both should be screened for fat-soluble vitamin deficiency by determining levels of vitamin A, D and E, as well as a prothrombin time on a 6-monthly basis. Patients who have fat-soluble vitamin deficiency should receive adequate supplemental therapy.

Metabolic bone disease, particularly osteoporosis, has recently been shown to be a frequent complication in patients with advanced PSC who are undergoing assessment for liver transplantation. Indeed, bone mineral density levels are often severely decreased in this group of patients. Despite the fact that two-thirds of patients with PSC are of male gender, 50% have bone mineral density levels that are below the fracture threshold. Bone mineral densities of the lumbar spine in general do not correlate with serum levels of bilirubin, 25-hydroxyvitamin D levels, fecal fat excretion or the presence or absence of inflammatory bowel disease. Histomorphometric examination of bone from several PSC patients has shown increased bone reabsorption, reduced bone formation, and moderate-to-severe osteopenia with little or no evidence of osteomalacia.

The pathophysiology of osteopenia remains unknown and currently no effective therapy has been found to prevent or manage osteopenia associated with chronic cholestatic liver disease. One hypothesis suggests that there is suppression of osteoblast function by a substance retained in cholestatic plasma, such as serum bilirubin. In a recent in vitro study, serum bilirubin was shown to inhibit the activity in human osteoblast-like cells, suggesting a possible pathogenic role of hyperbilirubinemia as the cause of osteoporosis in PSC patients. Further studies will be needed to confirm these findings.

Treatment for bone disease associated with PSC often includes supplemental vitamin D and calcium, along with adequate exercise and avoidance of excessive alcohol and tobacco consumption

(Table 20.7). Overall, this approach does not appear to be of major benefit to patients with cholestatic-induced osteoporosis. Other treatments such as the use of calcitonin, which inhibits bone reabsorption, and the use of sodium fluoride, which can enhance spine density and increase bone formation, have been shown to be of limited benefit in a small number of PSC patients.

The use of estrogen therapy, which has been shown to be of benefit in the treatment of postmenopausal osteoporosis, has

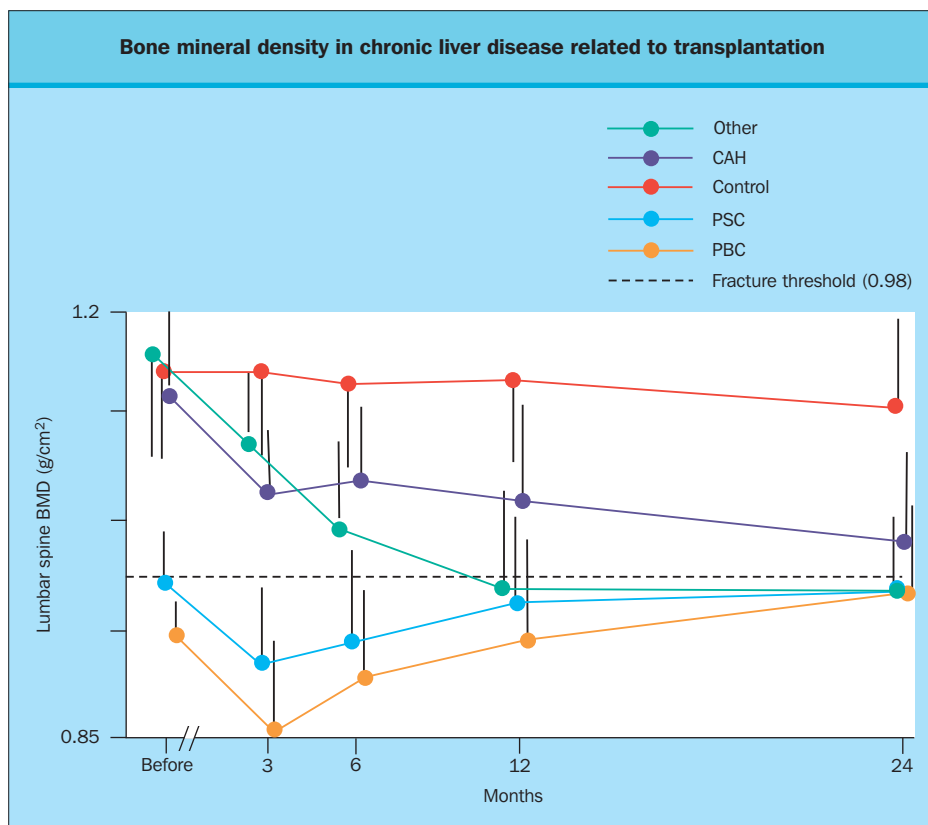
Treatment of osteopenia associated with PSC	
Avoid alcohol	
Avoid smoking	
Increase ambulation	
Vitamin D (maintain serum hydroxyvitamin D levels)	
Calcium	
Estrogen	
Calcitonin	
Fluoride	
Bisphosphonates	
Liver transplantation	

**Table 20.7 Treatment of osteopenia associated with primary sclerosing cholangitis (PSC).** Factors that may improve bone density in patients with PSC.

also been shown to be of benefit in patients with osteoporosis associated with cholestasis. However, bisphosphonate compounds such as etidronate and alendronate have been shown to be of much greater value than any of the previously listed therapies for the treatment of osteopenia associated with both chronic cholestatic liver disease and post-transplant bone disease. They can be particularly useful in patients prior to liver transplantation as they can raise the bone density to levels above the fracture threshold and thus help prevent spine fractures in the early post-transplant period. However, caution should be exercised with the use of oral bisphosphates in patients with portal hypertension because of the potential for esophageal ulceration with these drugs.

Following liver transplantation for PSC, the rate of bone loss is accelerated in the PSC patient. This is probably due to a combination of factors, including immobilization, the use of high doses of corticosteroids and increased circulating levels of certain cytokines. Recent studies from the Mayo Clinic have shown that bone mineral density of the lumbar spine decreases for the first 3–6 months post-transplant, during which time there is an increase in pathologic fractures (Fig. 20.5). However, bone mineral density subsequently increases to levels above baseline by 1-year post-transplantation, and generally by 2 years post-transplantation, bone density has been shown to be well above pretransplantation levels. The use of antireabsorptive drugs, such as bisphosphonates, has been shown to be effective in preventing the early bone loss after liver transplantation.

A high incidence of avascular necrosis in PSC patients post-transplant, which frequently requires joint replacement, has also been reported. Avascular necrosis is most likely related to the



**Figure 20.5 Bone mineral density in chronic liver disease related to transplantation.**

Measurements (mean  $\pm$  SD) of the lumbar spine using dual photon absorptiometry pretransplantation, at 3 months, 6 months, 12 months and 24 months after liver transplantation in patients with chronic liver disease and in an age- and sex-matched control population. CAH, chronic active hepatitis. Reproduced from Porayko et al (91) by permission of Elsevier.



use of high-dose corticosteroids, but the advent of new and more powerful immunosuppressive agents and the trend toward early steroid withdrawal should reduce the incidence of both pathologic fractures and avascular necrosis in the early post-transplant period.

**Complications characteristic of primary sclerosing cholangitis**  
These are included in Table 20.5. Bacterial cholangitis is an unusual presenting symptom of PSC, but frequently occurs in patients who have had previous bile duct surgery or in whom intervention with radiologic or endoscopic dilatation has been carried out for an obstructing dominant stricture of the large bile ducts. Back pressure resulting from extensive biliary stricturing and the presence of concurrent debris or stones in the large bile ducts is believed to be partially responsible for the deterioration in liver function and is frequently associated with bacterial cholangitis. Progression of the disease to cirrhosis and liver failure may be delayed if obstruction is relieved during the early stages of the disease. In managing such cases, endoscopic decompression is an attractive, temporary therapeutic alternative because it can be performed with relative ease, can facilitate dilatation of multiple strictures at the same time the procedure is performed, and eliminates surgical therapy that might ultimately complicate the liver transplant procedure. The results of trials suggest that balloon dilatation and stenting can alter the usually protracted course of PSC and may delay the timing of, or even the necessity for, liver transplantation (Table 20.8). Patients undergoing endoscopic decompression, or who have severe intrahepatic disease associated with bacterial cholangitis, should receive prophylactic broad-spectrum antibiotic therapy to prevent recurrent bouts of bacterial cholangitis. Preference is for quinolone antibiotics because of their high biliary concentration and broad-spectrum action against both Gram-positive and Gram-negative bacteria. In addition, one study has suggested that the combination of ciprofloxacin and ursodeoxycholic acid may be beneficial in preventing sludge formation in those patients undergoing biliary dilatation with stent placement.

Cholelithiasis and choledocholithiasis occur in up to 30% of patients with PSC. Chronic cholestasis predisposes to the

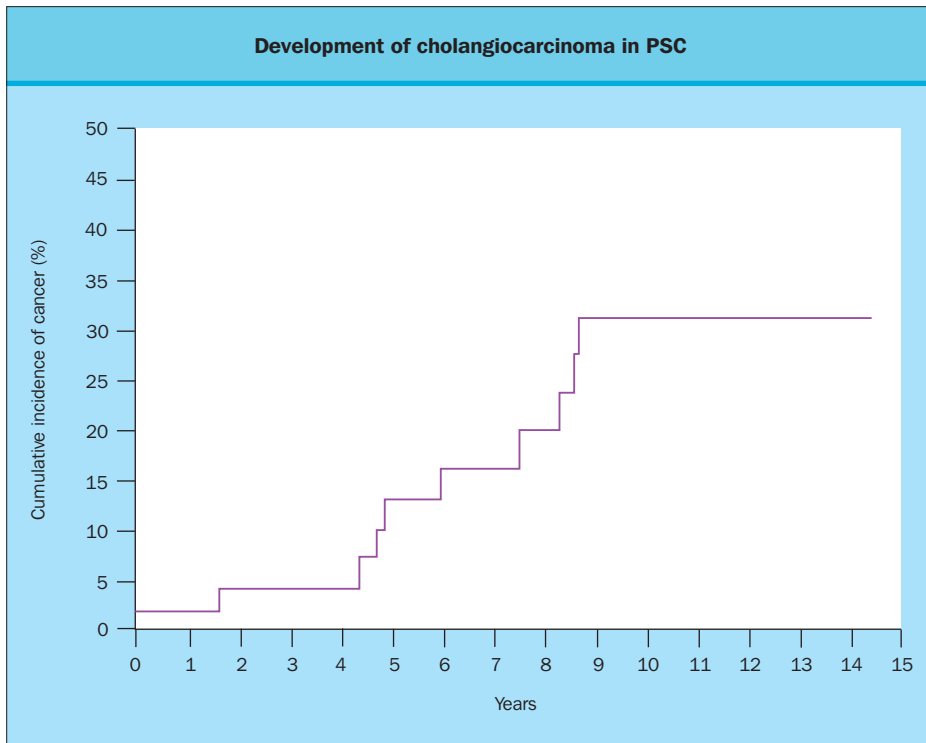
formation of cholesterol gallstones and bacterial cholangitis, causing bile stasis, predisposes to the formation of pigment stones. Indeed, diagnosing choledocholithiasis can be extremely difficult in patients with PSC. Therefore, a cholangiogram is essential for eliciting the cause of new onset jaundice or bacterial cholangitis. If choledocholithiasis is documented, stone extraction or surgical intervention is indicated.

Approximately 20% of patients with PSC develop a dominant stricture of the biliary tree. Frequently, the site of the stricture is in the biliary hilum, but strictures can also occur in the common bile duct and the common hepatic duct. This complication is often associated with the acute onset of jaundice, pruritus and/or fever related to bacterial cholangitis. If a dominant stricture is found on cholangiography, cytologic specimens should be obtained in order to exclude bile duct carcinoma, and balloon dilatation should be performed with the use of either a transhepatic or endoscopic retrograde approach, depending on the site of the stricture. In general, balloon dilatation of dominant strictures and removal of biliary sludge, while the patient is receiving antibiotics, is an effective treatment in alleviating the recent onset of jaundice or pruritus related to this complication. Approximately 50% of patients with PSC who undergo balloon dilatation of dominant strictures will have improvement for up to 2 years. Although some centers have advocated a surgical approach for biliary strictures related to PSC, the absence of prospective controlled data makes it difficult to assess the effect of the surgical treatment on the natural history of PSC. Because PSC is generally a progressive disease, surgical intervention should be considered only as a palliative measure aimed at relieving symptoms and excluding a concomitant diagnosis of cholangiocarcinoma. Surgical intervention has little or no value for PSC patients with cirrhosis or for those who have severe intrahepatic bile duct disease on cholangiography. Biliary tract surgery also seems to increase the difficulty and risk of liver transplantation.

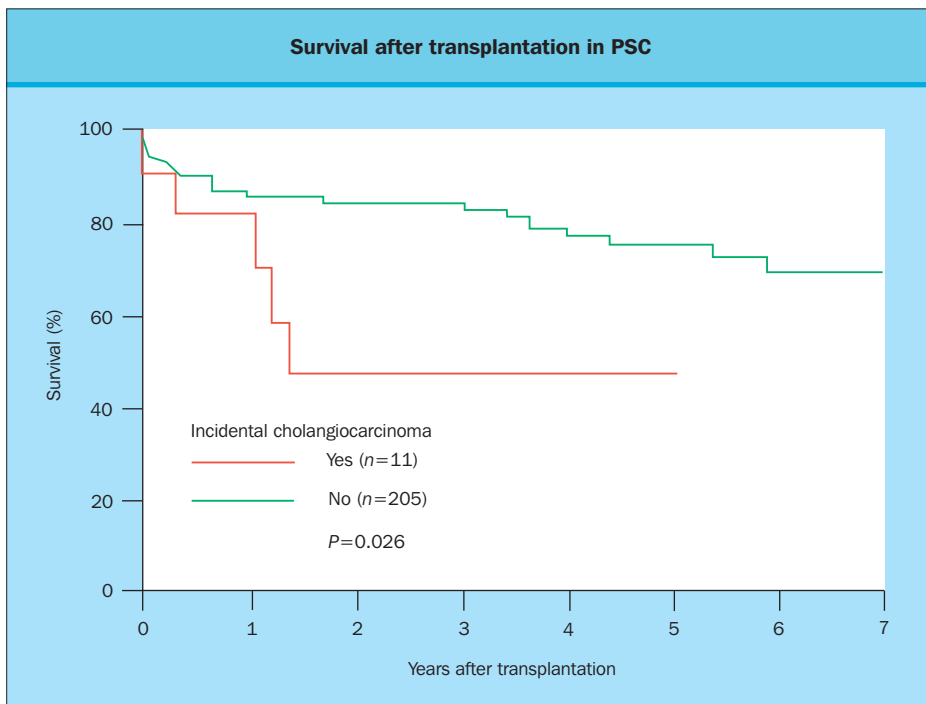
Several recent series of PSC patients followed long term reported that cholangiocarcinoma may develop in 6–30% of patients with PSC over a period of 10–30 years (Fig. 20.6). PSC should therefore be considered a premalignant condition of the biliary tree just as CUC is considered a premalignant condition of the colon. Cholangiocarcinoma in the setting of PSC is difficult to diagnose because of the lack of serologic markers and the fact that biliary cytologic and histologic studies for the most part have been insensitive in diagnosing cholangiocarcinoma. This is illustrated by the fact that 10% of patients with PSC undergoing liver transplantation are found to have an unsuspected cholangiocarcinoma. A persistent increase in serum bilirubin levels in patients with PSC may be an indication of the development of cholangiocarcinoma. Newer techniques examining cellular detail may be useful in the diagnosis of biliary tract malignancies. Examining bile duct cells obtained by brushing at ERCP for high cellular DNA content by techniques such as DIA has improved the diagnosis of cholangiocarcinoma when compared with routine cytology. Other investigators have used flow cytometry to detect DNA aneuploidy in cholangiocarcinoma from patients with PSC. In one study, these tumors displayed DNA aneuploidy significantly more often when compared to benign strictures. However further studies are needed before these newer techniques can be incorporated into routine clinical practice.

Summary of endoscopic therapy for PSC				
Year	n	Procedure	Follow-up (months)	Result
1997	23	Balloon dilation and ursodiol	45	Improved survival
1996	25	Dilation and stenting	29	Improved symptoms 57%
1995	53	Dilation and stenting	31	Improved symptoms 77%
1993	42	Dilation and stenting	52	Improved symptoms 70%
1991	35	Dilation and stenting	35	Improved symptoms 85%

**Table 20.8 Summary of endoscopic therapy in primary sclerosing cholangitis (PSC).** Summary of the outcome of five studies reported between 1991–97.



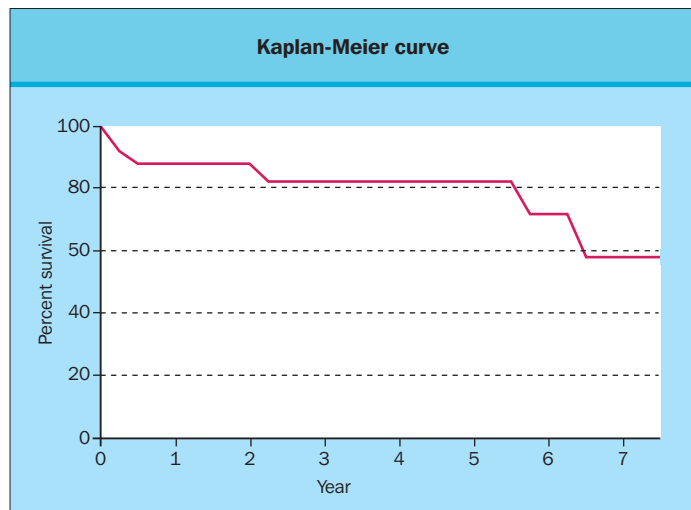
**Figure 20.6 Development of cholangiocarcinoma in PSC.** Cumulative actuarial incidence of cholangiocarcinoma from the time of onset of PSC. Reproduced from Farges et al (95) by permission of Mosby.



**Figure 20.7 Survival after transplantation in PSC.** Kaplan-Meier survival plot after transplantation in patients with PSC with and without detection of an incidental cholangiocarcinoma in the excised liver. The difference was statistically significant. Five-year survival rates  $0.47 \pm 0.17$  and  $0.75 \pm 0.04$ , respectively. Reproduced from Abu-Elmagd KM et al (93) by permission of the American College of Surgeons.

Patients with PSC and cholangiocarcinoma that was diagnosed before transplantation have very poor prognosis with liver transplantation with less than 10% surviving for more than 2 years. Even patients in whom an incidental cholangiocarcinoma is found at the time of transplantation have a 2-year survival rate of only a 40%, which is far below that of PSC patients who do not have a concurrent cholangiocarcinoma (Fig. 20.7). Thus, most

liver transplant centers consider a diagnosis of cholangiocarcinoma at least a relative and often an absolute contraindication to liver transplantation. However, a few centers are employing multimodal adjuvant therapeutic protocols (i.e. radiation and chemotherapy) to improve the results of liver transplantation for this complication and preliminary results have been encouraging. In a study from the Mayo Clinic, highly select patients with



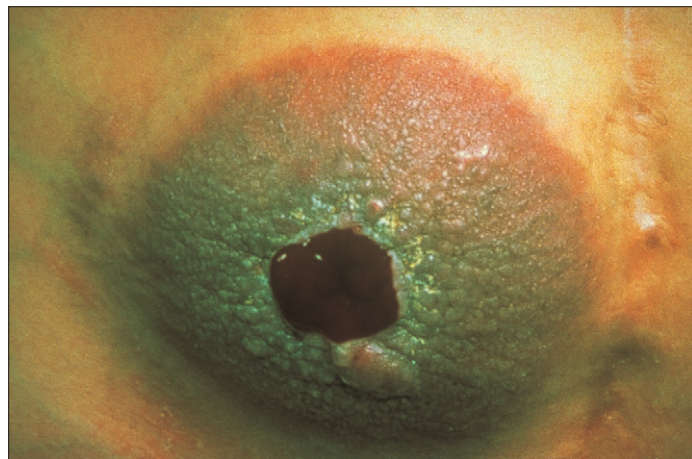
**Figure 20.8 Kaplan-Meier curve.** A Kaplan-Meier curve showing survival rate after liver transplantation in select patients with unresectable hilar cholangiocarcinoma.

cholangiocarcinoma undergoing orthotopic liver transplantation after neoadjuvant chemotherapy and radiotherapy had a 1-year actuarial survival of 88% and 5-year actuarial survival of 82% (Fig. 20.8). This compares favorably with 86% 1-year survival and 72% 5-year survival among deceased donor liver transplant recipients in the USA. Operative management (hepatic resection), chemotherapy and radiotherapy have not been shown to be useful in the treatment of bile duct cancer complicating PSC.

Because of the serious implications of a concomitant cholangiocarcinoma in the PSC patient, it has been important to identify which patients are at a high risk for developing this complication. There are conflicting data on the correlation between the duration of CUC or cirrhotic-stage PSC and the risk of developing cholangiocarcinoma. The use of tumor markers, such as CA 19-9, and cytologic techniques are helpful in diagnosing an existing cholangiocarcinoma, but have not been shown to date to be helpful in the early diagnosis or in screening for cholangiocarcinoma in PSC patients. Clearly, further studies will be needed to better identify those PSC patients at high risk for developing cholangiocarcinoma.

Hepatocellular carcinoma can also develop during the course of PSC. The patients at risk appear to be those who have advanced-stage PSC with extensive fibrosis, cirrhosis and diffuse nodular regeneration of the liver. The question of screening PSC patients for hepatocellular cancer has arisen; however, in the absence of well-established advanced cirrhosis, screening cannot be recommended at this time.

Finally, gallbladder cancer can also arise in patients with PSC. In a retrospective study of 102 patients with PSC who underwent a cholecystectomy, 57% of patients with a mass lesion in the gallbladder had adenocarcinoma and 33% of patients with benign lesions also had associated epithelial cell dysplasia. The presence of dysplasia may therefore indicate a dysplasia–carcinoma sequence in PSC similar to that seen in ulcerative colitis. Patients with PSC and gallbladder polyps should be considered for cholecystectomy. If a cholecystectomy is not performed, careful interim follow-up is warranted.



**Figure 20.9 Peristomal varix.** A peristomal varix surrounding the abdominal ileostoma in a patient with PSC. Reproduced by permission of the Mayo Foundation for Medical Education and Research.

### Complications of cirrhosis and portal hypertension

Advanced PSC is generally associated with complications of portal hypertension. In addition to ascites, encephalopathy and bleeding from esophageal or gastric varices, one special complication is the development and bleeding from peristomal varices in patients who have undergone proctocolectomy with the creation of an ileostomy (Fig. 20.9). Treatment for bleeding peristomal varices has been the creation of a transjugular intrahepatic portosystemic shunt, which has usually been quite successful in decreasing the number and severity of bleeding episodes. Non-selective  $\beta$  blockade may also be useful in this situation. As in other forms of chronic liver disease, esophageal variceal bleeding is best managed by variceal band ligation or sclerotherapy. Another option is the use of a transjugular intrahepatic portosystemic shunt. If possible, surgical shunting procedures should not be performed to avoid the risks associated with a subsequent liver transplant if indicated.

Ascites is best treated with sodium restriction, large-volume paracentesis and diuretic therapy. Administration of albumin should be considered in patients who have serum albumin levels below 2.6 g/L. In PSC, spontaneous bacterial peritonitis is a complication often related to biliary sepsis and cholangitis. In this situation, the use of oral selective bowel decontamination solution or chronic antibiotic therapy is especially appropriate to prevent recurrence of this severe life-threatening complication. Hepatic encephalopathy is managed with the use of lactulose. Dietary protein is restricted only if absolutely necessary.

### Associated diseases

The syndrome of PSC is associated with a variety of autoimmune-type diseases (Table 20.9). These include inflammatory bowel disease, celiac disease, retroperitoneal fibrosis, rheumatoid arthritis, thyroiditis and a host of other diseases that are thought to be of autoimmune origin. Of importance, inflammatory bowel disease occurs in up to 80% of patients with PSC. The majority of these patients have CUC and a minority has Crohn colitis. In general, inflammatory bowel disease is diagnosed several years before PSC. However, inflammatory bowel disease can be diagnosed simultaneously with PSC or years after the diagnosis of



PSC is made. In fact, the onset of inflammatory bowel disease has been demonstrated up to 6 years after a PSC patient had undergone liver transplantation, and PSC has been diagnosed for the first time many years after a proctocolectomy for CUC.

In patients with PSC and CUC, the mean age at onset of inflammatory bowel disease was 25 years and the mean age at the time of diagnosis of PSC was 39 years. Most patients (94%) had experienced symptoms related to inflammatory bowel disease at the time the diagnosis of PSC was made. Quiescent or

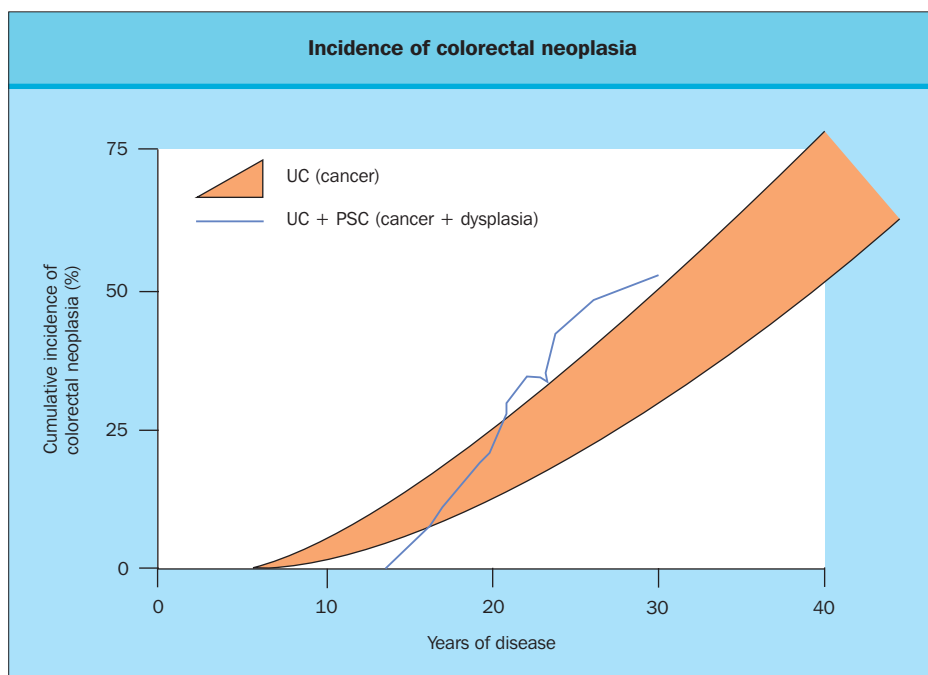
mild disease based on symptoms and colonoscopic examination was found in 80% of patients with PSC. Patients with PSC and inflammatory bowel disease may be at a higher risk for developing colorectal neoplasia. In a recent study, 25% of patients with PSC and ulcerative colitis developed colorectal cancer or dysplasia compared to 5.6% of patients with isolated ulcerative colitis. These patients may therefore warrant aggressive surveillance for colon cancer. A recent study has also shown that ursodeoxycholic acid decreases the risk of developing colorectal dysplasia or cancer in patients with PSC and CUC and may be useful adjunctive therapy (Fig. 20.10). Furthermore, it appears that patients with PSC who have associated CUC remain at increased risk for colon cancer even after they have undergone liver transplantation. These patients remain at increased risk for developing colon cancer and should undergo yearly colonoscopic surveillance and screening because CUC is frequently quiescent, particularly in patients receiving immunosuppression, and because CUC can develop for the first time following liver transplantation. If moderate or severe dysplasia is found in the colon after liver transplantation, a total proctocolectomy has been recommended as a prophylactic measure.

Patients with PSC also have a higher incidence of pouchitis following total proctocolectomy and ileal pouch-anal anastomosis (IPAA). In a study of 1097 patients with ulcerative colitis who underwent an IPAA following proctocolectomy, one or more episodes of pouchitis occurred in 32% of patients without PSC and 63% of patients with PSC. The cumulative risk of pouchitis after 1, 2, 5 and 10 years IPAA was 15.5, 22.5, 36 and 45% for the patients without PSC and 22, 43, 61 and 79% for patients with PSC. In another study, pouchitis in PSC patients was more frequently seen in those who underwent an IPAA compared to a Brooke ileostomy.

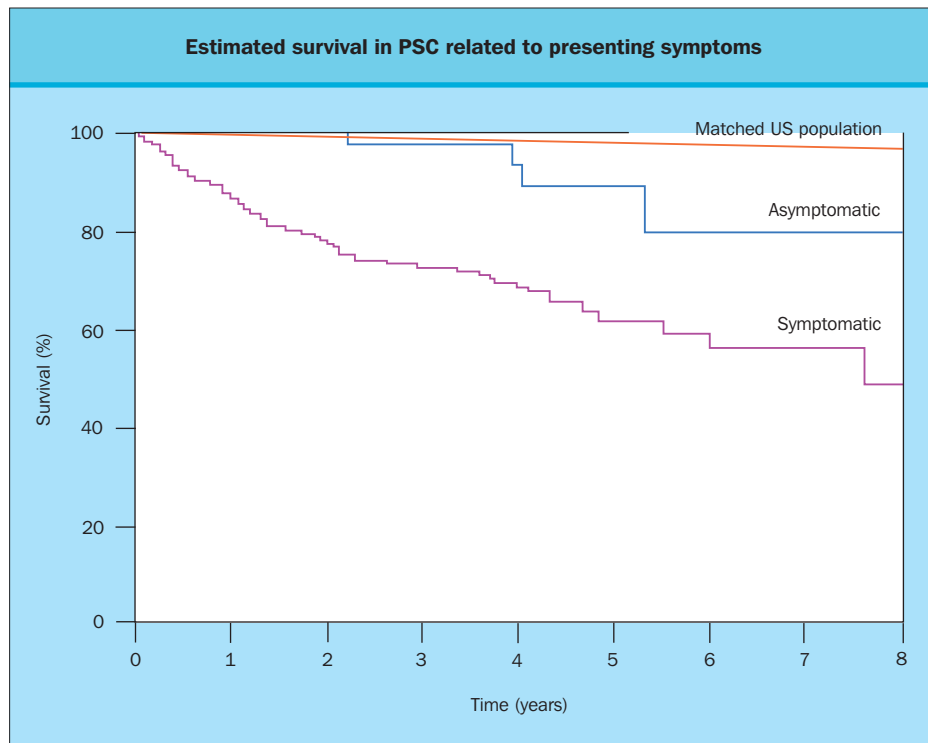
Autoimmune hepatitis may occur concurrently in patients with PSC. Diagnostic criteria for definite AIH may be present in 2% of patients with PSC. Autoimmune hepatitis shares

Diseases associated with PSC-like bile duct changes	
Inflammatory bowel disease	Peyronie disease
Celiac disease	Bronchiectasis
Retroperitoneal fibrosis	Systemic sclerosis
Thyroiditis	Membranous nephropathy
Sjögren syndrome	Pseudotumor of orbit
Autoimmune chronic active hepatitis	Autoimmune hemolytic anemia
Lupus erythematosus	Angioblastic lymphadenopathy
Vasculitis	Cystic fibrosis
Immune thrombocytopenic purpura	Eosinophilia
Histiocytosis-X	Sarcoidosis
Gallbladder disease	Nephropathy
Chronic pancreatitis	Mast cell disease
Rheumatoid arthritis	

**Table 20.9 Diseases associated with primary sclerosing cholangitis (PSC).** These include many autoimmune diseases, diseases linked to biliary disease, and miscellaneous associations.



**Figure 20.10 Incidence of colorectal neoplasia.** Cumulative incidence of colorectal neoplasia (carcinoma and dysplasia) over time in ulcerative colitis (UC) patients with PSC compared with the range of cumulative incidence of cancer over time in UC patients alone. Reproduced from Devroede in Winawer (80) by permission of Raven Press.



**Figure 20.11 Estimated survival in PSC related to presenting systems.** Kaplan-Meier estimated survival curves for asymptomatic and symptomatic patients with PSC. For comparison, survival curves in US population matched for age, sex, and race to asymptomatic patients are also shown. Difference in survival: asymptomatic versus control subjects,  $P < 0.001$ ; symptomatic versus asymptomatic patients,  $P < 0.003$ . Reproduced from Wiesner et al (89) by permission of Wiley-Liss, Inc.

genetic risk factors with PSC (HLAB8, DR3 and DRW52) which may explain similarities in clinical expression or transition between the two diseases. Corticosteroid therapy may be useful in the small subset of patients with PSC who also have features to suggest autoimmune hepatitis.

In addition to the conditions listed above, PSC can be associated with a number of other diseases, most of which are thought to be autoimmune diseases and are related to abnormalities in immune regulation.

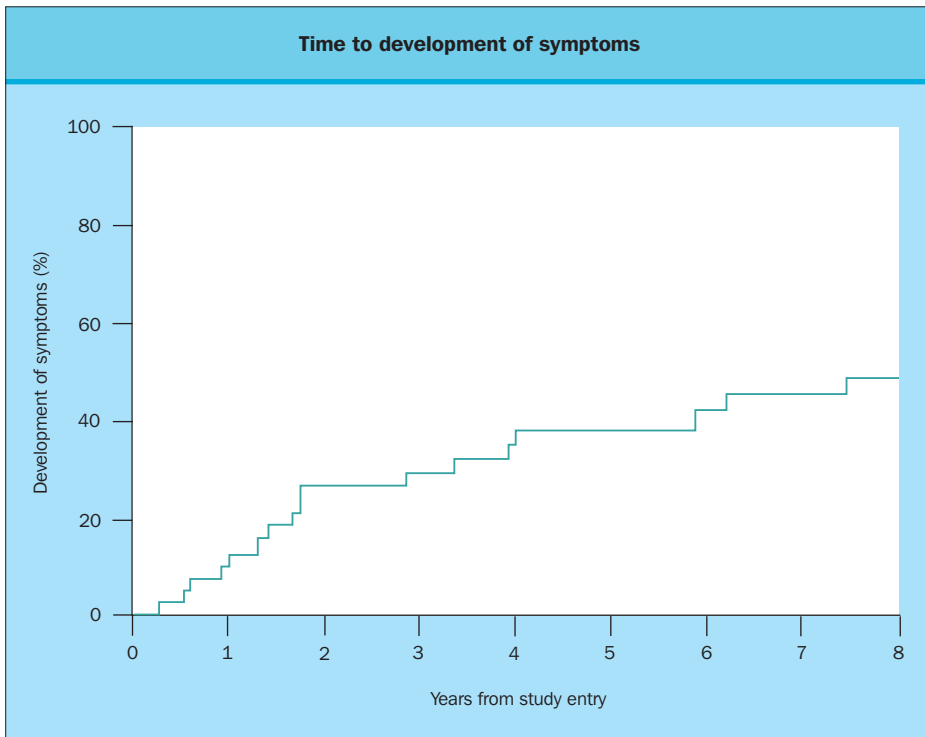
## NATURAL HISTORY AND PROGNOSIS

The majority of PSC patients who are asymptomatic at the time of diagnosis develop symptoms of fatigue and pruritus within a number of years. Several recent studies show that PSC is usually a progressive disease. The largest of these studies comes from the Mayo Clinic in which 174 PSC patients were followed for 6 years. Median survival from the time of diagnosis of PSC was 11.9 years, and both symptomatic and asymptomatic patients at the time of diagnosis had a decreased survival as compared with a US population matched for age, sex, and race (Fig. 20.11). These findings have been confirmed by several other centers which have reported the median survival from the time of diagnosis of PSC to be between 9 and 17 years. A subgroup of 45 PSC patients who were asymptomatic at the time of diagnosis warrants special mention. These patients were prospectively followed for a mean of 6.25 years. During this period of time, 76% experienced progression of liver disease based on clinical and/or pathologic findings (Fig. 20.12). Furthermore, 31% developed liver failure resulting in either death or the need for a life-saving liver transplant.

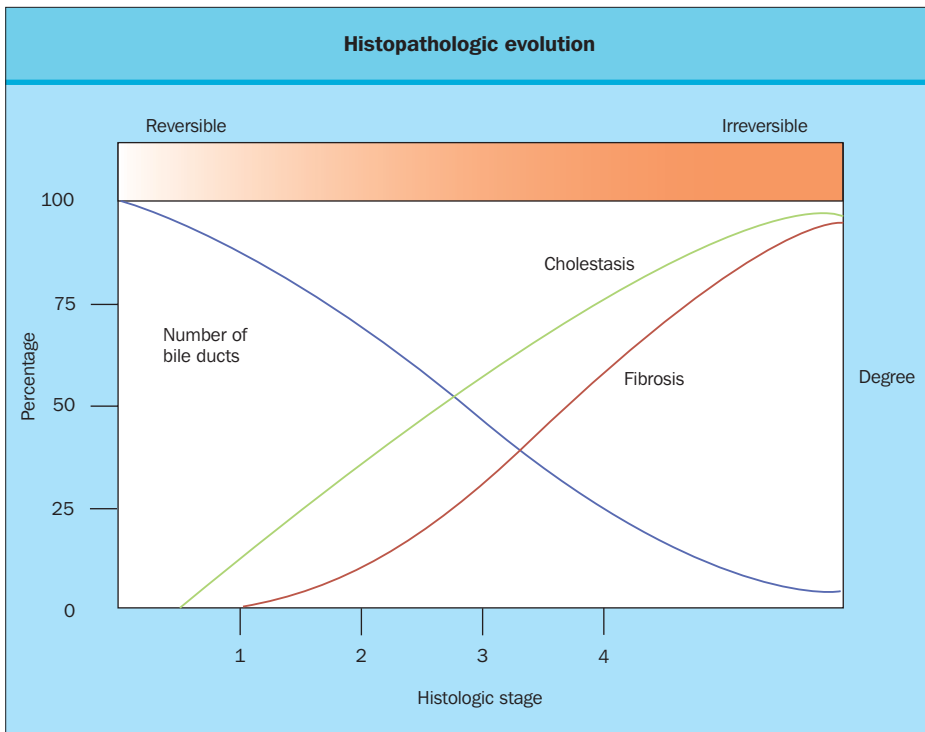
By contrast, some studies have found PSC to be a more benign disease. Studies from Norway estimated the mean survival

of patients with PSC to be 17 years after the initial diagnosis was made. Reasons for these differences have recently been put into perspective. First, PSC progresses silently for several years and can be detected only by prospective follow-up with serial liver tests and hepatic histologic examinations, which were not performed in all studies. Secondly, early detection of PSC would appear to influence favorably the mean survival estimates, such as in the Norwegian studies in which patients with inflammatory bowel disease who had minimal abnormalities in liver tests were aggressively assessed with cholangiography and diagnosed early in the course of their disease. Thirdly, the starting point for survival analysis is important for determining the overall survival time. The comparison of one group of patients in whom follow-up began at the time of cholangiographic diagnosis of PSC and a second group of patients in whom the diagnosis of PSC was back-dated to the time of the first onset of symptoms or biochemical abnormalities will provide different survival times. Therefore, when these differences in study design are taken into account, the weight of evidence indicates that PSC in most patients is a progressive disease.

Histopathologically, PSC evolves into a ductopenic syndrome in which there is progressive stricturing of both large and small bile ducts. The overall result is that the interlobular and septal bile ducts become obliterated and functionally lost. In the histopathologic evolution of PSC (Fig. 20.13), when a critical number of interlobular bile ducts are lost, cholestasis occurs and leads to portal fibrosis and eventually irreversible biliary cirrhosis. Thus, it seems logical that therapeutic intervention would be most successful when it is instituted early in the course of the disease before severe ductopenia and irreversible damage to the liver occurs. Unfortunately, at this time there is no medical, radiologic or surgical therapy of proven efficacy.

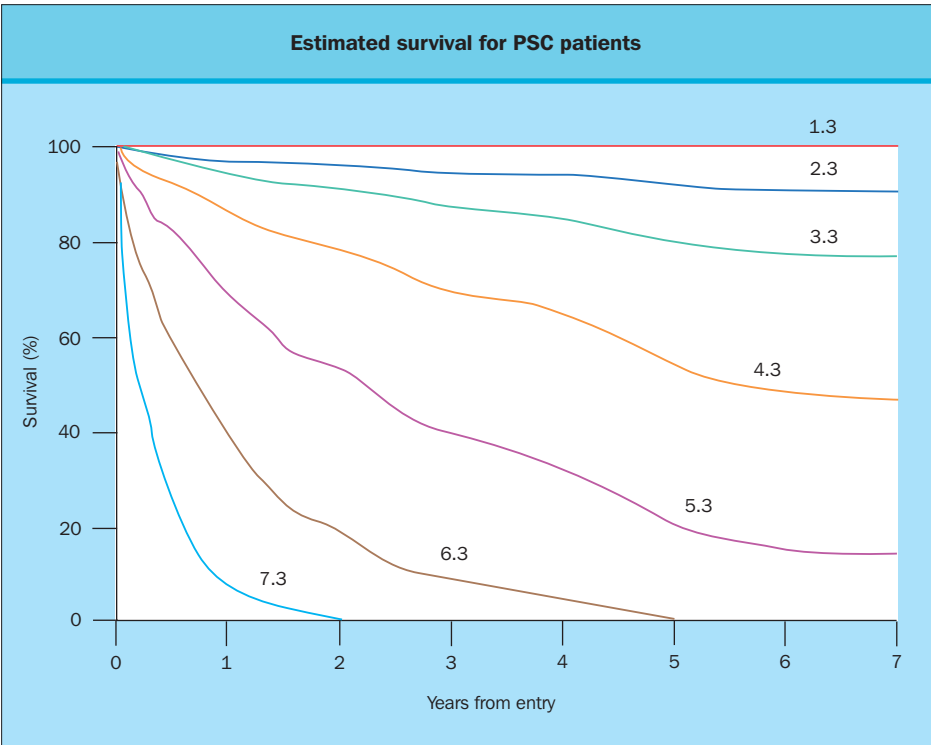


**Figure 20.12 Time to development of symptoms.** The estimated time to development of symptoms using the Kaplan-Meier method with Mayo Clinic diagnosis of PSC as the starting point. Reproduced from Porayko et al (90) by permission of the American Gastroenterological Association.



**Figure 20.13 Histopathologic evolution.** Histopathologic evolution of PSC from reversible stage, during which time early bile duct damage occurs and which should respond to medical therapy, to irreversible fibrotic-cirrhotic stage, which is resistant to medical intervention. Reproduced from Wiesner et al by permission of the Mayo Foundation for Medical Education and Research.





**Figure 20.14 Estimated survival for PSC patients.** Estimated survival for PSC patients by translating Mayo PSC risk score into a survival function. Increasing risk score leads to decreased survival. Reproduced from Wiesner et al (96) by permission of the American Association for the Study of Liver Diseases.

Prognosis of PSC					
Mayo Clinic	Multicenter	New Mayo	Kings College	Norway	Sweden
Bilirubin	Bilirubin	Age	Hepatomegaly	Age	Age
Age	Histologic stage	Bilirubin	Splenomegaly	Bilirubin	Bilirubin
Histologic stage	Age	Albumin	Alkaline phosphatase		Histologic stage
Hemoglobin	Splenomegaly	Variceal bleed	Histologic stage		
Inflammatory bowel disease		Aspartate aminotransferase			

**Table 20.10 Prognosis of PSC.** The prognostic models have identified a range of variables, but histologic criteria were not used in all models.

Prognostic survival models

A better understanding of the history of PSC is important in determining the rate of disease progression and the estimated survival for an individual patient at any specific time during the course of PSC. Predicting survival on the basis of clinical, biochemical and histologic features is important for monitoring therapeutic interventions and as an aid for the timing of liver transplantation in PSC patients. The use of sophisticated statistical approaches, most notably Cox multivariate regression analysis, has facilitated the development of a prognostic model based on independent clinical variables in which a survival risk score can be calculated and translated into a survival function for estimating survival for an individual PSC patient (Fig. 20.14). Several models have been developed based on long-term follow-up of a large

number of PSC patients in whom important clinical predictors of prognosis have been determined (Table 20.10). In the Mayo model, patient age, serum bilirubin level, hemoglobin concentration, hepatic histologic stage and presence or absence of inflammatory bowel disease were identified as independent prognostic variables. In the King’s College model, the presence of hepatomegaly, splenomegaly, degree of elevation of alkaline phosphatase and histologic stage were found to be independent predictors. In Norway, investigators using multivariate analysis have found that patient age and serum bilirubin levels were independent risk factors for survival. In a fourth model, based on the data from five major liver transplant centers throughout the world (Mayo Clinic in Rochester, MN; Oxford, UK; King’s College, UK; Tufts University, Boston, MA; and Thomas Jefferson

University, Philadelphia, PA), 426 PSC patients with a mean follow-up of 3 years were studied. In this study, serum bilirubin level, histologic stage, patient age and splenomegaly were identified as independent prognostic variables. With these variables, a severity risk score was determined and translated into a survival function so that survival of any PSC patient at any point in the disease process could be estimated. Subgroup analysis from these various liver transplant centers indicates that this model is generalizable to a wide spectrum of patients who have PSC. However, the new model also requires the need for a liver biopsy, which often limits the utility of the model as a clinical tool.

This deficit was addressed in a study from the Cleveland Clinic where the authors evaluated the Child-Pugh classification as a prognostic indicator for survival in PSC. The authors further compared the Child-Pugh model with the Mayo disease-specific PSC model. The Child-Pugh model utilizes the following clinical factors:

- serum bilirubin level;
- serum albumin level;
- prothrombin time;
- presence or absence of ascites; and
- grade of portosystemic encephalopathy.

The study demonstrated that the age-adjusted Child-Pugh model predicted survival before liver transplantation with accuracy similar to that of the Mayo PSC model. It also had the advantage of not requiring a liver biopsy and avoided complex mathematical computations. Furthermore, it is ideal for use in formulating minimal listing criteria for liver transplantation for the United Network for Organ Sharing (UNOS). This essentially places PSC patients on a level playing field with all chronic liver disease patients in whom the Child-Pugh classification is utilized for liver transplantation listing purposes and avoids having different criteria for each disease.

However, there are obvious shortcomings with regard to all of these models in that none of them has been prospectively evaluated, they all use historic data, and they do not take into account certain risk factors that are important in determining the timing for liver transplantation in the PSC patient, such as variceal bleeding, quality of life and the possibility of developing a hepatobiliary malignancy. Nevertheless, these models have been useful in counseling patients and have been important in advising the appropriate timing for liver transplantation for patients with this disease. Additional ongoing studies will be needed to assess prospectively the models and their applications toward further optimizing the timing of liver transplantation. In addition, the challenge remains to refine further these models so that they might be applied to monitoring the effect of experimental therapy on disease progression.

## MANAGEMENT

Primary therapy for PSC aimed at interrupting the disease process can be subdivided into medical, radiologic, and surgical approaches (Table 20.11).

### Medical therapy

Over the past 20 years, various medical treatments have been used in PSC patients in the hope of improving survival. The

diversity of medical therapies includes the use of cupruritic, immunosuppressive, antifibrogenic and choleretic agents, which suggests their ineffectiveness and reveals a lack of complete understanding of the pathophysiology of the disease.

### Cupruritic therapy

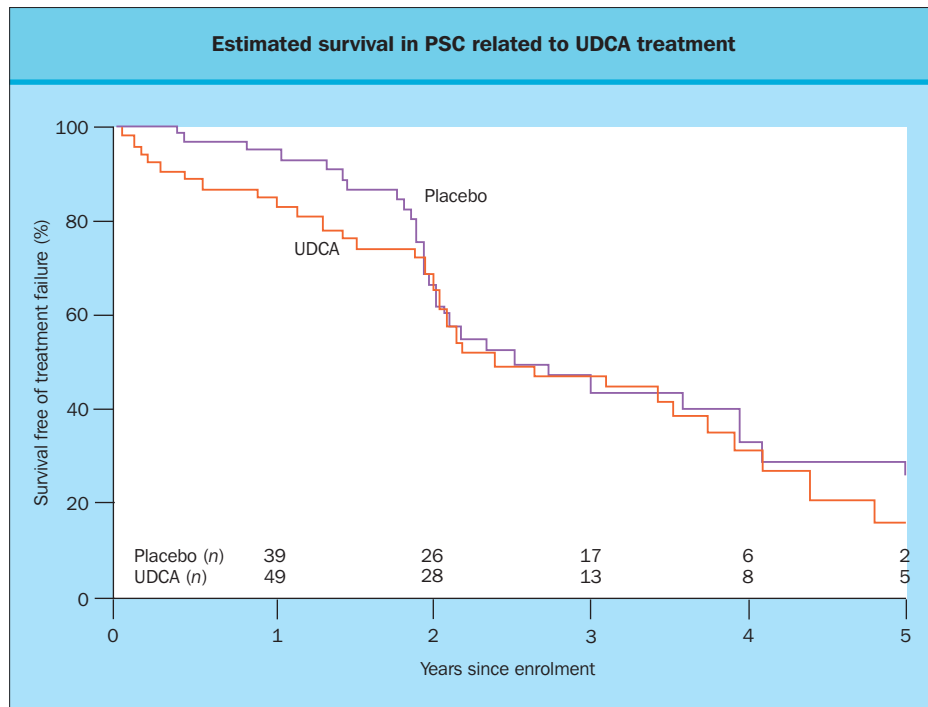
The evidence of increased hepatic copper in PSC provided the main rationale for evaluating D-penicillamine as a therapeutic agent for PSC. In a prospective randomized, double-blind, placebo-controlled trial, D-penicillamine had no favorable effect on PSC progression and patient survival. This was determined after 36 months of follow-up based on careful analysis of clinical and biochemical profiles, radiologic findings, liver histology and survival data between the two groups of PSC patients. Furthermore, major adverse effects of D-penicillamine including proteinuria and thrombocytopenia, led to the discontinuation of the drug in 20% of treated patients.

### Immunosuppressive therapy

The use of immunosuppressive agents in the therapy of PSC was based on the concept that PSC is an autoimmune disease. In uncontrolled observations, oral corticosteroids have been shown

Treatment of PSC
<b>Medical</b>
Cupruritic:
D-penicillamine
Immunosuppressive:
Prednisone
Azathioprine
Cyclosporin
Methotrexate
Tacrolimus
Mycophenolate mofetil
Antifibrotic:
Colchicine
Etanercept pentoxifylline
Choleretic:
Ursodeoxycholic acid
<b>Radiological</b>
Cholangioplasty
Biliary infusion therapy
<b>Surgical</b>
Reconstructive surgery
Proctocolectomy
Liver transplantation

**Table 20.11 Treatment of (PSC).** The treatments include drugs, radiologic and surgical approaches. All are aimed at retarding disease progression.



**Figure 20.15 Estimated survival in PSC related to UDCA treatment.** Kaplan-Meier analysis of survival free of treatment failure among study patients. Treatment failure was defined as one or more of the following: death, liver transplantation, histologic progression by two stages, progression to cirrhosis, development of esophageal varices, development of ascites or encephalopathy, sustained quadrupling of the serum bilirubin concentration, marked worsening of fatigue or pruritus, inability to tolerate the drug and voluntary withdrawal from the study. UDCA, ursodeoxycholic acid. Reproduced from Lindor et al (97) by permission of the Massachusetts Medical Society.

to improve the biochemical profile in PSC patients; however, long-term or controlled studies of corticosteroids are lacking. In a small, controlled study in PSC patients, topical corticosteroid application in the biliary tree via nasobiliary lavage provided no advantage over placebo. Other immunosuppressive agents that have been employed in the treatment of PSC include:

- azathioprine – the experience has been mostly anecdotal;
- cyclosporin – the results were negative;
- methotrexate – a randomized, double-blind, placebo-controlled trial showed no benefit in the treatment group; and
- tacrolimus – a small open-label study showed only improvement in liver enzymes.

These attempts at immunosuppressive therapy lead to the conclusion that further studies are needed to define the immunosuppressive effects in PSC patients. In addition, an open trial of prednisone and colchicine in PSC patients demonstrated no benefit on progression of hepatic histology and liver biochemical tests over a 2-year period. A pilot study combining ursodeoxycholic acid and methotrexate for the treatment of PSC was associated with toxicity and without improvement in liver tests compared with ursodeoxycholic acid therapy alone.

### Antifibrotic therapy

Due to the fact that PSC leads to liver cirrhosis, antifibrotic agents, such as colchicine, were suggested as a therapeutic option for PSC. Recently, in a prospective, double-blind study, 84 PSC patients were randomized to receive 1 mg of colchicine versus placebo daily for a period of 3 years. The results of this multicenter study revealed that colchicine had no beneficial effect on patient symptoms, liver biochemistry, hepatic histology or survival.

### Choleretic therapy

The enthusiasm for the use of ursodeoxycholic acid, a hydrophilic bile acid, in the treatment of PSC emerged from favorable

data on its use for PBC and from promising clinical and biochemical improvement reported in PSC patients receiving ursodeoxycholic acid in small uncontrolled studies. Subsequently, a prospective, randomized, double-blind, placebo-controlled trial involving 14 PSC patients was performed to examine further the efficacy of ursodeoxycholic acid. The authors of that study concluded that PSC patients treated with ursodeoxycholic acid at 13–15 mg/kg of body weight per day for 1 year demonstrated marked improvement in serum liver tests and histology on liver biopsy. The proposed benefit of orally administered ursodeoxycholic acid in cholestatic patients is thought to be related to:

- its accumulation in the endogenous bile pool which leads to replacement of endogenous potentially toxic bile acids in the cholestatic liver;
- its protective effect at the cellular and subcellular levels of the hepatocyte and cholangiocyte;
- its hypercholeretic activity; and
- its immunomodulatory effect.

However, in the recently published largest randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid treatment for PSC, no clinical benefit was found. The study included 105 patients with well-established PSC given ursodeoxycholic acid 13–15 mg/kg of body weight/day compared with placebo. The mean follow-up was 2.2 years. In this study, ursodeoxycholic acid, but not placebo, demonstrated improvement in liver tests at 1 and 2 years of treatment. Nevertheless, the primary outcome (time to treatment failure), as defined by the authors, and survival were not significantly different in the two groups of PSC patients (Fig. 20.15). However, in a randomized, placebo-controlled study of 26 patients followed over 2 years, the use of high dose ursodeoxycholic acid (20 mg/kg/day) was found to result in improved liver biochemistries, cholangiographic appearances and fibrosis. Another pilot study of high dose ursodeoxycholic acid (25–30 mg/kg/day) in 30 patients with PSC also demonstrated improved bio-



chemistries and the Mayo risk score, translating into a significantly better survival compared to patients on a placebo or low dose ursodeoxycholic acid. These promising results need to be explored further in patients with PSC.

In summary, to date, effective pharmacologic treatment for the underlying hepatobiliary disease in PSC is lacking. The data do not support the use of any mentioned agent except in the context of well-designed experimental studies. Future therapeutic trials for PSC must be centered on the asymptomatic patient because, when ductopenia occurs, the disease appears to be irreversible since bile duct epithelial cells are unable to regenerate.

### **Surgical therapy**

Until the advent of liver transplantation, surgical therapy for PSC was focused on alleviation of pruritus and jaundice, exclusion of cholangiocarcinoma, and the prevention of colonic adenocarcinoma by performing a proctocolectomy in patients with concurrent PSC and CUC.

#### **Biliary surgery**

Since PSC is a progressive disease, biliary surgery was introduced primarily to improve symptoms. Biliary enteric anastomoses with biliary stenting and reconstruction of the hepatic duct bifurcation with long-term transhepatic stents have been performed. In two retrospective studies evaluating biliary tract reconstructive surgery, cirrhotic PSC patients showed a significantly higher mortality rate compared to the non-cirrhotic patients. Thus, it is now widely accepted that biliary surgery should be avoided in PSC patients with cirrhosis or severe, diffuse intrahepatic disease. Instead, these patients should be considered for liver transplantation.

Although biliary reconstruction may benefit non-cirrhotic PSC patients with extrahepatic disease, the lack of prospective, controlled data makes it impossible to assess the long-term effects of this procedure on the natural history of PSC and the subsequent risk of developing cholangiocarcinoma. In our view, the value of biliary surgery is limited to selected PSC patients without cirrhosis who are not candidates for liver transplantation when extrahepatic dominant strictures are not amenable to endoscopic or transhepatic dilatation.

#### **Proctocolectomy**

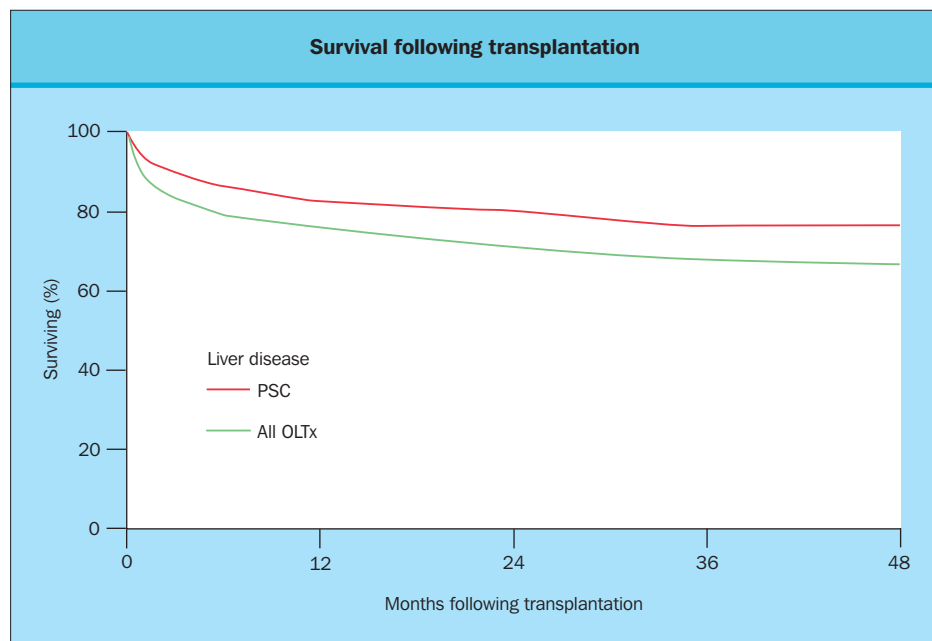
In patients with both PSC and CUC, proctocolectomy is frequently performed due to indications relevant to inflammatory bowel disease. The possible effect of proctocolectomy on the natural history of PSC was examined in a retrospective analysis involving 45 patients with coexisting PSC and CUC, of whom almost half had undergone proctocolectomy and the rest had not. This study revealed that the clinical signs, biochemical profile, histologic progression on liver biopsy, and survival were not different in the two groups. Moreover, the fact that PSC can occur years after proctocolectomy argues against any beneficial effect of this procedure on the progression of PSC. Nonetheless, patients with both PSC and CUC remain at high risk for

the development of adenocarcinoma of the colon; thus, an annual surveillance colonoscopy is strongly indicated, particularly in patients with PSC who have undergone liver transplantation. Proctocolectomy should be performed for intractable symptoms of inflammatory bowel disease that are not amenable to medical treatment and for colonic dysplasia and malignancy. In patients with PSC and CUC, if proctocolectomy is indicated, an IPAA is the procedure of choice since this procedure prevents the development of peristomal varices. Of note, a complication of the IPAA is pouchitis, a non-specific inflammation of the ileal reservoir that occurs more frequently in patients with coexisting PSC and CUC than in CUC patients alone. Frequently, treatment with metronidazole or other antibiotics will effectively control this condition.

### **Orthotopic liver transplantation**

Currently, the only suitable life-saving treatment for patients with end-stage PSC is orthotopic liver transplantation. Over the past decade, the outcome of liver transplantation in PSC patients with end-stage disease has improved significantly, and liver transplantation has emerged as the treatment of choice for these patients. Indeed, retrospective analysis of PSC patients using the Mayo PSC natural history model has shown that liver transplantation significantly improves the survival rate at all risk stratifications compared with the estimated survival and the absence of liver transplantation. Recently, the 1-year and 5-year patient survival after liver transplantation for PSC have been reported to be 90–97% and 85–88%, respectively (**Fig. 20.16**), based on independent studies from three major liver transplant centers. In patients with end-stage PSC, one of the major clinical challenges is selecting the ideal time for liver transplantation. It is known that patients with high Mayo PSC risk scores have an increased risk of death after orthotopic liver transplantation compared with patients with low Mayo risk scores. In addition, risk factors specific to PSC that affect the outcome of liver transplantation have been recognized. Recently, a prognostic model to predict the post-liver transplant morbidity in PSC and PBC has been described. In PSC patients, the guidelines for liver transplantation should include intractability of symptoms, signs and complications of portal hypertension. However, careful consideration of each patient's needs and quality of life remain important in this disease.

Despite a favorable outcome, liver transplantation for PSC is associated with specific complications. Post-liver transplant complications include an increased incidence of acute and chronic rejection frequently leading to graft loss, biliary strictures and leakage, hepatic artery thrombosis and lymphoproliferative disease. Recurrence of PSC after liver transplantation has been controversial primarily due to the lack of gold standard criteria for the diagnosis of recurrent PSC. When a cohort of patients was evaluated for recurrent disease based on biochemical, cholangiographic and histologic criteria, 20% were found to have evidence of recurrent disease at a mean of 55 months after liver transplantation. A number of these patients have required retransplantation, and the impact of the recurrence of PSC is yet to be determined.



**Figure 20.16 Survival following transplantation.** Survival in patients undergoing liver transplantation for PSC in comparison with overall survival for all indications for orthotopic liver transplantation. Patients undergoing liver transplantation for PSC had significantly improved survival ( $P < 0.01$ ) on the basis of 1992 data from UNOS. Patients with PSC ( $n = 499$ ); other indications ( $n = 4332$ ). Reproduced with permission from Aegean communications (94) Contemp Internal Med.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 21

## Alcoholic Liver Disease

Timothy R. Morgan and Samuel W. French

### INTRODUCTION

*'There is no more evidence for a toxic effect of pure ethyl alcohol upon liver cells than there is for one due to sugar.'* (Best 1949)

We have come far since Charles Best, winner of the Nobel Prize in medicine for the discovery of insulin, summarized the opinion of many physicians in the middle of the 20th century. At that time malnutrition rather than a direct toxic effect of alcohol was thought to cause liver disease. Indeed, the diagnosis 'nutritional cirrhosis of the alcoholic' was included in the International Classification of Diseases until the mid-1980s.

We now accept that chronic alcohol use commonly causes three types of liver disease: fatty liver, alcoholic hepatitis and fibrosis/cirrhosis. In addition to these three liver diseases, alcohol also causes foamy degeneration (microvesicular fatty liver disease) and hepatocellular carcinoma. Finally, alcohol contributes to liver damage in patients with other liver diseases, especially hepatitis C.

### ALCOHOL

Ethanol is a two-carbon alcohol produced by yeast during fermentation of carbohydrate. The two primary alcoholic beverages in the West are wine, a fermentation product of fruit (usually grapes) and beer, a fermentation product of grains, malt, and hops. Distilled spirits ('hard liquors') are distillations of primary alcoholic products and contain higher concentrations of ethanol. Different cultures and regions of the world use their endogenous grain to produce spirits. For example, corn (bourbon), potatoes (vodka), sugar cane (rum), blue agave (tequila), rice (sake), malts/grains (scotch), sap from coconut palm fonds (arrak), and wine (brandy), are used by various industries to make distilled drinks.

The alcohol content of beer is approximately 5% (range of 4–9% volume of ethanol per volume of beverage) while wine is about 12% (range 8–13% v/v). Distilled spirits usually contain approximately 40% ethanol, but can contain more than 80% ethanol. In the USA, 'proof' is defined as twice the ethanol content by volume. Thus, 100 proof whiskey contains 50% ethanol by volume. In the UK, proof is defined differently such that 100 proof whiskey contains 57% ethanol by volume.

For the purposes of research studies, drinks of alcohol are converted to grams of ethanol. For example, 4 ounces of wine (120 mL) contains approximately 11.4 g of ethanol (120 mL  $\times$  0.12 mL ethanol/mL  $\times$  0.79 g of ethanol/mL ethanol). A standard drink in the USA is 12 ounces of beer, 4 ounces of wine or

1.5 ounces of 80 proof spirits (in the USA, 1 fluid ounce is 29.57 mL) and contains 11–14 g of ethanol (Table 21.1). In the UK, the standard drink is defined as containing 8 g of ethanol (half-pint of beer (8 ounces, 284 mL), 6 ounces of wine or 25 mL of spirits). However, the actual amount of alcohol in one drink can vary considerably, perhaps related to who is pouring the drink and who is reporting the event.

Alcohol is the most commonly used drug in the USA. More than two-thirds of adults drink alcohol each year, 30% have had a 'binge' (five or more drinks at one setting or in a relatively short period of time) within the past month and 7.4% of adults (11% of men and 4% of women) regularly drink more than two drinks per day. More than 14 million adults in the USA meet the diagnostic criteria for alcohol abuse or dependence. Per capita alcohol consumption in the USA increased between 1960 and 1980, decreased 20% between 1980 and 1998, and has slightly increased to 6.7 L/year in 2000. In the UK, the per capita ethanol consumption increased approximately 150% from 1950. Currently in the UK, 27% of men and 13% of women drink more than the recommended limit (30 g/day for men and 20 g/day for women). Per capita ethanol consumption, especially in males, has increased dramatically in Asia during the past 40 years. Per capita alcohol consumption for various countries of the world is shown in Table 21.2.

In the USA chronic liver disease is the 10th most common cause of death in adults, the 7th most common cause of death among men aged 45–64, and the 4th most common cause of death in males aged 25–64 living in urban areas. Alcoholic

Average alcohol content of beverages		
Name	Description: Volume (oz; mL)	Alcohol content: Grams of ethanol/100 mL Volume of ethanol/100 mL
Beer	Glass	8–9 g
	8 oz; 284 mL	3–4%
Wine	Half glass	9–11 g
	4–5 oz; 100–120 mL	11–13%
Spirits	1 shot	12–14 g
	1.5 oz; 40–44 mL	40–50%
Fortified wine	Half glass	15–20 g
	4–5 oz; 100–120 mL	14–24%

Table 21.1 Average alcohol content of beverages

Per capita alcohol consumption in adults in various countries in 2000	
Country	Liters of ethanol
Luxembourg	12.3
Romania	12.1
Republic of Ireland	10.8
France	10.5
Germany	10.5
Spain	10.0
United Kingdom	8.4
Russia	8.1
Australia	7.8
Italy	7.5
USA	6.7
Canada	6.6
Japan	6.5
Argentina	6.3
Data obtained from Institute of Alcohol Studies (www.ias.org.uk)	

Table 21.2 Per capita alcohol consumption in adults in various countries in 2000

cirrhosis accounts for approximately 40% of cirrhosis deaths. In the UK, cirrhosis mortality rate doubled between 1993 and 2000, rising from 6.0 to 12.7/100 000 persons per year. Cirrhosis mortality rate parallels per capita alcohol consumption, when examined over time in one country (as in the USA during prohibition) or when examined at one time in different countries (Fig. 21.1). Every liter increase in per capita alcohol consumption raises cirrhosis mortality rate 15% in males and 8% in females.

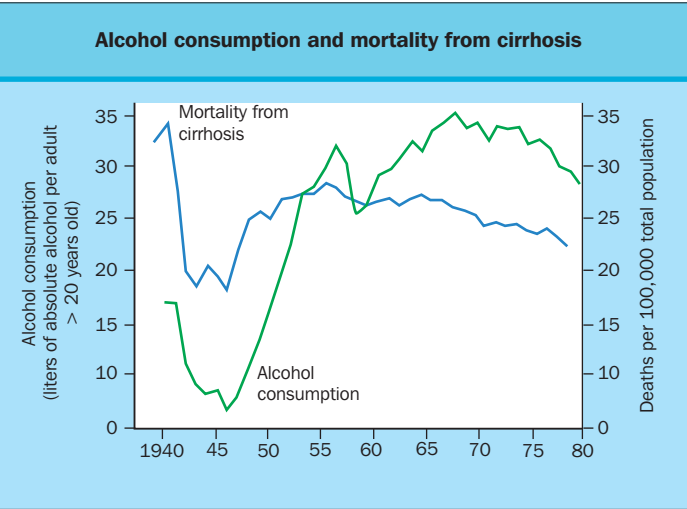


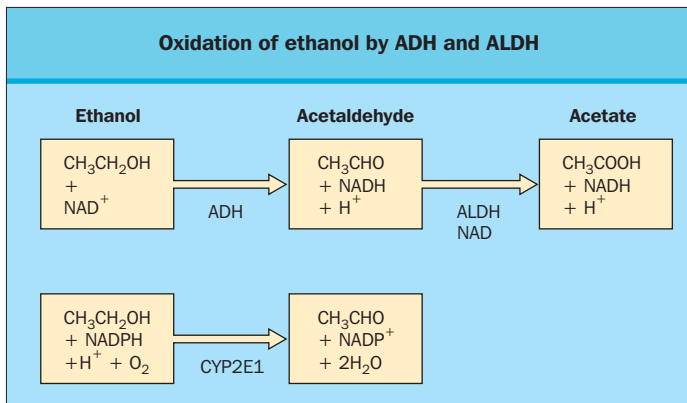
Figure 21.1 Alcohol consumption (adults) and mortality from cirrhosis in France: 1939–1980. Modified from Leibach (85).

The risk of cirrhosis increases when men consume >60 g of ethanol/day, while women are at increased risk consuming more than 20–30 g/day. It is generally stated that daily alcohol intake at this level for at least 10 years is required to produce cirrhosis. In reality, most patients with decompensated alcoholic cirrhosis have drunk more than 150 g, and frequently more than 200 g, of ethanol daily for more than 20 years. However, only a minority of patients, estimated at 10–25%, drinking excessive alcohol will develop cirrhosis. Leibach, in the 1980s, reported the highest rate of cirrhosis (50%) in chronic drinkers after 20 years of heavy daily alcohol use. A recent population based study in Italy found cirrhosis in 13.5% of patients drinking more than 120 g of ethanol/day. Risk factors for cirrhosis included drinking more than one type of alcohol and drinking at times other than meals (Table 21.3).

Ethanol is absorbed in the stomach (10%) and the small intestines (90%). It is metabolized in the liver (90%) or excreted unchanged by the lungs or kidneys (10%). In the liver, the two main alcohol metabolizing pathways are the alcohol and aldehyde dehydrogenases and the microsomal ethanol-oxidizing system (Fig. 21.2). The alcohol dehydrogenase–acetaldehyde dehydrogenase pathway metabolizes approximately 90% of ethanol. Alcohol dehydrogenase, the first enzyme in this pathway, is a cytosolic enzyme with a low  $K_m$  (high affinity) for alcohol and a moderate  $V_{max}$  (rate of alcohol metabolism). Oxidation of ethanol by alcohol dehydrogenase produces acetaldehyde and reduces nicotinamide dinucleotide (NAD) to NADH (reduced NAD). Alcohol dehydrogenase is found in hepatocytes throughout the hepatic lobule and does not increase in quantity with chronic alcohol use. Acetaldehyde is oxidized to acetate by aldehyde dehydrogenase. This reaction, which occurs primarily in mitochondria, also reduces NAD to NADH. Acetate, the end product of alcohol oxidation in this pathway, is converted to acetyl CoA and used for gluconeogenesis, fatty acid synthesis and in the citric acid cycle [Krebs cycle, which produces adenosine triphosphate (ATP)]. Oxidation of ethanol by alcohol dehydrogenase and aldehyde dehydrogenase consumes NAD and

Risk of developing cirrhosis with various amounts of daily alcohol consumption			
Alcohol intake (g/day)	No (n = 6442)	Cirrhosis (n = 35) (%)	Odds ratio (95% confidence interval)
Teetotallers	2501	1 (0.04)	—
1–30	2666	4 (0.15)	—
31–60	745	8 (1.0)	10.9 (3.6–33.5)
61–90	276	7 (2.3)	25.0 (7.9–79.3)
90–120	132	7 (4.9)	52.9 (16.6–169)
>120	122	8 (5.7)	62.3 (20.1–193)
Liver disease and lifetime daily alcohol consumption were determined in 6442 male and female adults living in two northern Italian towns. Patients with hepatitis B or hepatitis C were excluded from the analysis. Modified from Bellentani et al (97).			

Table 21.3 Risk of developing cirrhosis with various amounts of daily alcohol consumption



**Figure 21.2** Oxidation of ethanol by ADH and ALDH, and by cytochrome P4502E1. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

produces excess NADH, biochemical changes that may contribute to hepatic steatosis.

Cytochrome P4502E1, an enzyme in the microsomal ethanol oxidizing system, also oxidizes ethanol to acetate. CYP2E1 metabolizes ethanol when the blood alcohol level is significantly elevated (usually  $>150$  mg/dL). CYP2E1 differs from alcohol dehydrogenase in two respects. First, CYP2E1 is found in higher concentrations in the perivenular (zone 3) hepatocytes than in the periportal (zone 1) hepatocytes. Second, the activity of CYP2E1 increases with chronic alcohol use. During the metabolism of ethanol CYP2E1 can release oxygen free radicals (oxygen with an extra electron). CYP2E1 also metabolizes acetaminophen (Tylenol® and others), with production of the toxic metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI). In alcoholics relatively low doses of acetaminophen can lead to the rapid production of NAPQI, exhausting the antioxidant capacity of hepatocytes, with resulting hepatotoxicity.

## **PATHOPHYSIOLOGY**

As might be imagined in a disease that spans the spectrum from mild fatty change to inflammation, necrosis, apoptosis and fibrosis, there are numerous alterations in normal biochemical pathways and physiologic processes. Determining which changes are primary, and important in progressive liver disease, and which are secondary, is an area of active research.

### **Fatty liver**

Hepatocytes synthesize fatty acids, produce triglycerides (triacylglycerols), and secrete triglycerides in the form of very low density lipoproteins (VLDL). Hepatocytes also oxidize (break down) fatty acids by  $\beta$  and/or omega oxidation in the mitochondria, microsomes and peroxisomes, with  $\beta$  oxidation in the mitochondria being quantitatively the most important.

The altered redox state (increased NADH/NAD ratio) in hepatocytes as a result of ethanol oxidation contributes to fat accumulation in alcoholic fatty liver. The low concentration of NAD inhibits fatty acid oxidation in the mitochondria, a reaction that requires NAD. The increased NADH/NAD ratio also impairs gluconeogenesis from acetyl CoA resulting in shunting of the acetyl CoA towards synthesis of fatty acids. Ethanol may increase the supply of fatty acyl CoA esters and

glycerol-3-phosphate, the precursors of triglycerides as well as increasing the level of phosphatidic acid phosphatase, the rate-limiting step in the synthesis of triglycerides. Finally, alcohol inhibits secretion of VLDL by inhibiting microtubular assembly. Although the altered redox state in hepatocytes is believed to be the primary mechanism involved in fatty liver, cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 and IL-6 may also increase hepatocyte fatty acid production and inhibit mitochondrial fatty acid oxidation.

### **Alcoholic hepatitis**

Several pathways and processes have been implicated in the pathogenesis of alcoholic hepatitis. These are described below.

#### **Cytokines and oxidative stress**

Alcohol increases translocation of endotoxin [lipopolysaccharide (LPS)], a component of the cell wall of Gram negative bacteria, from the lumen of the small intestines to the portal circulation. Endotoxin binds to LPS binding protein in the blood, and then attaches to the CD14 receptor and the Toll-like receptor 4 (TLR4) on the surface of the Kupffer cell. LPS binding stimulates Kupffer cells to produce TNF- $\alpha$ , IL-1, IL-6, IL-8, monocyte chemo-attractant protein I (MCP-1), macrophage inflammatory protein I (MIP-1), transforming growth factor- $\beta$  1 (TGF- $\beta$  1) and other cytokines. These cytokines increase the inflammatory response in the liver, increase oxidative stress, and are important mediators for hepatic stellate cell activation, proliferation and collagen synthesis.

Oxidative stress increases within hepatocytes and in the extra-cellular space adjacent to hepatocytes. During the metabolism of ethanol CYP2E1 can release a hydroxyethyl free radical (free radical formed from ethanol) and a superoxide anion ( $\text{O}_2^{\bullet-}$ , oxygen with an extra electron). Mitochondria are another source of free radicals. Electrons 'leak' from complex I and complex III of the mitochondrial electron transport chain to molecular oxygen producing superoxide anions. Extrahepatic sources of oxidative stress include polymorphonuclear leukocytes (PMN) and monocytes that contain NAD(P)H oxidases and produce superoxide anion. In the presence of ferrous iron ( $\text{Fe}^{2+}$ ) and water, a superoxide anion can be converted to a hydroxyl radical, a potent oxidizing agent. The superoxide anion can combine non-enzymatically with nitric oxide ( $\text{NO}^*$ ) to form the powerful free radical, peroxynitrite ( $\text{ONOO}^-$ ). In addition to increased production of free radicals there are lower levels of glutathione, the primary intracellular antioxidant. In alcoholic cirrhosis there is a specific decrease in hepatic activity of methionine adenosyl-transferase, the enzyme producing S-adenosylmethionine, a precursor to glutathione, as well as a decrease in the activity of glutathione peroxidase, the enzyme which synthesizes glutathione.

Oxidative stress can damage DNA, proteins and lipids (lipid peroxidation), potentially altering multiple biochemical pathways and sensitizing cells to other injuring agents. Oxidative stress also interferes with hepatocyte function by altering intracellular calcium signaling, stress-activated protein kinases and transcription factors.

Mitochondrial function is altered in alcoholic liver disease. Mitochondrial oxidative phosphorylation (ATP synthesis) is decreased due to altered function or levels of the components of the respiratory chain. Glutathione transport into mitochondria



is decreased, reducing the antioxidant level in mitochondria, leaving mitochondria at increased risk of oxidative injury. Deletions in the mitochondrial DNA have been reported in humans with alcoholic liver disease.

### Apoptosis

Apoptosis is an active form of cell death characterized by nuclear and cellular fragmentation. In patients with alcoholic hepatitis, up to 5% of hepatocytes have histochemical markers of apoptosis. In particular, hepatocytes containing Mallory bodies often have markers of apoptosis. In alcoholic hepatitis, the number of apoptotic cells seen on liver biopsy correlates with bilirubin level and histologic severity, suggesting that apoptosis may be of pathophysiologic importance.

TNF- $\alpha$  released from activated Kupffer cells is a major stimulant of hepatocyte death. Binding of TNF- $\alpha$  to the TNF-1 receptor on the surface of the hepatocyte activates proteins that bind the death domain, including the TNF receptor-associated death domain protein (TRADD) and the Fas-activated death domain protein (FADD). These proteins lead to activation of caspase 8 and release of ceramide, both important in the apoptosis cascade. They also damage the mitochondrial outer membrane, an initial step in the release of proapoptotic mitochondrial proteins, especially cytochrome c. Although TNF- $\alpha$  also activates cytoprotective responses in hepatocytes, alcohol shifts the balance in favor of the proapoptotic response.

TNF- $\alpha$  can interact with other cells to increase oxidative stress. TNF- $\alpha$  binding to neutrophils stimulates IL-8 release, increasing neutrophil infiltration, and stimulates neutrophils to release free radicals including nitric oxide and superoxide anions. When there is extensive apoptosis, which overwhelms the ability of the liver to clear apoptotic cells, or when energy levels are insufficient for apoptosis, necrosis becomes the predominant form of death. In alcoholic liver disease, it is possible that a switch from apoptosis to necrosis stimulates fibrosis. Because of the presumed central role of TNF- $\alpha$  in alcoholic liver disease, treatments directed against this cytokine are currently being investigated.

TNF- $\alpha$  binding to its receptor is not the only pathway leading to apoptosis. Oxidative stress stimulates expression of Fas on hepatocyte cell membrane, sensitizing hepatocytes to Fas-ligand mediated apoptosis.

Acetaldehyde, produced by the metabolism of ethanol, can be toxic to hepatocytes. Acetaldehyde binds to lysine residues on proteins, lipids and DNA, potentially altering their function. Malondialdehyde and 4-hydroxynonenal, other aldehyde products of lipid peroxidation, can also bind to proteins. The acetaldehyde adducts may interfere with the normal function of the cell or produce new, antigenic proteins (neoantigens) which stimulate the host immune response. Antibodies against acetaldehyde adducts are found in patients with alcoholic liver disease, and the titer of the antibodies increases in patients with more severe injury.

### Immune system

In addition to proinflammatory cytokine activation of the innate immune system (e.g. TNF- $\alpha$ , IL-6, TGF- $\beta$ ), there is also activation of the adaptive immune response. Patients with alcoholic hepatitis and cirrhosis frequently have a broad-based

increase in serum level of  $\gamma$  globulins, and a specific increase in immunoglobulin A (IgA). Hepatocytes from alcohol-fed rats express CYP2E1-hydroxyethyl radical adducts, which are capable of stimulating antibody-dependent cell-mediated toxicity in vitro. Activated CD8<sup>+</sup> T cells are present in approximately 25% of patients with alcoholic hepatitis. Peripheral blood lymphocytes from patients with alcoholic liver disease can kill autologous hepatocytes in vitro suggesting a self-directed cellular immune response.

### Genetic factors

Long-term studies of twins suggest that genetic factors influence the risk of developing alcoholic cirrhosis. Association studies have evaluated single nucleotide polymorphisms (SNP) involving alcohol metabolizing enzymes (alcohol dehydrogenase, aldehyde dehydrogenase, CYP2E1), cytokines (TNF- $\alpha$ , IL-10, IL-4 receptor), fibrosis (type I collagen), oxidative stress (hemo-chromatosis gene, superoxide dismutase, glutathione S-transferase), DNA repair (XRCC1), steatosis (apolipoprotein E), and the immune system (CD14, TLR4, cytotoxic T lymphocyte antigen-4 [CTLA-4]). Studies of immune regulatory genes are of particular interest. Cytotoxic T lymphocyte antigen-4 is a T cell surface molecule which downregulates the adaptive immune response. The A to G polymorphism in exon 1 of the CTLA-4 gene is associated with autoimmune diseases, including autoimmune thyroid disease, diabetes type 1 and autoimmune liver disease. Studies have shown an increased risk for alcoholic cirrhosis in patients with the G allele, supporting a possible immune mechanism in alcoholic liver disease. Polymorphisms in the IL-10 promoter influence expression of this cytokine inhibitor of inflammation. In particular, the A allele at position -627 of the promoter is associated with decreased IL-10 transcription. Studies have also found a twofold increased risk of alcoholic liver disease in patients carrying the A allele at position -627 in the IL-10 promoter, suggesting that less inhibition (i.e. greater activation) of the immune system is associated with advanced alcoholic liver disease. These findings need to be confirmed in larger and more diverse populations.

### Fibrosis and cirrhosis

#### Hepatic stellate cells

Cirrhosis is the end result of the process of ongoing fibrosis. Alcoholic cirrhosis appears to result from a similar process that occurs with other forms of cirrhosis. The key event in the development of cirrhosis is activation of hepatic stellate cells (HSC) to myofibroblasts. During activation HSC undergo phenotypic changes that include loss of vitamin A and acquisition of features of smooth muscle cells (e.g.  $\alpha$  smooth muscle actin) as well as fibroblasts. Activated HSC undergo proliferation, migration and contraction. Platelet derived growth factor, probably secreted by activated Kupffer cells, is the primary stimulus for proliferation and migration, with other factors including endothelin-1, fibroblast growth factor (FGF), insulin-like growth factor and extracellular matrix. Activated HSC are the primary source of collagen and other extracellular matrix proteins as well as metalloproteinases that break down the native liver matrix proteins and tissue inhibitors of matrix metalloproteinases (TIMPs) that inhibit the action of matrix metalloproteinases.

Activated HSC secrete cytokines that have autocrine and paracrine effects. Among the cytokines with autocrine effects

are TGF- $\beta$  1, platelet derived growth factor, FGF, hepatocyte growth factor, platelet activating factor, and endothelin-1. With resolution of inflammation, activated HSC undergo apoptosis, leaving scar tissue. With time, scar tissue may resolve or the hepatic lobule may remodel, leaving nodules with altered blood flow surrounded by bands of scar tissue.

## CLINICAL: HISTORY, PHYSICAL EXAMINATION AND DIAGNOSIS

### Alcohol use and abuse

The CAGE (cutback, annoyed, guilty, eye-opener) questions can be used to screen for alcohol abuse (Table 21.4). Two positive answers indicate alcohol dependency, with a sensitivity of 70% and a specificity of 90%. Estimating the amount of alcohol consumed can be difficult. It may be helpful to ask about the amount of alcohol purchased per week rather than the amount consumed. In some instances family and friends may provide useful information. When in doubt, it is often wise to assume the patient may have an alcohol problem and to refer the patient to an alcohol treatment program.

When abnormal, serum  $\gamma$ -glutamyltransferase and mean corpuscular volume have a sensitivity of 30–40% in determining excessive alcohol use. Carbohydrate deficient transferrin is a specific, although not necessarily a sensitive, marker of excess alcohol use. It is not routinely available and is relatively costly. In practice, it is rarely used as a screening test for alcohol use.

### Fatty liver

Fatty liver is present in 80–90% of patients drinking more than 60 g of ethanol per day. Most patients are asymptomatic or have non-specific complaints such as mild nausea, fatigue or right upper quadrant discomfort. Patients with fatty liver disease often present to healthcare providers for treatment of alcoholism or trauma, or for routine medical care, and are incidentally found to have liver disease.

Approximately two-thirds of patients with fatty liver have hepatomegaly. The remainder of the physical examination is usually unremarkable. Aspartate aminotransferase (AST) may be raised, although rarely more than five times the upper limit of normal. As a rule alanine aminotransferase (ALT) is normal. Total bilirubin is frequently normal, but occasionally may be as high as 5 mg/dL. Typically, albumin is normal, as are the white blood cell (WBC) count, hemoglobin and prothrombin time. A reliable diagnosis of fatty liver is best made by liver biopsy, but

in practice liver biopsy is not often obtained unless there is a suggestion of more advanced disease.

### Foamy degeneration (microvesicular fatty liver disease)

Patients with foamy degeneration can present with jaundice, hepatic encephalopathy and marked elevation of AST and alkaline phosphatase. In general, the WBC count is not elevated and prothrombin time is normal. Liver biopsy is required to make the definitive diagnosis of microvesicular fatty liver disease. Patients with foamy degeneration usually recover quickly if they remain abstinent.

### Alcoholic hepatitis

Typically, patients with alcoholic hepatitis have consumed large amounts of alcohol for decades, although patients may have stopped drinking several weeks prior to admission to hospital, possibly because of the ill effects of the liver disease. Patients hospitalized for alcoholic hepatitis may have a variety of symptoms related to their inflammatory liver disease, including fever, nausea, anorexia, fatigue, pruritus, weakness, right upper quadrant pain and confusion (Table 21.5).

Physical examination is markedly abnormal in patients hospitalized with alcoholic hepatitis. Jaundice is the cardinal sign in alcoholic hepatitis and is present in all patients. Fever is frequently present (without infection). Common cutaneous signs include telangiectasias, spider angiomas and palmar erythema. Scratch marks, whitening of the nails and prominent venous collaterals in the periumbilical area are less common. Malnutrition, measured as decreased muscle mass, decreased serum proteins and decreased muscle strength, is present in more than 90% of hospitalized patients with alcoholic hepatitis. A minority have overt hepatic encephalopathy (i.e. asterixis), but most have subclinical hepatic encephalopathy. More than 75% of patients with severe alcoholic hepatitis have ascites and peripheral edema. Up to one-third have a hepatic bruit, heard over the liver either anteriorly or posteriorly, which is thought to be caused by increased blood flow through the hepatic artery. Splenomegaly is often present, but may not be detected by physical examination because of ascites.

Total serum bilirubin and conjugated bilirubin are elevated in all patients with alcoholic hepatitis and can exceed 30 mg/dL in patients with renal insufficiency. The WBC count is frequently elevated, with an increase in PMN and as well as increase in premature neutrophils ('bands'). Hemoglobin is mildly decreased (often to 11–12 g/L) due to dilution effect of increased plasma volume, decreased red blood cell (RBC) production, and increased RBC destruction. Variceal hemorrhage may contribute to decreased hemoglobin levels. AST (serum glutamic-oxaloacetic transaminase; SGOT) is elevated, although rarely more than five times the upper limit of normal. ALT (serum glutamic-pyruvic transaminase; SGPT) is often normal, but may be elevated above normal if the AST is more than threefold elevated. The AST/ALT ratio is greater than 1 in virtually all patients, and is generally close to 3. Alkaline phosphatase is moderately elevated, but is not a sensitive, specific or prognostic test in alcoholic hepatitis. Prothrombin time is elevated in most patients. Platelet count is decreased, but is generally  $>75\,000/\text{mm}^3$ . More often than not serum sodium is decreased (often less than 130 mEq/L) due to the reduced ability of the kidney to excrete free water. More often than not serum

#### CAGE questions

1. Have you ever felt like you should cut down on your drinking?
  2. Have people annoyed you by criticizing your drinking?
  3. Have you ever felt bad or guilty about your drinking?
  4. Have you ever had a drink first thing in the morning (eye-opener) to steady your nerves or get rid of a hangover?
- Answering 'yes' to two or more questions is suggestive of alcohol dependency.

**Table 21.4** CAGE questions

Relative frequency of various symptoms, physical findings and laboratory tests in patients admitted to hospital with alcoholic hepatitis	
Symptom	Frequency of abnormal finding (+, ++ or +++)
Weakness	+++
Anorexia	++
Fever	++
Confusion	+
Abdominal pain	+
Physical finding	
Fever	++
Jaundice	+++
Malnutrition, muscle wasting	+++
Telangiectasia, palmar erythema	+++
Hepatomegaly	++
Ascites	+++
Hepatic encephalopathy	+
Hepatic bruit	+
Abnormal laboratory test	
Bilirubin	+++
AST (usually <200 IU/mL)	+++
ALT (usually normal)	+
Albumin	+++
Creatinine	+
WBC, PMN	++
Hemoglobin	++
Platelet	+++
INR (prothrombin time)	++
Serum sodium (low)	++
Urine sodium (low)	+++
ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; PMN, polymorphonuclear leukocytes; WBC, white blood cell count.	

**Table 21.5** Relative frequency of various symptoms, physical findings and laboratory tests in patients admitted to hospital with alcoholic hepatitis

creatinine is normal, although creatinine clearance may be modestly reduced (60–80 mL/min). Elevated serum creatinine is a sign of hepatorenal syndrome and carries an ominous short-term prognosis. Urinalysis is unremarkable except for elevated bilirubin and urobilinogen. As a rule, urine sodium is low, often <10 mEq/L, even in patients without hepatorenal syndrome. The urine osmolality is higher than would be expected from the low serum osmolality. Excess protein is not present in the urine.

Up to 25% of patients are infected on admission to hospital, either in the ascites (spontaneous bacterial peritonitis), urine or blood. Cultures of ascites are recommended on admission to hospital and if the patient's hospital course deteriorates. If the patient is febrile, blood and urine cultures should be considered.

The diagnosis of alcoholic hepatitis is usually made on clinical grounds based on the presence of three or more of the following:

- serum total bilirubin >10 mg/dL,
- WBC count >10 000/ mm<sup>3</sup>,
- fever,
- ascites, and
- AST elevation in the appropriate range [i.e. AST:ALT ratio >1 (usually approximately 3), AST <10 upper limits of normal (ULN) (usually <5 × ULN)].

Transjugular liver biopsy is useful to confirm the diagnosis but is not available in many hospitals. A PMN count greater than 5500/mm<sup>3</sup> has an acceptable specificity and can be used to confirm the diagnosis of alcoholic hepatitis if a liver biopsy cannot be performed.

Patients may have milder forms of alcoholic hepatitis. In these patients, the symptoms, physical examination, and laboratory tests are less severely affected than those with severe alcoholic hepatitis although all patients have an elevated serum bilirubin level.

### Cirrhosis

Cirrhosis covers a broad spectrum of disease, from patients with 'early' cirrhosis to patients with decompensated cirrhosis, diagnosed by the presence of ascites, jaundice, encephalopathy or variceal bleeding. Patients with 'early' cirrhosis may be asymptomatic or complain of non-specific symptoms such as fatigue or weakness. As cirrhosis progresses, and especially in decompensated disease, patients are more likely to be symptomatic, often with fatigue, malaise and impotence.

The liver is firm and may be enlarged, especially the left lobe, or small and not palpable. The spleen is often, but not invariably, enlarged. The remainder of the physical examination may be normal in patients with early cirrhosis. Cutaneous signs of cirrhosis include palmar erythema and spider telangiectasias. Ascites is the most common form of decompensation in cirrhosis and is usually accompanied by peripheral edema. Overt hepatic encephalopathy (e.g. asterix) is infrequent, although subclinical hepatic encephalopathy is common in decompensated cirrhosis. Muscle wasting and decreased muscle strength are universal in advanced alcoholic cirrhosis.

All routine laboratory tests may be normal in 'early' cirrhosis. However, one or more laboratory tests are usually abnormal, with the degree of abnormality increasing in advanced cirrhosis. Albumin and hemoglobin levels may be decreased, in part because of the dilutional effect of increased plasma volume as well as decreased production. Typically, serum total bilirubin is normal, but it may be elevated several fold in advanced cirrhosis. Direct bilirubin is frequently mildly elevated in patients with normal total bilirubin, and is a sign of hepatic dysfunction. Prothrombin time may be elevated. Platelet count is decreased although usually not to the level in patients with hepatitis C cirrhosis. The serum AST is elevated if the patient is drinking alcohol, but may be normal in abstinent patients. Serum ALT is usually normal. Serum sodium is normal unless the patient has ascites. The WBC count may be decreased due to



hypersplenism. By and large urinalysis is normal. If patients have ascites, urine sodium is decreased, often to  $<20$  mEq/L.

A diagnosis of cirrhosis is likely in patients without alcoholic hepatitis who develop ascites or variceal hemorrhage. Making a diagnosis of cirrhosis in patients without these complications is difficult and requires liver biopsy to be certain. At present, laboratory tests, ultrasound and/or other radiologic imaging studies are not sufficiently sensitive or specific for the diagnosis of cirrhosis.

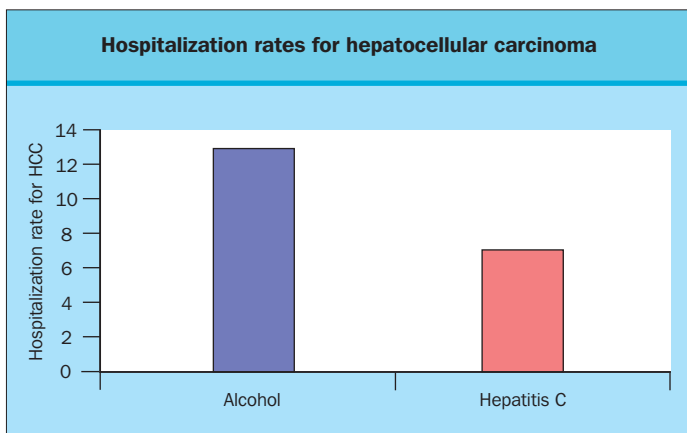
### Hepatocellular carcinoma

Alcohol may be the most common cause of liver cancer in the Western world. Alcohol is the underlying cause of 30–60% of liver cancers in Europe. In the USA, the age-adjusted hospitalization rate for hepatocellular carcinoma (HCC) associated with alcoholic cirrhosis was 70% higher than the rate for HCC associated with hepatitis C (Fig. 21.3). Chronic alcohol use accounts for 32% (USA) to 45% (Italy) of all HCC, which is higher than that due to chronic hepatitis C (22% in USA and 36% in Italy).

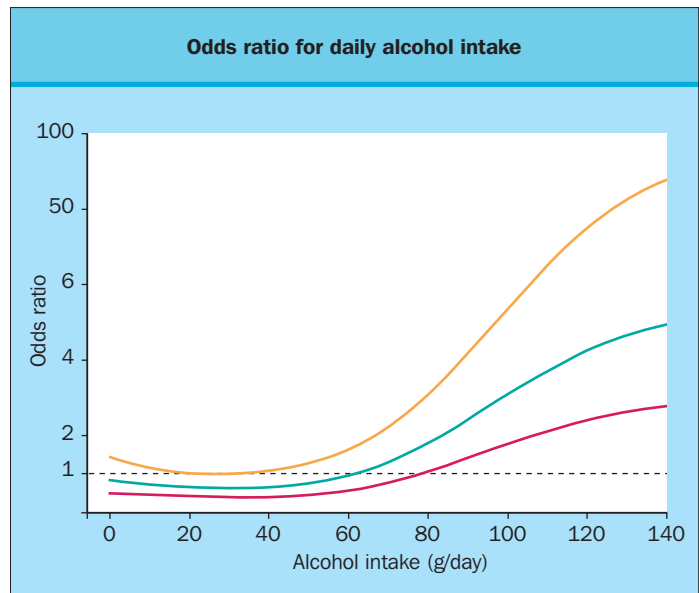
Risk for HCC is approximately fivefold higher in patients drinking more than 80 g/day as compared with abstinent patients (Fig. 21.4). Approximately 1% of males with decompensated (Pugh B or C) alcoholic cirrhosis develop HCC per year. As compared with the risk of HCC in actively drinking patients, the risk is increased four- to fivefold in patients who are abstinent for 1–10 years but returns to normal risk after patients have been abstinent for more than 10 years. Approximately 15% of HCC in alcoholics occur in non-cirrhotic livers. The diagnosis of HCC in alcoholic cirrhosis is similar to that for other types of cirrhosis except that serum  $\alpha$ -fetoprotein level is often normal or minimally elevated.

### Alcohol and hepatitis C

The combination of alcohol use and hepatitis C is exceedingly common. The prevalence of hepatitis C is 7–10-fold higher in alcoholics than in the general population, and up to 60% of patients with hepatitis C have a history of significant alcohol use. The prevalence of hepatitis C increases with more advanced liver disease, with 20–40% of patients with alcoholic cirrhosis



**Figure 21.3 Hospitalization rates for hepatocellular carcinoma (HCC).** Hospitalizations for HCC per 100 000 hospitalizations at VA Medical Centers in the USA, 1996–1998. Modified from El Serag et al. (00).



**Figure 21.4 Odds ratio for hepatocellular carcinoma (HCC) according to lifetime daily alcohol intake.** Odds ratio and 95% confidence intervals (dotted lines) for HCC in men living in Bresica, Italy, according to lifetime daily alcohol intake and adjusting for age, residence, HBsAg, hepatitis C. Reproduced from Donato et al (02) by permission of Oxford University Press.

having hepatitis C and 40–80% of alcoholic patients with HCC being infected with hepatitis C. Chronic alcohol use in patients with hepatitis C increases the risk of mortality from liver disease.

Patients with hepatitis C who drink more than five drinks per day (50 g of alcohol) have higher viral load and faster progression of fibrosis (Table 21.6). The effects of smaller amounts of alcohol use on fibrosis progression rate and hepatitis C viral load are unknown although it is unlikely that one drink per day is harmful. A prior history of heavy alcohol use decreases response to interferon monotherapy. Patients should not drink alcohol when they are receiving peginterferon and ribavirin.

### Odds ratio for the development of cirrhosis in patients without or with hepatitis C virus (HCV) categorized by the lifetime daily alcohol consumption

Lifetime daily alcohol intake (g/day)	Odds ratio	
	HCV negative	HCV positive
<25	1	11
25–50	1	10
75–100	9	25
>125	28	264

Lifetime daily alcohol consumption was determined in 285 patients with decompensated cirrhosis admitted to hospital and in 417 hospitalized control patients. Modified from Corrao et al (98).

**Table 21.6 Odds ratio for the development of cirrhosis in patients without or with hepatitis C virus (HCV) categorized by the lifetime daily alcohol consumption**

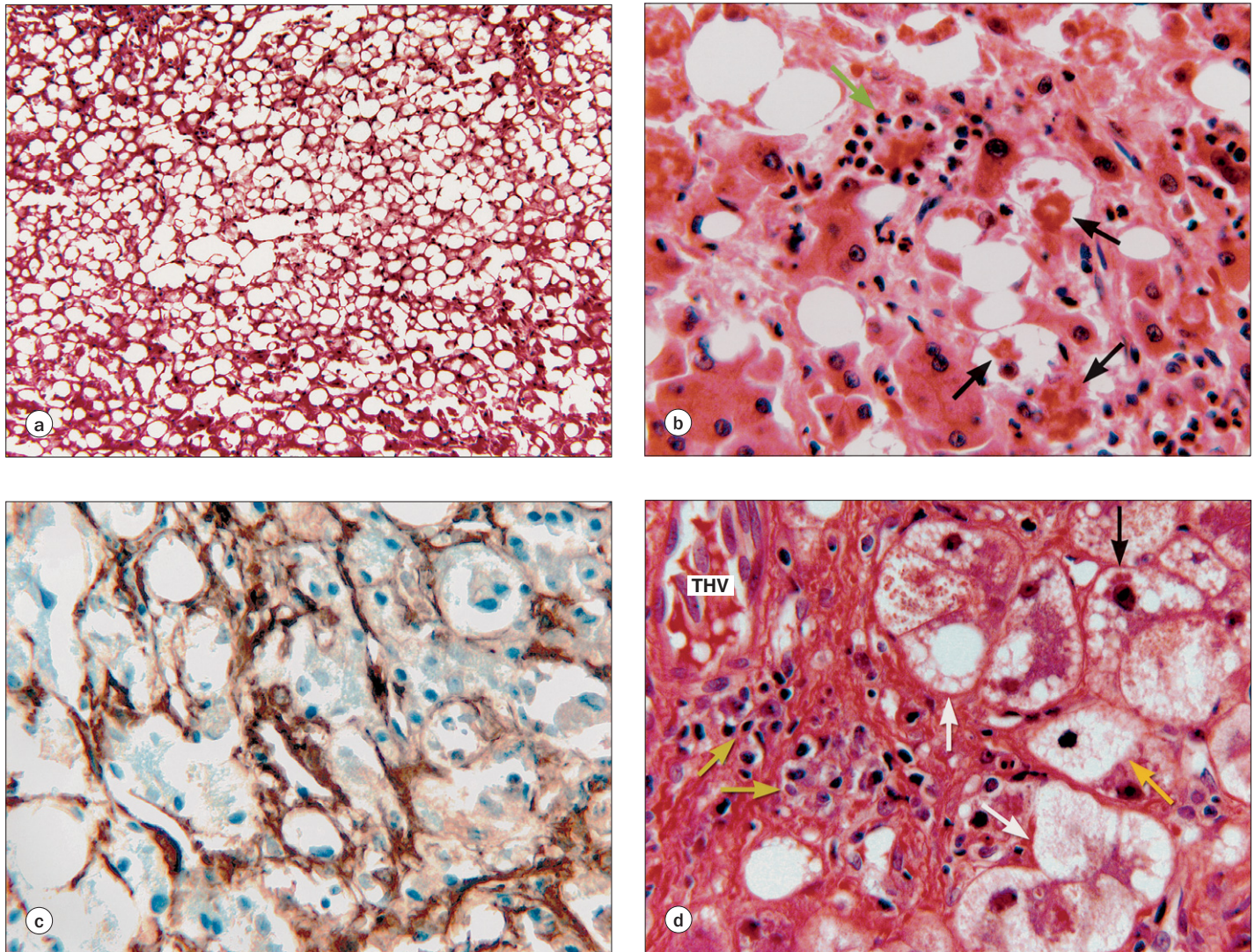
## PATHOLOGY

### Fatty liver

Fatty liver is present when more than 5% of hepatocytes contain fat droplets; in alcoholic fatty liver more than 50% of hepatocytes may contain fat (Fig. 21.5a). The fat is usually a single, non-membrane bound fat droplet that displaces the nucleus to the periphery of the cell. Perivenular hepatocytes (zone 3) are the first to acquire fat, followed by mid-zonal and, in severe cases, periportal hepatocytes. In foamy degeneration, microvesicular fat accumulates in the cytoplasm around a centrally placed nucleus. In both types of fatty liver, enlarged mitochondria (megamitochondria) can be found. Focal areas of inflammation around necrotic fat-filled hepatocytes (lipogranulomas) can be seen, but inflammation is not present elsewhere in the lobule. Small amounts of fibrosis are often present, especially in the centrilobular area.

### Alcoholic hepatitis

The pathologic findings of alcoholic hepatitis are characteristic and include fat, polymorphonuclear and lymphocytic inflammation, necrosis, apoptosis and fibrosis. PMN inflammation is found in the sinusoids and encircling necrotic hepatocytes ('satellitosis') (Fig. 21.5b). Although PMN are the most numerous inflammatory cells, lymphocytes may be present in the lobular inflammation. Necrosis is the predominant form of hepatocyte death. Interestingly, apoptosis is also present, and the degree of apoptosis correlates with patient survival. Perisinusoidal/pericellular fibrosis (i.e. in the space of Disse surrounding hepatocytes) leads to 'capillarization' of the sinusoids, a condition in which the endothelial cells lose their fenestrae and the loose, collagen type IV connective tissue normally found in the space of Disse is replaced by activated stellate cells which stain positive for  $\alpha$  smooth muscle actin (Fig. 21.5c). 'Chickenwire fibrosis' refers to extensive pericellular fibrosis, which on trichrome staining



**Figure 21.5 (a) Alcoholic fatty liver.** Fat is seen in more than 75% of the hepatocytes (hematoxylin and eosin stain, original  $\times 200$ ). **(b) Alcoholic hepatitis with satellitosis.** Polymorphonuclear leukocytes (PMN) surround a necrotic hepatocyte which contained Mallory hyaline (green arrow). There are multiple ballooning hepatocytes containing Mallory hyaline (black arrows) (hematoxylin and eosin, original  $\times 800$ ). **(c) Hepatic stellate cells.** Stain for smooth muscle actin (brown) demonstrates activated hepatic stellate cells in sinusoids surrounding hepatocytes (blue counter stain) in a patient with alcoholic hepatitis (immunostain for  $\alpha$  smooth muscle actin, original  $\times 800$ ). **(d) Alcoholic hepatitis.** Balloon cells (white arrows), megamitochondria (black arrow) and polymorphonuclear leukocytes (PMN) infiltrate in perivenular area (green arrows) are shown. A single cell contains microvesicular fat (yellow arrow). (THV, terminal hepatic vein; hematoxylin and eosin, original  $\times 1600$ ).



reveals blue-staining connective tissue surrounding individual hepatocytes. Fibrosis begins in the perivenular area (centrilobular fibrosis). In severe disease fibrosis can progress to obliteration of the central vein ('central hyaline necrosis') resulting in portal hypertension (and ascites) in the absence of cirrhosis. The portal areas are less involved in the inflammation and fibrosis, although they may have stellate fibrosis (or bridging fibrosis).

Hepatocytes are markedly abnormal in alcoholic hepatitis. Macrovesicular (and/or microvesicular) fat is usually present. Ballooning hepatocytes (large hepatocytes without extensive fat) are present in patients with severe alcoholic hepatitis (Fig. 21.5b, d). Ballooning is caused by an inability of hepatocytes to secrete proteins and ions, with consequent retention of water and swelling. Ballooned cells often contain Mallory bodies, eosinophilic cytoplasmic inclusions named after the Boston pathologist who described them in 1911 (Fig. 21.5b). Mallory bodies are a conglomerate of several intracellular proteins including cytokeratins (positive staining for cytokeratin 8 and 18) and heat shock proteins that accumulate because of a defect in protein degradation by the proteasome pathway. Megamitochondria are present in more than 50% of patients. On hematoxylin and eosin staining they appear as eosinophilic, globular inclusions within the cytoplasm (Fig. 21.5d).

### Fibrosis/cirrhosis

Chronic alcohol use can produce fibrosis without accompanying marked inflammation and necrosis. Three types of fibrosis are recognized in alcoholic liver disease:

- centrilobular fibrosis (vide supra),
- pericellular fibrosis (vide supra), and
- periportal fibrosis.

Periportal fibrosis begins as erosion of the limiting plate with ductular changes in the cells extending from the portal area. The proliferating ductules appear to arise from hepatic stem cells (oval cells) in or near the canal of Hering and stain positive for markers of both hepatocytes ( $\alpha_1$ -antitrypsin) and bile ducts (CK19). The ductule cells are associated with activated stellate cells and collagen, leading to the periportal fibrosis. With time, the fibrosis can extend to adjacent portal tracts or central veins, producing bridging fibrosis.

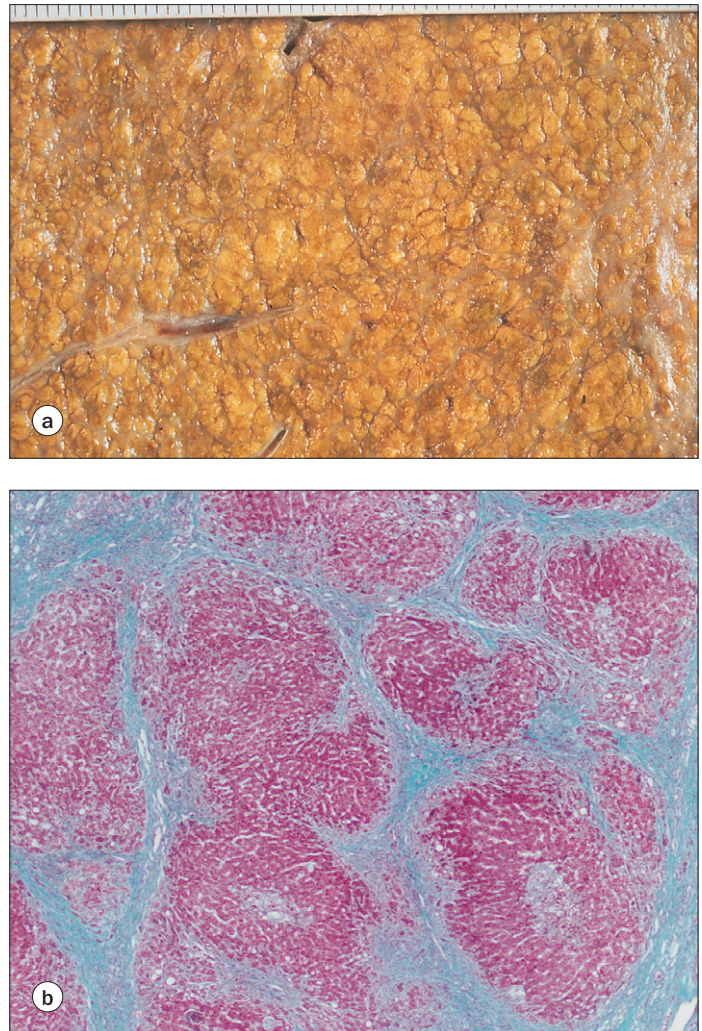
Cirrhosis refers to the disruption of normal liver architecture by scar tissue and the replacement of liver acini by regenerating nodules. In alcoholic cirrhosis, the nodules are usually less than 3 mm in diameter (micronodular) and may be seen on liver biopsy (Fig. 21.6a, b). With abstinence, larger nodules may form, with a resultant mixed micronodular and macronodular cirrhosis. Diagnosis of cirrhosis by liver biopsy may be more difficult in these cases since complete nodules may not be present on needle biopsy specimens.

## NATURAL HISTORY AND TREATMENT

### Alcoholism

Abstinence is essential for the treatment of alcoholic liver disease. Patients with alcoholic hepatitis, fibrosis or cirrhosis will develop progressive liver disease if they return to regular alcohol consumption.

Alcoholism is a chronic disorder in which a decrease in alcohol use is an acceptable alternative if total abstinence is not achievable. Gastroenterologists may achieve a significant beneficial effect



**Figure 21.6 Alcoholic cirrhosis.** (a) Cut surface of the gross liver showing alcoholic cirrhosis which shows multiple, small nodules. (b) Trichrome stain of alcoholic cirrhosis demonstrating broad bands of fibrosis (blue stain) surrounding hepatocytes (trichrome stain, original  $\times 40$ ).

for their patients by following the guidelines of a 'brief intervention' (Table 21.7). These include educating the patient about the risks involved with continued drinking (e.g. risk of progression to cirrhosis and complications of cirrhosis) and providing advice on how to change their drinking pattern. Brief interventions moderate drinking over the following year and are cost effective. Approximately 70% of patients participating in clinical trials of alcoholic liver disease are not drinking at any one time, although over the course of a year most patients will consume some alcohol. These studies suggest that many patients with alcoholic cirrhosis markedly reduce their alcohol consumption for prolonged periods.

Patients who continue to drink alcohol should be referred to an addiction specialist. Naloxone decreases the urge to drink and may be used in selected patients in conjunction with an addiction specialist. Acamprosate, an oral drug that reduces alcohol craving, is available in Europe but has not been approved by the Food and Drug Administration (FDA) for use in the USA.

### Fatty liver

No specific treatment for fatty liver disease is needed other than abstinence. Mortality is rare; with abstinence, fat resolves over



Brief intervention
Brief interventions are short counseling sessions (as short as 5 min) designed to assess a patient's drinking habits and to reduce his drinking habits. Brief interventions may need to be repeated at subsequent clinic visits. Brief interventions have six components.
1. Feedback of personal risk. Apprise the patient of his health risks from drinking. This may include liver damage (alcoholic hepatitis, cirrhosis) and/or abnormal lab tests [elevated aspartate aminotransferase (AST), bilirubin, etc.].
2. Responsibility of the patient. Emphasize the responsibility to change drinking habits rests with the patient. The final decision to stop drinking is up to the patient.
3. Advice to change. Express your professional opinion that the patient is seriously harming their health and your recommendation to change their behavior or they will develop more advanced liver disease.
4. Menu of ways to reduce drinking. Provide the patient with possible strategies to reduce drinking. This may include setting a limit on drinks per day, recognizing the setting in which drinking occurs, pacing the rate of drinking, etc. Encourage patients to set a limit to their drinking. Patients with alcohol dependence (rather than alcohol abuse) must clearly be advised to be abstinent, and not to reduce drinking.
5. Empathetic counseling. A warm and understanding style by the health professional delivering the brief intervention increases the chance of success.
6. Self-efficacy (or optimism of the patient). Encourage patients to rely on their own resources and to be optimistic about their ability to change their drinking behavior.

Table 21.7 Brief intervention.

several weeks. Patients with fatty liver who continue to drink more than 40 drinks per week have a 1% annual risk of developing cirrhosis during the next 10 years. Independent predictors of progressive fibrosis are mixed micro- and macrovesicular fat and megamitochondria on the initial liver biopsy.

Alcoholic hepatitis

The first step in the treatment of alcoholic hepatitis is determination of the severity using the Maddrey Discriminant Function (DF) using the formula:

$$DF = \text{serum bilirubin (mg/dL)} + \{[\text{patient prothrombin time (s)} - \text{control prothrombin time (s)}] \times 4.6\}.$$
 (Table 21.8)

Untreated patients with a DF >32 have a 30-day mortality

Discriminant function
$DF = \text{serum total bilirubin (mg/dL)} + \{[\text{patient prothrombin time (s)} - \text{control prothrombin time (s)}] \times 4.6\}$
A patient with a serum total bilirubin of 14.7 and a prothrombin time of 17.2 (control prothrombin time 12.8) would have a DF of: $14.7 + (4.4 \times 4.6) = 35$
To convert bilirubin in $\mu\text{mol/L}$ to $\text{mg/dL}$ , divide by 17.1

Table 21.8 Discriminate function.

rate of approximately 33% and a 6-month mortality rate of 50%. Patients with a DF <32 have a >90% chance of 6-month survival with routine medical care. With the exception of patients with hepatic encephalopathy, patients with a DF <32 are not candidates for specific treatment for their alcoholic hepatitis.

Despite the lack of survival benefit with nutritional therapy (see below), maintaining adequate caloric and protein intake is essential in these malnourished and often anorectic patients. Patients with alcoholic hepatitis who ingest inadequate amounts of calories have an extremely high mortality rate. Enteral feeding with a small-bore nasogastric tube is reasonable if patients do not spontaneously consume at least 35 kcal/kg/day. Protein intake of at least 1.5 g/kg body weight is recommended. Protein intake rarely precipitates hepatic encephalopathy and should not be reduced unless other measures to control encephalopathy are unsuccessful. Sodium in the diet should be limited to 2 g (88 mEq). Alcoholic patients are often deficient in B vitamins probably due to a combination of poor intake and decreased intestinal absorption. Intravenous multivitamins (especially thiamine) should be given for the first few days. Thiamine should be given prior to administration of intravenous glucose to prevent the further depletion of thiamine and precipitation of Wernicke encephalopathy. Oral multivitamins (without iron) should be continued daily while the patient is hospitalized, and for several months thereafter.

Up to 25% of patients with severe alcoholic hepatitis are infected on admission to hospital, with up to 40% having infection during their hospital stay. Routine culturing is recommended. Some experts recommend treatment with a broad-spectrum antibiotic such as cefotaxime 1 g t.i.d. until the results of the cultures are known. There is no data to support the continued use of antibiotics in patients in whom the cultures are negative. The peripheral WBC count is often increased without infection, but a rising WBC count necessitates frequent reculturing to ensure that the patient has not developed an infection.

Blood tests for liver function, renal function, electrolytes, calcium, magnesium, phosphorus, complete blood count (CBC) with differential, and prothrombin time should be obtained several times per week during hospitalization. Urine sodium and creatinine, either as a spot measurement or, preferably, as a 24-h determination, is useful to determine the patient's degree of sodium retention. Nutritional status (i.e. malnutrition) can be estimated from the creatinine height index or mid-arm muscle circumference. Deficiencies in potassium, magnesium and phosphorus can be replaced orally in most patients. Most patients require intravenous administration of fluids (or antibiotics). Some experts prefer a central venous catheter that can also be used to exclude intravascular volume depletion. Diuretics (e.g. spironolactone and furosemide) can be given to induce a diuresis provided the serum creatinine is monitored closely to prevent excessive intravascular volume depletion. Some experts are concerned about the possibility of inducing hepatorenal syndrome and prefer to manage ascites with dietary sodium restriction and paracentesis (with replacement of ascites removed with intravenous albumin).

Treatments aimed at interrupting pathophysiologic pathways have been attempted in alcoholic hepatitis, but few treatments are accepted as beneficial, and none is approved by the FDA. Nutritional support to maintain adequate caloric and protein intake is widely recommended. Prednisolone treatment is

Treatment of patients hospitalized with alcoholic hepatitis		
<b>Accepted treatment</b>		
Corticosteroids	Prednisolone 40 mg p.o. QAM, 28 days	Indicated if DF >32 or hepatic encephalopathy Contraindicated if sepsis, bleeding, renal failure
Nutritional supplementation	Oral or enteral Calories: 35 kcal/kg/day Protein: 1.5 g/kg/day Sodium: 2 g/day (88 mEq)	Does not improve survival Unlikely to worsen hepatic encephalopathy
<b>Possibly effective treatment</b>		
Anticytokine	Pentoxifylline 400 mg p.o. t.i.d.	Single study Reduced hepatorenal syndrome
<b>Not accepted treatment</b>		
Anti-TNF	Infliximab Etanercept	Possibly effective in small, uncontrolled trials Increased mortality with infliximab in RCT
Anabolic steroids	Oxandralone Testosterone	May improve nutritional status, does not improve survival
Anti-thyroid	Propylthiouracil	Does not improve survival
Antioxidants	Vitamins, selenium, N-acetylcysteine, etc.	Does not improve survival
Hepatic mitogens	Insulin and glucagon	Several deaths from hypoglycemia
Other treatment	Colchicine, verapamil, penicillamine	Do not improve survival
QAM; quality addiction management; RCT, randomized controlled trial; TNF, tumor necrosis factor		

**Table 21.9 Treatment of patients hospitalized with alcoholic hepatitis.**

recommended by the American College of Gastroenterology in patients with severe alcoholic hepatitis (DF >32). Specific treatments are reviewed below (Table 21.9).

### Corticosteroids

Corticosteroids inhibit the inflammatory response, in part by inhibiting activation of NF- $\kappa$ B, a transcription factor that activates TNF- $\alpha$  and other proinflammatory cytokines. Corticosteroids have been evaluated in at least 17 clinical trials enrolling more than 900 patients. Two randomized controlled trials published in the 1990s, one from France and the other from the USA, demonstrated improved 1-month survival with corticosteroid treatment. These authors combined their primary data with data from a third, negative study, to evaluate survival in a total of 215 patients (102 placebo and 113 corticosteroid). Survival at day 28 was 85% in patients receiving corticosteroids and 65% in placebo patients ( $p < 0.001$ ). Survival was significantly higher in the corticosteroid treated group at 6 and 12 months, but not at 24 months. Patients with a decrease in serum total bilirubin 7 days after starting corticosteroid treatment have an 82% survival at 6 months compared with a 23% survival in patients whose total bilirubin increases during the first 7 days of corticosteroid treatment. The latter group, which represents 28% of patients, and which cannot be determined from pretreatment assessment, is the group of patients in whom other forms of treatment are needed.

The recommended treatment is prednisolone 40 mg orally every morning (or methylprednisolone 32 mg/day intravenously in patients unable to tolerate oral medications). Treatment is restricted to patients with a DF >32 or those with overt hepatic

encephalopathy (e.g. asterixis). It is important to exclude infection prior to starting treatment, during treatment, and after treatment has stopped. Despite the theoretical risk for infection, only one study reported increased mortality from infection, and that occurred in the 11 months after corticosteroids were stopped. It seems reasonable to start corticosteroids if patients have received adequate antibiotic treatment for at least 48 h. Recent (past 2 weeks) upper gastrointestinal bleeding is commonly listed as a contraindication to starting corticosteroid treatment based on the (now incorrect) belief that corticosteroids cause duodenal ulcers. In practice corticosteroids may be started when patients have not bled for at least 48 h. Corticosteroids are not recommended if the serum creatinine is >2.0 mg/dL since these patients are likely to die from hepatorenal syndrome, a condition that is not improved by corticosteroid use.

Despite these recommendations, there continues to be controversy about the use of corticosteroids in alcoholic hepatitis. Only 7 of the 17 studies were randomized controlled trials that were evaluated on an intention to treat basis. In these seven studies, the relative risk of death with steroids was 1.06 (95% confidence interval of 0.87–1.31). The clinical trials have been the subject of three meta-analyses, two of which found corticosteroid treatment to be beneficial while the third meta-analysis performed using a different approach, found corticosteroids to not be beneficial. In addition, there may be a publication bias towards studies with positive results, especially trials with a small number of patients. Finally, one of the concerns with corticosteroids is the exclusion of patients with infection, upper gastrointestinal bleeding, or renal insufficiency.

Up to 40% of patients with alcoholic hepatitis might be excluded from treatment using these criteria. Additional studies with corticosteroids would be desirable, but are unlikely to be performed.

### **Pentoxifylline**

Pentoxifylline (PTX) is an oral phosphodiesterase inhibitor which decreases expression of TNF- $\alpha$  (and other proinflammatory cytokines) and which may inhibit apoptosis. Based on its anti-cytokine activity, a placebo controlled trial was undertaken in 103 patients with alcoholic hepatitis and a DF >32. Pentoxifylline 400 mg p.o. t.i.d. for 28 days decreased mortality during the index hospitalization from 46.1% in the placebo patients to 24.5% in the PTX group ( $p < 0.05$ ). The reduction in mortality was due to a decrease in the number of patients dying with hepatorenal syndrome. No significant side-effects were reported in patients receiving PTX. This study needs to be confirmed, preferably in comparison with corticosteroids, before use of PTX can be recommended in patients with alcoholic hepatitis. Nevertheless, experts frequently use PTX, especially if there are contraindications to corticosteroid use since PTX has minimal side-effects and few other treatments are available.

### **Nutritional therapy**

The universal presence of malnutrition, as well as the prior belief that alcoholic liver disease was a consequence of malnutrition rather than alcohol, prompted multiple studies of nutritional therapy for alcoholic hepatitis. The earliest studies, performed more than 20 years ago, involved parenteral feeding and demonstrated an improvement in nitrogen balance or serum albumin, but no improvement in survival. Five randomized controlled trials of oral/enteral feeding have confirmed improvement in nutritional status but have failed to show improved survival. A recent trial suggests enteral feeding decreases mortality from infections. This study compared corticosteroid treatment (with 2000 kcal standard hospital diet) to enteral feeding with a 2000 kcal diet (without corticosteroid treatment) in 71 patients with DF >32. Mortality at 28 days was similar in the corticosteroid group (25%) and in the enteral feeding group (31%). Mortality during the next 11 months was higher in the corticosteroid group (37%) than in the enteral feeding group (8%) primarily due to fewer infections in patients receiving enteral feeding. Overall survival at 12 months was similar in both groups (39% in corticosteroid versus 62% in enteral feeding,  $p = 0.26$ ). Although this study suggests that enteral feeding may be superior to corticosteroid treatment, especially after the first month of treatment, the overall survival in the corticosteroid group (39% at 12 months) was considerably worse than the survival rate in other randomized controlled studies with corticosteroids. Overall, enteral nutrition during the initial hospitalization with severe alcoholic hepatitis improves markers of nutritional status but has not been shown convincingly to improve survival.

### **Anti-tumor necrosis factor**

TNF- $\alpha$  plasma levels are increased in patients with alcoholic hepatitis and correlate with disease severity. TNF- $\alpha$  stimulates the proinflammatory cytokines IL-6 and the neutrophil chemotactic cytokine IL-8. In animal studies, knocking out the TNF receptor, or administration of TNF antibodies, reduces

alcoholic liver damage. Given the effectiveness of anti-TNF treatment of rheumatoid arthritis and Crohn disease, these agents have been evaluated in several small studies in patients with alcoholic hepatitis. Two small, non-randomized studies of infliximab (chimeric human-mouse monoclonal antibody against TNF- $\alpha$ ) treatment and one study of Etanercept (a TNF receptor: Fc fusion protein that binds to TNF- $\alpha$ ) reported no significant adverse events with anti-TNF treatment. Survival could not be evaluated since the studies were not randomized. There has been one prospective, randomized, controlled trial of anti-TNF treatment in alcoholic hepatitis. Patients with a DF >32 received treatment with prednisolone for 28 days. Patients were randomized with respect to additional treatment with infliximab (10 mg/kg at day 0, 14 and 28) or placebo. The study was discontinued prematurely because of increased mortality in patients receiving infliximab (39%) as compared with patients receiving placebo (18%) at 2 months ( $p =$  not significant). Furthermore, the rate of serious bacterial infections was significantly higher in patients receiving infliximab (13 patients) than in placebo patients (three patients,  $p < 0.002$ ). Further studies of these agents are planned; their use is not recommended outside of clinical trials.

### **Anabolic steroids**

Anabolic steroids improve nitrogen balance and were studied because of the universal malnutrition in patients with severe alcoholic hepatitis. In the largest study to date, 273 patients were randomized with respect to treatment for 3 months with both oxandralone and a branched chain amino acid supplement or placebo (oxandralone and a low-calorie, low-protein food supplement). Overall survival was similar at 28 days, although survival was higher in patients receiving oxandralone after 6 months of follow-up. Subsequent analysis suggested the improved survival was due to better nutritional intake in patients receiving oxandralone, rather than to the effects of oxandralone itself. Anabolic steroids have not been compared with corticosteroid treatment. A Cochrane review did not demonstrate significant beneficial outcome with anabolic steroid use in patients with alcoholic liver disease. Anabolic steroids are not currently used to treat alcoholic hepatitis.

### **Propylthiouracil**

Alcohol induces a hypermetabolic state in the liver similar to the hypermetabolic state found in hyperthyroidism. Propylthiouracil (PTU) has been used as a treatment for alcoholic hepatitis based on its ability to reduce hepatic oxygen consumption. Several randomized, placebo-controlled clinical trials have evaluated PTU treatment in hospitalized patients, although patients were not selected on the basis of their DF score. In one study PTU treatment led to a more rapid improvement in liver tests, but no study demonstrated an improvement in survival with PTU. Based on these negative studies, and rare hepatotoxicity, PTU is not recommended for alcoholic hepatitis.

### **Antioxidants**

There is increased oxidative stress and reduced levels of several antioxidants in alcoholic hepatitis. A clinical trial compared corticosteroids to an antioxidant cocktail (vitamins A, C, E, selenium, allopurinol, desferrioxamine and *N*-acetylcysteine) in



101 hospitalized patients with alcoholic hepatitis. The study was terminated prematurely because of increased mortality in patients receiving antioxidant treatment. A second study evaluated antioxidant treatment (*N*-acetylcysteine intravenously for 1 week, followed by vitamins A–E, biotin, selenium, zinc, manganese, copper, magnesium folic acid and Coenzyme Q daily for 6 months) in patients with advanced alcoholic hepatitis. Corticosteroids were used if prescribed by the attending gastroenterologist. Antioxidant treatment showed no benefit and did not improve 6-month survival, either alone or in combination with corticosteroid treatment. Based on these studies, currently available antioxidant treatments do not improve survival and are not recommended in alcoholic hepatitis.

### Hepatic mitogens

Insulin and glucagon stimulate hepatic regeneration following partial hepatectomy in rats and improved survival in a mouse model of fulminant hepatitis. In an effort to stimulate hepatic regeneration, five randomized controlled trials have evaluated intravenous insulin and glucagon treatment in alcoholic hepatitis. Two studies reported improved short-term survival while three found no difference in survival. Hypoglycemia occurred in several patients receiving insulin and glucagon, with two deaths due to hypoglycemia. Insulin and glucagon are not recommended for patients with alcoholic hepatitis.

### Other treatments

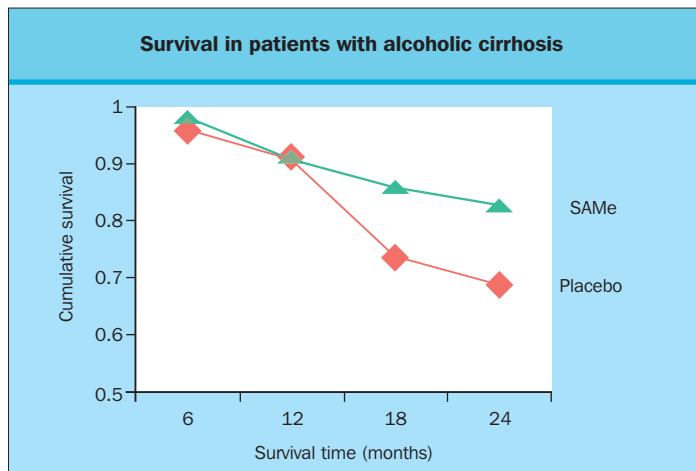
Colchicine, penicillamine and the calcium channel blocker, verapamil, have been used but are ineffective in alcoholic hepatitis.

### Cirrhosis

The natural history of cirrhosis is influenced by abstinence and the severity of the liver disease. Five-year survival is greater than 90% in non-decompensated patients who are abstinent but drops to less than 70% if patients continue to consume alcohol. In various studies, mean survival after the development of a complication of cirrhosis varies from 24 to 48 months. Established alcoholic cirrhosis is rarely, if ever, reversible to a state of minimal liver fibrosis, even if patients are abstinent for many years.

Several treatments have been evaluated in patients with alcoholic cirrhosis, although none is recommended for routine use at the current time.

S-adenosylmethionine (SAME) is a high-energy compound formed from methionine and ATP by the enzyme methionine adenosyltransferase (MAT). SAME is the primary methyl donor for biochemical reactions involving DNA methylation, and is a precursor of glutathione, a major intracellular antioxidant. Hepatic levels of MAT are decreased in cirrhosis, probably due to oxidative damage, leading to decreased SAME synthesis. Because of the known abnormalities in SAME metabolism, oral SAME has been studied as a treatment for alcoholic cirrhosis. A total of 123 patients with alcoholic cirrhosis (25% of whom also had viral hepatitis) were entered into a randomized controlled trial of SAME 1200 mg/day versus placebo. Survival at 24 months was higher in patients receiving SAME, although this did not reach statistical significance unless the eight patients with Pugh C cirrhosis were excluded from analysis (88% versus 71%,  $p < 0.025$ ) (Fig. 21.7). Side-effects of SAME treatment were minimal (nausea, diarrhea). SAME is moderately expensive



**Figure 21.7 Survival in patients with alcoholic cirrhosis.** Patients received either placebo or S-adenosylmethionine (SAME) 1200 mg/day orally for 24 months. Modified from Mato et al (99).

(approximately \$0.50 to 1.00/400 mg tablet) and would have to be taken for at least 2 years in order to obtain survival benefit. Although there are few side-effects of SAME treatment, it is premature to recommend SAME treatment until a confirmatory study has been conducted.

Colchicine treatment prolonged survival and reversed cirrhosis in a study of 100 patients with cirrhosis of various etiologies (45% had alcoholic cirrhosis) conducted in the 1970s and 1980s. However, a recent randomized, controlled trial of 549 VA (Veterans' Affairs) patients with Pugh B or C alcoholic cirrhosis found no improvement in survival with colchicine treatment. A Cochrane review conducted prior to the recent VA study did not recommend colchicine use in patients with alcoholic liver disease. Based on the negative results of the large VA clinical trial, colchicine treatment cannot be recommended for alcoholic cirrhosis.

Phosphatidylcholine (PPC), an extract of soy beans containing high amounts of dilinoleoyl phosphatidylcholine, prevented liver disease in alcohol-fed baboons and stimulates collagenase activity in isolated stellate cells. A placebo-controlled trial of 789 patients with alcoholic fibrosis, but not cirrhosis, was undertaken to determine whether oral PPC improved liver histology in humans with alcoholic fibrosis. In the 412 patients who had a repeat liver biopsy after 24 months of treatment, PPC was not more effective than the placebo in reducing the progression of liver disease. Thus, PPC treatment has not been shown to be effective in preventing progression of fibrosis in humans with alcoholic liver disease although the clinical trial was limited by the large number of dropouts and the low alcohol consumption in patients remaining in the study.

Nutritional therapy may be beneficial in hospitalized patients with alcoholic cirrhosis. A randomized, controlled trial compared an enteral diet containing 2115 kcal with an isocaloric hospital diet in 35 patients hospitalized with cirrhosis. The enteral diet contained branched-chain amino acids, medium-chain triglycerides (with adequate amounts of long-chain triglycerides) and maltodextrin. The in-hospital mortality rate was significantly lower in patients receiving enteral nutrition (12%) than in patients receiving standard hospital diet (47%). However, several other studies have failed to show a survival

benefit with nutritional therapy in patients hospitalized with cirrhosis, although nutritional therapy did improve nutritional status in several of these studies. At the present time routine enteral feeding is not recommended in patients hospitalized with cirrhosis.

Nutritional therapy of outpatients with alcoholic cirrhosis may reduce complications and hospitalizations. Two randomized, controlled clinical trials in patients with cirrhosis of various etiologies reported fewer complications of liver disease in patients receiving nutritional supplementation in addition to a standard diet. Similar to the findings of nutritional treatment in alcoholic hepatitis, mortality was not improved with dietary supplementation of outpatients with cirrhosis. A late evening snack has a positive effect on nitrogen balance on patients with cirrhosis when compared with an equicaloric diet without a late-evening meal. These studies suggest that oral nutritional support, when added to a regular diet, may improve nutritional status and reduce complications of liver disease in outpatients with advanced alcoholic cirrhosis. Due to differences in study design and diet used, no recommendations can be made regarding routine nutritional supplementation for outpatients with alcoholic cirrhosis. Further studies are needed to determine which nutritional supplementation is ideal and whether nutritional therapy is cost effective and improves survival.

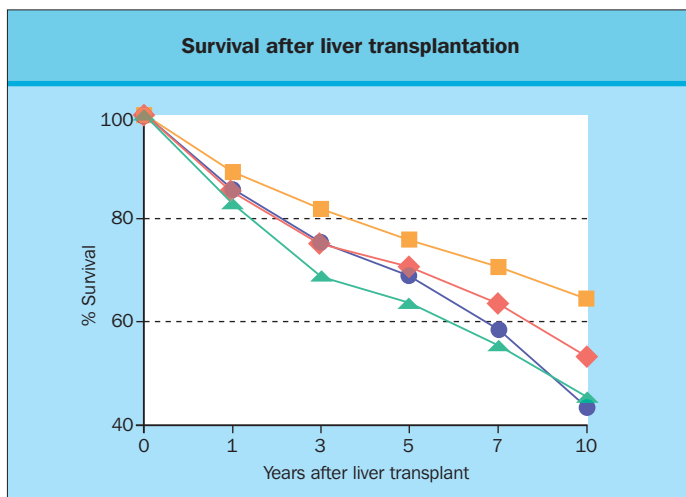
A randomized, placebo controlled trial compared propylthiouracil (PTU) treatment with placebo in 310 outpatients with alcoholic liver disease. Overall, PTU reduced 2-year mortality from 25 to 13% ( $p < 0.05$ ). In patients with more advanced liver disease, mortality declined from 55 to 25% with PTU treatment. PTU was not effective if patients continued to drink alcohol. Despite this positive study, PTU has not gained acceptance as a treatment for alcoholic liver disease, and is not currently used by the authors of this study. A Cochrane review of six randomized controlled trials failed to demonstrate an improvement in survival, liver-related mortality, complications of liver disease, or liver histology with PTU treatment.

Silymarin is an antioxidant derived from milk thistle and is a commonly used complementary and alternative medicine in the USA. Two long-term studies of silymarin have been performed in Europe. The first study randomized 140 patients with cirrhosis with respect to treatment with placebo or silymarin (140 mg three times/day) for 24 months, with continued follow-up for up to 6 years. Silymarin improved survival in the subgroup of patients with Pugh A cirrhosis although mortality in the placebo patients (50%) was much higher than would be expected in patients with Pugh A cirrhosis. Survival was not improved in a subsequent 5-year study of silymarin (150 mg t.i.d.) of 200 patients with alcoholic cirrhosis.

Treatment of ascites, esophageal varices, spontaneous bacterial peritonitis and hepatorenal syndrome associated with alcoholic liver disease can be found in other chapters.

## LIVER TRANSPLANTATION

Liver transplantation is now widely accepted as treatment for end-stage liver disease due to alcohol and alcoholic liver disease is the second most common indication for liver transplantation in adults in the USA, and the most common indication for liver transplantation in Europe. All transplant centers require a 6-month period of pretransplant sobriety, usually with participation in a substance abuse program and random testing for alcohol use. Indications for liver transplant are the same in alcoholic liver disease as in other liver diseases. Most transplant centers do not require additional cardiac evaluation to exclude alcoholic cardiomyopathy unless patients have clinical signs of heart failure. Several studies have shown that the 5–10 year survival of patients transplanted for alcoholic liver disease is similar, if not slightly better, than the survival for patients transplanted for hepatitis C (Fig. 21.8). The rate of recidivism after liver transplant is up to 30% at 5 years post-transplant, but alcohol leads to graft dysfunction in less than 15% of patients and to mortality in less than 5%. Nevertheless, post-transplant care by a specialist in addiction as well as strict abstinence is recommended in all patients receiving a liver transplant for alcoholic liver disease. At the current time, acute alcoholic hepatitis is not an indication for liver transplant because of the high risk of recidivism.



**Figure 21.8 Survival after liver transplantation.** Long-term survival for patients receiving liver transplant for alcoholic cirrhosis (blue diamonds) is similar to survival for patients receiving transplant for hepatitis C (green squares) or for hepatitis B (yellow triangles). Survival is best for patients receiving transplant for cholestatic liver disease/autoimmune liver disease (pink X). Modified from Jain et al (03).

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 22

## Fatty Liver, Non-alcoholic Steatohepatitis

Brent A. Neuschwander-Tetri

The accumulation of fat in hepatocytes, or hepatic steatosis, is a common histologic finding. As specific causes of liver disease have been identified and characterized, more attention has focused on the causes and consequences of non-alcoholic fatty liver disease (NAFLD), and especially non-alcoholic steatohepatitis (NASH), because these syndromes remain relatively enigmatic.

This chapter reviews the current knowledge about NAFLD and its worrisome subset, NASH. Most patients with NAFLD have insulin resistance as the primary cause of triglyceride accumulation in hepatocytes. Because the inciting factors that cause some patients to develop NASH in the setting of NAFLD are not presently known, the cumulative clinical knowledge is necessarily descriptive. As specific causes of these disorders such as insulin resistance are identified, we will begin to recognize NAFLD and NASH as histologic manifestations of specific metabolic defects, nutritional deficiencies, or possibly even unrecognized infections.

### EPIDEMIOLOGY

#### Benign steatosis

Body habitus is the primary predictor of the presence of benign hepatic steatosis. Whereas hepatic steatosis is found in 21% of lean healthy males, almost all morbidly obese individuals will have hepatic steatosis. In the less obese, including those with body mass higher than 10% above lean body weight, about 75% have some degree of hepatic steatosis. How fat is distributed on the body predicts the presence of hepatic steatosis. Analogous to coronary artery disease risk, body fat distributed about the hips is probably not a risk factor; however, a high ratio of abdominal fat to hip fat is a significant risk factor for hepatic steatosis. Youth is not protective; hepatic steatosis is found in obese children and just as frequently as in adults.

#### Non-alcoholic steatohepatitis

The risks factors for developing NASH are similar to the risks for benign steatosis. Because little is known of the underlying causes of NASH, efforts have been directed towards characterizing the recognizable risk factors for developing NASH. Although patients with NASH were once characterized as being obese, diabetic, female, and hyperlipidemic, not all patients fit this description. In fact, recent surveys have underscored the relatively high prevalence of NASH in patients without these 'classic' risk factors (Table 22.1).

#### Obesity

Obesity is clearly the most significant risk factor for the development of NASH. The overall prevalence of NASH in obese patients has been estimated in both surgical series and autopsy studies. *Lobular hepatitis* (which in retrospect probably rep-

Risk factors for hepatic steatosis and non-alcoholic steatohepatitis			
	<i>Steatosis</i>	<i>NASH</i>	<i>Cirrhosis</i>
Obesity (centripetal)	+++	++	++
Type 2 diabetes	++	+	+
Drugs	++	++	+/-
Female gender	+	-	-
Hypertriglyceridemia	+/-	+/-	-
Hypercholesterolemia	-	-	-
NASH, non-alcoholic steatohepatitis; +, minor risk factor; ++, moderate risk factor; +++, major risk factor; -, no risk			

**Table 22.1 Risk factors for hepatic steatosis and non-alcoholic steatohepatitis**

resents NASH) was found in 8.7% of patients in a large series of morbidly obese individuals about to undergo surgical small bowel bypass as a treatment for obesity; however, at autopsy, NASH was found in 18.5% of obese patients. Careful exclusion of coexisting risks (e.g. alcohol use, diabetes, protein malnutrition and drug toxicity) has confirmed that obesity alone is a major risk for the development of this syndrome.

#### Diabetes

Diabetes mellitus is also frequently associated with hepatic steatosis. Steatosis is found in about one-third of non-obese persons with type 2 diabetes at autopsy. As a group, patients found to have hepatic steatosis by ultrasound examination are more likely to have glucose intolerance and elevated baseline insulin levels than patients without ultrasonographic evidence of hepatic steatosis. Chronically elevated circulating insulin levels typically found in type 2 diabetic patients may be at least partly responsible for the accumulation of fat in the liver because of insulin's role in regulating intrahepatic fat synthesis and metabolism. In contrast, hepatic steatosis is relatively uncommon in type 1 diabetes, a condition characterized by primary loss of insulin-secreting  $\beta$  cells in pancreatic islets and manifested by episodes of hyperglycemia and low to normal insulin levels. When steatosis is present in patients with type 1 diabetes, it seems to correlate with poor glycemic control.

#### Hyperlipidemia and lipodystrophies

Hyperlipidemia, a condition that broadly includes elevations of serum cholesterol, serum triglycerides, or both, has been thought

to be a risk factor or clinical marker for the development of hepatic steatosis and NASH. In reality, this association lacks solid epidemiologic evidence because establishing hypertriglyceridemia as a risk for the development of NASH has been confounded by the dominant effects of obesity and dietary habits. Nonetheless, genetic disorders of serum lipid metabolism are often characterized by hepatic steatosis. Additionally, the lipodystrophies are definitely associated with the development of NASH and the progression to end-stage liver disease. These rare diseases are characterized by the abnormal mobilization of fat from peripheral stores, which greatly increases the flux of fatty acids through the liver. Whether the liver demonstrates abnormal fat metabolism in the lipodystrophies or is a passive target of the unusually high traffic of fatty acids is not known.

Gender

Of the risk factors for NASH identified in early surveys, female gender was overemphasized and has been refuted. The prevalence of NASH is equal among men and women at autopsy and the prevalence of hepatic steatosis found by computed tomography (CT) imaging is equal among men and women. Recent surveys of patients with NASH have accordingly demonstrated an equal gender distribution.

Drugs

Several drugs have been implicated in the development of benign steatosis and NASH (Table 22.2). The most common drugs implicated have been corticosteroids, estrogens and tamoxifen. Estrogens increase hepatic triglyceride synthesis, possibly outpacing the secretory capacity. Seemingly paradoxical

is the association between the use of tamoxifen and the development of NASH. Well-characterized cases have been described in women using it as an estrogen antagonist and as adjuvant therapy for breast cancer. This is explained by the observation that, although tamoxifen is used as an estrogen receptor antagonist, it can display weak estrogenic activity depending on the target tissue. This activity may be responsible for its association with NASH.

PATHOPHYSIOLOGY

Hepatic steatosis is caused by an imbalance between the delivery or synthesis of fat in the liver and its subsequent secretion or metabolism. In other words, fat accumulates when the delivery of fatty acids to the liver, either from the circulation or by de novo synthesis within the liver, exceeds that capacity of the liver to metabolize the fat by  $\beta$ -oxidation or secrete it as very low-density lipoprotein (VLDL). These are the two major mechanisms of fat delivery and two major mechanisms of fat disposal. Derangements in any of these pathways alone or in combination cause fat to accumulate in the liver and are discussed later in detail. Figure 22.1 shows how fat cycles between the liver and peripheral stores and the influence of major regulatory factors in governing the flux between the liver and the peripheral sites.

Delivery of fatty acids from peripheral stores to the liver

Triglycerides, or triacylglycerol (TAG), are stored in adipose tissue and released as free fatty acids into the circulation through the actions of lipoprotein lipases. The hydrolysis of TAG in adipocytes is controlled by intracellular cyclic adenosine monophosphate (cAMP) levels. Therefore, hormonal and pharmacologic stimuli that increase adipocyte cAMP cause the release of free fatty acids from peripheral stores and increase the delivery of fat to the liver. Epinephrine, norepinephrine, adrenocorticotrophic hormone (ACTH), glucagon, corticosteroids, and methylxanthines (e.g. caffeine) are agents that stimulate adenylyl cyclase and potentially increase adipocyte lipolysis via a hormone-sensitive lipase. The increased lipolysis brought about by corticosteroids may reflect a protective response to prolonged starvation.

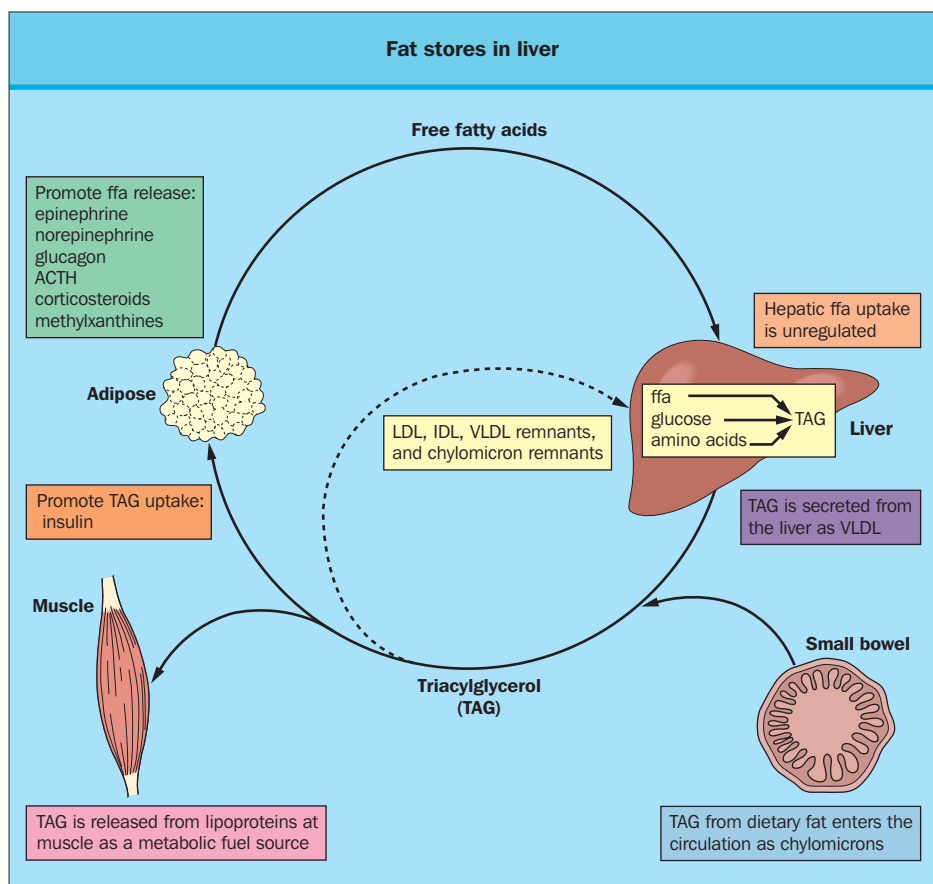
In the fed state, insulin exerts the opposite effect by increasing phosphodiesterase activity, thus depleting intracellular cAMP and preventing peripheral lipolysis. Insulin is also required for the reincorporation of fatty acids into triglyceride in both the liver and peripheral stores. Thus, after feeding, fat release from peripheral stores is normally inhibited and fat release from the liver is promoted. In states of insulin resistance, the normal post-prandial inhibition of lipolysis can be impaired leading to inappropriate release of free fatty acids from adipose tissues in the fed state.

Free fatty acids released from peripheral stores are hydrophobic and are strongly bound to circulating albumin. They are transported by albumin to tissues capable of taking up fatty acids and using them as metabolic substrates. The liver is the major site of fatty acid uptake, but the heart and skeletal muscle are also highly dependent on free fatty acids as a metabolic fuel and thus remove a fraction from the circulation.

Whereas the cAMP-mediated release of free fatty acids from the peripheral stores is highly regulated, the uptake of fatty

Causes of macrovesicular steatosis
Insulin resistance and hyperinsulinemia Obesity (especially centripetal) Type 2 diabetes
Drugs Corticosteroids Estrogens Tamoxifen (acting as a weak estrogen agonist) Amiodarone Chloroquine Nifedipine and diltiazem (case reports)
Metabolic Wilson disease
Nutritional Starvation Protein deficit (kwashiorkor, eating disorders) Choline deficiency Excess carbohydrate
Infections Chronic hepatitis C (especially genotype 3)
Miscellaneous Indian childhood cirrhosis Jejunioileal bypass

Table 22.2 Causes of macrovesicular steatosis



**Figure 22.1 Fat stores in the liver.** Fat cycles from peripheral stores to the liver as free fatty acids (ffa) bound to albumin and back to peripheral stores as triacylglycerol (TAG) as a component of very low-density lipoprotein (VLDL). TAG is also synthesized within the liver from excess glucose and amino acids and a small fraction of the total is taken up by hepatocytes in the form of lipoprotein remnants. Dietary fat is converted to free fatty acids within the bowel lumen. Whereas short chain fatty acids (<11 carbons) can be delivered directly to the blood, longer chain fatty acids are re-esterified into TAG, incorporated into chylomicrons, and delivered to the circulation via the lymphatics. Fatty acids are removed from the TAG in circulating VLDL and chylomicrons by vascular lipoprotein lipases. In energy-requiring tissues such as muscle, the released fatty acids serve as a source of fuel whereas adipose tissue resynthesizes TAG for storage.

The flux of fat through this cycle is highly dependent on the feeding state. During fasting, glucagon facilitates the release of TAG from peripheral fat stores. After a meal, insulin promotes the incorporation of fat into adipose tissue by locally upregulating vascular lipoprotein lipase activity. Insulin also promotes the formation of TAG from glucose within the liver and switches the trafficking of fatty acids from  $\beta$ -oxidation to TAG formation. ACTH, adrenocorticotrophic hormone; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein.

acids by the liver is passive and unregulated. In the absence of feedback regulation, the liver cannot decrease fatty acid uptake even when the capacity to catabolize fatty acids or re-esterify them and secrete them back into the circulation as VLDL is overwhelmed. Derangements in peripheral lipolysis thus have a direct impact on the flux of fat through the liver.

### Fatty acid synthesis within the liver

TAG is the most compact form of energy storage. Conversion of most excess dietary carbohydrate and protein to fatty acids and then to TAG is a primary responsibility of the liver. Excess glucose is converted to fat by both the liver and adipocytes. In the liver, the backbone of most amino acids can be converted to pyruvate and then to acetyl-coenzyme A (acetyl-CoA), which feeds directly into cytosolic fatty acid synthesis. For this reason, a recommendation for patients to adhere to a low fat diet when the calories are simply replaced by carbohydrate may not have the desired effect on reducing fat accumulation in the liver.

### Fate of fatty acids in the liver

#### $\beta$ -Oxidation

In the fasting state, adipocyte TAG is hydrolyzed to release free fatty acids, which are transported to the liver where they can serve as substrates for mitochondrial  $\beta$ -oxidation.  $\beta$ -Oxidation of fatty acids is a major source of energy needed to maintain liver viability during fasting. It is also the source of the ketone bodies, acetoacetate, acetone, and D-3-hydroxybutyrate. These are released into the blood and are essential fuel sources for

peripheral tissues, especially neurons, muscle and brain when glucose is in short supply because of the inability of neural tissues to use free fatty acids as fuel. Defects in hepatic  $\beta$ -oxidation cause microvesicular steatosis of the liver, intolerance to fasting, and in some cases a myopathy because of the muscle's normal requirement for fatty acids as an energy source.

The first step in any pathway that metabolizes fatty acids is the formation of esters with coenzyme A, also called acyl coenzyme A (acyl-CoA). Because acyl-CoA cannot enter the mitochondria directly, the fat moiety is transferred to carnitine and the acyl conjugates with carnitine are selectively transported into the mitochondria. This carnitine shuttle requires three enzymes:

- carnitine palmitoyl transferase I (CPTI), which forms the acyl carnitines;
- a translocase, and
- CPTII, which reverses the first step to form acyl-CoA from acylcarnitine within the mitochondria.

Once inside the mitochondria, acyl-CoA undergoes progressive shortening, two carbons at a time, to generate nicotinamide adenine dinucleotide in reduced form (NADH) and acetyl-CoA. This is the process of  $\beta$ -oxidation. The electrons carried by the NADH drive the production of adenosine triphosphate (ATP) and the acetyl-CoA produced is further catabolized by the Krebs cycle to form more ATP and other key metabolic intermediates, such as the ketone bodies. Different enzymes required for the chain shortening have relative preferences for the newly delivered long chain fatty



acids, the partly processed medium chain fatty acids, and the almost completely processed short chain fatty acids.

Defects in the function of the enzymes responsible for  $\beta$ -oxidation are increasingly recognized and associated with liver disorders that commonly present with some degree of microvesicular steatosis. Severe defects, such as CPTI deficiency, typically present during early childhood with fatty liver and intolerance to fasting. A defect in medium chain acyl-CoA dehydrogenase (MCHAD) has been found in some infants with sudden infant death syndrome. Other defects may manifest themselves during adulthood. One example is the association between acute fatty liver of pregnancy and a maternal partial defect in long chain hydroxyacyl-CoA dehydrogenase (LCHAD).

Certain drugs and toxins are known to impair mitochondrial  $\beta$ -oxidation and cause microvesicular steatosis. Valproic acid can undergo  $\beta$ -oxidation to intermediates that are toxic to mitochondria. Methylenecyclopropylalanine (hypoglycin) is a natural product found in unripe ackee fruit (*Blighia sapida*), which potently inhibits  $\beta$ -oxidation. Ackee is a popular fruit in Jamaica, and several deaths characterized by prodromal vomiting and hypoglycemia have been reported after the ingestion of unripe ackee.

The reason why impaired mitochondrial  $\beta$ -oxidation causes microvesicular steatosis has not been established. In general, the conditions under which  $\beta$ -oxidation is required (i.e. the non-fed state) are the same circumstances under which peripheral mobilization of fat is increased. Thus, there may be increased delivery of fatty acids to a liver which is relatively energy deprived with respect to its ability to generate ATP. Potentially, the impaired mitochondrial function per se with the attendant ATP deficiency and other resulting metabolic abnormalities may impair the complex process of packaging and secreting TAG as VLDL.

An oxidation pathway of secondary importance in the flux of fatty acids through the liver is the peroxisomal pathway. Peroxisomes oxidize very long chain fatty acids (>22 carbons) and dicarboxylic acids to shorter chain lengths, which can then be managed by mitochondrial  $\beta$ -oxidation. Importantly, peroxisomal oxidation of fatty acids generates hydrogen peroxide and thus peroxisomes may be an important source of oxidant stress in hepatocytes overburdened by a high flux of free fatty acids.

### Formation and secretion of VLDL

In the fed state,  $\beta$ -oxidation of fatty acids is not required as an energy source, and fatty acids delivered to the liver are mainly converted to TAG. Insulin regulates the metabolic path that fatty acids take in the liver. Without insulin, CPTI commits fatty acids to mitochondrial  $\beta$ -oxidation; when insulin levels are high, glycerol-3-phosphate acyltransferase commits fatty acids to the formation of TAG. Whether this partitioning is altered in states of insulin resistance has not been established.

In the fed state, the liver also efficiently converts excess glucose and the backbone of many amino acids to TAG. Energy storage as TAG is probably preferred because of its compactness; TAG energy density is 9 kcal/g and can be stored as a relatively dehydrated mass. Carbohydrates and amino acids are inherently less energy dense (3 and 4 kcal/g dry weight, respectively) and require solvation with a substantial amount of water,

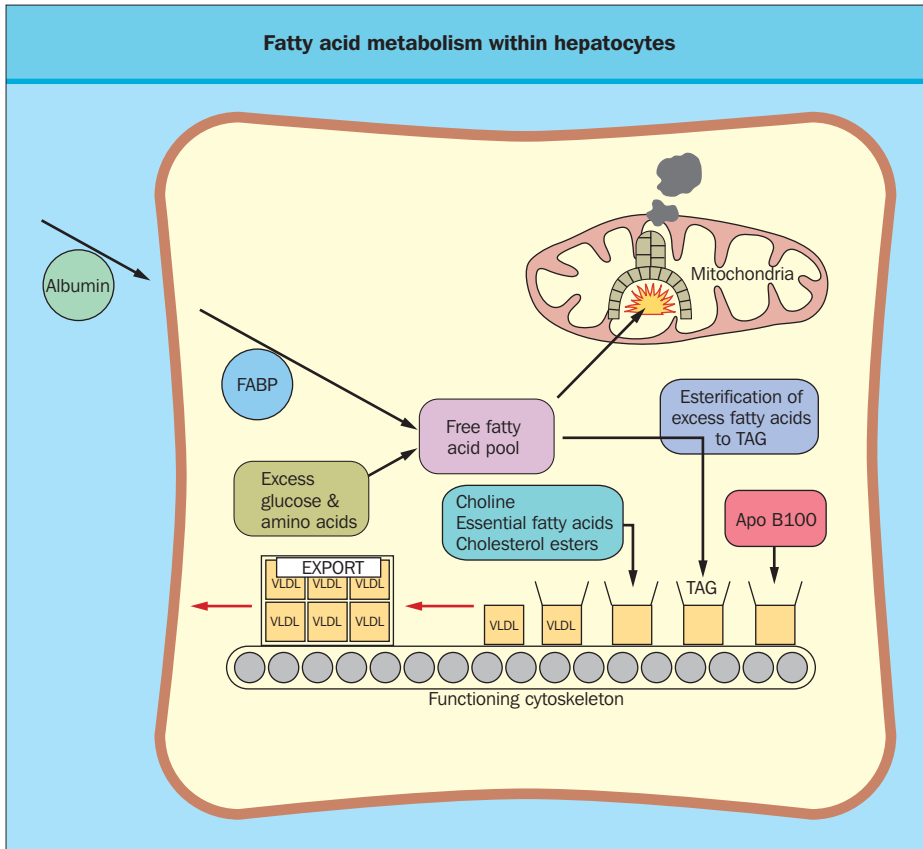
which only further reduces the energy density on a per weight basis.

Although delivery of fatty acids to the liver and fatty acid synthesis in the liver are the major contributors to liver TAG, not all liver TAG is derived from fatty acids. A fraction of liver TAG is taken up directly by endocytosis of lipoproteins and lipoprotein remnants such as low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), VLDL remnants and chylomicron remnants. After binding to the LDL receptor and endocytosis, the TAG contained within these particles is released in a lysosomal compartment and mixed with newly synthesized TAG.

The secretion of TAG into the circulation in the form of VLDL is the only mechanism of disposing of TAG from the hepatocyte. Because of its complexity, the synthesis and secretion of VLDL from the liver is the rate-limiting step in the cycling of fat between the liver and peripheral stores. Through a process of combining TAG with apolipoprotein B-100, phosphatidylcholine, cholesterol, and cholesterol esters, functional VLDL is formed and secreted by exocytosis (Fig. 22.2). Much can go awry in the complex process of VLDL secretion. The result is TAG accumulation and its common clinical correlate, hepatic steatosis (Table 22.3). This simple fact explains why the many seemingly unrelated causes of steatosis have but this one clinical manifestation.

Although VLDL may acquire minor amounts of other lipoproteins once it enters the circulation, when it is secreted from the liver apolipoprotein B-100 (apoB-100) is the only protein component. ApoB-100 is coded by a single gene on chromosome 2, and the gene structure is defined by 29 exons (expressed sequences) and 28 introns (intervening sequences). The product of mRNA translation is a 4536 amino acid protein with a molecular weight of 512 kDa and an amino terminus LDL receptor-binding domain. Post-translational processing includes N-glycosylation, acylation, and phosphorylation to achieve the functional protein. The synthesis of apoB-100 is rapid; protein synthesis requires less than 15 min and incorporation into VLDL followed by secretion requires another 30 min. Incorporation of TAG into nascent VLDL also requires microsomal triglyceride transfer protein (MTTP) and genetic defects in MTTP are associated with fatty liver and NASH. Much newly synthesized apoB-100 never reaches the circulation and is instead subject to intracellular degradation. This excess synthesis may occur when the supply of TAG is inadequate to form VLDL. Conversely, the constitutive rate of apoB-100 synthesis can be outpaced by excessive free fatty acid delivery to the liver and hepatocellular TAG accumulation develops.

Defects in apoB-100 synthesis and function cause TAG to accumulate in the liver. A simple deficiency of amino acids can prevent adequate apoB-100 synthesis, which may explain the steatosis commonly found in protein malnutrition (kwashiorkor). ApoB-100 is rich in threonine, and its synthesis is thus particularly sensitive to a deficiency of this amino acid. Certain apoB-100 gene mutations result in partial or complete loss of functional protein synthesis. Complete absence of functional protein (abetalipoproteinemia) presents in infancy with severe fat malabsorption and can lead to cirrhosis. The fat malabsorption associated with abetalipoproteinemia is probably due to the obligate deficiency of apoB-48, the intestinal lipoprotein analog of apoB-100 that is required for chylomicron assembly. ApoB-48 is



**Figure 22.2 Fatty acid metabolism within hepatocytes.** In hepatocytes, free fatty acids are primarily delivered from peripheral stores bound to albumin or synthesized from excess carbohydrate or amino acids. Fatty acids within hepatocytes are bound to the carrier protein, fatty acid-binding protein (FABP) and they are either shuttled to the mitochondria to generate energy [adenosine triphosphate (ATP)] and ketone bodies or esterified into triacylglycerol (TAG) and packaged in very low-density lipoprotein (VLDL) for secretion back into the blood. The packaging of TAG and the export of VLDL requires multiple cellular functions. An adequate supply of precursor amino acids and unimpaired protein synthesis are needed to make apolipoprotein B-100 (apoB-100) and microsomal triglyceride transfer protein (MTP); choline, essential fatty acids, and cholesterol esters are required to assemble VLDL. Finally, a functioning cytoskeletal network is then needed to export vesicles containing the VLDL particles from the hepatocyte. Defects or deficiencies in any one of these components can impair VLDL secretion and lead to fat accumulation within the cell.

Defects in very low-density lipoprotein (VLDL) synthesis	
Defect	Comments
ApoB gene defect	Homozygous abetalipoproteinemia is a severe disease of infancy; heterozygous hypobetalipoproteinemia varies in its phenotypic expression
MTP polymorphisms	MTP is required for lipidation of apolipoprotein B-100 during the formation of VLDL and polymorphisms of its gene have been associated with NAFLD
Dietary protein deficiency	VLDL synthesis is especially sensitive to threonine deficiency
Choline deficiency	Required to form phosphatidylcholine, an essential component of VLDL Methionine may be able to replace choline as a methyl donor May be a cause of TPN-induced steatosis
Essential fatty acid deficiency	Essential fatty acids are required for phosphatidylcholine synthesis
Cholesterol esterification defect	Cholesterol esters are required for VLDL assembly
Altered cytoskeleton	Impairs exocytosis
ApoB, apolipoprotein B; MTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver disease; TPN, total parenteral nutrition; VLDL, very low-density lipoprotein	

**Table 22.3 Defects in very low-density lipoprotein (VLDL) synthesis.**

required for intestinal fat absorption and is coded by the same gene as apoB-100 (see later). Partial loss of functional apoB-100, or hypobetalipoproteinemia, has a more diverse phenotypic expression depending on the degree of protein absence, but deficiencies have been identified in adults with NASH.

### Sources of plasma triacylglycerol

In the fasted state, most plasma triglyceride is found within VLDL secreted from the liver, whereas the small remaining fraction is distributed among various VLDL degradation products (see later). In contrast, in the fed state, the majority of

triglyceride is found within chylomicrons. There are two reasons for the dominance of chylomicron TAG over VLDL TAG in the fed state. The most obvious is that dietary fat absorbed from the small intestine is wholly incorporated into chylomicrons. In addition to the postprandial increase in circulating chylomicrons, there is a decrease in hepatic VLDL secretion because of the inhibitory effects of insulin on VLDL synthesis and secretion. States of insulin resistance are associated with compensatory hyperinsulinemia, an abnormality which may impair secretion of VLDL from the liver.

Chylomicron synthesis in the small bowel epithelial cells is analogous to hepatic VLDL synthesis and secretion in most respects. However, in humans, the predominant lipoprotein incorporated into nascent chylomicrons in enterocytes is apoB-48, whereas the liver incorporates apoB-100 into VLDL. Interestingly, apoB-48 is a protein with 2152 amino acids with a final molecular weight of 248 kDa coded by the amino terminus of the apoB-100 gene; the 2153rd codon of the apoB-48 gene is a stop codon that leads to the smaller protein product. During the final assembly of chylomicrons, smaller amounts of the A lipoproteins are also incorporated, and the final product is secreted into the lymphatics. Short chain fatty acids less than 10 carbons can bypass this route of absorption and pass directly into the circulation.

### **Fate of plasma triacylglycerol**

#### ***Lipoprotein modification***

Circulating lipoprotein complexes undergo several modifications before they release TAG at peripheral sites. Both VLDL and chylomicrons acquire C lipoproteins and apoE from circulating high-density lipoprotein (HDL). Neither VLDL nor chylomicrons lose apoB-100 or apoB-48 respectively to other lipoproteins, presumably because of the extensive hydrophobic interactions between these two lipoproteins and the lipid cores of the entire particles.

#### ***Release of fatty acids***

Very little of the TAG in the circulation is absorbed by the liver. Depending on the levels of circulating insulin, circulating TAG is consumed either by adipose tissue or by energy-requiring tissues such as muscle. In the fed state, high insulin levels favor uptake by adipose tissue. In the fasted state, lower insulin levels favor utilization by muscle and other tissues able to release fatty acids from lipoprotein-bound TAG. The lack of direct uptake of TAG or fatty acids from VLDL or chylomicrons may explain the paradoxical lack of a strong correlation between hyperlipidemic states and hepatic steatosis.

The uptake of fat from lipoproteins requires release of free fatty acids from TAG by lipoprotein lipase (LPL) at the vascular endothelium. LPL is an enzyme that belongs to a multigene family that includes pancreatic lipase. Although it is synthesized by many tissues, LPL is found predominantly in muscle and adipose tissue, which correlates with the major sites of TAG consumption. The enzyme attaches to vascular endothelial cells by its interaction with the glycoprotein heparan sulfate. The enzyme is not produced by vascular endothelial cells but instead is synthesized by adipocytes and myocytes. Because of their disparate roles in fat metabolism, fat and muscle express LPL under different conditions. LPL expression by the adipocyte is increased in the fed state by insulin, whereas expression in muscle is constitutively high and increases modestly with fasting.

Through the action of LPL, TAG is removed from VLDL and chylomicrons. Removal of TAG from VLDL sequentially generates VLDL remnants, IDL, and finally LDL in an increasing order of density. The increasing density reflects the progressively decreasing TAG content. The removal of TAG from VLDL is relatively rapid; after 15–60 min, most TAG is removed from circulating VLDL. About half of VLDL is processed to LDL, whereas the other half is less fully defatted and generates VLDL remnants and IDL. Chylomicrons are depleted of their TAG even more rapidly, with a substantial fraction of TAG removed within 5 to 10 min after entering the circulation. As a result, chylomicron remnants contain 10–20% of their original TAG and all of the dietary cholesterol originally incorporated into this gut-derived lipoprotein.

The end products after TAG is removed from VLDL and chylomicrons (namely VLDL remnants, IDL, LDL and chylomicron remnants) are taken up by the liver. How much this uptake of these relatively dense lipoproteins containing residual TAG contributes to the triglyceride pool in the liver is unknown but is probably minor compared with the intrahepatic synthesis of fatty acids and the delivery of fatty acids to the liver from peripheral stores. Uptake of lipoproteins is carried out by the hepatic LDL receptor. The receptor usually interacts with the apoE component of VLDL remnants, but in the more TAG-depleted and apoE-depleted LDL, the interaction is with apolipoprotein B-100. The LDL receptor has a relatively low affinity for LDL, and this weak interaction is probably one reason why LDL has a long circulating half-life of 3 days compared with its precursor VLDL. The role of defects in LDL clearance as a cause of vascular disease remains controversial. ApoB-100 and apoE polymorphisms have been identified and these decrease binding of LDL to the hepatic receptor and its subsequent clearance from the circulation. Although these genetic abnormalities are associated with hypercholesterolemia and vascular disease, the relative importance of defects in each of the lipoproteins has not been resolved, and their role in contributing to hepatic steatosis is uncertain.

### **Other forms of hepatic steatosis**

#### ***Microvesicular steatosis***

Microvesicular steatosis is distinguished from macrovesicular steatosis on well-defined morphologic grounds and also by pathophysiologic mechanisms. Whereas macrovesicular steatosis is caused by an imbalance in the hepatic synthesis and export of TAG, all causes of microvesicular steatosis are related to defects in mitochondrial function (Table 22.4). The reason why the accumulated TAG of microvesicular steatosis is retained in smaller vesicles distributed pancellularly rather than coalescing into large droplets characteristic of macrovesicular steatosis is unknown.

#### ***Alcoholic steatosis***

The pathogenesis of alcoholic liver disease is reviewed in Chapter 21. The accumulation of TAG in the liver caused by alcohol, even in normal individuals with modest alcohol consumption, has not been fully explained. Hepatic TAG synthesis is increased after the consumption of alcohol. Fat may accumulate simply as a result of excessive TAG synthesis, or there may be a concomitant defect in the secretion of VLDL that contributes synergistically to the accumulation of fat in the liver.



<b>Causes of microvesicular steatosis</b>	
<i>Cause</i>	<i>Mechanism</i>
<b>Drugs</b>	
Valproic acid	Depletion of mitochondrial CoA; $\beta$ -oxidation and blockade of $\beta$ -oxidation enzymes by P-450 drug metabolites
Tetracycline (high dose)	Inhibited $\beta$ -oxidation; impaired hepatic triglyceride secretion
Aspirin	Uncoupling oxidative phosphorylation; depletion of extramitochondrial acetyl-CoA, which prevents transport of fatty acids into the mitochondria
2-Arylpropionate NSAID (pirprofen, naproxen, ibuprofen, ketoprofen)	Inhibit $\beta$ -oxidation of medium- and short-chain fatty acids
Amineptine	Inhibit $\beta$ -oxidation of medium- and short-chain fatty acids
Nucleoside analogs (zidovudine, didanosine, zalcitabine, fialuridine)	Inhibition of mitochondrial DNA replication
<b>Toxins</b>	
Ethanol	Diminished mitochondrial $\text{NAD}^+$ impairs $\beta$ -oxidation; mitochondrial oxidant stress damages mtDNA
Hypoglycin (unripe ackee)	Impaired $\beta$ -oxidation
<i>Bacillus cereus</i> emetic toxin	Impaired $\beta$ -oxidation
Toxic shock syndrome	Unknown bacterial toxins
<b>Genetic defects</b>	
Acute fatty liver of pregnancy	Defects in the $\beta$ -oxidation; LCHAD defect in a subset of patients
$\beta$ -Oxidation defects:	
CPT I	Inadequate substrate for $\beta$ -oxidation
CPT II	Inadequate substrate for $\beta$ -oxidation
Others	Inadequate substrate for $\beta$ -oxidation
Ornithine transcarbamylase deficiency	Inhibition of long- and medium-chain fatty acid $\beta$ -oxidation by ammonium
Alpers disease	Unknown mitochondrial defect
<b>Other</b>	
Reye syndrome	Combined acquired and genetic defects in $\beta$ -oxidation or ureagenesis
Cholestasis (foamy degeneration)	Impaired mitochondrial function by bile acids
CoA, coenzyme A; CPT, carnitine palmitoyl transferase; NSAID, non-steroidal anti-inflammatory drugs; LCHAD, long-chain hydroxyacyl-CoA dehydrogenase	

**Table 22.4 Causes of microvesicular steatosis.**

Alcohol is also a major mitochondrial toxin, which explains features seen in severe alcoholic hepatitis such as megamitochondria and microvesicular fat. Hepatic lipid peroxidation occurs during alcohol metabolism by the liver, and oxidant stress may play a key role in the development of hepatic steatosis and the development of the inflammatory response characteristic of alcoholic hepatitis.

### Phospholipidoses

Phospholipid accumulation in the liver causes steatohepatitis and can cause a histologic picture similar to the steatosis caused by TAG accumulation. The phospholipidosis syndromes are caused by the accumulation of phospholipids in lysosomes. The expansion of membranes by excess phospholipids results in the development of enlarged lysosomes with a redundant membrane that is folded over repeatedly to form 'lamellar bodies'. These laminar bodies can be identified by electron microscopy because of their characteristic membranous whirl appearance.

The phospholipidoses are caused by impaired breakdown of phospholipids within lysosomes, leading to the accumulation of these amphipathic lipids in the lysosomal membranes. Genetic

deficiencies of enzymes that metabolize phospholipids can cause a lethal accumulation of phospholipids within lysosomes. Wolman disease occurs in childhood and is caused by the absence of lysosomal acid lipase. Cholesterol ester storage disease is caused by defective expression of the same enzyme, yet small residual activity can allow survival past childhood. Lysosomal sphingomyelinase is deficient in patients with Niemann-Pick disease.

Drugs that impair the lysosomal enzymes that metabolize phospholipids or diminish the lateral mobility of phospholipids within membranes are the major causes of the phospholipidoses (Table 22.5). Generally, drugs that cause phospholipidosis are polycationic (meaning that they have more than one positive charge) and amphipathic (meaning that in addition to a polar charged region of the molecule, much of the drug is non-polar or hydrophobic). These properties allow such drugs to bind phospholipids within membranes and prevent their normal mobility and enzymatic degradation. The lysosomes are probably a target for such amphipathic drug accumulation because of their lower intraorganelle pH, which favors compartmental accumulation. The same mechanism is also responsible for

Hepatic phospholipidoses
Metabolic diseases Niemann-Pick disease Wolman disease Cholesterol ester storage disease
Drugs (after long-term use) Amantadine Amiodarone Amitriptyline Chloroquine Chlorphenteramine Chlorpromazine Diltiazem Fenfluramine Imipramine Nifedipine Oral contraceptives Propranolol Thioridazine Gentamicin

Table 22.5 Hepatic phospholipidoses.

mitochondrial accumulation of similar drugs where they can be concentrated and impair mitochondrial function.

Because of the slow accumulation rate in the lysosomal membrane compartment, drugs that cause clinically apparent phospholipidosis are typically administered for prolonged periods before a significant amount of phospholipid accumulates. Thus, antibiotics such as gentamicin, ketoconazole and azithromycin, which cause phospholipidosis after chronic administration experimentally, are not major causes of clinically evident phospholipidosis, because of their typically short treatment regimens. By contrast, the now abandoned antianginal drug perhexiline maleate and the antiarrhythmic drug amiodarone cause clinically relevant phospholipidosis because of their need for prolonged use.

The relationship between phospholipidosis and injury to the liver is unclear. Although there are reports of idiosyncratic-type reactions to amiodarone with clinically evident liver injury, this occurs only in a minority of cases compared with the frequent development of phospholipidosis. Steatohepatitis, as distinguished from phospholipidosis, can be found following amiodarone treatment but does not appear to be a direct consequence of phospholipidosis.

**Steatosis and inflammation**

The inflammatory response characteristic of NASH appears to develop only in the setting of pre-existing steatosis. The converse – the development of the characteristic mixed cellular infiltrate throughout the acinus without steatosis – is not a known histopathologic condition. One hypothesis is that the fat within the steatotic liver serves as a readily available target for lipid peroxidation. Lipid peroxidation produces highly toxic intermediates that promote inflammation and directly compromise cellular integrity. This hypothesis is supported primarily by animal data. Evidence in humans has been less convincing. Alternative explanations include hepatocellular toxicity of excess free fatty acids, mitochondrial dysfunction

and hepatocellular ATP deficiency, and injury mediated by hepatic Kupffer cells and circulating endotoxin.

**CLINICAL FEATURES**

**Symptoms**

Hepatic steatosis is usually asymptomatic. The most common complaint in patients with benign steatosis or NASH, particularly in the obese, is dull right upper quadrant pain, which may be caused by a distended liver capsule. A minority of patients may have quite severe pain. A variety of less common non-specific constitutional symptoms such as weakness, fatigue and malaise are offered by patients with NASH. The prevalence and severity of these same constitutional symptoms is much greater in patients with alcoholic hepatitis, despite the similarity of biopsy findings and aminotransferase elevations.

**Physical examination**

Palpable hepatomegaly can signify a liver enlarged by benign steatosis or NASH. However, hepatomegaly can be difficult to detect by palpation in many patients because of the coexistent obesity. There are no other specific physical findings that would suggest hepatic steatosis. Findings such as jaundice, spider angiomas, muscle wasting, ascites, and palmar erythema point to the development of cirrhosis. Many patients with NAFLD have underlying insulin resistance and two findings characteristic of the metabolic syndrome, hypertension and acanthosis nigricans, can be found in patients with NAFLD.

**Laboratory evaluation**

Most patients who come to medical attention for the evaluation of subclinical liver disease do so because of incidental laboratory abnormalities. This fact biases all clinical surveys that attempt to establish the sensitivity of laboratory testing for identifying patients with NASH. Progression of NASH can occur with normal laboratory testing; this has been shown in reports of the preoperative evaluation of patients undergoing surgical treatment for obesity. Nonetheless, among the laboratory tests available, the aminotransferases are the most likely to be abnormal. Other biochemical abnormalities that are common in alcoholic hepatitis (e.g. hypoalbuminemia, hyperbilirubinemia and prothrombin time elevations) are not observed in NASH unless the disease has progressed to cirrhosis.

**Aminotransferases**

Patients with NASH typically have serum aminotransferases that range from normal to a maximum of four times the upper limit of normal (Table 22.6). The prevalence of elevated aminotransferases in patients with NASH is unknown. This is mainly because the presence of liver disease is not routinely pursued in patients with normal aminotransferases, even when imaging studies suggest hepatic steatosis. The presence of elevated aminotransferases cannot be used to distinguish benign steatosis from NASH.

One way in which aminotransferase elevations can be useful in identifying patients with NASH is the ratio of alanine aminotransferase (ALT) to aspartate aminotransferase (AST) activities. Whereas patients with alcoholic hepatitis typically have serum AST levels that exceed the ALT, patients with NASH often have an ALT that is greater than the AST level in the absence

Laboratory findings of steatosis and NASH	
Aminotransferases (ALT and AST)	Normal values do not exclude steatosis or NASH Typically normal to 250 U/L. ALT is greater than AST in most patients (helpful in distinguishing from alcoholic steatosis/steatohepatitis; AST is usually greater than the ALT in alcoholic liver disease and cirrhosis from any cause)
Alkaline phosphatase	Typically normal but can be up to two times the upper limit of normal
$\gamma$ -Glutamyltranspeptidase (GGT)	Can be elevated
Triglycerides	Frequently elevated in children with steatosis and NASH but not consistently elevated in adults
Cholesterol	No correlation; abnormally low cholesterol may indicate hypobetalipoproteinemia as a cause of steatosis/NASH
Total bilirubin, prothrombin time, albumin	Normal in the absence of cirrhosis
ALT, alanine aminotransferase; AST, aspartate aminotransferase; NASH, non-alcoholic steatohepatitis	

**Table 22.6** Laboratory findings of steatosis and NASH.

of cirrhosis. Identification of the intracellular origin of serum aminotransferases has not proved helpful in distinguishing NASH from alcoholic hepatitis. In both diseases, the ratio of mitochondrial AST to total AST is elevated, possibly reflecting a shared pathogenetic mechanism of mitochondrial injury. Patients with cirrhosis from any cause, including NASH, typically have serum AST levels that exceed ALT levels. For this reason, using the AST/ALT ratio is only helpful in patients without cirrhosis.

### Other biochemical markers

$\gamma$ -Glutamyltranspeptidase (GGT) elevations may correlate with increases in hepatic fat content, yet elevations of this enzyme are rarely isolated when substantial and are rarely worth pursuing further when trivial. Serum triglyceride elevations are found in only a few adults with NASH but are common in children.

### Estimating insulin resistance

Insulin resistance, and its resulting state of hyperinsulinemia, may be a primary underlying disorder in most patients with NAFLD. Insulin resistance is best measured by rigorous techniques such as the euglycemic insulin clamp method. Because performing this test is difficult in the routine clinical environment, simpler tests have been sought. Several generally accepted methods of approximating insulin sensitivity have been developed that are based on the product of the fasting insulin multiplied by the fasting glucose. Two of the most commonly used, the homeostasis model (HOMA-IR) and quantitative

Insulin resistance	
<b>QUICKI:</b> $= \frac{1}{\log(\text{gluc}) + \log(\text{ins})}$ $= \frac{1}{\log(\text{gluc} \times \text{ins})}$ Units: gluc = mg/dL ins = mU/L Normal: >0.358–0.387	<b>HOMA-IR:</b> $= \frac{\text{gluc (mM)} \times \text{ins}}{22.5}$ $= \frac{\text{gluc (mg/dL)} \times \text{ins}}{405}$ Units: ins = mU/L Normal: <1.57

**Figure 22.3 Insulin resistance.** Insulin resistance can be estimated by calculating the product of the fasting insulin times the fasting glucose. Two commonly used formulas, the QUICKI and the HOMA-IR, are based on this product. The cutoffs for normal ranges of these values may depend on the method used to measure insulin; the values given are derived from published studies and may not apply to every laboratory.

insulin sensitivity check index (QUICKI), rely on this product and are mathematically related (Fig. 22.3). An even simpler method is that a product of fasting insulin (mU/L)  $\times$  fasting insulin (mg/dL) >700 may indicate insulin resistance. Unfortunately, lack of standardization of insulin assays makes these values approximate and creates difficulties in comparing values from different laboratories. The value of estimating insulin resistance in patients with NAFLD is that it may provide patients with an additional incentive to adopt lifestyle modifications that include increased physical activity and caloric restriction.

### Imaging

The major abdominal imaging modalities – ultrasound, CT, magnetic resonance imaging (MRI) and radionuclide techniques – can each contribute to the identification of focal or diffusely distributed hepatic steatosis (Table 22.7). Each has the ability to indicate the presence of hepatic steatosis, yet none of these techniques has the ability to distinguish benign hepatic steatosis from NASH. Generally, NASH is non-focal, and thus areas of focal fat or areas of focal sparing in a fatty liver can create difficulties in distinguishing these lesions from malignancy. Several circumstances responsible for focal involvement or sparing have been described. Patients with diabetes and renal failure who receive insulin in their peritoneal dialysis fluid can develop subcapsular steatosis, which can progress to NASH that is confined to the periphery of the liver exposed to high concentrations of insulin. Additionally, a minority of patients has pancreatic venous drainage into a localized area of the liver and focal NAFLD can sometimes be found in this region. The opposite anatomic anomaly can also exist; some patients have gastric venous drainage, which contains less insulin than the portal blood flow, directed into a localized hilar region. If the remainder of the liver is fatty in such patients, the region spared of insulin is also spared of fatty infiltration. Such observations may provide important insights into the role of insulin in



Imaging findings of steatosis and NASH		
	Ultrasonography	Computed tomography
Diffuse steatosis and NASH	Echogenic liver parenchyma Loss of contrast between the bile duct wall and liver parenchyma Hemangiomas may appear hypoechoic compared with the surrounding liver parenchyma	Low-density liver parenchyma compared with the spleen
Focal steatosis	Often a geometrically shaped echogenic area No mass effect Can be transient, absent on follow-up studies	Single or multiple low-density areas (often with the appearance of metastatic disease)
Focal sparing	Area of decreased echogenicity Often in caudate lobe or adjacent to the gallbladder	Regions of normal density in an otherwise low-density liver
NASH, non-alcoholic steatohepatitis		

Table 22.7 Imaging findings of steatosis and NASH.

the accumulation of hepatic fat and the importance of fat as a precursor lesion to NASH.

### Ultrasonography

Compared with the normal liver, the fatty liver is diffusely echogenic or 'bright' (Fig. 22.4). However, ultrasonography of the liver is a reliable technique for the detection of hepatic steatosis only when the degree of steatosis is substantial. Thus, when biopsy results are compared with ultrasonographic findings, a significant amount of fat must be present for the liver to appear sonographically dense. The finding of a diffusely echogenic liver is also not entirely specific for steatosis because cirrhosis can cause a similar appearance albeit with a subjectively coarser texture.

### Computed tomography

CT scanning of the fatty liver typically reveals an abnormally low-density liver parenchyma compared with the spleen (Fig. 22.5). A low-density liver is a relatively common finding, occurring in up to 10% of all abdominal CT scans. Most commonly, the low attenuation is diffuse. However, in up to one-third of the patients, the involvement is focal and appears as one or more low-density lesions in an otherwise normal liver. Focal fat can be found anywhere in the parenchyma or can be localized only to the periportal region. Focal sparing in an otherwise fatty liver can also be seen, and it has the appearance of a high-density lesion that can be confused with a malignancy. A comparison of the density of the focal area of sparing with its surrounding liver and with the spleen is helpful to identify its nature.

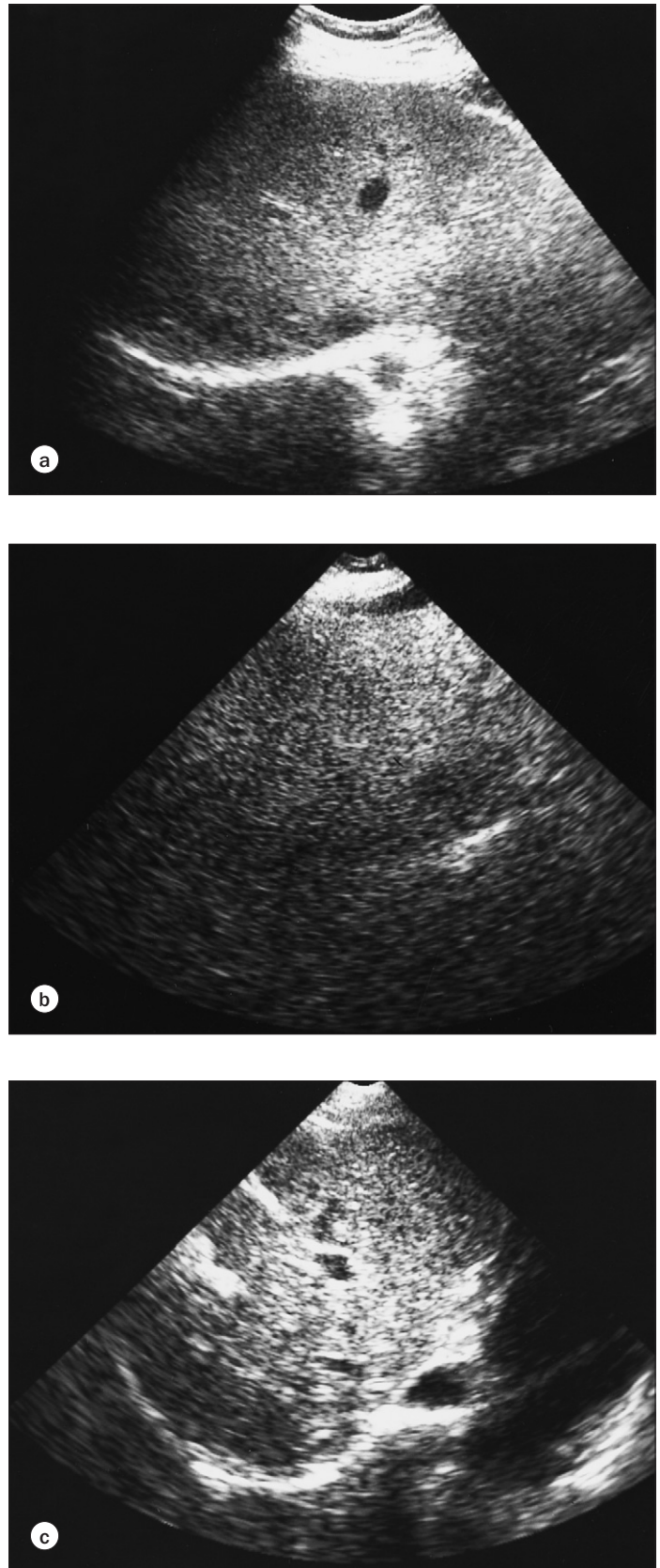
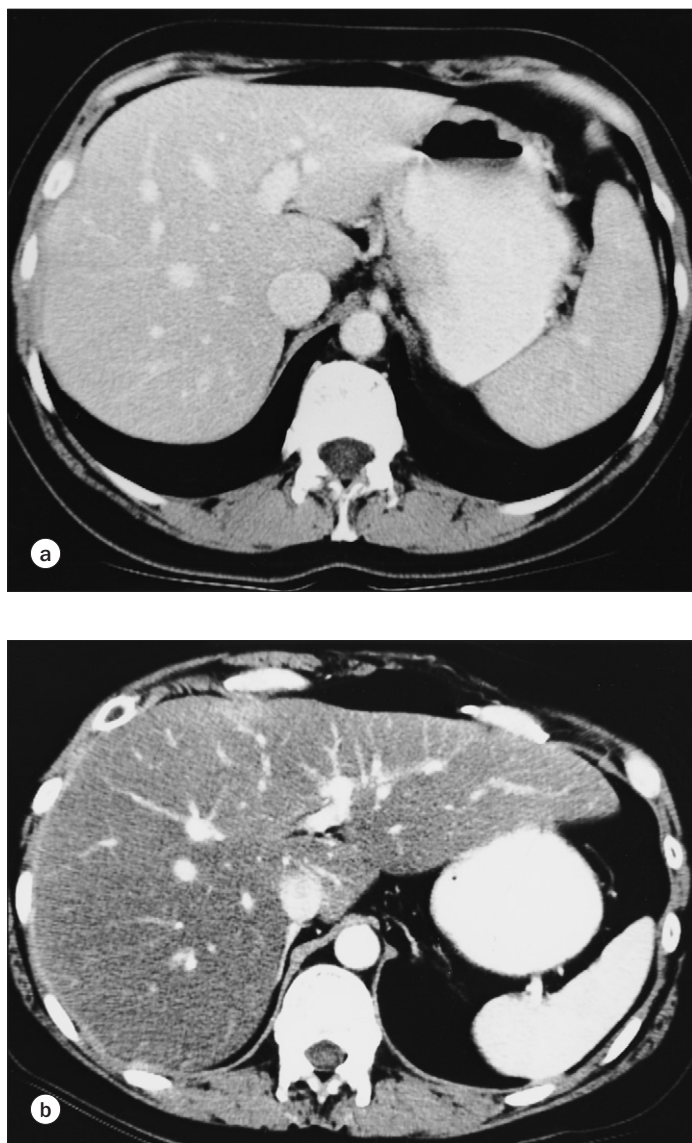


Figure 22.4 Ultrasound images of the liver from patients with a biopsy proven normal liver (a), severe steatosis (b), and cirrhosis caused by primary sclerosing cholangitis (c). Increased echogenicity is evident in both the steatotic and cirrhotic liver images compared with the normal liver.



**Figure 22.5 Abdominal computed tomography (CT).** Images demonstrate the appearance of a normal liver (a) and steatotic liver (b) after the administration of intravenous contrast solution. The normal liver density is comparable to the density of the spleen. The steatotic liver has a much lower radiographic density compared with the spleen, and the contrast-filled hepatic vasculature is accentuated by the surrounding low-density liver parenchyma. The image of the steatotic liver was obtained from a non-drinking woman who had received tamoxifen therapy for 3 years after the resection of a breast cancer. She complained of dull pain in the right upper quadrant of the abdomen. A liver biopsy showed severe steatosis, Mallory hyaline, and the presence of inflammation characteristic of non-alcoholic steatohepatitis (NASH).

### **Magnetic resonance imaging**

MRI can detect diffuse hepatic steatosis with a similar sensitivity to CT. MRI may not be particularly useful for further evaluating focal lesions suspected to be fat by CT, although phase shifting can be used to identify focal fat by its loss of intensity on  $T_1$ -weighted images. A variant is MR spectroscopy, a technique that has the greatest sensitivity for non-invasively detecting and quantifying liver fat.

### **Radionuclide imaging**

Radionuclide imaging using  $^{99m}\text{Tc}$  sulfur colloid identifies focal areas of fat within the liver as focal defects, a finding that contributes little if anything to making the distinction between benign and malignant etiologies. On the other hand, areas of focal sparing in the otherwise diffusely fatty liver will absorb tracer, and this can be helpful when evaluating such lesions. One radionuclide technique that has not been used often is the measurement of hepatic  $^{133}\text{Xe}$  retention. Although this test offers a highly sensitive measure of hepatic steatosis, its clinical usefulness has not been extensively evaluated.

### **Imaging problems**

#### **Focal fat**

The incidental discovery of lesions in the liver caused by focal fat or focal sparing can be challenging. Even after using more than one imaging modality, focal areas of fat in the liver can be difficult to distinguish from primary or metastatic malignancies. The resultant uncertainty can lead the clinician down a path of escalating diagnostic invasiveness in children and adults alike. A few clues can point to focal fat, such as the typically aspherical or geometric appearance of focal fat without exerting a mass effect on adjacent structures. However, this is not always the case, and fine-needle aspiration biopsy of problematic lesions may be required to definitively exclude malignancy, especially in patients with a history of prior malignancy. If the level of concern is lower, especially in children, then repeated imaging within 3–6 months is a reasonable and more conservative approach.

#### **Focal sparing**

Focal sparing, representing focal areas of normal liver, can be seen occasionally in an otherwise fatty liver. Focal sparing appears as a relatively hyperdense region by CT with corresponding hypodense areas identified by ultrasound. These areas, such as the commonly spared caudate lobe, tend to receive aberrant portal venous drainage with isolation of the spared segments of the liver from intestinal venous blood. The location and geometric shape of focal sparing generally distinguishes focal sparing from malignancy in a fatty liver, but a fine-needle biopsy for histologic confirmation may be necessary in unusual circumstances.

### **Identification of other lesions in a fatty liver**

Fat within the liver also creates problems in properly identifying other pathology, especially by ultrasonography. Hemangiomas are typically echodense, yet these benign lesions may appear as relatively hypodense lesions when surrounded by the much more echodense fatty parenchyma. Dynamic CT scanning and tagged red blood cell radionuclide imaging are the best tests for establishing the identity of such lesions when there is significant doubt. Detection of dilated intrahepatic bile ducts in the fatty liver can also present problems, because the usual contrast between the normal liver and the echodense bile duct wall is lost.

## **DIAGNOSIS**

### **Liver biopsy**

The diagnosis of NASH can be established only by liver biopsy. The clinical setting, the risk factors, the laboratory data, and the

imaging studies can only be suggestive. In fact, all of these together remain insensitive and non-specific for the diagnosis. Moreover, none of these can distinguish benign steatosis from NASH.

### Indications

Because biochemical abnormalities and imaging findings have poor sensitivity in signaling the presence of NASH, the threshold for obtaining a liver biopsy to evaluate patients suspected of having NASH should be low. Early recognition of NASH by biopsy can provide the necessary incentive to achieve sustained lifestyle modifications and weight loss, which probably results in improved histology and prevention of progressive liver disease in selected patients. The decision of whom to biopsy is individualized and general recommendations cannot be made.

### Biopsy findings

The histologic findings in NASH are substantially similar to those typically found in alcoholic hepatitis and are reviewed in detail in Chapter 7. Characteristic lesions in both conditions are hepatocellular steatosis, lobular inflammation and ballooning degeneration; sometimes Mallory bodies and fibrosis are also found. The steatosis found in NASH is predominantly macrovesicular ( $>2\ \mu\text{m}$ ), with fat globules that laterally displace hepatocyte nuclei. By contrast, microvesicular fat is characterized by  $<1\ \mu\text{m}$  globules that do not displace the nucleus peripherally. The lobular distribution of fat can vary in NASH, with panlobular and perivenular localization being the most common patterns. In situations where identification of fat is essential to convincingly identify microvesicular steatosis, a properly processed biopsy must be stained with a fat-specific stain such as Oil red O. Oil red O specifically highlights fat as dark red globules on a pale background.

Inflammation is an essential component in NASH. The absence of the characteristic inflammation in a fatty liver is the hallmark of benign steatosis. The inflammatory cells of NASH are typically a mixture of neutrophils and mononuclear cells and are found throughout the lobule. Although the presence of neutrophils is a characteristic finding, neutrophils are not usually the predominant inflammatory cell type. Nonetheless, focal neutrophilic infiltrates can be found in areas of focal necrosis.

The presence of Mallory bodies and glycogen nuclei are variable findings in NASH biopsies. Mallory bodies are aggregates of cytoskeletal proteins and can be found in some NASH biopsies. Mallory bodies appear as eosinophilic, stranded structures in the hepatocyte cytoplasm and are identified most easily in ballooned hepatocytes. The presence of Mallory bodies is not a required histologic feature of NASH, and they are often small compared with the Mallory bodies found in alcoholic hepatitis. The presence of large or antler-shaped Mallory bodies should raise the suspicion of surreptitious alcohol abuse, because these more obvious accretions are typical of alcoholic liver disease. In contrast, glycogen nuclei are usually seen more frequently in NASH than in alcoholic liver disease. The central accumulation of glycogen in hepatocyte nuclei makes the nuclei appear ring-like around a clear central region. The cause of glycogen nuclei is uncertain, but the identity of the nuclear abnormality has been confirmed by electron microscopy. It is a non-specific finding common in diabetes, Wilson disease and other disorders.

The identification of fibrosis in NASH is undoubtedly the most worrisome finding, because it predicts the potential for progression to cirrhosis in a given patient. The pattern of early fibrosis in NASH is similar to that seen in alcoholic liver disease, with collagen around the central vein and in the zone 3 perisinusoidal spaces producing a chicken-wire appearance. The overall prevalence of fibrosis in patients with NASH is uncertain; most earlier surveys were conducted before hepatitis C could be excluded serologically as a contributing factor; however, recent studies indicate that fibrosis is found in approximately 15–30% of patients.

## TREATMENT AND PROGNOSIS

### Lifestyle modification

Lifestyle modification means adopting a daily routine that includes more physical activity. The Diabetes Prevention Program trial clearly demonstrated the benefit of lifestyle modification in people at risk for developing diabetes. The goal in patients with NAFLD is to improve insulin sensitivity, reduce the degree of hyperinsulinemia, and thereby reduce hepatic fat accumulation. How much exercise is needed to achieve this salutary effect is debated. Achievable goals such as 0.5–1 h of an activity strenuous enough to raise the heart rate has been proposed as a means of preventing and treating diabetes and reducing the risk of vascular disease. However, the studies performed to date also suggest that more exercise is even better. The key is for every person to find a level of regular activity that can be adopted indefinitely as a lifestyle while taking into account physical disabilities, availability and costs of various options, and personal preferences.

### Weight reduction

Weight loss can effectively normalize aminotransferases and reduce liver fat in obese adults and children who present with elevated aminotransferases and hepatic steatosis. The benefit of reducing or eliminating hepatic steatosis is twofold. If symptoms such as right upper quadrant abdominal pain are present, these can be improved or eliminated. More significantly in terms of overall prognosis is the potential to eliminate the risk of NASH and progressive liver disease.

The degree of weight loss needed to achieve normalization of serum aminotransferases and improvement in hepatic steatosis has not been rigorously established. Several studies have shown that only modest weight reduction is needed, but exact numbers are hard to come by.

The means by which weight is lost is also very important. Gradual weight reduction achieved while providing adequate nutrients such as essential amino acids in the form of complete protein, essential fatty acids and vitamins is crucial. Substantial caloric deficit can yield short-term weight loss, but the flux of fat from peripheral stores to the liver in the setting of inadequate essential amino acids and other key nutrients cause the delivery of fat to the liver to exceed its capacity to synthesize and secrete VLDL. The net result is worsening of the hepatic fat accumulation.

### Management of diabetes

The role of improved glycemic control to prevent NASH and progressive liver disease in patients with type 2 diabetes has



been overstated in the past. Weight reduction is much more important, and in the absence of significant weight reduction, type 2 diabetic patients typically fail to have improved liver enzymes despite treatment with insulin to achieve better control of blood glucose. The current understanding of the causative role of hyperinsulinemia in the pathogenesis of NASH is consistent with this observation. When choosing an initial treatment regimen for patients with diabetes and NASH, a reasonable choice may be agents that improve insulin sensitivity.

Prevention of frequent hyperglycemic episodes is effective in reducing hepatic steatosis in patients with type I diabetes. However, NASH is not a significant problem for these patients, and thus the need for better glycemic control with respect to liver disease is to reduce symptomatic hepatomegaly caused by the benign accumulation of hepatic triglycerides.

### **Additional management recommendations**

Several important issues commonly confront clinicians during the evaluation of management of NAFLD. A relatively common problem is that patients with NAFLD require potentially hepatotoxic medications such as the HMG-CoA inhibitors ('statins') to control hypercholesterolemia. There is no data that directly documents the safety of this class of drugs in patients with pre-existing NAFLD. However, there is also no evidence that such patients are at increased risk for idiosyncratic hepatotoxicity and the improved survival related to diminished vascular disease is clearly a benefit. A recommended approach is to use statins in these patients to treat hypercholesterolemia and to measure the aminotransferases at regular intervals as in any other patient. If the values rise substantially above the pre-existing elevations, then alternative means of controlling cholesterol elevations can be considered.

Acetaminophen is another potentially hepatotoxic drug often prescribed in patients with any form of underlying liver disease. Because hepatotoxicity has not been observed in patients taking less than 2000 mg of acetaminophen daily, instructing patients to not exceed this dose is a reasonable recommendation.

Hepatitis A rarely causes liver failure (see Ch. 14), but many of the patients who develop such severe liver injury with hepatitis A may have pre-existing liver disease. This higher risk has led to the recommendation that all patients with any form of chronic liver disease be vaccinated against hepatitis A. Therefore, a reasonable management strategy is to vaccinate all patients with NAFLD against hepatitis A.

### **Liver transplantation**

Liver transplantation is an option for selected patients who progress to decompensated cirrhosis. Between 2 and 10% of

transplants may be for patients with progressive liver disease caused by NASH if most patients with cryptogenic cirrhosis are included. Because the characteristic findings of NASH can resolve after the development of cirrhosis, patients may present with bland cryptogenic cirrhosis after decades of having NASH. Additional evidence linking abnormalities of hepatic fat metabolism and the development of cirrhosis is provided by studies showing a disproportionately high prevalence of mutations in the apoB-100 lipoprotein gene in patients with cryptogenic cirrhosis. Moreover, there are now case reports of NASH developing as a recurrent disease following liver transplantation, suggesting that peripheral metabolic abnormalities or infectious causes may contribute to NASH.

### **Unproved treatment strategies**

The increasingly frequent identification of patients who are lean and do not have identifiable and correctable risk factors for NASH has led to the search for alternative treatment strategies with the aim of preventing progressive liver disease. The use of antioxidants has been evaluated in several small trials. Vitamin E can normalize aminotransferases in children, but the experience in adults has been less encouraging. Betaine, a methyl donor that may serve as an antioxidant, showed some benefit in a small pilot study. Initial interest in ursodeoxycholic acid (UDCA) has been dampened by the negative findings of a multicenter, placebo-controlled trial. Because of the complexity of fat secretion from the liver, there are likely to be many different causes of steatosis (and by inference NASH) that will each require its own specific treatment. For example, choline is required for the synthesis of lecithin, a phospholipid necessary for VLDL formation and triglyceride secretion. Choline deficiency was a major cause of hepatic steatosis during prolonged administration of parenteral nutrition and supplementation of total parenteral nutrition (TPN) solutions with additional choline appears to prevent this adverse consequence. Pantothenic acid deficiency has also been proposed as a cause of hepatic steatosis. Pantothenic acid is a necessary nutritional precursor for the synthesis of CoA, and CoA is essential for hepatic fat metabolism. In one unblinded study, pantothenic acid supplementation was thought to cause histologic improvement in hepatic steatosis. Well-designed clinical studies are needed to define the people who might benefit from such therapeutic interventions.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 23

## Hereditary Hemochromatosis

Bruce R. Bacon

Our understanding of several aspects of hereditary hemochromatosis (HH) has greatly expanded over the last several years with the identification and cloning of the hemochromatosis gene (*HFE*), the use of genetic testing for diagnosis, family screening and population studies, the improved understanding of the normal physiology and pathophysiology of *HFE* and the discovery of several other new genes and proteins involved in iron homeostasis. Also, it has become increasingly apparent that iron plays an important role in various liver diseases other than hemochromatosis. For example, it is well established that there are abnormalities in serum markers of iron metabolism in about 40–50% of patients with alcoholic liver disease, porphyria cutanea tarda (PCT), chronic hepatitis C, and non-alcoholic steatohepatitis (NASH). Furthermore, a high prevalence of C282Y heterozygosity has been identified in patients with PCT and in some series of patients with NASH. This chapter reviews new developments in how patients with HH are diagnosed, the pathophysiologic mechanisms that have been elucidated by the recently identified gene and its gene product, and briefly the role of mild secondary iron overload in several additional liver diseases.

### IRON OVERLOAD SYNDROMES (Table 23.1)

The term hereditary hemochromatosis refers to the inherited syndromes that have been described over the last several years. In 1996, the gene for HH, called *HFE* (6p21.3) was identified allowing for genetic testing for the two major mutations (C282Y, H63D) that are responsible for *HFE*-related HH. Subsequently, several additional genes/proteins involved in the regulation of iron homeostasis have been identified contributing to a better understanding of cellular iron uptake and release. All of these inherited syndromes result in the inappropriately high absorption of iron by the gastrointestinal mucosa which leads to the pathologic deposition of excessive iron in the parenchymal cells of the liver, heart, pancreas and other organs. In all of these syndromes, the long-term complications of parenchymal iron deposition are cell and tissue damage, fibrosis and functional insufficiency. The liver is always the principal recipient of the majority of the absorbed iron, and the liver is always involved in symptomatic hemochromatosis.

The other inherited forms of iron overload which do not involve mutations in *HFE* include juvenile hemochromatosis and iron overload resulting from mutations in transferrin receptor 2, ferroportin 1 and African iron overload. Juvenile hemochromatosis is characterized by rapid iron accumulation. Mutations in two different genes have been shown to cause forms of juvenile hemochromatosis. The more common of these occurs in *HJV*, found on chromosome 1q21; this gene encodes a

protein called hemojuvelin whose function is currently unknown. Mutations in the hepcidin gene (*HAMP*; 19q13.1) also produce a form of juvenile HH; hepcidin is a hepatic peptide that acts to downregulate iron absorption. Mutations of the transferrin receptor 2 (*TfR2*) gene (7q22) produce an autosomal recessive form of HH that is clinically similar to *HFE*-related HH. It is not yet known how these *TfR2* mutations result in iron overload, but it is possible that they cause abnormal iron sensing by hepatocytes, the predominant site of *TfR2* expression. A rare autosomal dominant form of HH results from missense mutations in the ferroportin 1 gene (*SLC40A1*, 2q32). While iron loading of periportal hepatocytes is observed, there is also iron accumulation in reticuloendothelial cells. The mechanism of iron overload caused by mutated ferroportin 1 is unclear. African iron overload occurs primarily in sub-Saharan Africa and is now considered to be the result of a non-*HFE*-related genetic trait that can be exacerbated by dietary iron loading. Some individuals manifesting African iron overload consume an iron-rich fermented maize beverage, but iron overload can also occur in individuals 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 reticuloendothelial iron is greater and the ratio of splenic iron concentration to hepatic iron concentration is higher in African iron overload. In some cases, iron loaded Kupffer cells are prominent in African iron overload while they are relatively spared in *HFE*-related HH. It has been suggested that a similar form of iron overload may occur in African-Americans, but further investigations are needed to determine the genetic basis, prevalence, and clinical consequences of this condition. It has been reported that approximately 20% of a cohort of Africans and African-Americans with iron overload have a novel mutation in the ferroportin 1 gene, suggesting that this mutation may contribute to iron loading.

There are several other clinically distinct syndromes of iron overload that should be distinguished from these inherited syndromes of iron overload. These are grouped as secondary iron overload where there is an increase in absorption of intestinal iron that is promoted by an underlying condition other than HH. Examples of such underlying conditions are anemia from ineffective erythropoiesis such as in thalassemia, aplastic anemia and sideroblastic anemia. Parenteral iron overload is always iatrogenic and is caused by red blood cell transfusions or iron dextran injections. Some patients who have ineffective erythropoiesis and thus secondary iron overload on that basis can be complicated further by having parenteral iron overload when they require blood transfusions. Many patients with various types of chronic liver disease have mild to moderate degrees of secondary iron overload. Occasionally, this is related



Classification of iron overload syndromes
<b>Hereditary hemochromatosis</b>
• <i>HFE</i> -related (Type 1)
– C282Y/C282Y
– C282Y/H63D
– Other <i>HFE</i> mutations
• Non- <i>HFE</i> -related
– Juvenile HH
• <i>HJV</i> -Hemojuvelin (Type 2, subtype A)
• <i>HAMP</i> -Hepcidin (Type 2, subtype B)
– <i>TfR2</i> -related HH (Type 3)
– Ferroportin 1-related HH (Type 4)
– African iron overload
<b>Secondary iron overload</b>
• Iron-loading anemias
– Thalassemia major
– Sideroblastic anemia
– Chronic hemolytic anemia
– Aplastic anemia
– Pyruvate kinase deficiency
– Pyridoxine-responsive anemia
• Parenteral iron overload
– Red blood cell transfusions
– Iron-dextran injections
– Long-term hemodialysis
• Chronic liver disease
– Porphyria cutanea tarda
– Hepatitis C
– Hepatitis B
– Alcoholic liver disease
– Non-alcoholic steatohepatitis
– Following portocaval shunt
• Dysmetabolic iron overload syndrome
<b>Miscellaneous</b>
• Neonatal iron overload
• Aceruloplasminemia
• Congenital atransferrinemia

**Table 23.1** Classification of iron overload syndromes.

to heterozygosity for *HFE* mutations, but often mutation analysis is unrevealing.

Neonatal iron overload is a rare disorder that has been recognized over the last 15 to 20 years. It is thought by some to

be caused by an intrauterine hepatic viral infection that results in an excessive uptake of iron into the fetal liver, which, in the absence of mature cytoprotective mechanisms, can result in liver failure, and is often fatal without liver transplantation.

## ***HFE*-RELATED HEREDITARY HEMOCHROMATOSIS**

*HFE*-related hereditary hemochromatosis is a common disorder of iron metabolism that affects approximately 1 in 200–250 individuals of northern European descent. With the advent of genetic testing, *HFE*-related HH is frequently identified in asymptomatic probands and in presymptomatic relatives of patients with the disease. Accordingly, a genetic diagnosis can be legitimately applied to individuals who have not yet developed any of the toxic consequences of iron overload in tissues. In fact, recent population studies have demonstrated that up to 30% of *HFE*-related HH patients who are C282Y homozygotes, have no phenotypic expression. This observation has led to the definition of different stages of recognition of hemochromatosis.

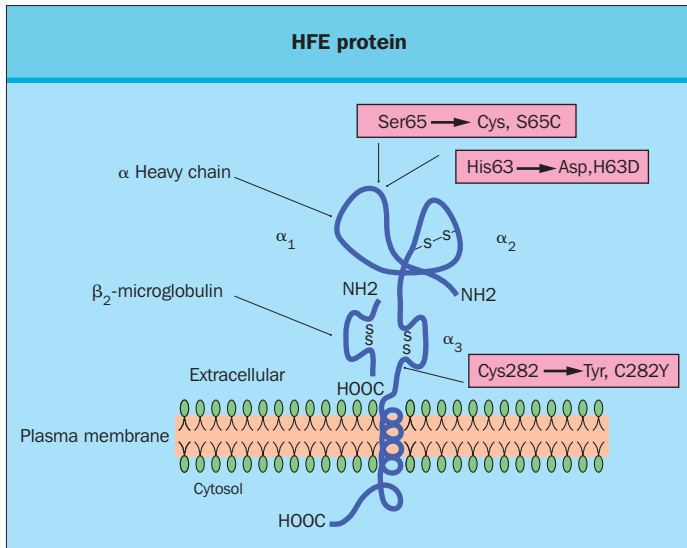
- Stage 1 refers to those patients with the genetic disorder and no increased iron stores.
- Stage 2 refers to those with the genetic disorder who have phenotypic evidence of increased iron stores but without tissue damage.
- Stage 3 refers to those who have what is mentioned above in stages 1 and 2 but who also have organ damage.

Thus, it is not justified to confine the diagnosis of hemochromatosis only to those individuals who are either symptomatic or who manifest organ damage such as cirrhosis, diabetes, heart failure, arthritis or skin pigmentation. Over the past several years, this concept has increased awareness of the disease, leading to earlier diagnosis and treatment, and presumably this has led to reduced morbidity and increased survival for patients with HH.

## **GENETICS OF *HFE*-RELATED HH**

*HFE* encodes for a major histocompatibility complex (MHC) class I-like molecule that requires interaction with  $\beta_2$ -microglobulin ( $\beta_2m$ ) for normal presentation on the surface of cells. The protein has a signal peptide binding domain, an immunoglobulin-like domain, a single transmembrane region, and a short cytoplasmic tail (Fig. 23.1). At least three missense mutations have been identified in *HFE*. One results in a change of cysteine to tyrosine at position 282 (C282Y); the second results in a change in histidine to aspartate at amino acid position 63 (H63D); the third results in a change of serine to cysteine at amino acid position 65 (S65C).

In the original study by Feder et al (1996) describing the identification of the *HFE* gene, 83% (148 of 178) of typical phenotypic patients with HH were homozygous for the C282Y mutation. There were an additional eight patients (4%) who were compound heterozygotes where one allele contained the C282Y mutation and the other allele contained the H63D mutation. Numerous additional studies have confirmed these observations showing homozygosity for the C282Y mutation in 64–100% of patients with typical phenotypic HH from the USA, France, Australia, Italy, Canada, Austria and other parts



**Figure 23.1 HFE protein.** Model based on its homology with major histocompatibility complex (MHC) class-I molecules. The HFE protein is a single polypeptide with three extracellular domains that would be analogous to the  $\alpha_1$ ,  $\alpha_2$  and  $\alpha_3$  domains of the other MHC class-I proteins.  $\beta_2$ -Microglobulin is a separate protein and interacts with the HFE gene product in a non-covalent manner in the  $\alpha_3$  homologous region. The approximate locations of the C282Y (Cys282Tyr), H63D (His63Asp) and S65C (Ser65Cys) mutations are shown. Modified from Feder et al (96).

of the world. In a case series from Australia, 100% of patients with HH who had a family history of iron overload were homozygous for the C282Y mutation. Conversely, in the study from Italy, only 64% of patients were homozygous for C282Y; however, the patient population evaluated in this study included patients with thalassemia, and with chronic liver disease due to hepatitis C and alcoholism, suggesting that perhaps some of these patients, in fact, had secondary iron overload. Alternatively, the frequency of C282Y may not be as high in southern Italian populations. Compound heterozygotes comprise approximately 3–5% of patients in most studies of hemochromatosis patients. It is generally agreed that when surveying white patients with iron overload, about 85–90% will be C282Y homozygotes. A few will be compound heterozygotes and the remainder most likely will have another genetic or acquired cause for developing iron overload. Routine genetic testing is not yet readily available for mutations in ferroportin 1, transferrin receptor 2, hepcidin or hemojuvelin to determine if any of these patients have one of these other mutations. Additionally, other abnormalities may be identified that result in alterations in iron homeostasis.

In prospective population studies, C282Y homozygosity is found in about 1 in 250 (0.4% of the population) individuals (Table 23.2). Compound heterozygosity (C282Y/H63D) is about four times more common (1–2% of the general population); in these prospective population studies, no C282Y/H63D patient had a significant degree of iron loading. Thus, compound heterozygotes can become iron loaded but the frequency with which this occurs is very low. The frequency with which the S65C mutation is found is not completely understood.

**Prevalence of C282Y homozygotes without iron overload in screening studies**

Population sample	Country	n	C282Y homozygotes	C282Y homozygotes with a normal ferritin (%)
Electoral roll	New Zealand	1064	1 in 213	40
Primary care	USA	1653	1 in 276	50
Epidemiologic survey	Australia	3011	1 in 188	25
Blood donors	Canada	4211	1 in 327	81
General public	USA	41 038	1 in 270	33
Total		50 977	1 in 255	46

**Table 23.2 Prevalence of C282Y homozygotes without iron overload in screening studies.**

## **PATHOPHYSIOLOGIC MECHANISMS IN HFE-RELATED HH**

The major mutation responsible for HFE-related HH results in the substitution of tyrosine for cysteine at amino acid 282 in the  $\alpha_3$  loop (C282Y) of HFE and abolishes the disulfide bond in this domain (see Fig. 23.1). Loss of this disulfide bond interferes with the interaction of HFE protein with  $\beta_2m$ , and the C282Y mutant protein demonstrates decreased presentation at the cell surface, increased retention in the endoplasmic reticulum and accelerated degradation. The HH-like phenotype of  $\beta_2m$ -knockout mice provides independent evidence of the importance of the HFE protein– $\beta_2m$  association for normal HFE protein function. The second (H63D) and third (S65C) mutations associated with HFE have little biologic impact when compared with C282Y.

The first mechanistic link between HFE protein and cellular iron metabolism resulted from the observation that HFE protein with  $\beta_2m$  forms a complex with the transferrin receptor (TfR1). The physical association of HFE protein with TfR1 has been observed in cultured cells and in duodenal crypt enterocytes, the site of regulation of dietary iron absorption. The observation that HFE protein and TfR1 are physically associated has led to a number of investigations on the effect of HFE protein on TfR1-mediated iron uptake and cellular iron status. These studies have yielded somewhat conflicting results concerning the effects of HFE protein on cellular iron metabolism, possibly due to differences in methodology; additional research will be required to resolve this issue especially in important sites such as the duodenum, liver and in the reticuloendothelial system.

An increase in intestinal iron absorption is a characteristic feature of HFE-related HH. Nearly all absorption of dietary iron occurs in the duodenum, where iron may be taken up either as ionic iron or as heme. Both forms of iron absorption are increased in patients with HFE-related HH. Ionic iron crosses the enterocyte in two stages:

- uptake across the apical membrane, and
- transfer across the basolateral membrane.

Prior to uptake, ionic iron is reduced from the ferric to the ferrous state. This is accomplished by the Dcytb ferric reductase which is expressed on the luminal surface of duodenal enterocytes. The ferrous iron crosses the apical membrane via the divalent metal transporter 1 (DMT1). Iron taken up by the enterocyte may be stored as ferritin or transferred across the basolateral membrane to the plasma. This latter process occurs via the iron transporter ferroportin 1. The basolateral transfer of iron requires oxidation of iron to the ferric state by the molecule hephaestin. In addition to an increased uptake of iron from the diet, patients with *HFE*-related HH demonstrate increased basolateral transfer of iron from the enterocyte to the plasma. Data suggest that the increased basolateral transfer of iron may be the driving force behind the increased intestinal iron absorption observed in *HFE*-related HH. Some studies on patients with *HFE*-related HH and *Hfe* knockout mice have demonstrated increased expression of mRNAs encoding Dcytb, DMT1 and ferroportin 1.

Two different (but not mutually exclusive) hypotheses have been proposed to explain the effect of mutant *HFE* on intestinal iron absorption:

- the hepcidin hypothesis, and
- the duodenal crypt cell programming hypothesis.

These hypotheses differ in the proposed cell type in which loss of functional *HFE* protein leads to downstream changes in body iron homeostasis. For the hepcidin hypothesis, the cell type is the hepatocyte, while for the crypt cell programming hypothesis, it is the duodenal crypt enterocyte. However, it is possible that *HFE* expression in another cell type (e.g. Kupffer cells) is also important.

Patients with *HFE*-related HH have excessive dietary iron absorption relative to body iron stores. This suggests that normal function of at least a component of the iron stores regulator is abnormally regulated in HH. Several lines of evidence point to hepcidin as a candidate. Mice in which the hepcidin gene has been knocked out develop a phenotype very similar to *Hfe* knockout mice, including hepatic iron loading, elevated transferrin saturation, and reticuloendothelial iron sparing. Furthermore, hepcidin is an acute-phase reactant, and plays a central role in the hypoferremia of inflammation (i.e. anemia of chronic disease). Interestingly, the changes in iron homeostasis observed during inflammation are the converse of those observed in *HFE*-related HH. Indeed, patients with *HFE*-related HH have decreased hepatic expression of hepcidin, as do *Hfe* knockout mice, despite excess hepatic iron stores. Furthermore, patients carrying mutations in the hepcidin gene have a severe form of juvenile HH. Why hepcidin is inappropriately under expressed with loss of functional *HFE* protein is uncertain. One possibility is that *HFE* protein is a participant in the process by which hepatocytes sense iron status. The preponderance of current data indicates that abnormally low levels of hepcidin play an important role in the pathogenesis of *HFE*-related HH.

An alternative hypothesis to explain the increase in iron absorption in *HFE*-related HH is the duodenal crypt cell programming hypothesis. Expression of *HFE* protein in association with TfR1 has been demonstrated in duodenal crypt cells. These observations have led to the proposal that *HFE* protein

influences the sensing of circulating iron by the duodenal crypt cells. This model postulates that normal *HFE* protein in duodenal crypt cells facilitates the uptake of plasma iron by the TfR1-mediated pathway, and that mutant *HFE* protein lacks this facilitating effect. Functional loss of *HFE* protein may thereby decrease the regulatory iron pool in crypt cells, resulting in a relatively iron-deficient state in these cells. In turn, this could result in the increased expression of iron transport genes in daughter villus enterocytes and lead to increased dietary iron absorption.

The precise role of *HFE* in regulating iron homeostasis remains an area of intensive investigation. Ultimately, testing these two hypotheses will require an examination of the consequences of mutant *HFE* protein specifically in hepatocytes and in duodenal crypt enterocytes.

CLINICAL FEATURES OF HH

HH is still infrequently recognized and under diagnosed mainly because physicians often feel that it is a rare disorder that may only be manifested by the classical triad of clinical findings consisting of increased skin pigmentation, diabetes, and cirrhosis. C282Y homozygosity is found in about 1 in 250 individuals of northern European descent (Table 23.2) with the heterozygote frequency of approximately 1 in 10 individuals. Thus, hemochromatosis is a very common inherited abnormality in Caucasian populations. Our understanding of HH has evolved dramatically over the last 50 years. Series of HH patients reported in the 1950s to the 1980s described most patients with the classic symptoms and findings of advanced hemochromatosis (Table 23.3). Then in the 1990s, HH was increasingly identified by evaluating patients who had abnormal iron studies on routine serum chemistry panels or by performing iron studies in a patient who had a family history of HH. When series of patients were described who were identified in this way, the majority of them (approximately 75%) were asymptomatic, and did not exhibit any of the end-stage manifestations of

Symptoms in patients with hereditary hemochromatosis
Asymptomatic <ul style="list-style-type: none"><li>• Abnormal serum iron studies on routine screening chemistry panel</li><li>• Evaluation of abnormal liver tests</li><li>• Identified by family screening</li><li>• Identified by population screening</li></ul>
Non-specific, systemic symptoms <ul style="list-style-type: none"><li>• Weakness</li><li>• Fatigue</li><li>• Lethargy</li><li>• Apathy</li><li>• Weight loss</li></ul>
Specific, organ-related symptoms <ul style="list-style-type: none"><li>• Abdominal pain (hepatomegaly)</li><li>• Arthralgias (arthritis)</li><li>• Diabetes (pancreas)</li><li>• Amenorrhea (cirrhosis)</li><li>• Loss of libido, impotence (pituitary, cirrhosis)</li><li>• Congestive heart failure (heart)</li><li>• Arrhythmias (heart)</li></ul>

Table 23.3 Symptoms in patients with hereditary hemochromatosis.



hemochromatosis. About 25% of patients had increased fatigue which improved with treatment and a lesser proportion had arthritis, liver disease or diabetes. We now know, with numerous prospective population studies that have recently been described, that up to 30% of C282Y homozygotes have no phenotypic expression at all (stage 1), with normal iron studies and no symptoms (Table 23.2). Moreover, in some studies, fewer than 10% of C282Y homozygotes have evidence of iron mediated disease (i.e. tissue damage) of any kind. Nonetheless, it is important for clinicians to realize that because HH is so common that when a patient does have the symptoms of HH and/or an elevated transferrin saturation level or an elevated ferritin level they should be considered for the diagnosis and be appropriately evaluated.

When patients do present with symptoms, consideration of hemochromatosis should be given for symptoms of fatigue, right upper quadrant abdominal pain, arthralgias, impotence, decreased libido, symptoms of heart failure or diabetes (Table 23.3). Similarly, findings of hepatomegaly, cirrhosis, extra-hepatic manifestations of chronic liver disease, testicular atrophy, signs of congestive heart failure, skin pigmentation, and arthritis should raise the suspicion of HH (Table 23.4). Needless to say, many of the aforementioned symptoms and signs are also indicative of disease processes other than HH, but when systematic studies of arthritis clinics or diabetes clinics have looked specifically for HH, previously undiagnosed cases have been identified, often to the surprise of the clinician caring for those patients. These studies illustrate the need to consider HH in patients who present with the symptoms and signs known to occur in established HH. Currently, many new patients come to medical attention because of screening, either by dedicated primary care physicians or because of a positive family history.

In older series of patients with HH, when patients were identified by symptoms or findings of the disease, women typically presented about 10 years later than men and there

were about 10 times the number of men presenting as women, presumably because of a 'protective effect' of menstrual blood loss and iron loss during pregnancy. More recently, when greater proportions of patients were identified by screening blood studies or by family screening studies, the age of diagnosis for women was found to be approximately equivalent to that for men and the number of men identified was roughly equivalent to the number of women identified. In the face of abnormal iron studies, clinicians should not wait for typical symptoms or findings of HH before considering the diagnosis of HH.

## DIAGNOSIS

Once the diagnosis of HH is considered, either by an evaluation of abnormal screening iron studies, in the context of family studies, in a patient with an abnormal genetic test, or in the evaluation of a patient with any of the aforementioned symptoms or clinical findings, definitive diagnosis is relatively straightforward. Delayed diagnosis most often comes as a result of a physician not considering HH as a possibility, thinking that it is a rare disorder. Once considered, fasting transferrin saturation [serum iron divided by total iron binding capacity (TIBC) or transferrin  $\times$  100%] and ferritin levels should be obtained. Both of these will be elevated in a symptomatic patient (Table 23.5). Transferrin saturation should be measured in the fasting state, because serum iron levels have a diurnal variation and can be increased following a meal. Unfortunately, the sensitivity and specificity of transferrin saturation and ferritin levels become problematic when young individuals are being evaluated or when patients have abnormal iron studies in the context of other diseases. In the absence of HH, serum iron studies (predominantly ferritin) can be abnormal in about 40–50% of patients with chronic viral hepatitis, NASH, and alcoholic liver disease. Additionally, because ferritin is an acute phase reactant, other inflammatory disorders (e.g. rheumatoid arthritis) and various neoplastic diseases (e.g. lymphoma, other cancers) can cause elevated serum ferritin levels in the absence of iron overload. Thus, serum iron studies alone have many false-positive and false-negative results in predicting iron stores and reliance on these studies by themselves for diagnosis of HH can result in errors of diagnosis. As a result, the development of a widely available genetic test has contributed to better characterization of those patients with underlying liver disease and abnormal serum iron studies. Nonetheless, in the absence of any other medical illness, the combination of an elevated transferrin saturation level and an elevated ferritin level is approximately 90% sensitive and specific for the diagnosis of HH.

Currently, if either a fasting transferrin saturation or ferritin level is elevated, regardless of what has led to it being obtained, mutation analysis for *HFE* should be performed. If the patient is a C282Y homozygote or a compound heterozygote (C282Y/H63D) with normal liver enzymes and abnormal iron studies, then a diagnosis of *HFE*-related HH can be made and phlebotomy therapy can be initiated. If the patient's liver enzymes are elevated or if the serum ferritin is  $>1000$  ng/mL, then the patient should be considered for a liver biopsy. Several studies have shown that significant degrees of hepatic fibrosis (stage 3 or stage 4) will be missed by not performing a liver biopsy in patients who have ferritin levels  $<1000$  ng/mL and liver enzymes that are normal. Conversely, not all patients with

### 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, etc.
- Joints
  - Arthritis
  - Joint swelling
- Heart
  - Dilated cardiomyopathy
  - Congestive heart failure
- Skin
  - Increased pigmentation
- Endocrine
  - Testicular atrophy
  - Hypogonadism
  - Hypothyroidism

**Table 23.4** Physical findings in patients with hereditary hemochromatosis.

Laboratory findings in patients with hereditary hemochromatosis			
Measurements	Normal subjects	Patients with hereditary hemochromatosis Asymptomatic	Symptomatic
Blood (fasting)			
Serum iron level (µg/dL)	60–80	150–280	180–300
Serum transferrin level (mg/dL)	220–410	200–280	200–300
Transferrin saturation (%)	20–50	45–100	80–6000
Serum ferritin level (ng/mL)			
Men	20–200	150–1000	500–6000
Women	15–150	120–1000	500–6000
Genetic (HFE mutation analysis)			
C282Y/C282Y	wt/wt	C282Y/C282Y	C282Y/C282Y
C282Y/H63D*	wt/wt	C282Y/H63D	C282Y/H63D
Liver			
Hepatic iron concentration			
µg/g, dry weight	300–1500	2000–10 000	8000–30 000
µmol/g, dry weight	5–27	36–179	140–550
Hepatic iron index **	<1.0	>1.9	>1.9
Liver histology			
Perls' Prussian blue stain	0–1+	2+ to 4+	3+, 4+
* Compound heterozygote			
** Hepatic iron index (HII) is calculated by dividing the hepatic iron concentration (in µmole/g, dry weight) by the age of the patient (in years). With increased knowledge of genetic testing results in patients with iron overload, the utility of HII has diminished.			

Table 23.5 Laboratory findings in patients with hereditary hemochromatosis.

either elevated liver enzymes or a ferritin >1000 ng/mL, will have advanced fibrosis on biopsy. When liver biopsy is performed, a histochemical stain for iron (Perls' Prussian blue stain) should be requested, and it is reasonable to request a biochemical determination of hepatic iron concentration (HIC). With the measured HIC, the hepatic iron index (HII) can be calculated. The HII is the HIC (in µmol/g dry liver) divided by the age of the patient (in years). HII is no longer as useful as it was prior to the availability of genetic testing when an HII of >1.9 was considered diagnostic of HH. Additionally, liver biopsy can provide an assessment of the degree of fibrosis and whether or not cirrhosis is present and of other histologic abnormalities such as steatosis. From a prognostic standpoint, the presence of advanced fibrosis or cirrhosis is important to establish, since the risk of hepatocellular carcinoma is significantly increased in those patients who have established cirrhosis.

Histologically, iron deposition is found in higher levels in periportal (zone 1) hepatocytes and there is a periportal to pericentral gradient (Fig. 23.2). On higher power, iron is seen predominantly in parenchymal cells, with sparing of Kupffer cells (Fig. 23.3). When early diagnosis is missed and treatment is not performed, patients can progress to micronodular cirrhosis and liver failure (Fig. 23.4). In symptomatic patients, the HIC will typically be above 10 000 µg/g dry weight (normal <1500 µg/g dry weight) and values as high as 40 000 µg/g can be seen. In patients with secondary iron overload related to alcoholic liver disease, chronic viral hepatitis or NASH, the HIC is rarely as high as 10 000 µg/g and is more commonly in the 2000 to 3000 µg/g range. With genetic testing now identifying patients prior to the development of any phenotypic evidence of iron overload (normal transferrin saturation and normal ferritin) liver biopsy is less frequently performed. In this setting, if liver

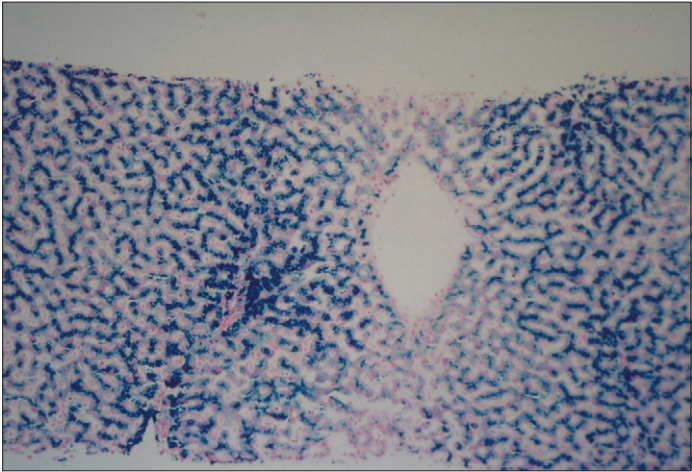


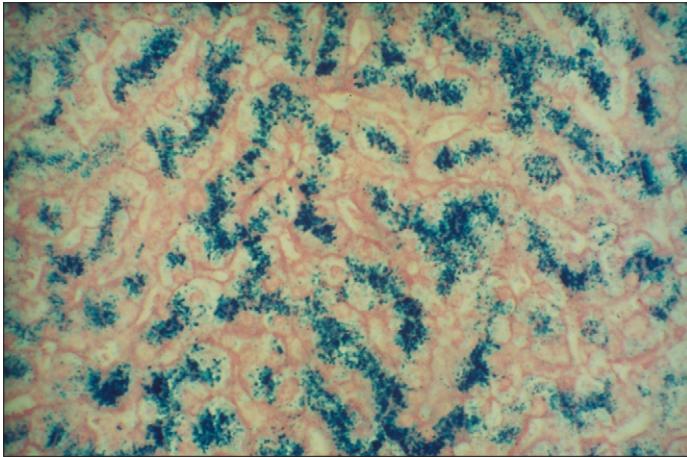
Figure 23.2 Liver histology in hereditary hemochromatosis. At low power, increased iron deposition can be seen in a periportal distribution (Perls Prussian blue stain, original magnification × 40).

biopsy was performed, it is expected that iron stains would be completely normal or negative.

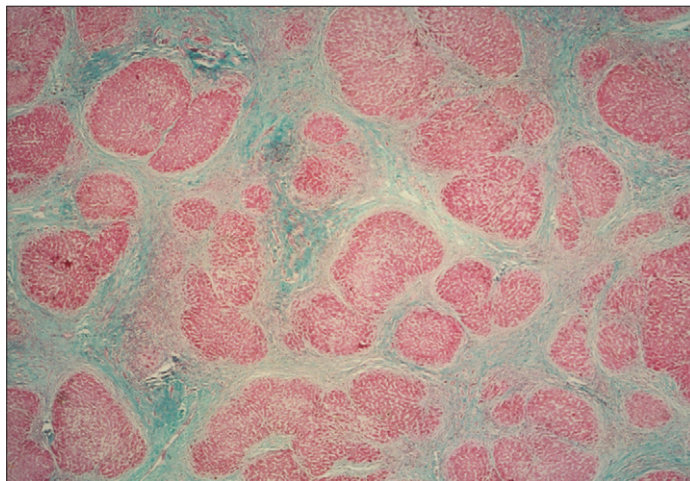
TREATMENT OF HH

Once the diagnosis of HH is established, physicians must ensure that patients are adequately treated and that family screening is performed. Treatment is simple, effective, inexpensive and safe. Patients should be encouraged to initially have weekly therapeutic phlebotomy of 500 mL of whole blood. This is equivalent to approximately 200–250 mg of iron, depending





**Figure 23.3 Liver histology in hereditary hemochromatosis.** At higher power, the iron deposits can be seen predominately in hepatocytes in a pericanalicular distribution (Perl's Prussian blue stain, original magnification  $\times 200$ ).



**Figure 23.4 Liver histology in hereditary hemochromatosis.** At low power, micronodular cirrhosis is seen in this sample from an explant liver from a patient who received an orthotopic liver transplant for complications of end-stage liver disease due to hemochromatosis (Masson trichrome stain, original magnification  $\times 10$ ).

#### Response to phlebotomy therapy in hereditary hemochromatosis

- Reduction of excessive tissue iron stores
- Improved survival, if diagnosis and treatment occur before development of cirrhosis and diabetes
- Improved sense of well-being, energy level
- Improved cardiac function
- Improved control of diabetes
- Reduction in abdominal pain
- Reduction in skin pigmentation
- Normalization of elevated liver enzymes
- Reversal of hepatic fibrosis (approximately 30% of cases)
- No reversal of established cirrhosis
- Reduction in portal hypertension in cirrhotics
- No (or only minimal) improvement in arthropathy
- No reversal of testicular atrophy

**Table 23.6 Response to phlebotomy therapy in hereditary hemochromatosis.**

rather, the desired endpoint is for them to be depleted of their excess iron stores. Most patients tolerate therapeutic phlebotomy quite well and actually have a sense of improved well-being after the initial phlebotomies have been completed. If liver enzymes have been abnormal, they will characteristically return to normal once iron stores have been depleted; however, established cirrhosis will not reverse. There are some reports of portal fibrosis improving with phlebotomy therapy. Other benefits of therapeutic phlebotomy include: reduction of skin pigmentation, improvement in cardiac function, reduction in insulin requirements in those patients who are diabetic, reduction in portal hypertension in those patients who are cirrhotic, reduction in abdominal pain, and an improved energy level and sense of well being (Table 23.6). Conditions that characteristically do not reverse with phlebotomy include testicular atrophy, established cirrhosis and arthropathy. Furthermore, the risk of hepatocellular carcinoma does not decrease with phlebotomy if the patient already has cirrhosis.

Once the initial therapeutic phlebotomy has been completed and patients have been successfully depleted of their excess iron stores, then most individuals will require maintenance phlebotomy of one unit of blood to be removed every 2 to 3 months. Because most patients with HH absorb approximately 2 to 3 mg of iron per day in excess of their daily requirements, they will accumulate an excess of approximately 250 mg of iron over a 3-month period. This is balanced by the 250 mg of iron that is removed with a single phlebotomy; thus, maintenance phlebotomy every 3 months is usually adequate. Some patients absorb more than the 2–3 mg of iron per day and thus require maintenance phlebotomy more often. It is unusual for patients to require maintenance phlebotomy more often than every 2 months. Occasionally, a few patients who have been accurately diagnosed (homozygosity for the C282Y mutation) do not reaccumulate iron for reasons that are unclear. These patients are usually older and the presumption is that their efficiency of iron absorption has been diminished with age; however, when this situation occurs, patients should be evaluated for occult blood loss from the gastrointestinal tract.

upon the hemoglobin concentration of the blood removed. Some patients can tolerate twice weekly phlebotomy, and reports from older literature describe patients who tolerated phlebotomy three times per week; however, this is tedious and often inconvenient. Monthly phlebotomy should not be recommended unless patients cannot tolerate phlebotomy more frequently. Therapeutic phlebotomy should be performed until patients develop iron-limited erythropoiesis, which is identified by the failure of the hemoglobin or hematocrit level to recover before the next phlebotomy. It is reasonable to monitor transferrin saturation and ferritin levels periodically to predict the return to normal iron stores and to provide a method of encouragement to patients who are undergoing phlebotomy. Therapeutic phlebotomy is continued until the transferrin saturation is  $<50\%$  and serum ferritin levels are  $<50$  ng/mL; some clinicians favor bringing the ferritin level down to  $<20$  ng/mL. It is not necessary for patients to become anemic;



## SCREENING FOR HH

It is recommended that all first degree relatives of an identified proband be screened for hemochromatosis, including siblings, parents and children. Certainly, this recommendation could be extended to include aunts, uncles and cousins as well. Genetic testing with *HFE* mutation analysis has taken the place of human lymphocyte antigen (HLA)-haplotyping and is now used as the preferred method for family screening. Many physicians now obtain transferrin saturation, ferritin, and *HFE* mutation analysis all at the same time. If there is an elevated ferritin level indicating increased iron stores in a C282Y homozygote or a compound heterozygote, it is reasonable to proceed to therapeutic phlebotomy to deplete excess iron stores without a liver biopsy as long as liver enzymes are normal and the ferritin is <1000 ng/mL.

Similarly, when patients are identified by a positive genetic test in population screening outside of the context of a family history when there is an elevated transferrin saturation and/or ferritin level, they should be treated accordingly. If there is an increased ferritin level in the absence of elevated liver enzymes, patients should be offered treatment with therapeutic phlebotomy. A liver biopsy is not needed in this situation.

It has been debated as to whether genetic testing should be used as a general population screening technique. Several recent studies have shown that when *HFE* mutation analysis as well as iron studies have been done prospectively in large general populations, the prevalence of phenotypic expression in C282Y homozygotes has been around 50% (Table 23.2). Thus, a plan to screen the entire population would identify many individuals and 'label' them as C282Y homozygotes with the potential for genetic disease, which might never be realized. Theoretically, this could lead to genetic discrimination with possible psychological and/or economic consequences. At present, there are no recommendations for screening of the general population.

## IRON AND OTHER LIVER DISEASES

As mentioned previously, abnormally elevated serum iron studies have been described in about 40–50% of patients with alcoholic liver disease, chronic viral hepatitis and NASH. Additionally, it has been known for years that iron plays an important role in PCT. Some of these patients will have a mild increase in hepatic iron stores which is usually not to the degree of iron loading seen in typical *HFE*-related HH. Serum and hepatic iron studies are generally normal in patients with cholestatic liver diseases.

It has been recognized for years that serum ferritin levels are elevated in many patients with alcoholic liver disease in the absence of *HFE*-related HH. Liver biopsy with measurement of HIC and calculation of HII has been necessary in the past to distinguish HH from alcoholic siderosis. With the advent of genetic testing, it has been shown that the prevalence of either C282Y homozygosity or heterozygosity is no different in

alcoholic patients with or without secondary iron overload compared to controls. There has been no benefit derived from phlebotomy therapy to deplete iron stores in alcoholic patients with mild secondary iron overload.

It has been reported that 40–50% of patients with PCT have at least one allele with the C282Y mutation and many patients are in fact C282Y homozygotes. Thus, when evaluating someone with PCT, it is reasonable to request *HFE* mutation analysis. Regardless of these results, therapeutic phlebotomy remains a cornerstone in the therapy of PCT.

In patients with NASH, some studies have shown that about 30–40% of patients have at least one allele with the C282Y mutation indicating the importance of genetic testing in this disorder, particularly when there are abnormal blood iron studies. Perhaps of greater importance in the NASH studies, a higher frequency of fibrosis was found in those patients who were C282Y positive with mild degrees of iron overload, suggesting a synergistic effect between tissue iron stores and the development of increased fibrosis in these patients. Nonetheless, phlebotomy therapy has not yet been rigorously evaluated in patients with NASH.

Numerous studies of *HFE* mutation analysis in patients with chronic hepatitis C have shown that C282Y heterozygotes are found in the same frequency as in controls, and are often associated with abnormal iron studies (elevated ferritin and transferrin saturation). Some studies have shown a synergistic effect on the development of increased hepatic fibrosis. These findings raise the question of whether or not therapeutic phlebotomy would be beneficial for treatment of patients with chronic hepatitis C. Phlebotomy therapy has been used in the past as an adjunctive treatment in chronic hepatitis C in patients who failed to respond to interferon monotherapy and it has repeatedly been shown to result in a reduction in serum alanine aminotransferase (ALT) levels; however, phlebotomy does not have an antiviral effect. Beneficial effects of phlebotomy on liver histology in chronic hepatitis C are mild at best.

## SUMMARY

In summary, the discovery of *HFE* has led to an enhanced ability for clinicians to accurately diagnose patients with HH. The HII is no longer used to confirm a diagnosis of HH; instead, the diagnosis is made and confirmed by genetic testing with *HFE* mutation analysis. At some point, genetic testing should be commercially available for mutations in ferroportin, transferrin receptor 2, hepcidin, hemojuvelin, and other genes of iron metabolism. The need for liver biopsy in diagnosis is markedly reduced compared to before the availability of *HFE* mutation analysis. Treatment of HH remains straightforward, inexpensive, effective and simple. Interactions between iron and hepatitis C and NASH are still being clarified. The pathophysiologic mechanisms that occur in HH are increasingly being understood and at some time in the future it will be possible to apply this information to other liver diseases.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 24

## Wilson Disease

Peter Ferenci

### DEFINITION

Wilson disease is an autosomal recessive inherited disorder of copper metabolism resulting in pathologic accumulation of copper in many organs and tissues. The hallmarks of the disease are the presence of liver disease, neurologic symptoms and Kayser-Fleischer corneal rings. The familial nature of Wilson disease was recognized in the original description of this disease by Samuel Alexander Kinnier Wilson.

### EPIDEMIOLOGY

Until very recently, Wilson disease was believed to be very rare. By a population-based approach, the incidence of Wilson disease was estimated to be at least 1:30 000–50 000 (Ireland: 17/10<sup>6</sup> live births, former East-Germany: 29) with a gene frequency of 1:90 to 1:150. However, these estimations were mostly based on adolescents or adults presenting with neurologic symptoms. More recent data however indicate that neurologic symptoms occur only in about half of Wilson disease patients. Thus, the incidence of Wilson disease was underestimated by these studies. Furthermore, in selected populations (Jews from Uzbekistan, China) Wilson disease seems to be even more common. Among selected groups of patients Wilson disease is certainly more frequent. About 3–6% of patients transplanted for fulminant hepatic failure and 16% of young adults with chronic active hepatitis of unknown origin have Wilson disease.

### PATHOGENESIS

The basic defect in Wilson disease is the impaired biliary excretion of copper resulting in the accumulation of copper in various organs including the liver, the cornea and the brain. The consequence of copper accumulation is the development of severe hepatic and neurological disease. Copper's unique electron structure allows these cuproenzymes to catalyze redox reactions, but causes ionic copper to be very toxic, readily participating in reactions that promote the synthesis of damaging reactive oxygen species. Copper overload particularly affects mitochondrial respiration and causes a decrease in cytochrome C activity. Damage to mitochondria is an early pathologic effect in the liver. Damage to the liver has been shown to result in increased lipid peroxidation and abnormal mitochondrial respiration both in copper loaded dogs and in patients with Wilson disease. The mechanism(s) triggering copper-induced lipid peroxidation are unknown. However, it is conceivable that, due to hepatic copper accumulation, patients with Wilson disease are particularly sensitive to any oxidative stress.

The pathogenesis of neurologic disease is less clear. Neuronal damage is mediated by copper deposition in the brain. Copper may be directly toxic to neurons or may exert its effects by selective inhibition of brain monoamine oxidase A (MAO-A). Copper accumulation in the brain may be secondary to liver damage, but this hypothesis is inconsistent with the clinical observation that many patients with neurologic disease have only mild liver disease, and that conversely patients with advanced liver failure have no neurologic symptoms. Furthermore the preferential affection of basal ganglia cannot be explained. The discovery of the Wilson disease gene may help to better understand the pathophysiology of copper metabolism. ATP7B is also expressed in the brain, but its function is unknown. It is conceivable that increased copper uptake into the brain is a direct result of a certain mutation resulting in specific functional alterations of cerebral ATP7B.

### The Wilson disease gene

The Wilson disease gene was localized to human chromosome 13 by cosegregation of the disease with the red cell enzyme marker, esterase D, in several large Middle Eastern kindred. Further linkage analysis of additional families, using more polymorphic DNA markers defined a region close to the retinoblastoma locus as the candidate region for the Wilson disease gene. By positional cloning strategies, the Wilson disease gene was identified by three independent groups in 1993. Final identification was established with the use of conserved regions of the previously discovered Menkes disease (another disease with impaired copper metabolism) gene. The Wilson disease gene codes for a copper transporting P-type adenosine triphosphatase (ATPase), ATP7B.

Wilson disease protein contains the following functional domains and is thought to form an ion channel: six copper binding regions [Cu] containing Cys-X-X-Cys motifs at the amino terminal end; and adenosine triphosphate (ATP) binding, aspartyl kinase and phosphorylation domains. Alternatively spliced forms of Wilson disease protein lacking transmembrane sequences 3 and 4 (exon 8) are expressed in brain.

The functionally important regions of the Wilson disease gene are six copper binding domains, a transduction domain (amino acid residues 837–864; containing a Thr-Gly-Glu motif) involved in the transduction of the energy of ATP hydrolysis to cation transport, a cation channel and phosphorylation domain (amino acid residues 971–1035; containing the highly conserved Asp-Lys-Thr-Gly-Thr motif), an ATP-binding domain (amino acid residues 1240–1291) and eight hydrophobic transmembrane sequences (1–8), in one of which (region 6) is the Cys-pro-Cys sequence found in all P-type ATPases.



Common mutations of the Wilson disease gene in various populations		
	Most common mutation (exon)	Other common mutations
Central-, eastern-, northwestern Europe*	H1069Q	3400delC, exon 8 (multiple), P969Q
Sardinia	-441/-421 del (5' UTR)	2463delC, V1146M
Canary Islands	L708P	
Turkey	R969Q, A1003T	Exon 8, H1069Q,
Brazil	3400delC	
Saudi Arabia	Q1399R	
Far East	R778L	
* Russia, Bjelorus, Poland, Bulgaria, former Yugoslavia, Czech Republic, Slovakia, Hungary, Germany, Benelux, Greece (Ferenci P, unpublished data)		

**Table 24.1 Common mutations of the Wilson disease gene in various populations.**

Molecular genetic analysis of affected patients reveals over 200 distinct mutations (database maintained at the University of Alberta -<http://www.medgen.med.ualberta.ca>). Mutations include missense and nonsense mutations, deletions and insertions. Some mutations are associated with a severe impairment of copper transport resulting in severe liver disease very early in life; other mutations appear to be less severe with disease appearance in mid adulthood. While most reported mutations occur in single families, a few are more common. The His1069Gln (H1069Q) missense mutation occurs in 30–60% of patients of eastern-, northern- and central-European origin. It is less frequent in patients of Mediterranean descent and only rarely seen in patients of non-European origin. About 10% of patients of French or British extraction have a Gly1266Lys mutation in the ATP hinge domain. The 2299insC mutation can be detected in some patients of European and Japanese descent. The Arg778Leu mutation is present in 27% of Taiwanese patients, but is not found in non-Asian patients. In Sardinia two frameshift mutations (1515insT and 2464delC) are found in about 20% of patients. These mutations were not found in other populations (Table 24.1).

The study of genotype–phenotype correlations is hampered by the lack of clinical data, the rarity of some mutations and the high frequency of the presence of two different mutations in individual patients (compound heterozygotes) (Table 24.2). In an ongoing study involving 820 patients with Wilson disease mostly from Europe, mutations on both chromosomes were identified in 58% of the patients (Table 24.2), with at least one mutation in 30%. Sufficient information is available only for the H1069Q mutation. Homozygosity for H1069Q is associated with late onset neurologic disease. In contrast, patients with mutations in exon 8 commonly present with liver disease.

#### Hepatic copper metabolism and the role of ATP7B

Copper is an essential nutrient needed for such diverse processes as mitochondrial respiration (cytochrome C), melanin

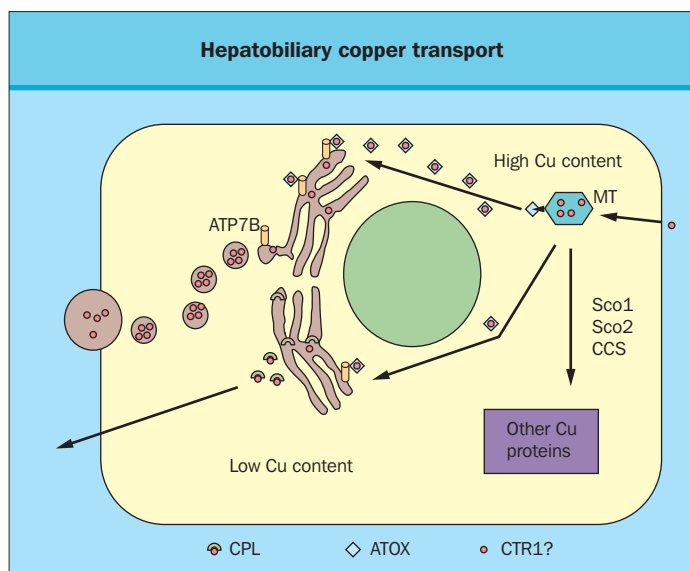
Phenotype-genotype correlation in 474 patients with both mutations of the Wilson disease gene identified			
Mutation	n	Percentage with liver disease	Mean age at onset
H1069Q/H1069Q	215	46	21.5 (liver:19.8; neuro: 24.6)
H1069Q/2299insC	24	70	17.4
H1069Q/3400delC	22	30	18.8
H1069Q/R969Q	14	92	24.1
H1069Q/Q1351X	11	37	18.9
H1069Q/W779X	10	60	19.5
G710S/G710S	8	50	17.9
H1069Q/G710S	6	82	22.2
H1069Q/K844Kfs	7	60	19.4

**Table 24.2 Phenotype-genotype correlation in 474 patients with both mutations of the Wilson disease gene identified.** (Ferenci, unpublished data.)

biosynthesis (tyrosinase), dopamine metabolism (DOPA- $\beta$ -mono-oxygenase), iron homeostasis (ceruloplasmin), antioxidant defense (superoxide dismutase), connective tissue formation (lysyl oxidase) and peptide amidation.

Dietary copper intake is approximately 1–2 mg/day. Quoted copper contents of foods are unreliable. While some foods, such as meats and shellfish, have consistently high copper concentrations, others such as dairy produce are consistently low in copper. However, the copper content of cereals and fruits varies greatly with soil copper content and the method of food preparation. Estimates of copper intake should include water copper content, the permitted upper concentration for copper in drinking water being 2 mg/L. The recommended intake in the first 6 months of life is 80 mg/kg/day. Thus, dietary copper intake far exceeds the trace amounts required. Approximately 10% of dietary copper is absorbed in the upper intestine and binds loosely to albumin, certain amino acids (histidine, cysteine and threonine) and peptides. Most of the ingested copper is taken up by the liver. The hepatic uptake of diet-derived copper appears to be an unsaturable, carrier-mediated, energy independent mechanism.

Specific pathways allow the intracellular trafficking and compartmentalization of copper, ensuring adequate cuproprotein synthesis while avoiding cellular toxicity (Fig. 24.1). After uptake by hepatocytes, copper is bound to metallothionein, a cytosolic, low molecular weight, cysteine-rich, metal binding protein. The copper stored in metallothionein can be donated to other proteins, either following degradation in lysosomes or by exchange via a glutathione (GSH) complex. Biliary excretion is the only mechanism for copper elimination, and the amount of copper excreted in the bile is directly proportional to the size of the hepatic copper pool. Trafficking of copper in hepatocytes is complex and involves several transport proteins: the copper transporter 1 (Ctr1) transports copper with high affinity in a metal-specific, saturable fashion at the hepatocyte plasma



**Figure 24.1 Hepatobiliary copper transport.** CTR1 = copper transporter 1, MT = metallothionein, CPL = ceruloplasmin. ATOX, Sco1, Sco2, CCS – copper chaperones.

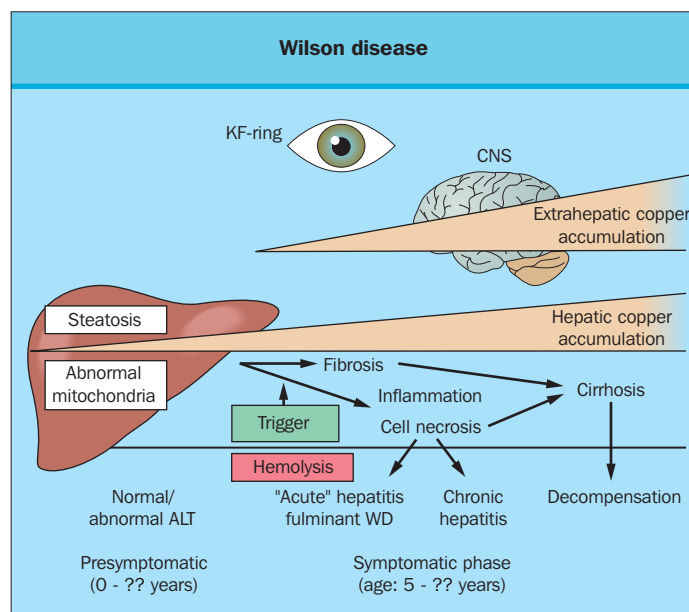
membrane. Metallothioneins, a group of cysteine-rich intracellular proteins capable of binding metal ions, including copper, cadmium, and zinc, have a critical role to protect intracellular proteins from copper toxicity. Metallochaperones transfer copper to the site of synthesis of copper containing proteins. The cytoplasmic copper chaperone atox1 is required for copper delivery to the Wilson ATPase (ATP7B) in the hepatocyte secretory pathway by direct protein–protein interaction. In the copper-limiting environment of the cell, the delivery of copper by atox1 is responsible for initiating the catalytic activity and the intracellular trafficking of ATP7B. ATP7B is the gene product of the Wilson disease gene (Fig. 24.1) located on chromosome 13 and is a polytopic membrane protein containing several motifs characteristic of P-type ATPases. The histidine residue in the SEHPL motif within the cytoplasmic loop is the site of the H1069Q missense mutation, the most common disease allele found in northern-, central- and eastern European populations with Wilson disease. In hepatocytes, this ATPase resides in the trans-Golgi network transporting copper into the secretory pathway for incorporation into apoceruloplasmin and excretion into the bile. Copper is incorporated into apoceruloplasmin at the level of the Golgi compartment. Ceruloplasmin contains six tightly bound copper atoms. Its main function is to carry copper to various tissues. Another important physiologic role of ceruloplasmin is to act as a ferro-oxidase, converting  $\text{Fe}^{++}$  to  $\text{Fe}^{+++}$ . Other chaperones (Sco1, Sco2, Cox17, lys7) carry copper for synthesis of the other cuproenzymes and do not require an interaction with ATP7B. A shortened 140 kDa Wilson disease protein probably lacking one or two copper binding sites resides in the mitochondrial membrane where it presumably passes copper into the mitochondrion to be incorporated into cytochrome oxidase.

Because hepatic uptake of dietary copper is not saturable, hepatic copper accumulation can easily be induced. Toxicity of copper, however, depends on its molecular association and

subcellular localization rather than on its concentration in the liver. Metallothionein-bound copper is non-toxic. Several metals including zinc can induce metallothionein synthesis.

## CLINICAL PRESENTATIONS

Wilson disease may present at any age, the oldest reported case was 76 years at the time of diagnosis. The clinical symptoms are highly variable, the most common ones being liver disease and neuropsychiatric disturbances (Fig. 24.2). Children usually present with liver disease, while in older patients neurologic disease is more common (Table 24.3). None of the clinical signs is typical and diagnostic. One of the most characteristic features of Wilson disease is that no two patients, even within a family, are ever quite alike. With increased awareness for Wilson disease, patients are generally diagnosed earlier, thus ‘late’ consequences of the disease like Kayser-Fleischer rings or severe neurologic symptoms are less frequently seen. Early symptoms, if present at all, are uncharacteristic and non-specific. Patients presenting with acute or chronic hepatic Wilson disease are indistinguishable from patients with liver diseases of other etiology. Early neurologic symptoms are also quite untypical, and may



**Figure 24.2 Wilson disease.** Clinical presentations in relation to copper accumulation.

Clinical presentation according to the age onset of symptoms				
Age (years)	<10	10–20	20–35	>35
Neurologic n (%)	6 ( 9.2%)	96 (36.6%)	140 (63.1%)	23 (53.5%)
Liver n (%)	59 (90.8%)	166 (63.4%)	82 (36.9%)	20 (46.5%)
All n	65	262	222	43

**Table 24.3 Clinical presentation according to the age onset of symptoms.** (Ferenci et al, unpublished data.)

Clinical symptoms in Wilson disease patients presenting with liver disease					
Author (Country)	Walshe (UK)	Stremmel (Germany)	Schilsky (USA)	Scott (UK)	Ferenci (Austria)
Number with liver disease (out of)	87 (>250)	? (51)	20* (320)	17* (45)	30 (64)
<b>Presenting symptom</b>					
Jaundice, anorexia, vomiting (%)	44	14	15	41	37
Ascites/edema (%)	26	14	50	24	23
Variceal hemorrhage (%)	6		10	6	3
Hemorrhagic diathesis (%)	8				3
Hemolysis (%)	20	10	5		10
Hepatomegaly/ splenomegaly (%)	16	49	15	29	17
Fulminant hepatic failure (%)	NA	NA	NA	NA	17
Asymptomatic <sup>§</sup> (%)		18	5		23
*Only cases with chronic active hepatitis; <sup>§</sup> elevated alanine aminotransferase (ALT) at routine testing, or accidental finding of cirrhosis or of Kayser-Fleischer rings.					

**Table 24.4 Clinical symptoms in Wilson disease patients presenting with liver disease.**

progress slowly over many years before diagnosis is made based on 'typical signs'. About half of the patients are referred for psychologic testing because of poor school performance or behavioral problems. Year-long psychotherapy is not uncommon in such patients before the diagnosis is made.

### Kayser-Fleischer rings

Characteristically, the ring starts as a small crescent of golden brown granular pigment seen at the top of the limbus. This is followed by the appearance of a lower crescent, and these two crescents gradually broaden, meet laterally and form complete rings (**Fig. 24.3**). The finding of a complete ring therefore suggests long-standing disease and is a useful indicator of severe copper overload. The ring is not always detected by clinical inspection. If doubt exists, the cornea should be examined under a slit lamp by an experienced ophthalmologist. Kayser-

Fleischer rings are present in 95% of patients with neurologic symptoms, in 50–60% of patients without neurologic symptoms, and only in 10% of asymptomatic siblings.

### Liver disease

Most patients with Wilson disease, whatever their clinical presentation or presymptomatic status, have some degree of liver disease (Table 24.3). Chronic liver disease (if undiagnosed and untreated) may precede manifestation of neurologic symptoms for more than 10 years. Patients can present with liver disease at any age. The most common age of hepatic manifestation is between 8 and 18 years, but cirrhosis may already be present in children below the age of 5. On the other hand, Wilson disease can also be diagnosed in patients presenting with advanced chronic liver disease in their 50s or 60s, without neurologic symptoms and without Kayser-Fleischer rings.

Depending on referral patterns, the proportion of patients presenting with liver disease alone varies from 20 to 46%. Liver disease may mimic various forms of common liver conditions, ranging from asymptomatic elevation of liver enzymes to acute hepatitis, fulminant hepatic failure (about one out of six patients with hepatic presentation), chronic hepatitis, and cirrhosis (about one out of three patients) with all of its complications (Table 24.4).

### Acute Wilsonian hepatitis and fulminant Wilson disease

Acute Wilsonian hepatitis is indistinguishable from other forms of acute (viral or toxic) liver diseases. It should be suspected in young patients with acute non-viral hepatitis. Liver histology often reveals the presence of cirrhosis. This initial episode of liver damage may be self-limiting and may resolve without treatment, and diagnosis is made retrospectively, when neurologic symptoms occur years later.

On the other hand the disease may rapidly deteriorate and resemble fulminant hepatic failure with massive jaundice,



**Figure 24.3 Kayser-Fleischer ring.** A 15-year-old patient with neurologic Wilson disease.



hypoalbuminemia, ascites, severe coagulation defects, hyperammonemia and hepatic encephalopathy. Hepatocellular necrosis results in the release of large amounts of stored copper. Hypercupremia results in hemolysis and severe hemolytic anemia complicates acute liver disease. Although Wilson disease is rare, in patients presenting with fulminant hepatic failure it is not uncommon and accounts for 6–12% of patients with fulminant hepatic failure referred for emergency liver transplantation.

Although fulminant and subfulminant liver failure due to Wilson disease has several distinctive features, rapid diagnosis may be very difficult. Serum aminotransferase activity is usually not increased above 10 times normal and thus the levels are much lower than the levels commonly recorded in fulminant hepatitis. The combination of anemia, marked jaundice and relatively low aminotransferase activities in young patients should always raise the suspicion of acute Wilson disease. The conventionally-used parameters of copper metabolism are of little use. Kayser-Fleischer corneal rings and neurologic abnormalities are absent in most patients presenting with acute liver disease. An alkaline phosphatase–total bilirubin ratio below 2.0 has been claimed to provide 100% sensitivity and specificity to diagnose Wilsonian fulminant liver failure, but the usefulness of this test has not been confirmed in larger series. The best diagnostic test is the quantification of copper in biopsy material or in the explanted liver. One puzzling feature of fulminant Wilson disease is the preponderance of the female sex (female:male ratio 3:1).

### **Chronic hepatitis due to Wilson disease**

Wilson disease may present, particularly in young patients, with a clinical syndrome indistinguishable from chronic active hepatitis of another etiology. Symptoms include malaise, fatigue, anorexia and vague abdominal complaints. Arthralgias, amenorrhea, delayed puberty, low grade jaundice may be present. Frequently Kayser-Fleischer rings are absent and plasma ceruloplasmin is in the normal range. Liver biopsy shows severe chronic active hepatitis but the diagnosis can be missed if hepatic copper content is not measured. Suspicion of Wilson disease should be high in young persons with chronic active hepatitis of unclear etiology. In this group Wilson disease is a common diagnosis. Without treatment, patients progressively deteriorate with ascites, edema and occasionally jaundice within a few months, and eventually die of liver failure.

Patients presenting with neurologic symptoms may also suffer from significant liver disease. In a substantial proportion of patients symptomatic liver disease predates the occurrence of neurologic signs. Previously it was believed that ‘cirrhosis is present in all patients with neurologic disturbances’ (Scheinberg & Sternlieb 1984). In most patients, cirrhosis was clinically asymptomatic and was only diagnosed by liver biopsy. However, if a liver biopsy is performed in all patients presenting with neurologic symptoms at diagnosis, the proportion of patients with cirrhosis is only about 25%, and about half of the patients have only minimal liver disease (Table 24.5).

### **Neurologic presentation**

Neurologic symptoms usually develop in the mid-teens or in the 20s. However, there are well documented cases in which neurologic symptoms developed much later (45–55 years). The

<b>Liver biopsy findings in 127 patients with Wilson disease</b>				
<i>Liver histology</i>	<i>Hepatic presentation</i>	<i>Neurologic presentation</i>	<i>Siblings</i>	<i>Total</i>
Cirrhosis	45 (54.2%)	14 (41.1%)	2 (11.1%)	61 (45.2%)
Fibrosis	9 (10.8%)	3 (8.8%)	3 (16.7%)	15 (11.1%)
Chronic hepatitis	12 (14.4%)	9 (26.5%)	6 (33.3%)	27 (20.0%)
Steatosis	13 (15.7%)	5 (14.7%)	5 (27.8%)	23 (17.0%)
Minimal changes	4 (4.8%)	3 (8.8%)	2 (11.1%)	9 (6.7%)
<b>Total</b>	<b>83</b>	<b>34</b>	<b>18</b>	<b>135</b>

**Table 24.5 Liver biopsy findings in 127 patients with Wilson disease.**  
All biopsies were obtained before initiation of therapy.

initial symptoms may be very subtle abnormalities such as mild tremor, speech and writing problems and are frequently misdiagnosed as behavioral problems associated with puberty. The symptoms may remain constant or progress steadily. The hallmark of neurologic Wilson disease is a progressive movement disorder. The most common symptoms are dysarthria, dysphagia, apraxia and a tremor-rigidity syndrome (juvenile Parkinsonism). Because of increasing difficulty in controlling movement, patients become bedridden and unable to care for themselves. Ultimately, the patient becomes helpless – usually alert, but unable to talk. In patients presenting with advanced liver disease, neurologic symptoms are mistaken as signs of hepatic encephalopathy.

### **Psychiatric presentation**

About one-third of patients initially present with psychiatric abnormalities. Symptoms can include reduced performance in school or at work, depression, a very labile mood, sexual exhibitionism and frank psychosis. Frequently, adolescents with problems in school or work are referred for psychological counseling and psychotherapy. Among our patients, two were hospitalized in psychiatric institutions for psychosis, one having committed several suicide attempts, and two for severe alcohol abuse before diagnosis of Wilson disease was made. The delay in diagnosis in one case was 12 years.

### **Other clinical manifestations**

*Hypercalciuria and nephrocalcinosis* can be the presenting signs in patients with Wilson disease. Hypercalciuria is possibly the consequence of a tubular defect in calcium reabsorption. Penicillamine therapy was accompanied by a decrease in urinary calcium excretion to normal values in half of the patients studied. Wiebers et al (1979) observed renal stones in 7 of 54 patients with Wilson disease.

*Cardiac manifestations* in Wilson disease include arrhythmias, cardiomyopathy, cardiac death and autonomic dysfunction. Of patients with Wilson disease, 34% have electrocardiographic abnormalities. Two cases of cardiac deaths were reported (one died of repeated ventricular fibrillation, the other of dilated cardiomyopathy). In one of them copper content in the myocardium was measured and found to be markedly elevated.

Routine tests for diagnosis of Wilson disease			
Test	Typical finding	False 'negative'	False 'positive'
Serum ceruloplasmin	Decreased	Normal levels in patients with marked hepatic inflammation overestimation by immunologic assay	Low levels in: – malabsorption – aceruloplasminemia – liver insufficiency – heterozygotes
24 h urinary copper	>100 µg/day	Normal: – incorrect collection – children without liver disease	Increased: – hepatocellular necrosis – contamination
Serum 'free' copper	>10 µg/dL	Normal if ceruloplasmin overestimated by immunologic assay	
Hepatic copper	>250 µg/g dry weight	Due to regional variation – in patients with active liver disease – in patients with regenerative nodules	Cholestatic syndromes
Kayser-Fleischer rings by slit lamp	Present	– in up to 40% of patients with hepatic Wilson disease – in most asymptomatic siblings	Primary biliary cirrhosis

**Table 24.6** Routine tests for diagnosis of Wilson disease.

The occurrence of *chondrocalcinosis* and *osteoarthritis* in Wilson disease may be due to copper accumulation similar to the arthropathy of hemochromatosis.

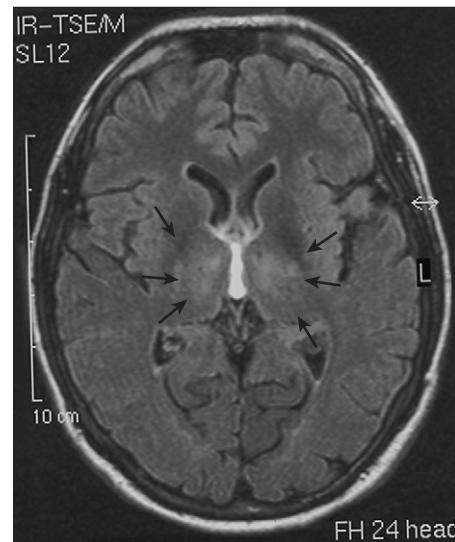
## DIAGNOSIS

The diagnosis of Wilson disease is usually made on the basis of clinical findings and laboratory abnormalities (Table 24.6). According to Sternlieb & Scheinberg (1979), diagnosis of Wilson disease can be made if two of the following symptoms are present: Kayser-Fleischer rings, typical neurologic symptoms and low serum ceruloplasmin levels.

### Patients with neurologic disease

In a patient presenting with typical neurologic symptoms with Kayser-Fleischer rings, the diagnosis is straightforward. Clinical neurologic examination is more sensitive than any other method to detect neurologic abnormalities. No further diagnostic procedures are necessary to establish the diagnosis. Kayser-Fleischer rings are rarely absent in neurologically symptomatic patients. However, there are a few well-documented cases of neurologic Wilson disease without demonstrable Kayser-Fleischer rings. In such patients, diagnosis is usually made by a low serum ceruloplasmin level.

Brain magnetic resonance imaging (MRI) is useful to document the extent of changes in the central nervous system. The most common abnormalities are changes in signal intensity of gray and white matter (Fig. 24.4), and atrophy of the caudate nucleus, brain stem, cerebral and cerebellar hemispheres. A characteristic finding in Wilson disease is the 'face of the giant panda' sign, but it is found only in a minority of patients. In Wilson disease, an abnormal striatum or an abnormal pontocerebellar tract correlates with pseudo-Parkinsonian symptoms, and an abnormal dentatothalamic tract with cerebellar signs. The presence of portosystemic shunting is strongly associated



**Figure 24.4** Cranial MRI. T<sub>2</sub>-weighted image, fluid attenuation inversion recovery (FLAIR), (by courtesy of Dr D. Prayer) of a Wilson disease patient with neurologic symptoms. Signal hyperintensity in the thalamus (arrows).

with abnormality of the globus pallidus. On treatment, some of the MRI abnormalities are fully reversible.

Auditory evoked brainstem potentials are helpful to document the degree of functional impairment and the improvement by decoppering treatment.

### Patients with liver disease and hemolytic anemia

Diagnosis is far more complex in patients presenting with liver diseases. None of the commonly used parameters alone allows for a certain diagnosis of Wilson disease. Usually a combination of various laboratory parameters is necessary to establish the diagnosis.

Kayser-Fleischer rings may be absent in up to 50% of patients with Wilsonian liver disease and even in a higher proportion in fulminant Wilson disease. On the other hand patients with

primary biliary cirrhosis may occasionally have Kayser-Fleischer rings.

### **Laboratory parameters**

#### **Routine laboratory parameters of liver disease**

In general, aminotransferases are only mildly increased, and deep jaundice combined with mild elevation of liver enzymes in non-surgical patients should raise the suspicion for fulminant Wilson disease. However, increases of aminotransferases may be indistinguishable from findings seen in acute hepatitis. Sometimes alkaline phosphatase activities are relatively low in patients with Wilson disease. A ratio of total serum bilirubin concentration to alkaline phosphatase activity of  $>2$  may differentiate fulminant Wilson disease from other forms of fulminant hepatic failure.

#### **Serum ceruloplasmin**

Serum ceruloplasmin can be measured by an immunologic assay or by the oxidase method. Since the immunologic ceruloplasmin assay can be automated by nephelometric methods, it is widely used in clinical laboratories. The oxidase method is only performed in specialized centers. Whereas serum ceruloplasmin is decreased in most patients with neurologic Wilson disease, it may be in the low normal range in up to 45% of patients with hepatic disease. On the other hand, even a low ceruloplasmin level is not diagnostic for Wilson disease in the absence of Kayser-Fleischer rings. It may be low in subjects with familial hypoceruloplasminemia, in severely malnourished subjects, and in heterozygous carriers of the Wilson disease gene. Very low levels were found in a patient with autoimmune hepatitis which increased following steroid treatment. Thus, in patients with liver disease a normal ceruloplasmin level cannot exclude the diagnosis, nor is a low level sufficient to make the diagnosis of Wilson disease.

There are several possible explanations for the high proportion of patients with hepatic Wilson disease with ceruloplasmin levels in the normal range. In view of the multiple mutations of the Wilson disease gene, differences in the functional impairment of the gene product, a Cu-ATPase, may be associated with variations in clinical and laboratory findings. Second, apoceruloplasmin may interfere with the immunologic assay for ceruloplasmin. Its contribution to the results obtained in patients with low ceruloplasmin values is negligible, but in those closer to the borderline, the additional apoceruloplasmin may push them into the normal range. An overestimation of serum ceruloplasmin can be suspected if the serum copper concentration is lower than expected by the measured ceruloplasmin (which contains 0.3% of copper) level (see below). Finally, ceruloplasmin is an acute phase reactant and its serum concentration increases as a consequence of inflammation. Most patients with a normal ceruloplasmin level have severe liver disease. The decrease in the ceruloplasmin level after initiation of decoppering treatment (Fig. 24.5) in parallel with the improvement of liver disease, supports this concept. Similarly serum ceruloplasmin may increase in pregnancy to high normal values.

#### **Serum copper**

In general, serum copper values parallel those of ceruloplasmin. Therefore, serum copper is frequently low in patients with Wilson disease. However, about half of patients have serum

copper levels in the normal range. Patients with fulminant Wilson disease and/or hemolytic anemia may even have markedly increased levels. Most of the copper in serum is bound to ceruloplasmin, and under normal condition less than 5% circulates as 'free copper'. In normal subjects it does not exceed 10  $\mu\text{g/dL}$ . The 'free' copper concentration can be calculated by subtracting the ceruloplasmin bound copper (ceruloplasmin  $\times$  3.3) from the total copper concentration. An increased free copper level ( $>10 \mu\text{g/dL}$ ) is considered a useful diagnostic test for Wilson disease (providing the measured ceruloplasmin level is correct, see above).

#### **Urinary copper excretion**

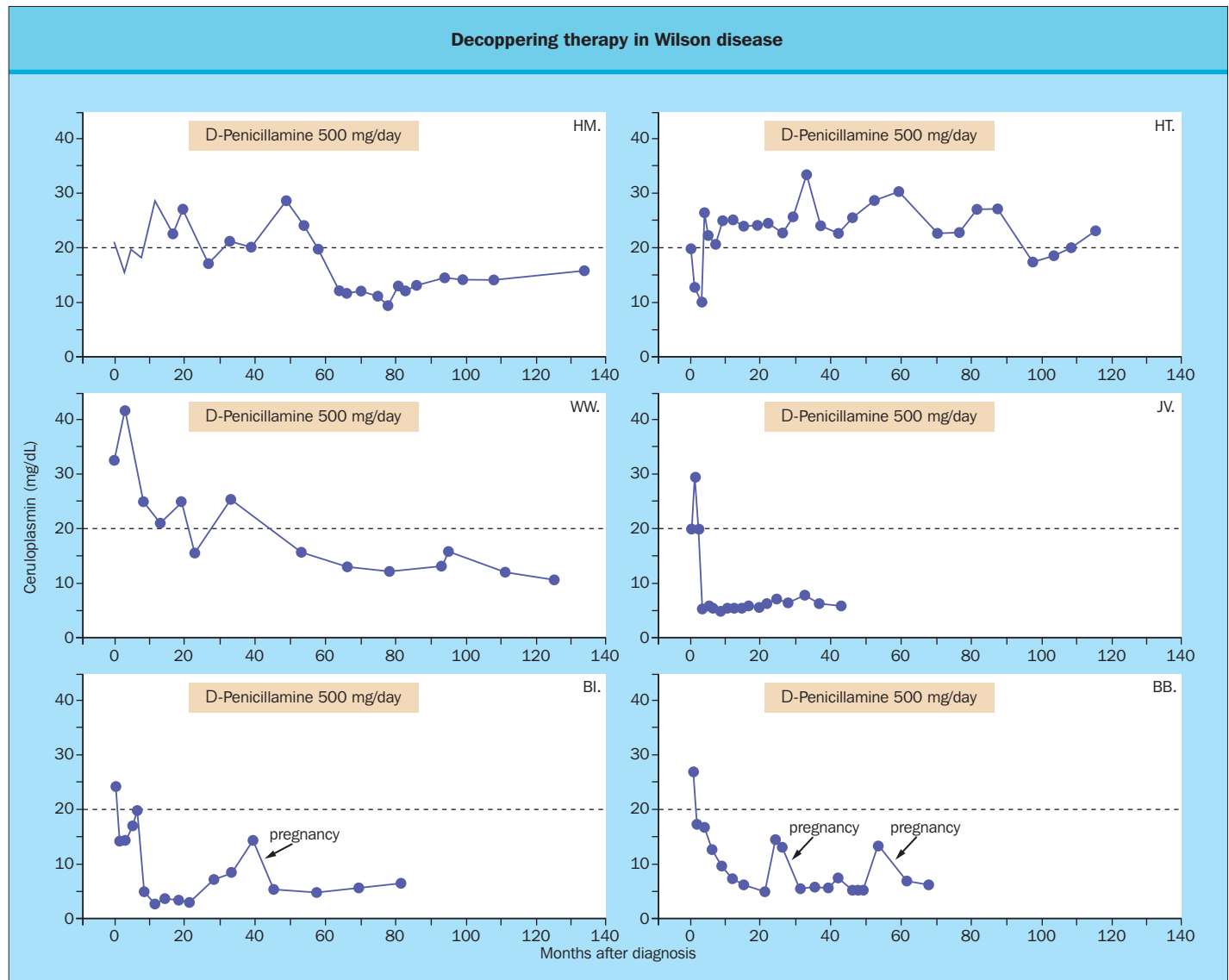
Urine copper excretion is markedly increased in patients with Wilson disease (88% in one study); however, its usefulness in clinical practice is limited. The estimation of urinary copper excretion may be misleading due to incorrect collection of 24 h urine volume or to copper contamination. In presymptomatic patients urinary copper excretion may be normal, but will increase after a D-penicillamine challenge. On the other hand, urinary copper excretion is increased in any disease with extensive hepatocellular necrosis.

#### **Hepatic copper content**

Hepatic copper content is increased in 82% of patients with Wilson disease and usually exceeds 250  $\mu\text{g/g}$  dry weight (normal: up to 50  $\mu\text{g/g}$ ). In the absence of other tests suggestive for abnormal copper metabolism, diagnosis of Wilson disease cannot be made based on an increased hepatic copper content alone. Patients with chronic cholestatic diseases, neonates and young children and possibly also subjects with exogenous copper overload have increased hepatic copper concentration  $>250 \mu\text{g/g}$ . On the other hand, hepatic copper content may be normal or borderline in about 10% of patients with unquestionable Wilson disease due to sampling given great regional differences in hepatic copper distribution, especially in the cirrhotic liver. In a recent study multiple biopsies taken from the explanted livers of two patients with fulminant hepatic failure revealed an up to a 500-fold variation in copper content. Only 2 of 14 and 3 of 16 specimens, respectively, were within the diagnostic range for Wilson disease, but in a cirrhotic liver all the specimens tested yielded diagnostic results. Thus, estimates from a single biopsy specimen may be misleading.

In a recent study hepatic copper content was measured in 106 liver biopsies obtained at diagnosis of Wilson disease, in 212 patients with a variety of non-cholestatic liver diseases, and in 26 patients without evidence of liver disease. Liver copper content was  $>250 \mu\text{g/g}$  dry weight in 87 (82%) of Wilson disease patients, between 50 and 250  $\mu\text{g/g}$  in 15, and in the normal range in four. Liver copper content did not correlate with age, the stage of fibrosis, or the presence of stainable copper. Liver copper content was  $>250 \mu\text{g/g}$  or between 50 and 250  $\mu\text{g/g}$  dry weight in 3 (1.4%) and 20 (9.1%) of 219 patients with non-cholestatic liver diseases, respectively. By lowering the cut off from  $>250 \mu\text{g/g}$  to 75  $\mu\text{g/g}$  dry weight, the sensitivity of liver copper content to diagnose Wilson disease increased from 81.2 to 96%, the negative predictive value improved from 88.2 to 97.1%, but the specificity (98.6 to 90.1%) and the positive predictive value (97.6 to 87.4%) decreased. Thus, although liver copper content is a useful parameter, it neither proves nor





**Figure 24.5 Decoppering therapy.** Effect of decoppering therapy on serum ceruloplasmin levels in patients with Wilson disease with normal or borderline ceruloplasmin levels at diagnosis. On treatment ceruloplasmin decreased in most patients to low levels, but in some (HT) it remained in the low normal range. Note the effect of pregnancy on serum ceruloplasmin levels (BI and BB)

excludes Wilson disease with certainty. Diagnosis requires a combination of a variety of clinical and biochemical tests. A diagnostic scoring system (Table 24.7) was developed at the 8th International Meeting on Wilson disease, Leipzig, Germany, 2001. The validity of this score has yet to be assessed prospectively.

### Liver biopsy

#### Light microscopy

Liver biopsy findings are generally non-specific and not directly helpful to make the diagnosis of Wilson disease. Liver pathology (Fig. 24.6) includes early changes like fatty intracellular accumulation, which often progresses to marked hepatic steatosis. At later stages, hepatic inflammation with portal and periportal lymphocytic infiltrates and the presence of necrosis and of fibrosis may be indistinguishable from other forms of

hepatitis. Some patients have cirrhosis without any inflammation. The detection of focal copper stores by the Rhodanin stain is a pathognomonic feature of Wilson disease, but is only present in a minority (about 10%) of patients.

### Electron microscopy

The ultrastructural abnormalities in Wilson disease include pathologic changes of mitochondria and peroxisomes. Hepatocellular mitochondria are pleomorphic, with varying combinations of abnormalities including enlargement, bizarre shapes, increased matrix density, separation of the normally apposed inner and outer membranes, widened intercrystal spaces, enlarged granules, and crystalline, vacuolated or dense inclusions. Sometimes peroxisomes are abnormally enlarged, rounded or misshapen, and contain a granular or flocculent matrix of varying electron density.

Scoring system developed at the 8th International Meeting on Wilson disease, Leipzig 2001.			
Typical clinical symptoms and signs		Other tests	
Kayser-Fleischer rings		Liver copper (in absence of cholestasis)	
Present	2	>5 × ULN (>250 µg/g)	2
Absent	0	50–250 µg/g	1
Neurologic symptoms		Normal (<50 µg/g)	-1
Severe	2	Rhodanine pos. granules*	1
Mild	1		
Absent	0	Urinary copper (in absence of acute hepatitis)	
Serum ceruloplasmin		Normal	0
Normal(>0.2 g/L)	0	1–2 × ULN	1
0.1–0.2 g/L	1	>2 ULN	2
<0.1g/L	2	Normal, but >5 × ULN after D-penicillamine	2
Coombs neg. hemolytic anemia		Mutation analysis	
Present	1	2 chromosome mutations	4
Absent	0	1 chromosome mutation	1
		No mutations detected	0
TOTAL SCORE	Evaluation:		
4 or more	Diagnosis established		
3	Diagnosis possible, more test needed		
2 or less	Diagnosis very unlikely		
* if no quantitative liver copper available; ULN, upper limit of normal			

**Table 24.7** Scoring system developed at the 8th International Meeting on Wilson disease, Leipzig 2001. (Reproduced from Ferenci et al (03).

### Magnetic resonance imaging of the liver

Hepatic T<sub>2</sub>-weighted MRI changes include multiple tiny low-intensity-nodules surrounded by high-intensity septa in about 60% of patients. Some patients also had low-intensity nodules in T<sub>1</sub>-weighted images. Patients with abnormal MRI findings had liver cirrhosis or advanced fibrosis. In contrast, patients without pathologic MRI liver morphology had only slight histopathologic changes of parenchyma and normal laboratory data. In patients with low-intensity nodules of the liver by MRI and an unknown cause of cirrhosis, laboratory data and histopathology should be checked when searching for disorders of copper metabolism.

### Radiocopper-test

The basis of this test is the biphasic plasma kinetics of copper. An oral dose of <sup>64</sup>Cu is given, and the blood radioactivity at 1, 2, 24 and 48 h is determined. Initially, labeled copper is taken up by the liver and is completely removed from the serum. Later, the label reappears bound to ceruloplasmin. This second peak is absent in Wilson disease patients. This test is rarely used today.

### Mutation analysis

#### Direct mutation analysis

Direct molecular genetic diagnosis is difficult because of the occurrence of many mutations, each of which is rare.

Furthermore, most patients are compound heterozygotes (i.e. carry two different mutations). Screening for mutations is done by denaturing high-pressure liquid chromatography (HPLC) analysis followed by direct sequencing of the exon suspected of carrying a mutation. This approach is impractical for clinical diagnosis. In contrast, when using allele-specific probes, direct mutation diagnosis is rapid and clinically very helpful, if a mutation occurs with a reasonable frequency in the population. In Austria, the H1069Q mutation is present in 61% of Wilson disease patients, and a two-step polymerase chain reaction (PCR)-based test for this mutation has become very useful. For example, the large family of an index patient homozygous for this mutation with more than 40 members was examined within 2 days. An asymptomatic affected sibling and several heterozygotes can be detected, even if first degree relatives are not available for testing (Fig. 24.7). A multiplex PCR for the most frequent mutations makes direct mutation analysis for diagnosis feasible.

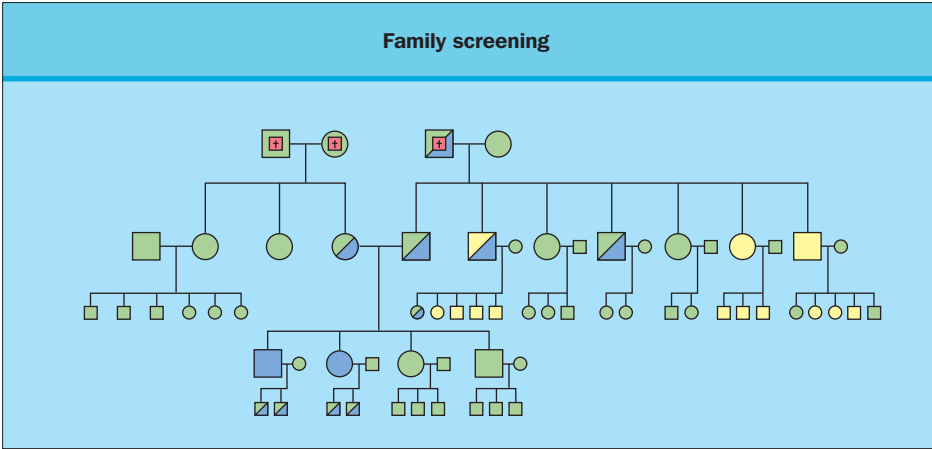
Figure 24.8 presents a suggested flow diagram for the diagnosis of Wilson disease.

#### Haplotype analysis

Because of the complexity in identifying the many mutations in Wilson disease, haplotypes can be used to screen for mutations

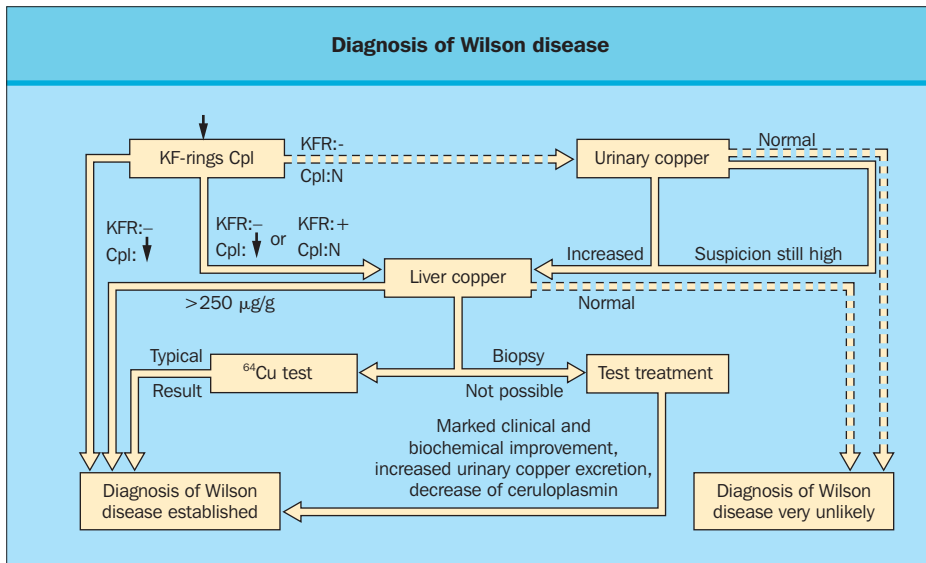
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Please refer to the printed publication.

**Figure 24.6 Liver histology in Wilson disease.** (a) Vacuolated nuclei (hematoxylin and eosin,  $\times 400$ ) and portal infiltration (grade 2) extending into the lobules in a 32-year-old asymptomatic sibling. (b) Marked steatosis, vacuolated nuclei (hematoxylin and eosin,  $\times 400$ ), and mild lobular infiltration (grade 1) in the 18-year-old asymptomatic sibling (H1069Q homozygote). (c) Copper granules in hepatocytes (Rhodanin,  $\times 200$ ), mild portal infiltration (grade 1) and fibrosis (stage 2) in a 15-year-old girl (compound heterozygote for His1069Gln) with neurologic Wilson disease. No biochemical evidence of liver disease. (d) Severe chronic active hepatitis (grade 4) with bridging necrosis and fibrosis stage 4 in a 24-year-old male (unknown mutation) presenting as severe acute hepatitis (hematoxylin and eosin,  $\times 100$ ). All photographs by Dr K Kaserer.



**Figure 24.7 Family screening.** A polymerase chain reaction (PCR)-based test is used to detect the His1069Gln mutation in a homozygous index case. (The gray symbols indicate subjects not tested.)



**Figure 24.8** Diagnosis of Wilson disease.

and to examine asymptomatic siblings of index patients. A number of highly polymorphic microsatellite markers have been described that closely flank the gene and are highly variable: D13S316, D13S314, D13S301 and D13S133. Where the markers are different at each locus in a patient, testing of at least one parent, or child of the patient is necessary to obtain the haplotype. The identification of unusual haplotypes can lend support to, but is not sufficient to confirm, the diagnosis of Wilson disease.

Microsatellite markers are also useful to study the segregation of the Wilson disease gene in most families. By this approach, diagnostic dilemmas in differentiating heterozygote gene carriers and affected asymptomatic siblings can be solved. For such analysis, at least one first degree relative and the index patient are required.

### Family screening

Once the diagnosis of Wilson disease is made in an index patient, evaluation of the family is mandatory. The likelihood of finding a homozygote among siblings is 25%, and among children is 0.5%. Testing of second degree relatives is only useful if the gene was found in one of the immediate members of his/her family. No single test is able to identify affected siblings or heterozygote carriers of the Wilson disease gene with sufficient certainty. Today, mutation analysis is the only reliable tool for screening the family of an index case with known mutations; otherwise haplotype analysis can be used. A number of highly polymorphic microsatellite markers that closely flank the gene allow tracing of the Wilson disease gene in a family. Haplotype analysis and restriction fragment length polymorphism of flanking genes (before the discovery of the Wilson disease gene) proved to be extremely useful for prenatal testing and firm diagnosis of affected siblings in Wilson disease families (Fig. 24.9).

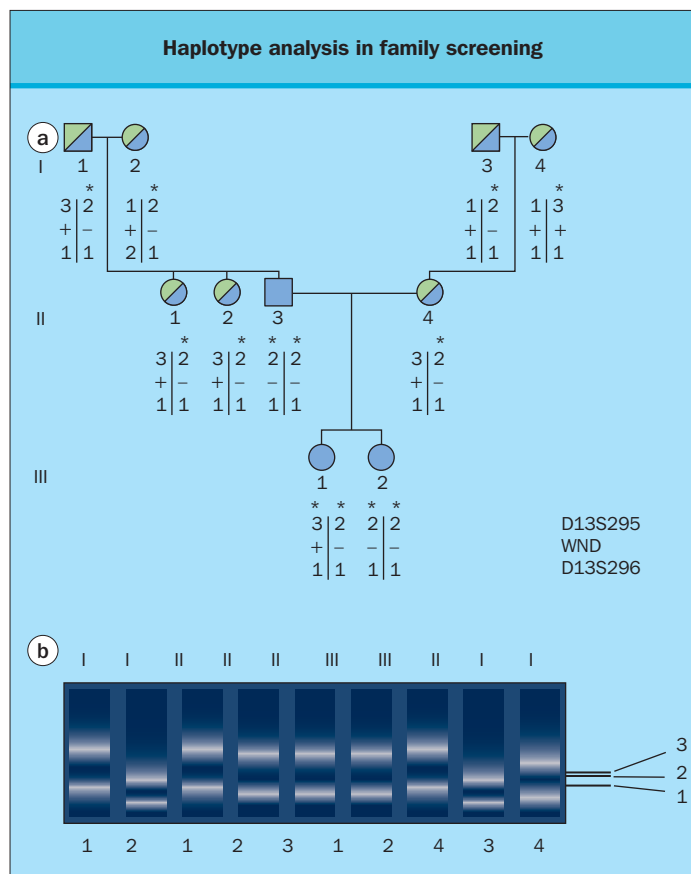
## TREATMENT

Treatment for Wilson disease progressed from the intramuscular administration of British anti-Lewisite (BAL) to the more easily administered oral penicillamine. Alternative agents to penicillamine like trientine were developed and introduced for patients with adverse reactions to penicillamine. Zinc was developed separately, as was tetrathiomolybdate, which was used for copper poisoning in animals. Today, the mainstay of treatment for Wilson disease remains lifelong pharmacologic therapy, but the choice of drug mostly depends on the opinion of the treating physician and is not based on comparative data.

According to the recent American Association for the Study of Liver Diseases (AASLD) practice guideline on Wilson disease, initial treatment for symptomatic patients should include a chelating agent (penicillamine or trientine). Treatment of presymptomatic patients or maintenance therapy of successfully treated symptomatic patients can be accomplished with the chelating agent penicillamine or trientine, or with zinc. Liver transplantation, which corrects the underlying hepatic defect in Wilson disease, is reserved for severe or resistant cases.

### Penicillamine

Penicillamine was first reported to be effective in treating Wilson disease by Walshe in 1956 and has been the 'gold standard' for therapy. Penicillamine acts by reductive chelation: it reduces copper bound to protein and thereby decreases the affinity of the protein for copper. Reduction of copper thus facilitates the binding of copper to the drug. The copper mobilized by penicillamine is then excreted in the urine. Within a few weeks to months, penicillamine brings the level of copper to a subtoxic threshold, and allows tissue repair to begin. The great majority of symptomatic patients, whether hepatic, neurologic or psychiatric, respond within months of starting



**Figure 24.9 Haplotype analysis in family screening.** (A) Pedigree with DNA haplotypes of microsatellite marker analysis. The numbers indicate allelic designations at microsatellite marker loci. \* = mutant alleles. (B) Separation of the polymorphic dinucleotide microsatellites on a polyacrylamide gel. The index case (II/3) was a 39-year-old man presenting with cirrhosis, Kayser-Fleischer rings and neurologic symptoms. His elder daughter (III/1, 10 years) had elevated alanine aminotransferase (ALT) level and a liver biopsy diagnostic for Wilson disease (WND). Her 4-year-old sister (III/2) was healthy and had normal ALT, normal ceruloplasmin, and normal urinary copper excretion. By haplotype analysis she was identified as homozygous carrier of the Wilson disease gene. Diagnosis was confirmed by liver biopsy. Reproduced from Maier-Dobersberger et al (95) by permission of The American Gastroenterological Association.

treatment. Among neurologic patients, a significant number may experience an initial worsening of symptoms before they get better.

The usual dose of penicillamine is 1–1.5 g/day. Initially, this dose will cause a large cupriuresis, but copper excretion later decreases to 0.5 mg/day. To prevent deficiency induced by penicillamine, pyridoxine (vitamin B<sub>6</sub>) should be supplemented (50 mg/week). Once the clinical benefit is established, it is possible to reduce the dosage of penicillamine to 0.5–1 g/day. A lower maintenance dose will decrease the likelihood of late side-effects of the drug.

A major problem of penicillamine is its high level of toxicity. In our series, 20% of patients had major side-effects and were switched to other treatments. Other series report even higher frequencies of side-effects. There are two broad classes of penicillamine toxicity: direct, dose-dependent side-effects, and immunologically-induced lesions. Direct side-effects are pyridoxine deficiency, and interference with collagen and elastin

formation. The latter results in skin lesions like cutis laxa and elastosis perforans serpingiosa. By routine skin biopsies 1 year after initiation of treatment we found signs of elastic and collagen fiber abnormalities in every patient, but none had developed symptomatic skin disease so far. These side-effects can be prevented or mitigated by decreasing the dosage of penicillamine. More recently, it was suggested that the total absence of holoceruloplasmin as the result of intensive decoppering is associated with impaired iron mobilization from the liver. Hepatic iron concentrations in the range of patients with hemochromatosis were found in a few patients with Wilson disease after year-long high-dose penicillamine treatment. Immunologically mediated side-effects include leukopenia and thrombocytopenia, systemic lupus erythematosus, immune complex nephritis, pemphigus, buccal ulcerations, myasthenia gravis, optic neuritis and Goodpasture syndrome. Immunologically mediated side-effects occur within the first 3 months of treatment and require immediate cessation of penicillamine. To diagnose these side-effects as soon as possible, patients should be monitored at weekly intervals during the first 6 weeks of therapy. If the drug is well tolerated, monitoring intervals can be gradually prolonged.

### Trientine

Trientine is a copper chelator, acting primarily by enhancing urinary copper excretion. Trientine is licensed for treatment of Wilson disease and is now generally available. Experience with trientine is not as extensive as with penicillamine. It seems to be as effective as penicillamine with far fewer side-effects. Its efficacy was evaluated in patients with intolerance to penicillamine. Discontinuation of penicillamine resulted in death from hepatic decompensation or fulminant hepatitis in 8 of 11 patients who stopped their own treatment after an average survival of only 2.6 years. In contrast, 12 of 13 patients with intolerance to penicillamine who switched to trientine (1–1.5 g/day) were alive at 2–15 years later. The remaining patient died accidentally. However, the efficacy of trientine was not compared with penicillamine as initial treatment of Wilson disease. Uncontrolled anecdotal reports and our own experience indicate that trientine is a satisfactory first line treatment for Wilson disease. In the early phase of treatment trientine appears to be more potent to mobilize copper than penicillamine, but cupriuresis diminishes more rapidly than with penicillamine. The cupriuretic power of trientine may be disappointing but is sufficient to keep the patient clinically well.

### Ammonium tetrathiomolybdate

This drug has two mechanisms of action. First, it complexes with copper in the intestinal tract and thereby prevents absorption of copper. Second, the absorbed drug forms a complex with copper and albumin in the blood and renders the copper unavailable for cellular uptake. There is very limited experience with this drug. Tetrathiomolybdate appears to be a useful form of initial treatment in patients presenting with neurologic symptoms. In contrast to penicillamine therapy, treatment with tetrathiomolybdate does not result in initial neurologic deterioration. This agent is particularly effective at removing copper from the liver. Because of its effectiveness, continuous use can cause copper deficiency. Also, bone marrow depression has been observed in a few patients treated with this drug.

## Zinc

Zinc interferes with the intestinal absorption of copper by two mechanisms. Both metals share the same carrier in enterocytes and pretreatment with zinc blocks this carrier for copper transport (with a half-life of about 11 days). The impact of zinc-induced blockade of copper transport by other carriers into the enterocyte was not studied. Second, zinc induces metallothionein in enterocytes, which acts as an intracellular ligand, binding metals which are then excreted in the feces with desquamated epithelial cells. Indeed, fecal excretion of copper is increased in patients with Wilson disease on treatment with zinc. Furthermore, zinc also induces metallothionein in the liver, protecting hepatocytes against copper toxicity.

Data on zinc in the treatment of Wilson disease are derived from uncontrolled studies using different zinc preparations (zinc sulfate, zinc acetate) at different doses (75–250 mg/day). The efficacy of zinc was assessed by four different approaches. First, patients successfully decoppered by D-penicillamine were switched to zinc and the maintenance of their asymptomatic condition was monitored. Most patients maintained a negative copper balance and no symptomatic recurrences occurred. Some patients, however, died of liver failure after treatment was switched to zinc. Stremmel et al (1991) observed the occurrence of severe neurologic symptoms in a 25-year-old asymptomatic sibling 4 months after switching from D-penicillamine to zinc.

A second group of symptomatic patients switched to zinc as an alternate treatment due to intolerance to D-penicillamine. Sixteen case histories have been published so far. Liver function and neurologic symptoms improved in three and five patients, respectively. One patient further deteriorated neurologically and improved on retreatment with D-penicillamine. The remaining patients remained in a stable condition. Follow-up studies in 141 patients demonstrated that zinc is effective as sole therapy in the long-term maintenance treatment of Wilson disease. In a third group zinc was used as first line therapy. About one-third were asymptomatic siblings and two-thirds presented with neurologic or hepatic symptoms. Most patients remained free of symptoms or improved. In 15% neurologic symptoms worsened and improved on D-penicillamine treatment. Three patients died of progressive liver disease. Finally, in a prospective study in 67 newly diagnosed cases, the efficacy of D-penicillamine and zinc was similar. This was not a randomized study; every other patient was treated with zinc. Zinc was better tolerated than D-penicillamine. However, two zinc-treated patients died of progressive liver disease.

It is unknown whether a combination of zinc with chelation therapy is useful or not. Theoretically these drugs may have antagonistic effects. Interactions in the maintenance phase of zinc therapy with penicillamine and trientine were investigated by copper balance studies and absorption of orally administered  $^{64}\text{Cu}$  as endpoints. The result on copper balance was about the same with zinc alone as it is with zinc plus one of the other agents. Thus, there appears to be no advantage to concomitant administration.

## Antioxidants

As discussed before, the main mechanism of hepatocellular injury by excess copper is the formation of free radicals resulting in lipid peroxidation and impaired mitochondrial respiration.

Thus, antioxidants such as  $\alpha$ -tocopherol may be important adjuncts in the treatment of Wilson disease. There are no large experiences with  $\alpha$ -tocopherol. A few observations indicate that this therapeutic adjunct may be useful in severe liver disease.

## Monitoring therapy

If a decoppering agent is used for treatment, compliance can be tested by repeated measurement of the 24 h urinary copper excretion. This approach is not useful if patients are treated with zinc. If in a compliant patient urinary copper excretion decreases over time and stabilizes at  $<500 \mu\text{g/day}$  the dose of D-penicillamine can be lowered.

Efficacy of treatment can be monitored by the determination of 'free' copper in serum, depending on the presenting symptoms. Liver disease can be assessed by routine liver tests. Repeated liver biopsies with measurement of hepatic copper content are not helpful. Improvement of neurologic symptoms can be documented by clinical examination. In addition, some of the MRI abnormalities are fully reversible on treatment. Auditory evoked brainstem potentials are also helpful to document improvement by decoppering treatment.

## Liver transplantation

Liver transplantation is the treatment of choice in patients with fulminant Wilson disease and in patients with decompensated cirrhosis. Besides improving survival, liver transplantation also corrects the biochemical defect underlying Wilson disease. However, the role of this procedure in the management of patients with neurologic Wilson disease in the absence of hepatic insufficiency is still uncertain.

Schilsky et al (1994) analyzed 55 transplants performed in 33 patients with decompensated cirrhosis and 21 with Wilsonian fulminant hepatitis in the USA and Europe. The median survival after orthotopic liver transplantation was 2.5 years, the longest survival time after transplantation was 20 years. Survival at 1 year was 79%. Non-fatal complications occurred in five patients. At the University of Pittsburgh 51 orthotopic liver transplants (OLT) were performed on 39 patients (16 pediatric, 23 adults). The rate of primary graft survival was 73% and patient survival was 79.4%. Survival was better for those with chronic advanced liver disease (90%) than it was for those with fulminant hepatic failure (73%). In the Mayo Clinic series 1-year survival ranged from 79 to 87%, with an excellent chance of long-term survival. The outcome of neurologic disease following OLT is uncertain. In a retrospective survey, four of seven patients with neurologic or psychiatric symptoms due to Wilson disease improved after OLT. Anecdotal reports document a dramatic improvement in neurologic function within 3 to 4 months after OLT. In contrast, central pontine and extrapontine myelinolysis and new extrapyramidal symptoms developed in a patient 19 months after OLT. Some patients transplanted for decompensated cirrhosis have had psychiatric or neurologic symptoms, which improved following OLT.

## PREGNANCY IN PATIENTS WITH WILSON DISEASE

Prior to the introduction of penicillamine, successful pregnancies in patients with Wilson disease were rare due to reduced



Outcome of pregnancy in different series						
	USA	UK	UK	USA	India	Austria*
Number of patients	18	10	7	19	16	11
Number on treatment (DPEN/Zn/TRI)	18 (+/-/-)	9 (+/-/-)	7 (-/+/+)	19 (-/+/+)	6 (+/-/-)	10 (+/-/+)
Mean duration of treatment (years)	6.6	11.7	5	5.1	5.2	5
Number of successful conceptions	29	15	11	26	59	19
Number of abortions (spontaneous/elective)	-	5 (3/2)	1 (1/-)	-	26 (24/2)	4 (4/-)
Type of delivery	NA	13/2	8/1	26/-	30/-	NA
Full-term normal/Premature	DPEN: D-Penicillamine, Zn: zinc, TRI: trientine NA: not assessed;* currently we have followed two additional pregnant patients with an uneventful course; one of them had a successful in vitro fertilization. Of the 120 live births, 117 were normal, one with isochromosome X was born prematurely, 1 required surgery for a heart defect at 6 months, 1 microcephalic baby died 1 h postpartum. The condition of all mothers remained unchanged during and after pregnancy. One patient was transplanted for deteriorating liver disease 2 years after delivery.					

**Table 24.8 Outcome of pregnancy in different series.** (In addition there are several case reports on successful pregnancies in patients with Wilson disease.)

fertility, menstrual irregularities and consequences of severe liver dysfunction. Since then, many case reports and series reported successful pregnancies in more than 100 patients with Wilson disease (Table 24.8). In general, the outcome both for mother and child is excellent. During pregnancy, serum copper and ceruloplasmin levels rise until 24 weeks and decline thereafter possibly due to copper uptake by the fetus.

### Assessment prior to pregnancy

Patients with Wilson disease wishing to become pregnant should be seen prior to conception for assessment of their hepatic function and copper status. Patients who are copper overloaded or who have esophageal varices should possibly avoid or delay pregnancy. Patients on well-established therapy should continue with this treatment through the pregnancy and compliance with treatment must be emphasized to the patient. If the patient has been decoppered, it is prudent to reduce the dose of penicillamine to 500 mg daily. Reducing the dose of penicillamine amongst patients who are not decoppered, however, places the patient at risk of neurologic and hepatic compromise and trientine should be considered as an alternative treatment.

### Drug therapy during pregnancy

Controversy over prescribing penicillamine in pregnant patients exists due to its possible teratogenic effects. Rare cases of birth defects including hydrocephalus and cerebral palsy have been reported in patients treated with penicillamine for a variety of diseases. However, the overall teratogenic risk of penicillamine is low and there is general support for continuing treatment throughout pregnancy to avoid the risk of relapse in the mother, although the optimal dosage of penicillamine is not known. Trientine appears to be an alternative to penicillamine with no

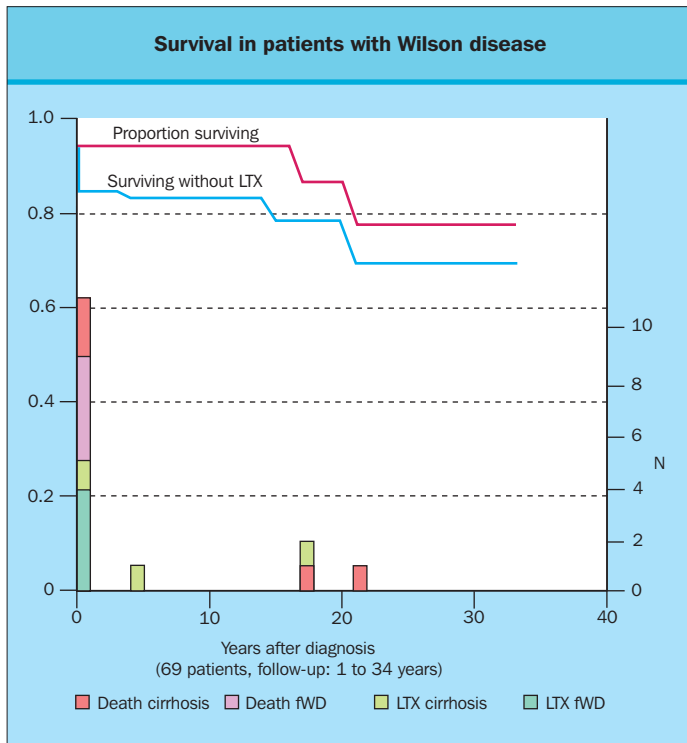
reported teratogenic effects, but the experience with this drug is limited. The use of zinc in pregnancy has not been associated with any fetal abnormality and possibly has a protective effect from some birth defects. The limited experience with zinc or trientine in pregnancy does not justify a change in drug therapy during pregnancy.

### PROGNOSIS

Untreated, symptomatic Wilson disease progresses to death in all patients. The majority of patients will die of complications of advanced liver failure, some of progressive neurologic disease. The overall mortality from Wilson disease treated medically (in most cases by D-penicillamine) has not been assessed prospectively. The mortality in 33 patients followed for 21 years by Scheinberg & Sternlieb (1987) was approximately 20%. This figure is close to the cumulative mortality in 69 Austrian patients (Fig. 24.10). In this group there was a substantial early mortality due to liver failure within the first 2 months after diagnosis. Only two patients died during the follow-up, one of them developed liver failure after a 2-year period of non-compliance. About an equal number of patients with severe liver failure survived following orthotopic liver transplantation. In a German study in 51 patients the cumulative survival was slightly reduced during the early period of follow-up but was not different from an age- and sex matched control population after 15 years of observation (96%).

### Liver disease

In general, prognosis depends on the severity of liver disease at diagnosis. In patients without cirrhosis or with compensated cirrhosis, liver disease does not progress after initiation of decoppering therapy. Liver function improves gradually (Fig.



**Figure 24.10 Survival in patients with Wilson disease.** The cumulative survival of 60 Austrian patients was followed. The broken lines indicate 'survival rates' if death or liver transplantation (LTX) are chosen as endpoints. The bars refer to the number of patients dying from or transplanted for fulminant Wilson disease (fWD) or decompensated cirrhosis.

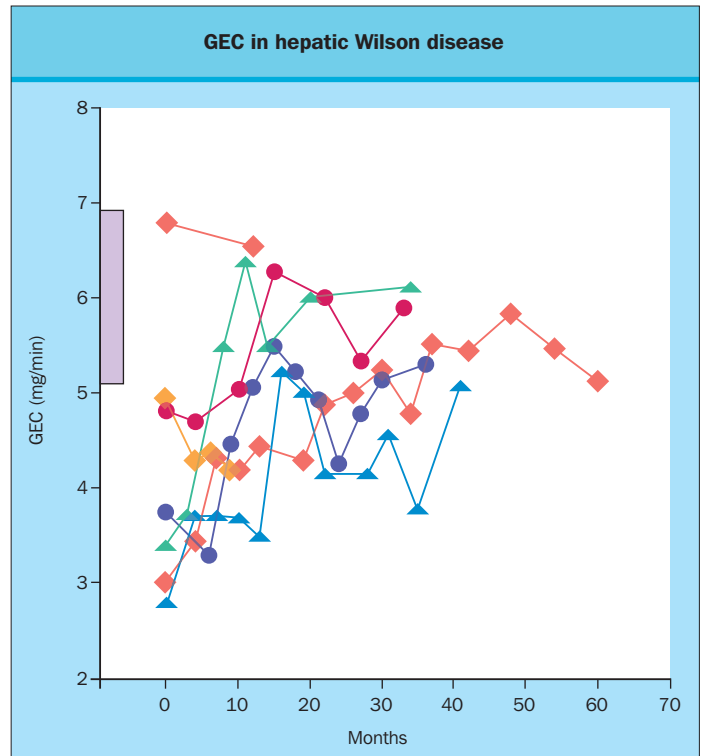
24.11) and liver function parameters (serum albumin, prothrombin time) will become normal in most patients within 1 to 2 years. In compliant patients treated with D-penicillamine or trientine, liver function remains stable and no progressive liver disease is observed.

Schilsky et al (1991) followed 20 patients with Wilsonian chronic active hepatitis. Treatment with D-penicillamine was promptly initiated in 19 patients. One refused treatment and died 4 months later. Treated patients received D-penicillamine or trientine for a total of 264 patient-years (median: 14). In 18 patients, symptomatic improvement and virtually normal levels of serum albumin, bilirubin, aspartate aminotransferase and alanine aminotransferase followed within 1 year. One woman died after 9 months of treatment. Two patients, who became non-compliant after 9 and 17 years of successful pharmacologic treatment, required liver transplants.

In patients presenting with fulminant Wilson disease, medical treatment is rarely effective. Without emergency liver transplantation, mortality is very high. In a group of 34 patients, a prognostic index based on serum bilirubin levels, aspartate aminotransferase activity, and prothrombin time (Table 24.9) was developed. A score  $>7$  was always associated with death. However, this prognostic score has not been validated prospectively. Nevertheless, it is a useful guide to assess short-term mortality in the setting of liver transplantation.

### Hemolytic anemia

If diagnosed and treated early, hemolysis subsides within a few days after initiation of D-penicillamine therapy. Spontaneous



**Figure 24.11 GEC in patients with hepatic Wilson disease.** Serial determination of galactose elimination capacity (GEC) in seven patients with hepatic Wilson disease.

Prognostic index in Wilson disease					
	0*	1*	2*	3*	4*
Serum bilirubin ( $\mu\text{mol/L}$ )	$<100$	100–150	151–200	201–300	$>301$
AST ( $\times$ ULN)	$<2.5$	2.6–3.5	3.6–5	5.1–7.5	$>7.5$
PTT (sec over control)	$<4$	4–8	9–12	13–20	$>21$
Modified from Nazer et al (77). * = score points; AST, aspartate aminotransferase; PTT, partial thromboplastin time; ULN, upper limit of normal A score = 7 is associated with high probability of death					

**Table 24.9 Prognostic index in Wilson disease.**

remissions may occur even without treatment but relapse usually within few months. Hemolysis associated with active liver disease may progress rapidly to fulminant Wilson disease.

### Neurologic disease

Patients presenting with neurologic symptoms have a better prognosis than those presenting with liver disease. None of our patients died; two required liver transplantation. In Brewer's series (Brewer et al 1992), 2 out of 54 patients died due to complications which were attributed to their impaired neurologic function. Neurologic symptoms are partly reversible. Improvement of neurologic symptoms occurs gradually over several months. Initially, neurologic symptoms may worsen,

especially on treatment with D-penicillamine. In some patients neurologic symptoms disappear completely, and abnormalities documented by evoked responses or MRI may completely resolve within 18–24 months.

Ten of our patients were followed prospectively for 5 years after diagnosis. Brain function was assessed by repeated recording of short latency sensory potentials, auditory brainstem

potentials and cognitive P300 evoked potentials. Electrophysiologic and clinical improvement was observed as early as 3 months after initiation of chelation therapy and continued until final assessment after 5 years. Three patients became completely normal but residual symptoms were detectable in seven. Such permanent deficits are unlikely to be improved by liver transplantation.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 25

## The Porphyrrias, $\alpha_1$ -Antitrypsin Deficiency, Cystic Fibrosis and Other Metabolic Diseases of the Liver

Herbert L. Bonkovsky and James H. Reichheld

### INTRODUCTION

In this chapter, we describe a number of disorders of intermediary metabolism that affect the liver. For the most part, these are due to hereditary inborn errors of metabolism that are manifested relatively early in life. Some such diseases (e.g. hereditary hemochromatosis, Wilson disease, and other inborn disorders of copper metabolism) are subjects of other chapters in this book.

Advances in molecular medicine have done much to advance our understanding of these disorders and are leading to promising new diagnostic and therapeutic interventions. Organ (liver and bone marrow) transplantation has already found its way into the therapy of some of these disorders. Identification of single enzyme defects has facilitated replacement enzyme therapy in Gaucher disease and avoidance of enzyme substrates in hereditary fructose intolerance. Identification of causative genes and their defects has facilitated appropriate genetic counseling, prenatal screening, and potential recombinant enzyme replacement therapy for several metabolic diseases of the liver. Capacity for curative treatment has been demonstrated in animal models of gene therapy, some of which are now leading to human trials.

### THE PORPHYRIAS

The porphyrias are diseases caused by defects in heme synthesis. They have been implicated in the popularized ‘madness’ of King George III of England and Scotland, in Vincent van Gogh’s bouts with psychiatric illness, and fancifully as a biologic basis for the legend of vampires. In all the porphyrias, specific enzyme defects in the biosynthetic pathway of heme result in the accumulation of toxic metabolites, manifested variably as disorders of the skin, nervous system and liver, depending on the enzyme affected and the pattern of accumulation of metabolic intermediates. Although hepatic effects of the porphyrias may be mild, systemic effects may be marked and varied. Indeed, the porphyrias may deceptively mimic other clinical syndromes; and, more than two generations ago, porphyria was called ‘the little imitator’ by Jan Waldenström, in deference to syphilis, known then as ‘the big imitator’.

The porphyrias are classified as *hepatic* or *erythropoietic*, based on the organ in which the major overproduction of heme precursors occurs (Table 25.1). The *acute hepatic porphyrias* may be associated with acute neurologic crises. Such crises can be exacerbated and even precipitated by an array of medications, such that many porphyric crises are unwittingly induced

and often iatrogenic. Although generally rare, the porphyrias are more common in specific populations, and they have been associated with an increased risk of hepatocellular carcinoma.

The diagnosis of porphyria is based on detection of porphyrins or porphyrin precursors (5-aminolevulinic acid, porphobilinogen) in blood or urine, and further distinction is based on the patterns of porphyrins (and/or precursors) in urine, stool or plasma. High concentrations of porphyrins within urine may impart a purple or red color, justifying the name ‘porphyria’, from the Greek *porphyra* for ‘purple’. Porphyric crises may be minimized by avoiding precipitants, and the symptoms of acute attacks can be managed by suppressing the over production of these potentially toxic precursors of heme.

### Epidemiology and pathophysiology

Different porphyrias may predominate in specific geographic areas. For example, among the acute porphyrias, variegate porphyria is the most common type found in South Africa, whereas the acute intermittent type is the predominant acute porphyria in Europe and the USA. Some are autosomal dominant, whereas others are recessive; and, for protoporphyria, the pattern of inheritance is apparently variable. Symptomatic acute porphyria typically presents only after the onset of puberty, and crises of acute porphyria affect women more frequently than men. Drugs are the most common precipitants of attacks in the acute hepatic porphyrias, inciting symptomatic disease in predisposed individuals. Although the list of potential offenders is extensive, common precipitants include barbiturates, hydantoins, sulfonamides, oral contraceptives, carbamazepine, and valproic acid. Ethanol may exacerbate the acute porphyrias, porphyria cutanea tarda (PCT), and protoporphyria.

An understanding of the pathophysiology of the porphyrias follows study of the biosynthetic pathway of heme (Fig. 25.1). Eight enzymatic steps are involved, and seven main defects are described. Deficiencies of enzymatic activities result in overproduction of toxic precursors by feedback derepression of  $\delta$ -aminolevulinic acid (ALA) synthase, the first and rate-controlling enzyme of the pathway. ALA synthase condenses glycine and succinyl coenzyme-A to form ALA, a probable neurotoxin. Heme normally provides repression of this enzyme (Fig. 25.2a); a deficiency of intracellular heme in hepatocytes results in elevated ALA production (Fig. 25.2b). Porphobilinogen (PBG), the metabolite following ALA, which is also produced in excess in all of the acute porphyrias, except for that due to severe deficiency of ALA dehydratase (PBG synthase) (Table 25.1), may similarly be neurotoxic, but there is less evidence for

The porphyrias with major hepatic manifestations – classification and features						
Disease	Enzyme defect	Autosomal inheritance	Clinical features	Metabolite profile with overt disease		
				Urine	Stool	Blood
<b>Acute Hepatic Porphyrias</b>						
ALA dehydratase deficiency (severe)	ALA dehydratase	Recessive	Neurovisceral	<b>ALA</b> <b>PBG</b> (URO) <b>COPRO</b>	Normal	<b>PROTO</b>
Acute intermittent porphyria	Porphobilinogen deaminase	Dominant	Neurovisceral	<b>ALA</b> <b>PBG</b> (URO) (COPRO)	(URO) (COPRO) <b>PROTO</b>	(URO) (COPRO) (PROTO)
Hereditary coproporphyria	Coproporphyrinogen oxidase	Dominant	Neurovisceral cutaneous	<b>ALA</b> <b>PBG</b> (URO) <b>COPRO</b>	(URO) <b>COPRO</b>	(COPRO)
Variegate porphyria	Protoporphyrinogen oxidase	Dominant	Neurovisceral cutaneous	<b>ALA</b> <b>PBG</b> (URO) <b>COPRO</b>	(URO) <b>COPRO</b> <b>PROTO</b>	(PROTO)
<b>Other Hepatic Porphyrias</b>						
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Dominant or sporadic	Cutaneous	(ALA) <b>URO</b> (COPRO)	<b>URO</b> <b>COPRO</b>	(URO)
Protoporphyria	Ferrochelatase	Dominant or recessive	Cutaneous	(URO) (COPRO)	(COPRO) <b>PROTO</b>	(COPRO) <b>PROTO</b>
ALA, 5-aminolevulinic acid; URO, uroporphyrin; COPRO, coproporphyrin; PROTO, protoporphyrin; PBG, porphobilinogen. Bold type indicates an elevated level; parentheses indicate that the level may be normal or slightly elevated. When a metabolite is not listed, its level is typically not elevated.						

Table 25.1 The porphyrias with major hepatic manifestations – classification and features.

this than for ALA. Neurologic effects may also be due to heme deficiency in neuronal tissue. The cutaneous features of the porphyrias are secondary to photosensitization by porphyrins, although the specific cutaneous effects of excess protoporphyrin differ from those of the other naturally occurring porphyrins.

In the acute hepatic porphyrias, the chief site of porphyrin production is the liver. Other than the enzymatic abnormalities, however, there is little structural or functional liver pathology. Nonetheless, with progressive disease, porphyrins may be directly hepatotoxic. Heavy alcohol use can precipitate or exacerbate the acute hepatic porphyrias. Iron overload associated with PCT may be responsible for hepatocellular damage, particularly in association with hepatitis C infection. An increased risk of the development of hepatocellular carcinoma has been associated with acute intermittent porphyria. PCT is also associated with an increased risk of development of hepatocellular carcinoma, although it is not yet clear whether such risk can be attributed to the accumulation of hepatic uroporphyrin per se or to other causes of chronic hepatitis or cirrhosis associated with PCT (e.g. alcohol, iron overload or chronic hepatitis C).

### Clinical features

#### Hepatic porphyrias

There are five hepatic porphyrias, four associated with acute neurologic crises, called *acute hepatic porphyrias*. They include

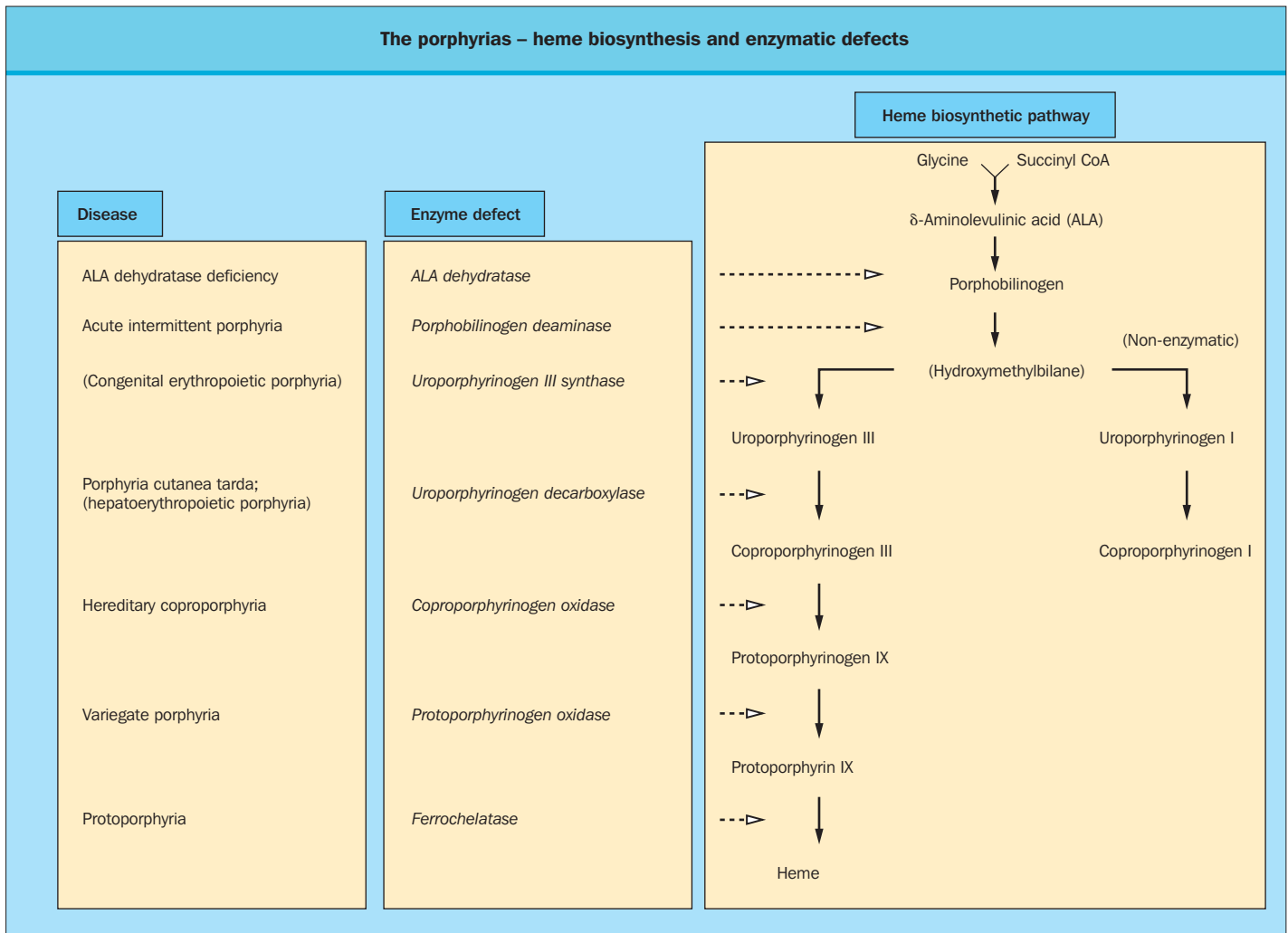
*5-aminolevulinic acid dehydratase deficiency (ADDP)*, *acute intermittent porphyria (AIP)*, *hereditary coproporphyria (HCP)*, and *variegate porphyria (VP)* (see Table 25.1 and Fig. 25.1). PCT involves hepatic accumulation of porphyrins and may be associated with liver disease, but it is not associated with acute crises. A syndrome resembling acute neurovisceral hepatic porphyria may be induced by lead toxicity. As discussed elsewhere in this chapter, hereditary tyrosinemia type 1, which is associated with overproduction of 4,6-dioxoheptanoic acid (succinylacetone), a potent inhibitor of ALA dehydratase (see Fig. 25.12), may also be associated with porphyric crises. Acute attacks are generally more common in women, usually after the onset of puberty, and are associated with the luteal phase of menstruation, such that some women suffer attacks monthly.

#### Acute hepatic porphyrias

##### Severe deficiency of ALA dehydratase

Severe deficiency of ALA dehydratase results in decreased conversion of ALA to PBG and, therefore, in greatly increased ALA formation without increased PBG formation. For unclear reasons, increased excretion of coproporphyrin may also occur. Recurrent, severe neurologic attacks may occur, marked by abdominal pain and even respiratory and bulbar paralysis. Some paresis may be permanent. In order for clinical manifestations to occur, a profound deficiency of ALA dehydratase (<10% of





**Figure 25.1 The porphyrias – heme biosynthesis and enzymatic defects.** ALA,  $\delta$ -aminolevulinic acid. Open-headed (horizontal) arrows indicate the sites of enzymatic defects; solid (vertical) arrows represent the normal synthetic pathway. Diseases in parentheses are erythropoietic porphyrias and are not discussed in this chapter.

normal) must be present, because normally the activity of this enzyme in the liver is present in great excess, compared with ALA synthase or PBG deaminase. Fortunately, profound deficiency of ALA dehydratase is rare. It may be hereditary (homozygous or compound heterozygous deficiency) or acquired (due to inhibitors such as 4,6-dioxoheptanoic acid or lead).

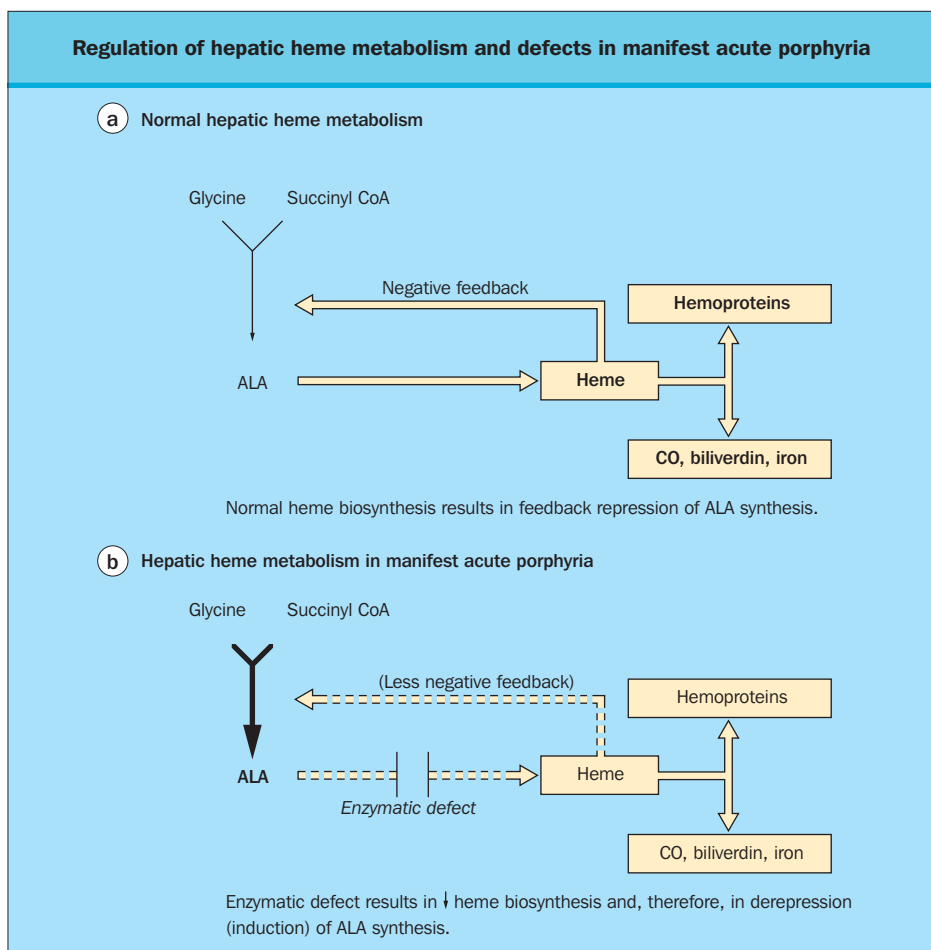
#### Acute intermittent porphyria

The most common form of acute porphyria in the USA, AIP is an autosomal dominant disorder of variable penetrance, which is due to a deficiency of PBG deaminase. The gene (located on chromosome 11q24.1-2) has been cloned and sequenced. Two closely similar forms of mRNA have been identified. The housekeeping form is larger than the erythroid form. The two forms differ in that exon 2 is skipped in the housekeeping form, whereas exon 3 is skipped in the erythroid form. Numerous genetic defects, including point mutations, splice defects and exon deletions, have been identified, all of them leading to the AIP phenotype. No clear phenotypic manifestations have been related to specific genotypic abnormalities.

In most parts of the world, it is thought that the defective gene is carried by 5–10/100 000 persons. Most commonly, women present in the third decade of life, men in the fourth; and attacks are precipitated by medications or stress. The most common symptom of AIP is colicky abdominal pain that may last days and is frequently accompanied by constipation, nausea and vomiting. Pain and paresthesias over the trunk and extremities are also common. Despite these sensations, neurological examination more typically reveals a motor neuropathy. An array of other neurologic abnormalities may be found on examination, including tachycardia and hypertension, bulbar findings and areflexia. Psychological effects may be manifested subtly as depression or anxiety or may rage as frank psychosis and delirium. Hyponatremia may occur, associated with the syndrome of inappropriate release of anti-diuretic hormone. Hyponatremia, along with the neurologic crisis, may result in seizures or coma. Death may follow infection associated with recurrent aspiration and/or pulmonary paralysis.

#### Hereditary coproporphyria

Estimates of the incidence and prevalence of HCP are made difficult by its more benign course and less severe presentation



**Figure 25.2 Regulation of hepatic heme metabolism and defects in manifest acute porphyria.** ALA,  $\delta$ -aminolevulinic acid.

than AIP. Deficiency of coproporphyrinogen oxidase results in elevated coproporphyrin III, excreted in stool and less so in urine. The gene for this enzyme has been cloned and sequenced, localized to chromosome 9, and numerous mutations have been described. Acute attacks, similar to those in AIP, may present as a vesiculobullous dermatitis covering the face, hands or other exposed skin. Coproporphyrin III is excreted in bile, and any cholestatic process will increase blood levels and promote active disease.

#### Variegate porphyria

Also called South African porphyria because of its high incidence there, VP is caused by deficiency of protoporphyrinogen oxidase, the gene for which has been localized to chromosome 1q22. Approximately 3 in 1000 South Africans of Dutch ancestry are affected by a single point mutation traced to a common founder who emigrated from the Netherlands to Cape Town in 1688. VP is usually dominantly inherited. Symptoms and signs are variable, as its name suggests, and patients may suffer attacks similar to AIP, photosensitivity similar to HCP, or may be entirely asymptomatic. Liver tissue fluoresces under ultraviolet light but shows no other characteristic changes. Recent data from South Africa suggest an association between VP and hepatocellular carcinoma, and it is likely that all forms of acute porphyria are associated with increased risk of this cancer.

#### Chronic hepatic porphyrias

##### Porphyria cutanea tarda

Because its principal enzymatic defect is in the liver, PCT is classified as a hepatic porphyria but not an acute porphyria because acute neurologic attacks do not occur. The enzymatic deficiency is in uroporphyrinogen decarboxylase. Three types of PCT have been identified. Type I is the most common, comprising about 75% of cases. It is caused by an apparently acquired or sporadic and potentially reversible deficiency of hepatic uroporphyrinogen decarboxylase (Uro-D) activity. The causes of the deficiency are often unclear and probably multifactorial. Recognized associations include heavy exposure to alcohol, halo-aromatic chemicals such as polychlorinated biphenyls or tetra-chloro-dibenzo-*p*-dioxin, exposure to estrogens, iron overload, and chronic hepatitis C.

Type II PCT is a form in which activity of uroporphyrinogen decarboxylase is decreased 50% in all tissues, including the liver. The cause of this deficiency is a hereditary defect. However, this degree of decrease per se is not sufficient to cause clinical or biochemical features of active PCT. This is not surprising because hepatic activity of uroporphyrinogen decarboxylase is much higher than that of PBG deaminase or ALA synthase. Thus, even a 50% decrease in uroporphyrinogen decarboxylase would not generally be sufficient to limit the rate of heme biosynthesis. Other factors, including those already listed for type I, are also important in the pathogenesis of clinical illness in type II PCT.

Type III PCT is a rare form in which an apparently inherited defect in uroporphyrinogen decarboxylase occurs, but only in the liver. Subjects with type III PCT thus have positive family histories, normal erythrocyte uroporphyrinogen decarboxylase, and decreased hepatic uroporphyrinogen decarboxylase. Because there is apparently only one gene for uroporphyrinogen decarboxylase in all tissues, it is far from clear how or why type III PCT occurs. The gene for uroporphyrinogen decarboxylase has been cloned and sequenced, localized to the short arm (p) of chromosome 1, and numerous mutations have been identified, all of which may give rise to a PCT phenotype. No clear phenotypic-genotypic associations have emerged to date.

PCT is the most common hepatic porphyria and has classically been associated with the Bantus of South Africa who used to drink an iron-laden traditional beer that had been brewed in iron pots. Indeed, some degree of hepatic iron loading (usually mild) is both a common finding and an important exacerbant of hepatic pathology, and there is an association between symptomatic PCT and hemochromatosis. Clinical findings include photosensitivity (**Fig. 25.3a**) similar to that in HCP or VP, and hepatic disease is consistently present, often in association with chronic hepatitis C, alcohol ingestion, iron overload or exogenous estrogens. Deposition of uroporphyrin may itself be toxic. Moderate elevations of serum aminotransferases are typical, whereas cholestatic indices are usually normal or mildly elevated. Some degree of glucose intolerance and insulin resistance is common, and frank diabetes mellitus is not unusual.

Liver biopsy demonstrates moderate siderosis and may show steatosis, fibrosis and necrosis. The tissue fluoresces brightly under ultraviolet light (**Fig. 25.4**). An association has been elucidated between patients with sporadic (versus familial) PCT and hepatitis C virus (HCV) infection, such that 60–90% of PCT patients test positive for HCV antibody or RNA. There is an association between both persistent serum aminotransferase

elevation and the severity of liver disease in PCT and the presence of HCV infection. HCV is a precipitant of PCT, and successful eradication of HCV infection may be sufficient to treat PCT although initial iron depletion is still the standard of care. Iron overload seems to potentiate liver disease in patients with either HCV or PCT, and hepatic iron overload in patients with PCT may predispose those with concurrent HCV infection to more severe hepatocellular disease. The main reason for increased hepatic iron in PCT is the C282Y mutation of the *HFE* gene (see Ch. 23). In some studies, the H63D mutation has also occurred with increased frequency in PCT patients compared with controls.

### **Erythropoietic porphyrias**

Our discussion here is limited to protoporphyria, which may produce severe hepatobiliary disease. Congenital erythropoietic porphyria, erythropoietic coproporphyria and hepatoerythropoietic porphyria are quite rare disorders due to autosomal recessive mutations.

### **Protoporphyria (erythropoietic protoporphyria)**

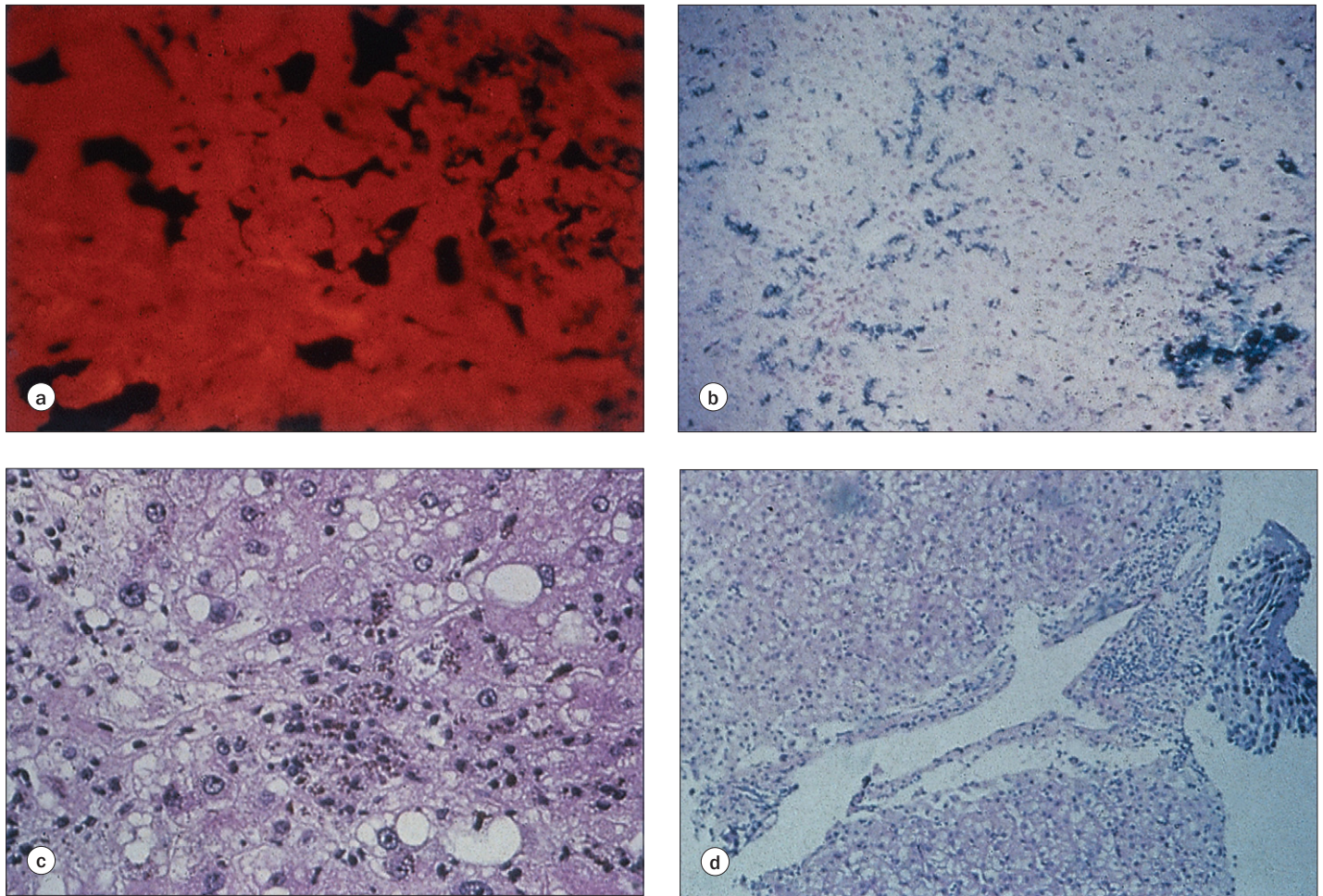
Protoporphyria (PP) is caused by a hereditary deficiency in the activity of ferrochelatase (see Figs 25.1, 25.2 and Table 25.1). Although this deficiency is present in all tissues, the major overproduction of PP occurs chiefly within developing erythrocytes. The gene for ferrochelatase has been cloned and sequenced, localized to chromosome 18q22, and numerous mutations associated with a PP phenotype have been described. Some of these appear to be more severe than others, with greater risks for development of liver damage, which is the most serious potential complication of the disease.

The inheritance of PP is complicated and variable among affected kindred. It is autosomal dominant with low penetrance in most, autosomal recessive in others; and it has been attributed in some to a genetic defect in the ferrochelatase gene



**Figure 25.3 The porphyrias: cutaneous findings.** (a) Severe ulceration of the hands in a patient with porphyria cutanea tarda (PCT). The cutaneous changes of PCT are not typically at such an advanced stage. (b) The hand of a patient with protoporphyria, showing subtle but characteristic thickening of the skin over the knuckles, so-called 'peau d'orange' (orange skin-like change).





**Figure 25.4 Porphyria cutanea tarda (PCT): liver histopathology.** (a) Unstained liver biopsy from a patient with PCT, viewed under fluorescence microscopy. (b) Photomicrograph of a liver biopsy from a patient with PCT showing siderosis, an early finding (hematoxylin and eosin,  $\times 250$ ). (c) Photomicrograph of a liver biopsy from a patient with PCT showing fatty infiltration of the liver. (d) Low-power photomicrograph showing fibrosis and the development of hepatoma in a liver biopsy from a patient with PCT (hematoxylin and eosin,  $\times 100$ ). [(a)–(d), courtesy of J Bloomer.]

coupled to a second genetic defect at another locus. Even in those with apparently autosomal dominant inheritance, the activity of ferrochelatase, measured in the liver, fibroblasts or lymphocytes, is much less than the 50% of normal that would be predicted for a defect in one of two alleles. Because ferrochelatase is a dimer, one interpretation of these results is that only dimers composed of two normal subunits retain activity. Evidence for this model has come from studies involving ultraviolet inactivation of ferrochelatase.

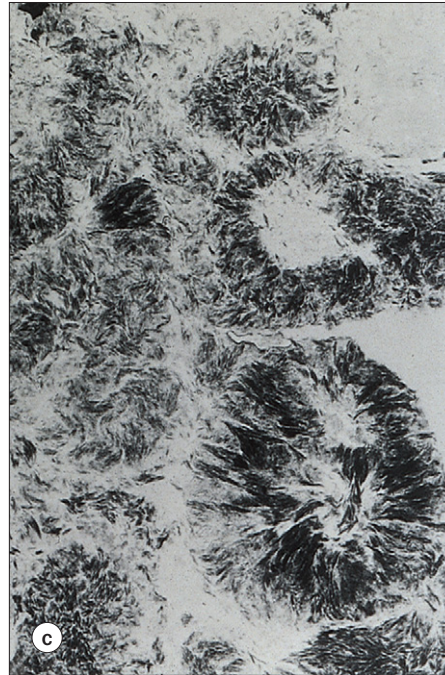
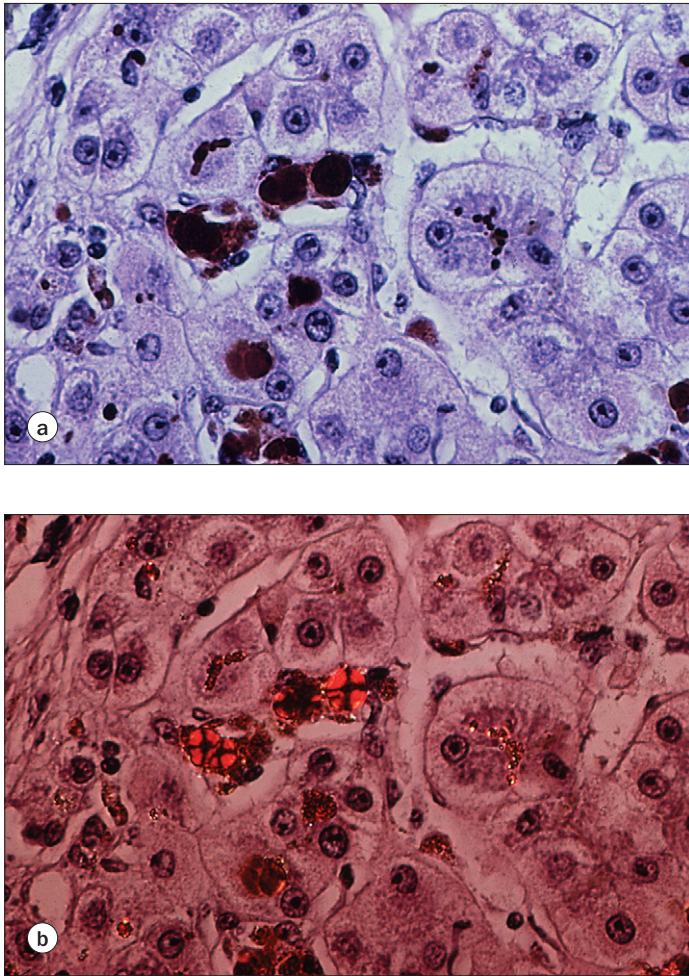
A large and still growing number of genetic abnormalities of ferrochelatase have been described, including missense mutations, nonsense mutations, intragenic deletions and splicing abnormalities. Exon skipping has been the most common abnormality. In addition to such defects in one of the two alleles of the ferrochelatase gene, a genetic polymorphism (a C $\rightarrow$ T transition at position -23) in intron 1 of the other allele has been found consistently in PP patients, especially those with more marked deficiencies of ferrochelatase activity and liver disease. Thus, the following have been proposed: Two mutant alleles or, much more commonly, one mutant allele plus the -23 C $\rightarrow$ T transition in the other allele, are needed for phenotypic expression of PP. The -23 C $\rightarrow$ T allele probably reduces ferrochelatase gene expression mildly and helps to account for

the variability in enzyme activities among patients and controls. However, even homozygosity for the -23 C $\rightarrow$ T transition is insufficient to produce PP. Of course, other genetic and environmental factors are also of potential pathophysiologic importance in PP.

PP was not clearly characterized until the 1960s despite its relative frequency. Although likely to be the second most common porphyria after PCT, PP may be missed because an excess of protoporphyrin, the biochemical hallmark, is detectable only in blood or stool, not urine. Therefore, testing, if limited to the urine, will miss the diagnosis.

In PP, skin photosensitivity is manifested acutely by stinging, burning, or itching of exposed skin, followed within several hours by erythema, edema, and, chronically, by scarring hypertrophic changes (see Fig. 25.3b). The chronic changes in PP are usually less severe than in PCT, HCP or VP. Marrow overproduction and accumulation of protoporphyrin results in characteristic fluorescence of young red cells forms. Mild hypochromic, microcytic anemia is common, often with low serum ferritin and transferrin saturation, suggesting iron deficiency. It is unknown why iron deficiency is so common. Pigmented gallstones, laden with protoporphyrin, are frequently found even in young patients. Deposition of protoporphyrin





**Figure 25.5 Protoporphyria: liver histopathology.** (a) Photomicrograph of a liver from a patient with protoporphyria, showing brown deposits of protoporphyrin IX within Kupffer cells, canalliculi, and hepatocytes (hematoxylin and eosin,  $\times 400$ , regular light). (b) The same tissue as (a), visualized under polarized light, showing characteristic birefringence of protoporphyrin IX (hematoxylin and eosin,  $\times 400$ , polarized light). [(a) and (b), courtesy of G Klatskin.] (c) Electron microscopy of protoporphyrin IX crystals within the liver of a patient with protoporphyria. (Courtesy of J Bloomer.)

crystals is found in hepatocytes and Kupffer cells, resulting in liver fluorescence and, under polarized light, a characteristic birefringence (Fig. 25.5). Hepatic pigment deposition also causes the disease's most severe effects: non-fatal hepatic fibrosis, cirrhosis or unremitting cholestasis and liver failure.

### Diagnosis

The porphyrias are difficult, if not impossible, to diagnose on the basis of clinical findings alone because of their protean manifestations, as outlined earlier. Still, while porphyria is often diagnosed only after an extensive clinical evaluation, most patients referred to centers specializing in the evaluation and care of patients with porphyria do not have the disease. It is important to think of the diagnosis of porphyria in patients with consistent clinical features. However, it is especially important not to jump to an unwarranted diagnostic conclusion unless biochemical findings are fully supportive. In this regard, the most important confounding entities are the secondary porphyrinurias.

Screening tests for porphyria rely on rapid detection of excess PBG or porphyrins in urine, blood or stool. The Hoesch and Watson-Schwartz tests detect urinary PBG, which reacts with Ehrlich reagent to form a visible red pigment. The Hoesch test is easy to perform and interpret at the bedside. One or two drops of urine are added to 2 to 3 mL of Ehrlich reagent (2 g

*p*-dimethylaminobenzaldehyde in 100 mL 6M HCl). The immediate development of a cherry red color on top of the solution is a positive test. Both the Hoesch and Watson-Schwartz tests may be confounded by pH indicators that are red in acid, by indoles, and by methyl dopa metabolites, each of which may lead to false-positive results. Then, too, conditions in which ALA dehydratase is greatly reduced will not produce an increase in urinary PBG. Quantitative measurements should, therefore, follow all positive qualitative test results.

The urine talc test and amyl alcohol test are qualitative tests for increased urinary porphyrins in which a Wood lamp is used to visualize a porphyrin-laden pink or red fluorescent phase. Quenchers of porphyrin fluorescence or dilute urine may cause a false-negative test result, and some non-porphyrin compounds may fluoresce. Positive test results are, therefore, confirmed by high-performance liquid chromatography (HPLC) or methods that quantify porphyrins and show their patterns of excretion.

PP does not result in increased urine porphyrin excretion and, therefore, cannot be diagnosed by examination of the urine. Through an acid extraction technique, erythrocyte protoporphyrin can be identified under a Wood lamp. PP and the other erythropoietic porphyrias will test positive, as will conditions such as lead poisoning or iron deficiency, in which erythrocytic zinc protoporphyrin levels are increased. This is because acids remove zinc from the chelate, leaving free

protoporphyrin. Direct fluorescence microscopy of erythrocytes can identify erythrocytes that contain increased porphyrin levels, though rapid fading, a problem particularly with protoporphyrin, may result in false-negative results. Stool porphyrins can be identified under a Wood lamp after extraction with amyl alcohol, glacial acetic acid and ether. Bacterial heme metabolism may result in false-positive results as may gastrointestinal bleeding. All such positive screening test results for porphyrins should be confirmed by specific quantitative testing. Similarly, with patients in whom the index of suspicion is high, apparently negative screening tests should also be confirmed by such testing.

A simple test of considerable value for diagnosis and differential diagnosis of the cutaneous porphyrias is the plasma porphyrin fluorescence pattern. In this test, the fluorescence emission spectrum of diluted plasma is measured. The exciting wavelength is the Soret band (400–410 nm). Uro- and coproporphyrin, which accumulate in PCT or HCP, have a peak at 618 nm and protoporphyrin, which accumulates in PP, has a peak at 636 nm. A protein–porphyrin complex unique to VP has a peak at 626 nm. The latter is nearly always present in post-pubertal VP patients, and is the simplest and most reliable method for making a presumptive diagnosis.

**Treatment**  
**Acute porphyrias**

The treatment of acute porphyria involves the careful avoidance of exogenous precipitants between attacks while giving supportive care and vigorously attempting to curb the production of heme precursors during an acute crisis. There are therapies beneficial to treatment of all the porphyrias, and others specific to a particular porphyria (Table 25.2).

**Treatment of acute attacks**

Initial management typically requires narcotic control of pain, which may be enhanced with the use of a phenothiazine, such as chlorpromazine. Tachycardia and hypertension may be controlled with  $\beta$ -blockade (nadolol, propranolol), and the

patient should be monitored carefully for possible progression of neuromuscular dysfunction, particularly the onset of respiratory paralysis.

By modifying enzyme activity, the production of heme precursors can be minimized. Through suppression of ALA synthase activity, administration of glucose intravenously may help to diminish symptoms. This ‘glucose effect’ takes advantage of observations in rodents and humans that fasting increases ALA synthase activity and that, to the contrary, carbohydrate administration reduces activity. A goal of 300 g/day or more of glucose or carbohydrate therapy is recommended in the acutely ill patient. Because of nausea, vomiting or poor gut motility, this may need to be given parenterally.

If promptly available, intravenous heme should be given immediately to any patient with acute porphyria who is sufficiently ill to require hospital admission. Treatment of acute porphyria with exogenous heme infusion was first done more than 35 years ago. By increasing the regulatory pool of heme and consequently promoting feedback repression of ALA synthase, administration of exogenous heme reduces ALA synthase activity. Although the biochemical effect to decrease hepatic ALA and PBG synthesis is fleeting, the clinical benefits of intravenous heme are usually more lasting. The usual dose is 3–4 mg/kg/day for 3–5 days. Potential side-effects include thrombophlebitis, coagulopathy and reversible renal tubular damage. In the USA, intravenous heme is available as Panhematin, the first developed and approved ‘orphan’ drug. It is now marketed by Ovation Pharmaceuticals (Deerfield, IL), and it can be ordered for immediate air shipment from O’Hare Airport 24 hours/day, 7 days/week by calling (888) 514-5204. Panhematin is a lyophilized powder cake. If it is dissolved in sterile water, as the manufacturer suggests, it must be administered within 1 h, because of complex polymerization or other reactions, which diminish its effectiveness and increase its toxicity. However, when redissolved in human serum albumin, hematin is stable for at least 24 h and has fewer side-effects as well.

In most European and some other countries, heme is available for human use in the form of heme arginate (Normosang, Leiras

The porphyrias: therapy for acute attacks and chronic disease			
Acute attacks	<ul style="list-style-type: none"><li>• Control pain with narcotics as warranted; consider phenothiazine</li><li>• Control hypertension and tachycardia with <math>\beta</math> blockade</li><li>• Monitor for neuromuscular/respiratory dysfunction</li><li>• Remove exacerbating drugs or chemicals</li><li>• Glucose/carbohydrate therapy, at least 300 g/day glucose</li><li>• Heme therapy as intravenous hematin, 3–4 mg/kg for 3–5 days</li><li>• LHRH analog for women with luteal phase recurrence</li></ul>		
Chronic or recurrent disease	General interventions	Porphyria cutanea tarda	Protoporphyria
	<ul style="list-style-type: none"><li>• Avoid alcohol, exacerbating drugs or chemicals</li><li>• Wear sunscreen, opaque clothing</li><li>• Treat skin infection promptly</li></ul>	<ul style="list-style-type: none"><li>• Diminish iron stores with phlebotomy</li><li>• Use chloroquine, hydroxychloroquine, or alkalization of urine to increase urinary porphyrin excretion</li><li>• Treat hepatitis C, if present</li></ul>	<ul style="list-style-type: none"><li>• Oral <math>\beta</math> carotene</li><li>• Erythrocyte transfusion, iron therapy</li><li>• Cholestyramine, activated charcoal</li><li>• Liver transplantation</li></ul>
LHRH, Leuteinizing hormone-releasing hormone			

Table 25.2 The porphyrias: therapy for acute attacks and chronic disease.



Pharmaceuticals, Helsinki, Finland). It, too, is thrombogenic and thrombophlebitic, and many experienced porphyriologists in Europe recommend diluting Normosang in human serum albumin, which decreases these adverse effects.

Women susceptible to luteal phase disease recurrence may benefit from a luteinizing hormone-releasing hormone (LHRH) analog (e.g. leuprolide acetate), which is typically continued for over a year. Side-effects include an initial worsening of symptoms and osteopenia. For the latter, the patient should prophylactically receive calcium and vitamin D supplements and, if warranted, bisphosphonates. Oral contraceptives have been used in place of LHRH analogs with apparent benefit, but they have the potential disadvantage of increasing ALA synthase activity and consequently precipitating attacks.

### Prevention of attacks

Factors known to be capable of precipitating or exacerbating acute attacks of disease should be avoided. The most important of these are alcohol (more than two drinks per day), other inducing drugs or chemicals [any agent capable of inducing hepatic cytochrome(s) P450], intercurrent infections, starvation, severe dieting or any dieting regimen that does not provide adequate daily intake of carbohydrate and protein.

All first-degree relatives of known patients should be evaluated carefully, with specific molecular testing (if the genomic abnormality has been identified in the index case of the family), with measurement of enzymatic activities in appropriate tissues (if such a defect has been characterized in the index case), or with quantitative measurement of urinary, fecal and plasma porphyrins (and, for urine, of the porphyrin precursors ALA and PBG). Such evaluations are best performed in a specialized center, because reliable assays and kindred analyses are not widely available. These studies are best done in children close to puberty, because acute attacks are rare before puberty, attesting to the role played by sex hormones in the pathogenesis of disease.

Reliable, validated assays of enzymatic activities deficient in the acute porphyrias are available only for ALA dehydratase and PBG deaminase, both of which are cytoplasmic and, therefore, present even in adult erythrocytes. In contrast, coproporphyrinogen and protoporphyrinogen oxidase are located in mitochondria and, therefore, are not present in mature erythrocytes. Reliable assays for the latter thus require the use of cells containing mitochondria (e.g. leukocytes or cultured skin fibroblasts).

All members of affected kindreds found to harbor the relevant enzymatic deficiency should be counseled to avoid the potential triggers of acute attacks (vide supra).

In Europe, there are now several government-funded centers that carry out sophisticated biochemical and molecular methods for differential diagnosis and genetic characterization of patients and families with porphyria. A listing can be found at [www.porphyrifoundation.com](http://www.porphyrifoundation.com). In the USA, several large commercial labs offer chemical testing of feces, plasma, and urine and erythrocytic enzymatic assays. However, to our knowledge, the plasma fluorescence pattern (vide supra) is offered only by K. Anderson, University of Texas Medical Branch. If a diagnosis of porphyria has been established, based upon clinical and biochemical-lab findings, efforts to identify specific genetic defects can be performed by R.J. Desnick (Dept

of Human Genetics, Mt Sinai School of Medicine, New York, NY, phone 212- 659-6700, <http://www.mssm.edu/genetics/>).

### Porphyria cutanea tarda and protoporphyria

Overt symptomatic PCT should be treated by removal of potential offending drugs and chemicals (e.g. estrogens, iron and alcohol). Although not clearly shown to exacerbate human PCT, P450-inducing drugs such as barbiturates or hydantoins clearly exacerbate the uroporphyria that occurs in experimental models of PCT and are also best avoided.

The cornerstone of therapy of PCT continues to be the removal of iron from the liver, accomplished most rapidly and economically by therapeutic venesection. Usually, 400–500 mL of blood is removed per week, continuing until near iron deficiency has been achieved (serum ferritin <20 ng/mL). Typically, patients with PCT have a mild-to-moderate degree of iron overload; some are heterozygous for hereditary *HFE*-linked hemochromatosis; and a few are homozygous for this disease. Removal of 8–16 pints of blood (ca 2–4 g iron) is generally needed. However, iron depletion induces remission of PCT even when iron stores are normal. Patients with anemia and PCT can often be venesected if recombinant erythropoietin is given. This is particularly useful in the growing numbers of subjects with PCT complicating chronic renal failure and dialysis.

An antimalarial agent (chloroquine, hydroxyquine) may be added if the patient is severely affected. By forming water-soluble complexes with uroporphyrins, chloroquine and hydroxychloroquine increase urinary porphyrin excretion. Treatment with these agents should be instituted at low doses (125 mg b.i.w.), and the patient should be monitored for retinopathy. Doses can be increased to 125–250 mg daily after several weeks. Alkalization of the urine also promotes urinary excretion of porphyrins.

The association between HCV and PCT described earlier suggests potential new interventions, and resolution of PCT after antiviral therapy of chronic hepatitis C alone has been reported in a few subjects. Iron reduction for HCV-infected PCT patients with liver pathology may prove to be particularly important because iron reduction in hepatitis C patients without PCT has shown potential benefit in several studies.

Active skin disease in PCT should be treated by avoidance of light exposure (opaque clothing and sunscreens) and trauma (because the skin is abnormally fragile). Typical sunblock products (e.g. *p*-aminobenzoic acid) are ineffective, because they do not block light of 400–415 nm, which, as already described, is the type of light that excites cutaneous porphyrins and, with oxygen, gives rise to the cutaneous manifestations of all the cutaneous porphyrias.

The management of PP chiefly involves efforts to:

- minimize PP overproduction,
- ameliorate cutaneous symptoms and protect the skin from damage,
- remove symptomatic PP-rich gallstones, and
- avoid hepatic injury caused by excess accumulation of PP.

Because iron deficiency exacerbates PP overproduction, it is important to be sure that such deficiency is not present. Most patients with PP are mildly anemic, due to the inborn error in heme synthesis. Thus, serum ferritin and transferrin saturation must be used to assess iron status. Hypertransfusion also

reduces PP overproduction, but its use is limited to the minority of patients with PP with unusually severe disease.

Amelioration of the cutaneous manifestations of PP involves the avoidance of sunlight, the use of wide-brimmed hats and clothing opaque to light, the use of opaque, barrier sunscreens, and the administration of  $\beta$ -carotene. The latter must be used in large doses (120–180 mg/day), which impart a yellowish tinge to patients' complexions. Although most patients feel that they do benefit from high doses of  $\beta$ -carotene, the benefit is modest at best, and some take it only in the summer. Secondary infection of skin should be treated promptly to avoid further complications.

Protoporphyrin-laden gallstones occur frequently in patients with PP, often at young ages. Stones causing symptoms should be removed by cholecystectomy.

The serious potential complication of PP is the development of pigmentary cirrhosis and liver failure. Anything that can cause or exacerbate cholestasis may potentiate and accelerate these processes. Therefore, all patients with PP who are not already immune should be vaccinated against hepatitis A and B, should be strongly counseled not to drink alcohol to excess (more than two drinks/day for men, more than one drink/day for women) and not to use drugs that have a high propensity to cause cholestasis (e.g. 17-alkylated androgens, phenothiazines). Although it was hoped that high doses of oral charcoal or colestyramine would enhance fecal excretion of PP and reduce its enterohepatic circulation, there is scant evidence for the benefit of such therapy, and its chronic use is unacceptable to most patients. Similarly, administration of bile salts [cheno- or ursodeoxycholic acid (UDCA)] has not led to convincing evidence of increased fecal excretion of PP or clinical benefit.

Patients with PP and cirrhosis should be considered for liver transplantation. They may unpredictably develop rapidly accelerating cholestasis and liver failure. Intravenous infusions of heme have led to the stabilization of a few such patients and served as a bridge to transplant. Regular plasmaphereses, followed by intravenous heme-albumin infusions, have helped patients with recurrent protoporphyrin hepatopathy after liver transplantation. Any confounding causes of cholestasis should be corrected. Transplantation in such patients poses formidable challenges due to their severe light sensitivity: operating lights should have filters that screen out light of 380–420 nm; some patients have developed severe neuropathy in the postoperative period. Because transplantation does not correct the erythropoietic enzyme defect, these patients require continued surveillance for recurrent liver disease. In theory, bone marrow transplantation should be curative, but has only rarely been performed in children with PP.

Prevention of PCT and PP involves avoidance of potential triggering factors and careful evaluation of relatives of index cases. Subjects at risk for development of PCT may be detected by assay of erythrocytic uroporphyrinogen decarboxylase (Uro-D), although, as already described, only about 25% of patients have a familial form in which activity of this enzyme is reduced. All first-degree relatives should be screened with at least random urinary measurements of porphyrin concentrations.

Prevention of overt PP involves kindred evaluation with measurement of erythrocytic and fecal PP concentrations. If they are elevated, such subjects should be checked for iron deficiency (which is to be avoided), should be counseled to

avoid exposure to hepatotoxins (e.g. alcohol, synthetic androgens and estrogens), and should be vaccinated to prevent viral hepatitis (A and B). This is because any intercurrent cholestatic liver disease may precipitate severe hepatic decompensation due to toxic effects of retained PP.

## $\alpha_1$ -ANTITRYPSIN DEFICIENCY

$\alpha_1$ -Antitrypsin ( $\alpha_1$ -AT) deficiency is a relatively common autosomal disease of Caucasians that may cause pulmonary disease and less frequently, hepatic disease.  $\alpha_1$ -AT deficiency remains the most common cause of pediatric metabolic liver disease. Inherited defects in the gene that encodes the  $\alpha_1$ -AT protein may cause lack of synthesis or alterations in the tertiary structure of the protein. Such structural abnormalities may disrupt the normal cellular  $\alpha_1$ -AT transport in hepatocytes, and the ensuing accumulation of the defective protein results in potentially severe liver disease and liver failure. The diagnosis is made by serum  $\alpha_1$ -AT quantification, protease inhibitor typing, DNA analysis and histologic analysis. Orthotopic liver transplantation is the definitive therapy for progressive disease but fortunately is required for only a few affected patients. It is important to consider  $\alpha_1$ -AT deficiency in the differential diagnosis of any patient with chronic hepatitis, cirrhosis or hepatocellular carcinoma of unclear etiology.

### Epidemiology and pathophysiology

$\alpha_1$ -Antitrypsin is a serine protease inhibitor, and its deficiency is therefore deemed one of the 'serinopathies', a heterogeneous group of diseases that share the common etiology of defective serine protease activity.  $\alpha_1$ -AT opposes the activity of trypsin and other proteases, most notably neutrophil elastase. It is normally synthesized in the liver and deficiency of  $\alpha_1$ -AT results in low serum levels. In the lung, low serum  $\alpha_1$ -AT prevents the normal repression of tissue elastase. Excess activity of this proteolytic enzyme results in excessive breakdown of normal elastin, resulting in diminished elastic recoil and abnormally high lung compliance. Ultimately, the patient suffers progressive obstructive pulmonary disease (emphysema) as the critical elastic component of lung recoil is diminished (**Fig. 25.6**). Whereas the likelihood of developing pulmonary disease is predictable from a patient's genotype, liver disease is less reliably predicted. Indeed, for unclear reasons, it is rare for patients to have both pulmonary and hepatic manifestations of disease.

A better understanding of the epidemiology of  $\alpha_1$ -AT deficiency can be gained by further examining the genetic basis for the disease. The  $\alpha_1$ -AT gene is on chromosome 14q3 and may be represented by several different alleles. These alleles have been identified by studying electrophoretograms of serum  $\alpha_1$ -AT, usually with comparison of relative rates of migration of the peptides. Each allele confers a different probability of developing clinical pulmonary or hepatic disease. Of more than 70 alleles, M (for 'main') is the most common normal allele, and S and Z are the most common mutant alleles. Homozygosity for the S ('slow') allele [ $P_i$ SS (protease inhibitor SS)] results in approximately half the normal serum level of  $\alpha_1$ -AT, with no associated disease. The most common Z ('slowest') mutant is caused by a point mutation resulting in a single base substitution (glutamine to lysine at amino acid 342), which produces



**Figure 25.6  $\alpha_1$ -Antitrypsin deficiency: chest roentgenogram.** Chest X-ray of a 39-year-old man with  $\alpha_1$ -antitrypsin deficiency showing advanced chronic obstructive pulmonary disease, with hyperinflated lungs and flattened diaphragms. This 41-year-old man developed clinical emphysema and cirrhosis in his 30s.

profound deficiency of serum  $\alpha_1$ -AT and pulmonary and hepatic disease in the homozygous patient,  $P_iZZ$  (protease inhibitor  $ZZ$ ). The  $P_iSZ$  genotype carries a low risk of clinical disease. Recent studies suggest heterozygotes for genotypes  $P_iMS$  and  $P_iMZ$  may also be at increased risk for cirrhosis and hepatocellular carcinoma, particularly when other concurrent liver insults are present, such as viral hepatitis or alcoholic liver disease.

Approximately 1 in 40 adult Caucasians (of North American or northern European origin) carry the  $Z$  allele for  $\alpha_1$ -AT, such that approximately 1 in 1600 live white births are  $P_iZZ$  genotype. However, only 10–20% of patients homozygous for the  $Z$  allele will develop hepatic disease. This discrepancy may well be related to the underlying mechanism of disease. Murine transgenic studies have shown that the *accumulation* of abnormal  $\alpha_1$ -AT in hepatocytes, not the *absence* of protease repression, leads to hepatic disease. The mutant amino acid sequence results in abnormal protein folding and thus adversely affects post-translational processing of  $\alpha_1$ -AT. The  $P_iZZ$  genotype appears to cause the reactive center of one  $\alpha_1$ -AT molecule to bind the defective and, therefore, inappropriately exposed  $\beta$ -pleated sheet of another. Successive binding of this type results in polymerization of  $\alpha_1$ -AT molecules. The transport of  $\alpha_1$ -AT from its site of synthesis, the hepatocellular rough endoplasmic reticulum, through the Golgi apparatus and into the circulating blood is severely impaired (Fig. 25.7). Polymerized  $\alpha_1$ -AT accumulates in hepatocytes and mediates damage by direct cytotoxicity and perhaps other mechanisms.

Studies of transduced human skin fibroblasts have demonstrated a marked delay in degradation of the abnormal protein in cells from  $P_iZZ$  patients with liver disease, compared with the protein from cells of patients without liver disease. This suggests that both a defect in export of abnormal protein and also a delay in its degradation may be required for the

development of hepatic disease. Whereas  $\alpha_1$ -AT deficiency in lung disease represents a disorder of enzyme dysfunction, liver disease due to  $\alpha_1$ -AT deficiency is instead a disorder of protein conformation, and is indeed a prototype for other recognized ‘conformational diseases’, such as Alzheimer and Creutzfeldt-Jakob disease, in which inappropriate aggregation of protein causes intracellular accumulation, subsequent cellular dysfunction and clinical disease.

### Clinical features

Hepatic disease occurs in 10–20% of individuals homozygous for the  $Z$  allele ( $P_iZZ$ ) and is commonly first manifested in infancy. In the first few months of life, jaundice and hepatomegaly may accompany direct hyperbilirubinemia and mild serum amino-transferase elevations. Less often, splenomegaly and ascites are present and synthetic function may be impaired.

This infantile cholestatic picture is typically not fatal and may mark the individual’s only liver disease until early adulthood. Indeed, a decade after initial presentation, a deficient individual may show no signs of clinical or biochemical disease. However, a subpopulation of individuals with clinical disease in infancy will present in late childhood or early adulthood with ascites, hepatosplenomegaly, variceal hemorrhage or other signs of portal hypertension. Risk factors for progression to cirrhosis have not been clearly defined.

Upon inquiry, patients presenting with hepatic disease as children or young adults frequently report a history of neonatal jaundice. Adult patients, in particular, may present with chronic hepatitis or with signs of portal hypertension. Whereas a quarter of cirrhotic individuals die during childhood, the decline of hepatic function is more typically slow, such that cirrhotic children or adults may survive many years before significant hepatic failure ensues.

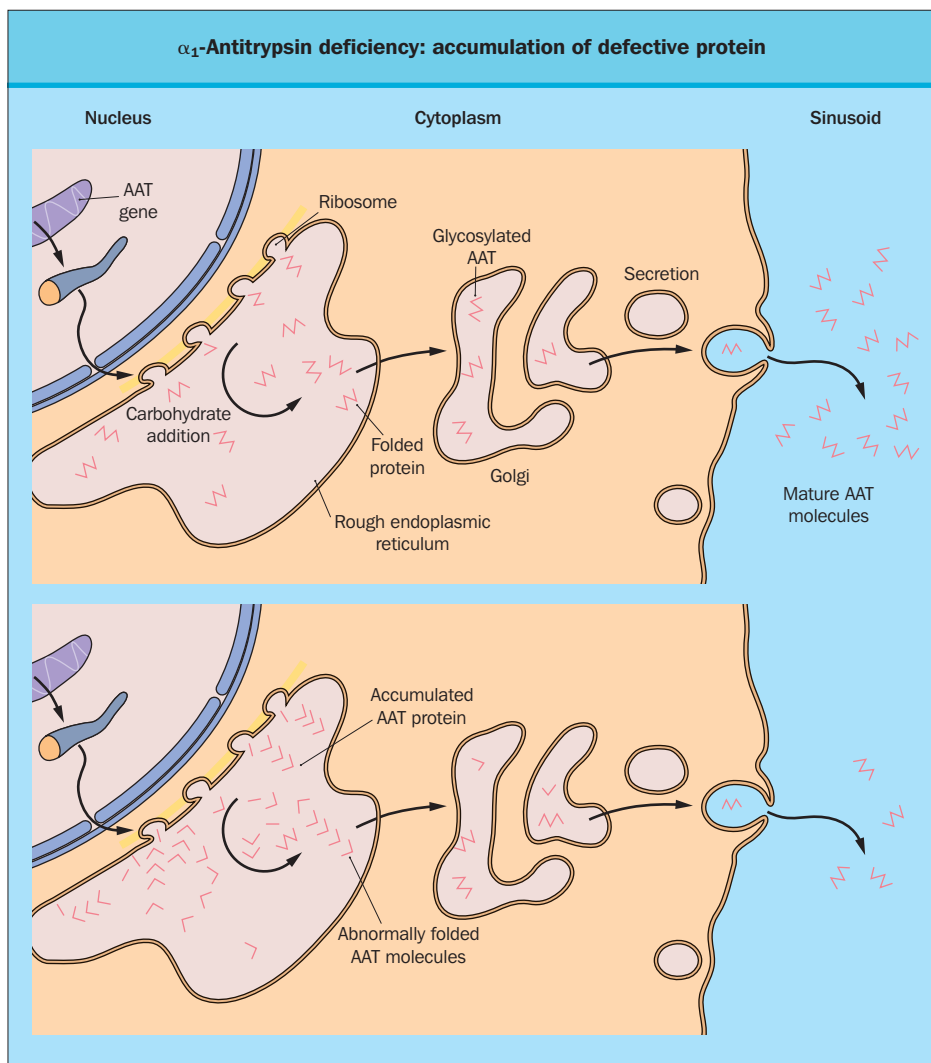
There is a strong correlation between liver disease associated with  $\alpha_1$ -AT deficiency and primary hepatocellular carcinoma, and  $\alpha_1$ -AT deficiency should be considered as a possible predisposing factor for any patient with hepatocellular carcinoma.

### Diagnosis

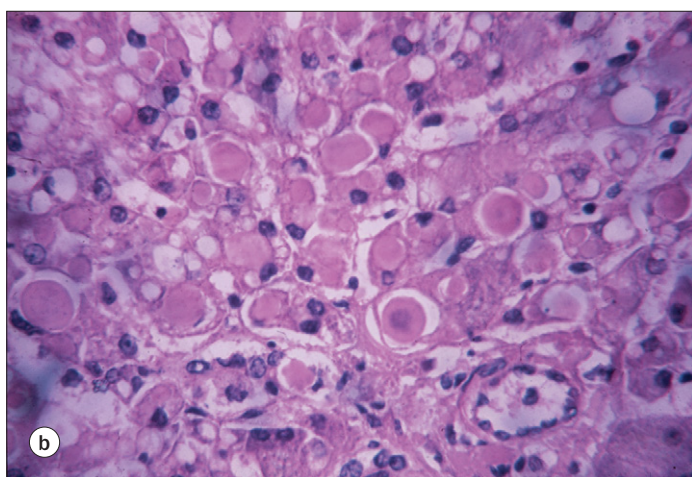
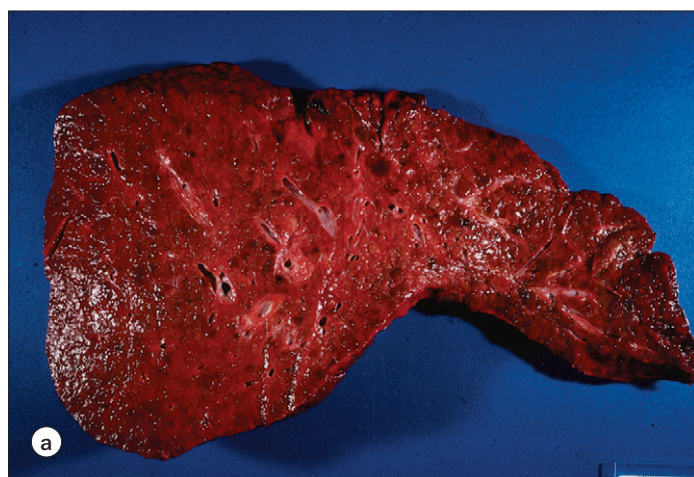
A serum quantitative  $\alpha_1$ -AT level should be measured in jaundiced neonates or in any patient with chronic hepatitis, cirrhosis or other liver disease of unclear etiology. Because it is an acute phase reactant, falsely high (‘normal’)  $\alpha_1$ -AT levels may accompany inflammatory states. After the first few months of life, deficient individuals will have liver histology marked by periportal hepatocytes with diastase-resistant, periodic acid-Schiff (PAS)-positive intracellular globules (Fig. 25.8). These globules are not specific to  $\alpha_1$ -AT deficiency, but in this disease they represent an intracellular accumulation of  $\alpha_1$ -AT, as evidenced by specific immunoperoxidase staining. They may also be found in Kupffer cells. Inflammation, periportal fibrosis or cirrhosis may be present. Accretion of  $\alpha_1$ -AT within the rough endoplasmic reticulum is evident on electron microscopy.  $\alpha_1$ -Antitrypsin deficiency and extrahepatic biliary atresia have similar clinical presentations in neonates, but liver biopsy in  $\alpha_1$ -AT deficiency reveals a marked paucity of intrahepatic bile ducts.

Subjects with low serum  $\alpha_1$ -AT levels should undergo phenotyping by isoelectric focusing or agarose gel electro-

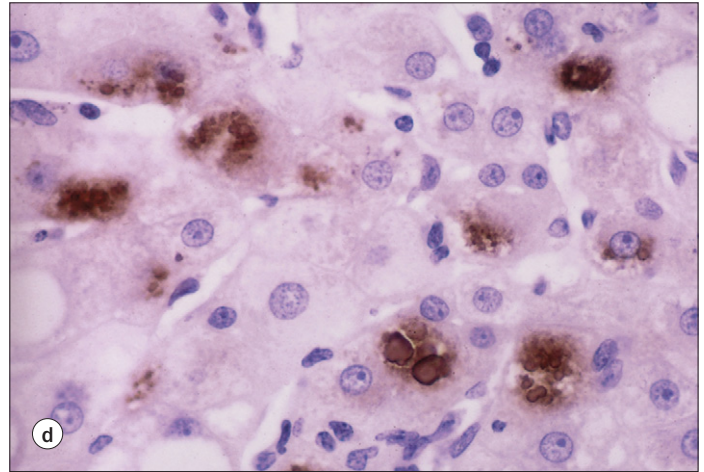
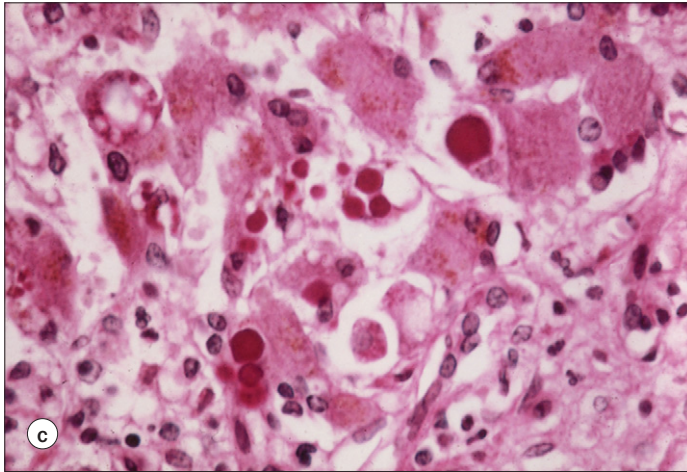




**Figure 25.7  $\alpha_1$ -Antitrypsin deficiency: accumulation of defective protein.** Schematic showing, above, normal  $\alpha_1$ -antitrypsin gene and protein synthesis with prompt glycosylation and unhindered secretion of normal protein. Below, patient with P<sub>ZZ</sub> genotype and synthesis of abnormal protein; transport from the rough endoplasmic reticulum is hindered, resulting in accumulation of the defective protein and hepatotoxicity.



**Figure 25.8  $\alpha_1$ -Antitrypsin deficiency: liver pathology.** (a) Cut surface of a liver from a patient who was transplanted for cirrhosis due to  $\alpha_1$ -antitrypsin deficiency. The entire surface consists of relatively small, closely packed regenerative nodules encompassed by fibrous bands. This appearance is typical of cirrhosis and is non-specific with regard to etiology. (b) Photomicrograph of a liver biopsy showing prominent eosinophilic dense globules in the cytoplasm of many hepatocytes (hematoxylin and eosin,  $\times 250$ ).



**Figure 25.8  $\alpha_1$ -Antitrypsin deficiency: liver pathology.** (Cont'd) (c) Photomicrograph showing that the cytoplasmic globules are periodic acid-Schiff (PAS)-positive and diastase-resistant (PAS-diastase,  $\times 250$ ). (d) Liver biopsy immunostained using  $\alpha_1$ -antitrypsin as primary antibody. A positive reaction is indicated by the brown color (ABC immunostain technique  $\times 400$ ). (Courtesy of Barbara Banner, University of Massachusetts Medical Center.)

phoresis. Although both P<sub>1</sub>ZZ and P<sub>1</sub>SZ patients are predisposed to hepatic disease, specific genotyping may be important for genetic counseling. Quantitative serum  $\alpha_1$ -AT may be helpful in determining heterozygosity in such patients, although parental phenotyping may be necessary. Prenatal diagnosis and phenotyping are possible by amniotic fluid analysis. Genetic testing is also possible now, especially if a causative mutation has already been uncovered in the kindred.

### Treatment

Several established and experimental therapies exist for pulmonary disease, including cessation of tobacco use and avoidance of other potentially toxic inhalants, and infusion or inhalation of purified plasma  $\alpha_1$ -AT. Some studies suggest that breast-feeding is beneficial for newborns with the disease during the first year of life, as functional antitrypsin is provided by maternal macrophages within the breast milk. Surgical shunting has been used to maintain patients with moderate histologic liver disease complicated by severe portal hypertension, and transjugular intrahepatic portosystemic shunts (TIPS) may serve as a bridge to transplant. Orthotopic liver transplantation has remained the definitive intervention for patients with progressive liver failure and portal hypertension. Hepatic decline may proceed slowly, however, and patients may be maintained for years with conservative measures before surgical intervention is warranted. Survival of subjects with  $\alpha_1$ -AT deficiency who have undergone orthotopic liver transplantation may be similar to that of patients with other liver diseases, with 1- and 5-year survival rates of approximately 80–70%, respectively. Recent retrospective reviews suggest survival rates at five years and beyond may surpass 90% in children requiring transplantation. The donor liver synthesizes normal protein and, therefore, the disease does not recur in the new liver, and serum levels of  $\alpha_1$ -AT become normal.

With the understanding that liver pathology in  $\alpha_1$ -AT results from polymerization of abnormal protein attempts have been made to either interfere with the formation of these aggregates

using competitive molecules, or induce rapid clearance of the aggregates using chemical 'chaperones'. These novel therapies may become useful in the treatment of  $\alpha_1$ -AT deficiency and other conformational diseases.

At this time, gene therapy does not seem promising for the treatment of hepatic disease in  $\alpha_1$ -AT deficiency. Such therapy aims for the re-expression of a normal  $\alpha_1$ -AT protein but will not prevent the accumulation of mutant protein within hepatocytes. Moreover, expression of the normal gene may be associated with up regulation of the synthesis of mutant protein, possibly promoting and accelerating the development of liver disease. By stimulating expression of abnormal protein, gene therapy of *pulmonary* disease might also promote the development of hepatic disease, as may treatment of pulmonary disease with purified plasma  $\alpha_1$ -AT. It appears that successful gene therapy of hepatic disease will mandate prevention of mutant protein expression while normal protein synthesis is promoted.

Interestingly, a recent analysis of transgenic P<sub>1</sub>ZZ mouse liver showed a proliferative advantage of hepatic cells without intracellular  $\alpha_1$ -AT aggregates over those encumbered with these aggregates. This suggests that the accumulation of defective  $\alpha_1$ -AT aggregates leads to a functional disadvantage at the cellular level. Such a selective functional and proliferative advantage of non-affected hepatocytes over those affected by abnormal  $\alpha_1$ -AT aggregates is intriguing as it may someday provide an opportunity to promote repletion and replacement of  $\alpha_1$ -AT encumbered cells with viable unaffected cells. Studies in a murine model of another metabolic liver disease, hereditary tyrosinemia type 1, have suggested a possible strategy for gene therapy providing adequate synthesis of normal protein and suppression of mutant protein. By conferring a genetic, selective advantage upon retrovirally corrected cells, in vivo propagation of corrected cells (producing normal protein) to the exclusion of mutant cells might be accomplished for  $\alpha_1$ -AT deficiency. Such strategies are discussed further in this chapter in the section on hereditary tyrosinemia type 1.



## CYSTIC FIBROSIS

Cystic fibrosis (CF) is a chronic disease of deficient chloride conductance manifested by tenacious, often obstructive epithelial secretions that result in clinical disease in several organ systems. Although pulmonary disease is frequently the most visible consequence of the disease (**Fig. 25.9**), CF is the most common cause of pancreatic insufficiency in young adults in the USA and is associated as well with hepatobiliary disease. Because of improved management, life expectancy for most patients with CF is greater than 30 years, and it is increasingly likely that the hepatic manifestations of this disease will be encountered. Indeed, liver disease is the second leading cause of death in patients with CF.

### Epidemiology and pathophysiology

CF is inherited as an autosomal recessive trait, affecting more than 1 in 2000 white births, making it the most common autosomal recessive disease expressed clinically in white children. The disease is associated with a defect of the CF transmembrane conductance regulator (CFTR), a cyclic adenosine monophosphate (cAMP)-activated chloride channel that is predicted to be a protein of 1480 amino acids coded for by a gene located on chromosome 7q31. The CFTR has 12 membrane-spanning segments clustered into two regions, two nucleotide binding folds, and a regulatory region. Nearly 1000

mutations of the CFTR gene have been described. Mutations causing clinical disease are chiefly found in the nucleotide binding folds, and more than 70% of North American and northern European CFTR mutations involve a deletion of a single phenylalanine, at position 508 (Delta F508) of the first nucleotide binding fold (**Fig. 25.10**).

Within the liver, the CFTR is located along the apical membrane of biliary epithelial cells. Defective CFTR results in severely diminished chloride conductance, insufficient luminal fluid and, consequently, viscous, inspissated bile. Bile flow is reduced; secretion of protective mucin is decreased; and the concentrations of bile acids in bile are increased. Pancreatic insufficiency is associated with changes in bile acid absorption, and the intraluminal bile acid pool is shifted from hydrophilic, taurine-conjugated bile acids to hydrophobic, glycoconjugated bile acids, which are hepatotoxic. The result is ductal obstruction and oxidative ductal injury, leading to disease of the intra- and extrahepatic biliary system.

### Clinical features

Hepatobiliary complications of CF may present as disease of the liver, biliary tract, or gallbladder (**Table 25.3**).

#### Liver

More than half of patients with CF may have hepatic involvement, but only about 10% of patients with hepatic disease are symptomatic. Fatty liver is common, particularly in infancy. Chronic ductal obstruction is eventually accompanied by ductal proliferation, inflammatory infiltrate, and periportal fibrosis, known as biliary fibrosis (**Fig. 25.11a**). Hepatocytes are spared at this point, and the architecture of the liver is mostly preserved. Involved portal tracts may be unevenly distributed, such that liver biopsy is subject to sampling error. With persistent insult, fibrosis progresses and multilobular biliary cirrhosis develops, initially leaving spared foci of structurally uninvolved hepatic tissue, but ultimately leading to a hard and nodular cirrhotic liver. Approximately 2–5% of patients, during their lifetimes, will develop cirrhosis as a complication of CF.

Although hepatic involvement in CF more typically presents in children with concurrent respiratory and pancreatic insufficiency, liver disease may be the first clinical feature, particularly in infants. More commonly, hepatomegaly is noted in an otherwise asymptomatic adolescents or young adults. Fatty liver is the most common cause of hepatomegaly in CF, but hepatomegaly may also be falsely suggested if the liver is displaced inferiorly by pulmonary hyperinflation. Alternatively, hepatomegaly may be caused by passive congestion secondary to pulmonary hypertension in CF. A hard, nodular liver is suggestive of cirrhosis. Associated splenomegaly may be present and may cause left upper quadrant pain. Peripheral stigmata of liver disease are not frequently seen, and the first presenting sign of portal hypertension may be variceal hemorrhage. The risk of developing portal hypertension increases with age and, interestingly, shows familial clustering.

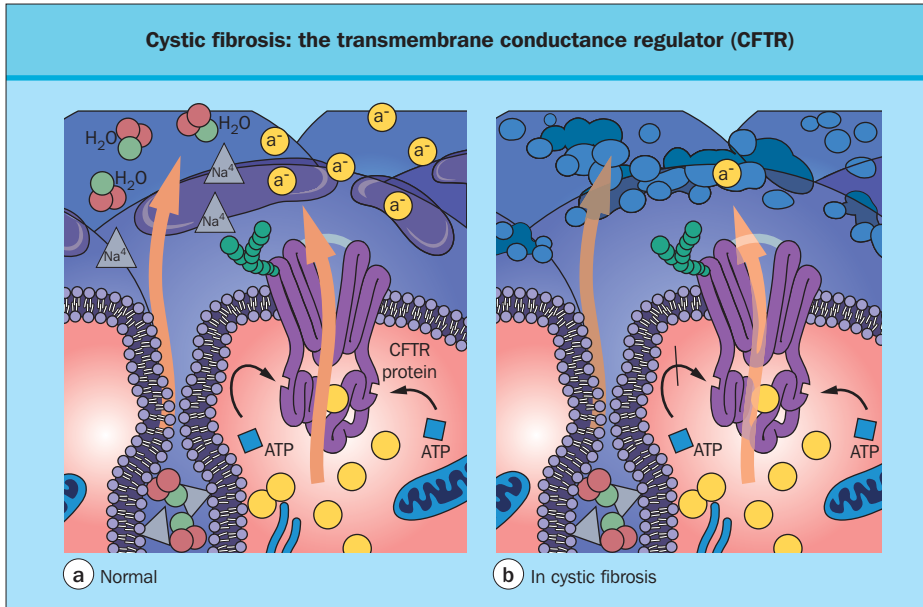
#### Biliary tract and gallbladder

Biliary tract involvement is common and may present independently of hepatic disease. In infants, CF may be manifested as jaundice because of obstructive liver disease, presenting similarly to biliary atresia. It is intriguing that neonatal jaundice



**Figure 25.9 Cystic fibrosis: chest roentgenogram.** Twenty-year-old man with cystic fibrosis showing early cystic changes as well as 'train tracking', parallel lines representing thickened, dilated bronchi. Patchy densities are evident in the upper lung fields, consistent with bronchiectasis. (Courtesy of the Department of Radiology, University of Massachusetts Medical Center.)





**Figure 25.10 Cystic fibrosis: the transmembrane conductance regulator (CFTR).** Schematic showing (left) normal transmembrane transport of chloride, mediated by active transport, requiring binding of adenosine triphosphate (ATP) at the nucleotide binding region of the CFTR. Water molecules follow chloride conductance. Deletion (right) of a single phenylalanine at position 508 (Delta F508) of the nucleotide binding site of the CFTR results in inadequate ATP binding and diminished chloride conductance. As a result, little water follows and secretions are inspissated.

Hepatobiliary features of cystic fibrosis		
Liver	Biliary tract	Gallbladder
Only 10% symptomatic although > half affected	May present independently of hepatic disease	Most disease asymptomatic
Fatty liver	Common duct stricture	Microgallbladder
Common in infancy and adulthood, most common cause of hepatomegaly	Caused by pancreatic fibrosis	Gallbladder atrophied and collapsed
Biliary fibrosis	Choledocholithiasis	Cholecystic dyskinesia
Hepatocytes spared; architecture preserved		Gallbladder filled with thick bile
Multilobular cirrhosis	Sclerosing cholangitis	Cholelithiasis
2–5% of patients, may be accompanied by portal hypertension and splenomegaly	1% of patients, ?chronic inflammation causative	10–25% of patients, most asymptomatic, calcium bilirubinate
Neonatal jaundice		
Not predictive of adult disease		
Comments:	Comments:	Comments:
Liver chemistries neither sensitive nor specific	Alkaline phosphatase likely most sensitive test but must be fractionated; ultrasound may be helpful	Ultrasound helpful
Uneven distribution of disease → sampling error on liver biopsy		

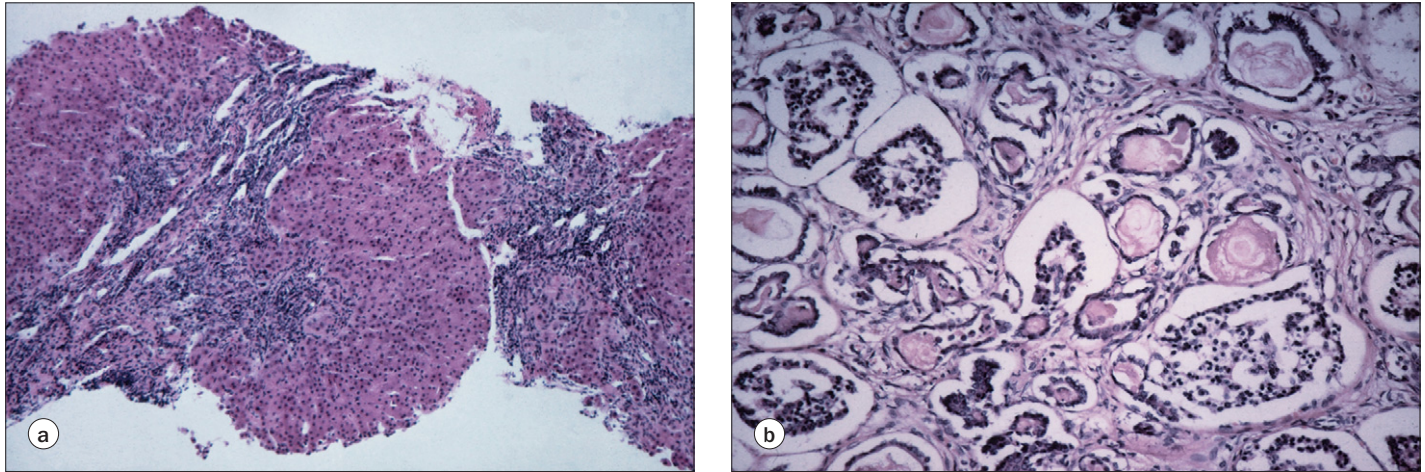
**Table 25.3 Hepatobiliary features of cystic fibrosis.**

does not seem to be predictive of future hepatic disease for patients with CF. As patients age, pancreatic fibrosis (Fig. 25.11b) can result in common bile duct stricture. Approximately 10–25% of patients with CF have gallstones, most of which remain asymptomatic. Although they were thought in the past to be composed of cholesterol, the stones are believed now to consist chiefly of calcium bilirubinate. The gallbladder may be dyskinetic and filled with thick, mucoid bile or may be

atrophied and collapsed ('microgallbladder'). Almost a third of patients may have such a gallbladder. About 1% of patients with CF develop sclerosing cholangitis, possibly because of chronic inflammatory changes within the biliary system.

### Diagnosis

The laboratory detection of early liver disease in CF may be difficult. More than 85% of patients with hepatomegaly will



**Figure 25.11 Cystic fibrosis: liver and pancreatic histopathology.** (a) Photomicrograph of a liver needle biopsy from a patient with cystic fibrosis showing portal triads greatly expanded as bands that extend across the biopsy and delineate nodules of residual parenchyma. These changes are typical of secondary biliary cirrhosis in cystic fibrosis (hematoxylin and eosin,  $\times 40$ ). (b) Photomicrograph of a pancreas from a child with cystic fibrosis. The pancreatic acini have been replaced by fibrosis. Dilated ducts are filled with inspissated eosinophilic secretions. The islets remain untouched but are closer together than normal because of the loss of parenchyma between them (hematoxylin and eosin,  $\times 100$ ). (Courtesy Barbara Banner, University of Massachusetts Medical Center.)

have abnormal liver chemistry test results, but the remainder may have entirely normal tests. Conversely, approximately 13% of patients with CF *without* histologic liver disease will have abnormal liver chemistries. Liver chemistry tests, therefore, are neither highly sensitive nor specific for underlying hepatic disease in CF. Serum alkaline phosphatase is probably the most sensitive chemistry for underlying cholestasis but must be fractionated because non-hepatic alkaline phosphatase may also be elevated in CF.

Underlying biliary disease may be revealed by diagnostic imaging. Ultrasound or oral cholecystography may demonstrate cholelithiasis, cholecystitis or microgallbladder. Ultrasound may be particularly useful and has a high specificity and sensitivity for detecting CF-related liver fibrosis at an early stage. Endoscopic retrograde cholangiopancreatography (ERCP) can additionally reveal common duct stricture or sclerosing cholangitis. Hepatic scintigraphy may be helpful in detecting early liver involvement, even when ultrasound has been unrevealing. Liver biopsy serves as an important method for staging intrahepatic disease and for following progression, despite the recognized possibility of sampling error due to uneven hepatic involvement, even when multilobular cirrhosis has developed. Sweat testing in the young adult or adolescent with unexplained cholestatic disease or portal hypertension may reveal CF and should always be performed in young patients with hepatobiliary disease of unclear origin.

Attempts to identify patients with CF at risk for developing liver disease have identified some predisposing factors. Foremost, hepatobiliary complications occur almost exclusively in patients with pancreatic insufficiency, although the degree of pancreatic insufficiency seems to have little significance. The prevalence of hepatobiliary disease is higher among males than females. Although a history of meconium ileus carries an increased risk, neonatal jaundice does not. Some studies have suggested a possible association between hepatobiliary disease in CF and certain human lymphocyte antigen (HLA) types (e.g. DQ6), but no characteristic genotype has been identified. While

severe genotype is a likely risk factor for liver disease, non-CF genes and exogenous factors may serve as important determinants in whether liver disease will develop and how severe it will be. The familial clustering of portal hypertension in CF-related liver disease suggests such factors may play a key role in its pathogenesis. Despite the potential severity of liver disease in CF, prospective studies of patients with CF-related liver disease have failed to show that liver disease confers a higher mortality, highlighting the difficulty in predicting clinical outcomes in this phenotypically heterogeneous disease.

### Treatment

The primary care for patients with CF is supportive. Avoidance and prompt, vigorous treatment of infection are continual challenges in patients with CF. Maintaining nutrition is of fundamental importance, with high-calorie, vitamin-enriched (particularly fat-soluble vitamins D, E and K), elemental dietary supplementation as well as pancreatic enzyme administration. Vitamin A may be toxic to the liver in CF and should be supplemented with caution.

UDCA has emerged as an important therapy in patients with CF with hepatobiliary disease. UDCA is the major component of bear bile, found in much lower concentrations in human bile as a derivative of chenodeoxycholic acid. It is hydrophilic and may have a cytoprotective effect on biliary epithelium by displacing more toxic hydrophobic bile acids. In addition, micelles of UDCA are smaller and facilitate choleresis. Administration of UDCA in CF has been associated with improved liver chemistries, suggesting reduced hepatotoxicity and diminished cholestasis; the latter is further supported by studies of biliary scintigraphy. Improvement in nutritional status is observed, perhaps by way of increasing fat absorption. UDCA has been associated with delayed progression of other cholestatic liver diseases such as primary biliary cirrhosis, and long-term prospective trials seem to confirm that the benefits of UDCA outlined above may well impart an improved prognosis. Ultrasonographic study of patients receiving UDCA has shown

dramatic arrest of nodular biliary cirrhosis, as well as halting and possibly reversal of focal biliary cirrhosis. Patients receiving UDCA are given taurine supplementation, because therapy with UDCA exacerbates chronic CF-associated taurine deficiency. Taurine administration has also been associated with augmented fat absorption in patients with CF.

In patients who have developed advanced liver disease, management of portal hypertension becomes critical. TIPS may help manage portal hypertension for several years, or serve as a bridge to transplant. Orthotopic liver transplantation (OLT) in CF presents particular challenges because of the multiorgan, systemic nature of CF. In such patients, pulmonary disease and nutritional deficiency may affect outcome, and subsequent immunosuppressive therapy must be managed especially carefully to balance the risk of infection with that of graft rejection. One- and 5-year survival rates of patients with CF who have undergone OLT have been approximately 85 and 70%, respectively.

There are promising potential therapeutic advances under development. Because heterozygosity for defective CFTR function is not associated with clinical hepatobiliary disease, it has been postulated that fewer than half of functional CFTR receptors are needed for normal hepatobiliary phenotype and that homozygous disease might thus be amenable to gene therapy. Unlike gene therapy for other genetic diseases of the liver, gene therapy for CF must target not hepatocytes primarily, but the biliary epithelial cells, which divide infrequently. Recombinantly attenuated adenoviral vectors can infect non-dividing cells and have been used successfully for gene transfer to biliary epithelial cells in human cell lines and are promising for future application in CF therapy. Endoscopic retrograde biliary access might be used to distribute adenoviral or similar vectors of normal CFTR genes to biliary epithelial cells. The study of CFTR conformation and genetics seems likely to afford a plethora of potential therapies for the future treatment of CF-related liver disease.

## HEREDITARY TYROSINEMIA TYPE I

Tyrosine is an essential aromatic amino acid, required for the synthesis of catecholamines, melanin and thyroid hormones. Impaired catabolism of tyrosine occurs in several genetic or acquired disorders that may result in elevated plasma concentrations ( $>200 \mu\text{M}$ ) of tyrosine (tyrosinemia). Four autosomal recessive inborn errors result from deficiencies in enzymes of the catabolic pathway of tyrosine: hereditary tyrosinemia (HT) types 1, 2 and 3, and alkaptonuria (see the Online Mendelian Inheritance in Man at [<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>]). Tyrosinemia is suspected in otherwise unexplained neurological abnormalities (seizures, developmental delay) or liver disease. If the latter is present, testing for HT1 is urgent. HT1 is associated with severe liver disease. It is due to an autosomal recessive deficiency of the enzyme fumaryl acetoacetate hydrolase. There is a very high risk of development of hepatocellular carcinoma and, although the disease is rare worldwide, there are populations in which the gene is common and where complications of HT1 account for almost one in three liver transplantations. Until recently, treatment has been limited to variably effective conservative measures such as dietary changes and liver transplantation.

Now, directed enzyme repression may serve as an important modality of non-surgical intervention and promising advances in gene therapy are being examined.

## Epidemiology and pathophysiology

HT1 presents early in life as either an acute disease of infants, which results in fatal liver failure within several months if left untreated, or as a chronic syndrome marked by progressive cirrhosis, renal tubular dysfunction, severe neurologic symptoms, and failure to thrive. Due to founder effects, the disease is geographically clustered and is notably prevalent in the Netherlands and French Canada. In the Saguenay-Lac St Jean region of Quebec more than 4% of individuals carry the defective gene, and the disease occurs in 1 in 1846 live births.

Several autosomal recessive mutations result in defective fumaryl acetoacetate (FAA) hydrolase activity, promoting the accumulation of toxic metabolites in hepatocytes and renal tubular cells. The defective enzyme's substrate, FAA, and the precursor maleyl acetoacetate increase in plasma concentration as do tyrosine, methionine, and sometimes phenylalanine (Fig. 25.12). Urinary concentrations of succinylacetone (SA, 4,6-dioxoheptanoic acid) are increased by the shunted metabolism of FAA and maleyl acetoacetate. Several different mutations of FAA hydrolase have been identified. For unclear reasons, affected members of the same family may have either the acute or chronic form, such that individuals with the same mutation may have different clinical diseases. This variable phenotypic expression suggests that other factors – environmental influences or the influences of other genes – may affect the severity and course of the illness.

An accumulation of ALA occurs as enzymatic shunting results in increased succinylacetone, which is a potent inhibitor of 5-aminolevulinic acid dehydratase. Increased 5-aminolevulinic acid is likely responsible for marked neuropathy resembling that seen with elevated 5-aminolevulinic acid in the hepatic porphyrias (vide supra).

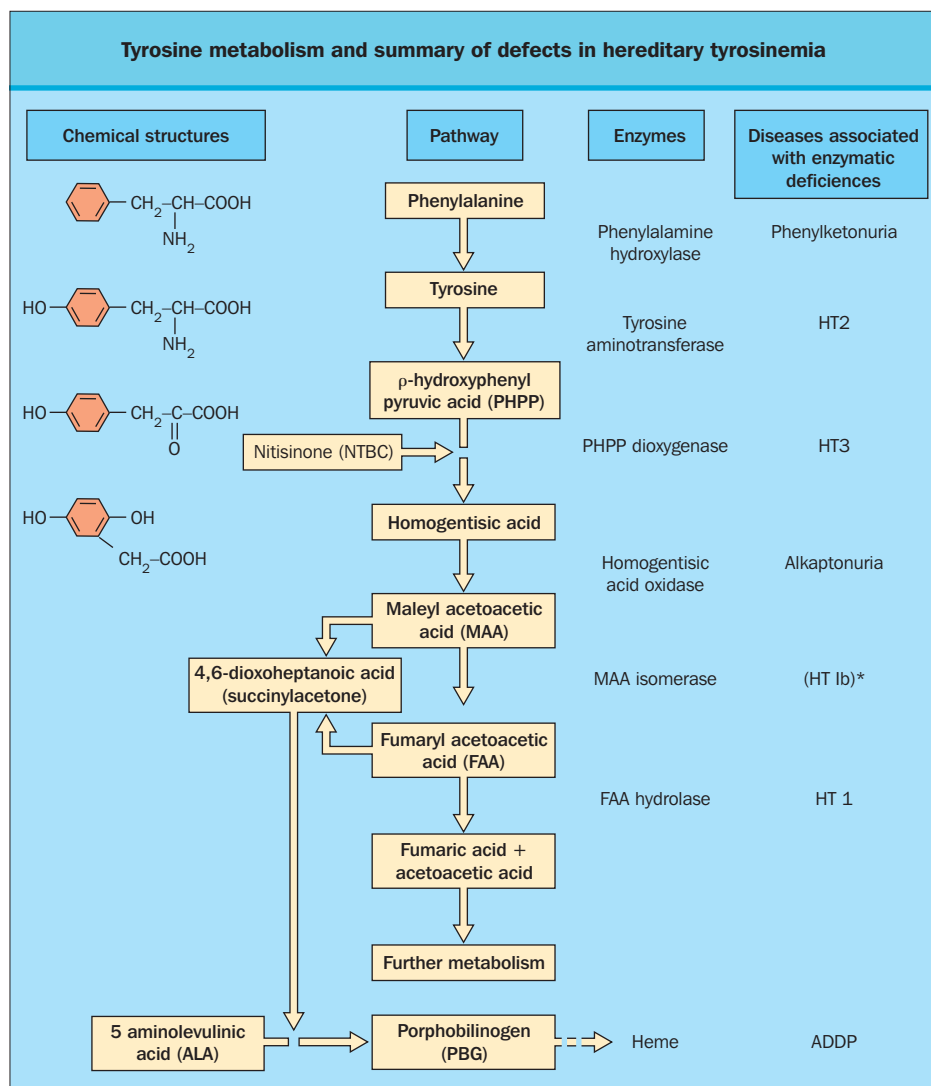
## Clinical features

The acute form of HT1 results in hepatic failure over the first several months of life. The chronic form presents with more gradually progressive disease. Failure to thrive may be followed by more focal findings, such as hepatomegaly and, as cirrhosis develops, splenomegaly, ascites, jaundice, easy bruisability, and peripheral edema may ensue. Vitamin D-resistant, hypophosphatemic rickets may develop, and peripheral neuropathy may occur. The risk of hepatocellular carcinoma increases monthly such that, by 2 years of age, the risk of hepatocellular carcinoma is approximately 40%.

## Diagnosis

Type 1 HT may be anticipated from a family history or genetic screening, or the diagnosis may be made prenatally. Post-gestationally, the diagnosis is typically made by the detection of biochemical abnormalities, including elevated urinary succinyl acetone and increased serum and urinary tyrosine, phenylalanine and methionine. Decreased FAA hydrolase activity may also be detected in cultured skin fibroblasts.  $\alpha$ -Fetoprotein elevation may reflect development of hepatocellular carcinoma, and more moderate elevations may also precede the development of neurologic disease.





**Figure 25.12 Tyrosine metabolism and summary of defects in hereditary tyrosinemia.**

The pathway of phenylalanine and tyrosine metabolism is outlined, and the enzymes responsible and diseases associated with defects in enzyme activities are shown. The effects of nitisinone (NTBC) to inhibit PHPP dioxygenase and 4,6-dioxoheptanoic acid (succinyl acetone) to inhibit ALA dehydratase, the second enzyme of the heme biosynthetic pathway (cf Fig. 25.1), are also shown. The most severe of the hereditary disorders of tyrosine metabolism is hereditary tyrosinemia type 1. Its treatment with nitisinone has led to much improvement. Adequate therapy with nitisinone converts hereditary tyrosinemia type 1 (HT1) into an HT2-type phenotype, which is not associated with excesses of the highly toxic mutagenic maleyl acetoacetate, FAA, or 4,6-dioxoheptanoic acid (succinyl acetone). \*Tentative designation. Abbreviations used: ALA, 5-aminolevulinic acid; FAA, fumaryl acetoacetic acid; MAA, maleyl acetoacetic acid; NTBC, nitisinone [2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione]; PBG, porphobilinogen; PHPP, *para*-hydroxy phenyl pyruvic acid.

### Treatment

Traditionally, HT1 has been treated by eliminating dietary intake of tyrosine or related precursors so as to prevent the production of toxic metabolites. By this means, variable control of the disease is obtained, and some patients may not respond. Until recently, early OLT has been the only means for more definitive treatment. Because of the progressive risk for hepatoma, transplantation in the first 2 years of life has been recommended to avoid progression to metastatic disease. The new liver has no defect in tyrosine metabolism, and hepatic manifestations thus do not recur. Although, the renal defect in FAA hydrolase persists as does renal tubular dysfunction, renal tubular defects persisting after hepatic transplantation do not appear to be clinically significant. Neurologic sequelae of HT1 may resolve gradually but entirely after successful liver transplantation.

The treatment of choice is nitisinone, formerly known as NTBC [2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione]. Nitisinone selectively inhibits 4-hydroxyphenylpyruvate dioxygenase, diminishing the formation of toxic metabolites (FAA and SA) and reducing hepato- and nephrotoxicity (see Fig. 25.12). It has improved and ultimately eliminated peripheral neuropathy, reduced the need for liver

transplantation, and reduced significantly the risk of hepatocellular carcinoma. The typical starting dose is 1 mg/kg/day, divided in two doses and given orally. Therapy is monitored by measuring plasma amino acids, blood and urinary SA, liver tests, complete blood cell count (CBC), and serum  $\alpha$ -fetoprotein. Goals include normalization of SA levels, which may require 3 months, and plasma tyrosine levels <500  $\mu$ M.

Promising possibilities for new, effective modes of gene therapy are being investigated and may have important implications for the treatment of hereditary tyrosinemia and other diseases subject to hepatic gene therapy. It has been observed that the resected livers of patients with HT1 are often a mosaic of cells, with cells expressing normal FAA hydrolase existing in regenerative nodules alongside mutant cells. It was postulated that this apparent spontaneous reversion to a normal allele might confer a selective advantage to these cells and promote their repopulation of the liver. The possibility of initiating and accelerating the repopulation of normal liver cells became a focus of attention.

In a murine model of HT1, transplantation of normal hepatocytes resulted in their seeding and repopulation of the liver, to the exclusion of mutant cells. Similarly, repeated portal vein infusion of retroviruses carrying the normal FAA hydrolase gene

afforded complete repopulation of the liver with normally functioning, retrovirally transduced cells. In this model, it appears that normal differentiated hepatocytes preferentially multiply and replace cells carrying the mutant enzyme. By improving the efficiency of gene transfer, similar manipulations may become possible for repopulating mutant human liver with genotypically normal cells. By combining within the retroviral vector both the gene for transfer and also a second gene that affords a selective advantage for hepatocytes, it eventually may be feasible both to initiate and promote the repopulation of human liver with functional hepatocytes and overcome or eliminate the enzymatic defect without the need for liver transplantation.

## ACID LIPASE DEFICIENCY (WOLMAN DISEASE AND CHOLESTEROL ESTER STORAGE DISEASE)

### Epidemiology and pathophysiology

Deficiency of acid lipase results in two phenotypically distinct syndromes, Wolman disease and cholesterol ester storage disease (CESD), each inherited as an autosomal recessive disorder. They are marked by diffuse deposition of cholesterol esters, resulting in hepatic and multiorgan disease. Wolman disease presents with severe acute decline in early infancy, whereas CESD has a more benign course throughout adulthood (Table 25.4).

The gene coding for acid lipase has been localized to chromosome 10 and has recently been cloned. Acid lipase is a widely distributed lysosomal enzyme that normally hydrolyzes cholesterol esters of low-density lipoproteins (LDL). While other lysosomal enzymes metabolize the protein component of LDL to peptides and amino acids, acid lipase frees LDL cholesterol from esterification, making it available for cellular metabolism and for feedback repression of extrahepatic cholesterol synthesis. Therefore, deficiency of the enzyme results

in an accumulation of cholesterol esters as well as increased cellular cholesterol synthesis. The liver, spleen, lymph nodes and intestine are typically laden with cholesterol esters and triglycerides. In Wolman disease, the adrenal glands are invariably affected as well.

### Clinical features

For unclear reasons, Wolman disease and CESD have different clinical courses despite an apparently similar enzyme deficiency. Wolman disease is characterized in early infancy by abdominal pain and distention, vomiting and diarrhea, and hepatosplenomegaly. Growth and neurologic development are retarded. Progressive hepatosplenomegaly and distention are accompanied by profound malabsorption, and death usually occurs within the first year of life. CESD has a more benign course and usually presents in childhood, although some patients present as young adults. The patient may be asymptomatic, but hepatomegaly is universal and typically progressive, with hepatic fibrosis leading ultimately to cirrhosis and complications of portal hypertension. Premature atherosclerosis accompanies hyperlipoproteinemia.

Liver chemistries (conjugated bilirubin and serum aminotransferases) are typically elevated in Wolman disease but are usually normal in CESD. Plasma cholesterol and triglycerides are normal in Wolman disease but usually elevated in CESD. Serum LDL may be elevated in CESD, whereas low hepatic synthesis in Wolman disease likely accounts for low LDL levels. Without exception, patients with Wolman disease have distinctive granular calcification diffusely involving enlarged adrenal glands, which is easily seen on abdominal roentgenograms. The adrenal glands are enlarged but not calcified in CESD.

Liver biopsy demonstrates steatosis with cholesterol ester-laden Kupffer cells that are swollen and vacuolated. Hepatic fibrosis and, ultimately, cirrhosis may occur. In CESD, hepatocytes show birefringent lipid globules. Kupffer cells do not show birefringence, probably owing to peroxidation of the Kupffer cell cholesterol esters.

### Diagnosis and treatment

A diagnosis is made from the characteristic clinical findings and from the demonstration of lysosomal acid lipase deficiency in cultured leukocytes or skin fibroblasts. Traditionally, intervention has been limited to supportive care. Inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (e.g. atorvastatin, lovastatin) may be used to diminish intracellular cholesterol synthesis. Recently, bone marrow transplantation has shown promise for patients with Wolman disease, and cloning of the gene for acid lipase has engendered hope for eventual recombinant enzyme replacement therapy and gene therapy.

## HEREDITARY FRUCTOSE INTOLERANCE

Hereditary fructose intolerance (HFI) is an autosomal recessive defect of aldolase B, an isozyme of fructose biphosphate aldolase. The enzyme is found in liver, intestine and kidney, where it normally cleaves fructose-1-phosphate into dihydroxyacetone phosphate and glyceraldehyde. Deficiency results in cytoplasmic accumulation of fructose-1-phosphate (Fig. 25.13).

The gene for aldolase B (ALDOB) is located on chromosome 9q; many mutations associated with the HFI phenotype have been described. More than half of North American and European

Acid lipase deficiency – Wolman disease and cholesterol ester storage disease	
Enzyme defect	Lysosomal acid lipase
Clinical features	Wolman disease: infant with abdominal pain and distention, vomiting, diarrhea, hepatosplenomegaly, mental retardation, adrenal calcification, and enlargement. CESD: may be asymptomatic; child/adult with hepatomegaly, cirrhosis, premature atherosclerosis, adrenal enlargement
Diagnosis	Acid lipase deficiency in cultured leukocytes or skin fibroblasts. Liver biopsy with cholesterol ester-laden Kupffer cells, fibrosis, cirrhosis. Birefringent hepatocytes in CESD
Treatment	Wolman disease: supportive care, death by age one. Bone marrow transplant under investigation. CESD: 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, supportive care of liver disease
CESD, cholesterol ester storage disease.	

**Table 25.4** Acid lipase deficiency – Wolman disease and cholesterol ester storage disease.

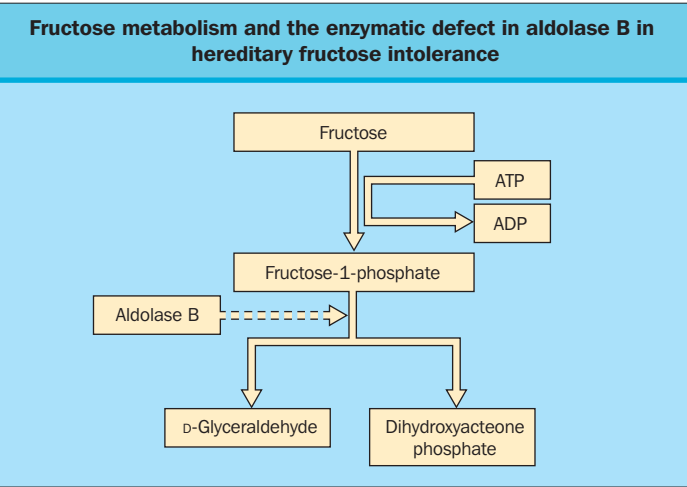


Figure 25.13 Hereditary fructose intolerance – enzymatic defect.

Hereditary fructose intolerance	
Enzyme defect	Aldolase B (fructose-1-phosphate aldolase)
Inheritance	Autosomal recessive
Clinical features	Hypoglycemia, abdominal pain, vomiting, hepatomegaly, renal tubular damage, hypophosphatemia, growth retardation
Diagnosis	Intravenous fructose challenge, liver biopsy, molecular diagnosis (DNA analysis)
Treatment	Avoidance of fructose-containing sugars; normal lifespan if treated

Table 25.5 Hereditary fructose intolerance.

allelic defects are accounted for by the A149P mutation. A strong founder effect is shown by linkage studies, suggesting that the distribution of the A149P mutation was likely by way of genetic drift, rather than by recurrent de novo mutation. HFI is most prevalent among northern Europeans.

Clinical findings include marked abdominal pain and vomiting after the ingestion of fructose, sucrose or sorbitol (Table 25.5). Hypoglycemia may follow and can be fatal. Chronic effects include growth retardation, hepatomegaly, renal tubular damage and hypophosphatemia. The diagnosis has traditionally been made by intravenous fructose challenge or by liver biopsy. However, as most enzymatic defects are coded for by just a few mutations, molecular diagnostic methods are leading to less invasive diagnosis. Genetic screening of affected kindred may be accomplished by similar means. Treatment involves the avoidance of fructose or sugars metabolized to fructose, and patients typically develop a strong distaste for sweet foods. Patients without chronic fructose exposure may anticipate a normal life span.

HEPATIC GLYCOGENOSES (GLYCOGEN STORAGE DISEASES)

Epidemiology and pathophysiology

The glycogenoses, or glycogen storage diseases (GSD), are caused by defects of glycogen metabolism, resulting in tissue

accumulation of normal or abnormal glycogen molecules and impaired conversion of glycogen stores to glucose. In most types, hypoglycemia ensues and may be severe or even fatal. Their inheritance is autosomal recessive, except for type VI, which is sex-linked. We limit our discussion to those affecting the liver (Table 25.6), which includes all but type V, which affects muscle, and type VII, which affects erythrocytes.

Type I glycogen storage disease

Type I, or GSD 1 (von Gierke disease), affects the liver and kidney. There are three subtypes. Type 1a is caused by deficiency of hepatic microsomal glucose-6-phosphatase. The gene coding for glucose-6-phosphatase has been cloned and is located on chromosome 17. Type 1b is caused by a defective translocase required for glucose-6-phosphatase activity at the endoplasmic reticulum, and type 1c by defective microsomal phosphate/pyrophosphate translocase T-2 (Fig. 25.14).

Clinical features

Clinical findings in infants include difficulty feeding and hypoglycemic seizures. Children have short stature and adiposity. Marked hepatomegaly is universal (Fig. 25.15a), but the absence of splenomegaly is a distinguishing feature from other storage diseases. Adults are short, often anemic, with proteinuria, microalbuminuria, renal calcifications and chronic renal failure. Osteopenia and related fractures are not uncommon. Hyperlipidemia may be manifest as xanthomata, and hyperuricemia as gout. Liver findings in adults commonly include adenomas (as many as 75% of patients), and there is an increased incidence of hepatocellular carcinoma. In contrast to hepatic adenomas in the general population, there is a strong male predominance in GSD 1. Notably, cirrhosis does not occur.

Type 1a (as well as types III, VI and IX) is associated with the polycystic ovary syndrome, which is found in almost all female patients older than 5 years of age and is likely a consequence of hyperinsulinemia. Type 1b is associated with defective neutrophil glucose-6-phosphatase transport and, as a result, bacterial infections and gingivitis are frequent problems. Platelet dysfunction can be seen in all subtypes. Hypoglycemia is evident on laboratory testing, as is metabolic acidosis. Serum amino-transferases are usually normal, but alkaline phosphatase and  $\gamma$ -glutamyltranspeptidase (GGTP) may be elevated. Increased serum cholesterol, triglycerides and uric acid levels are typical.

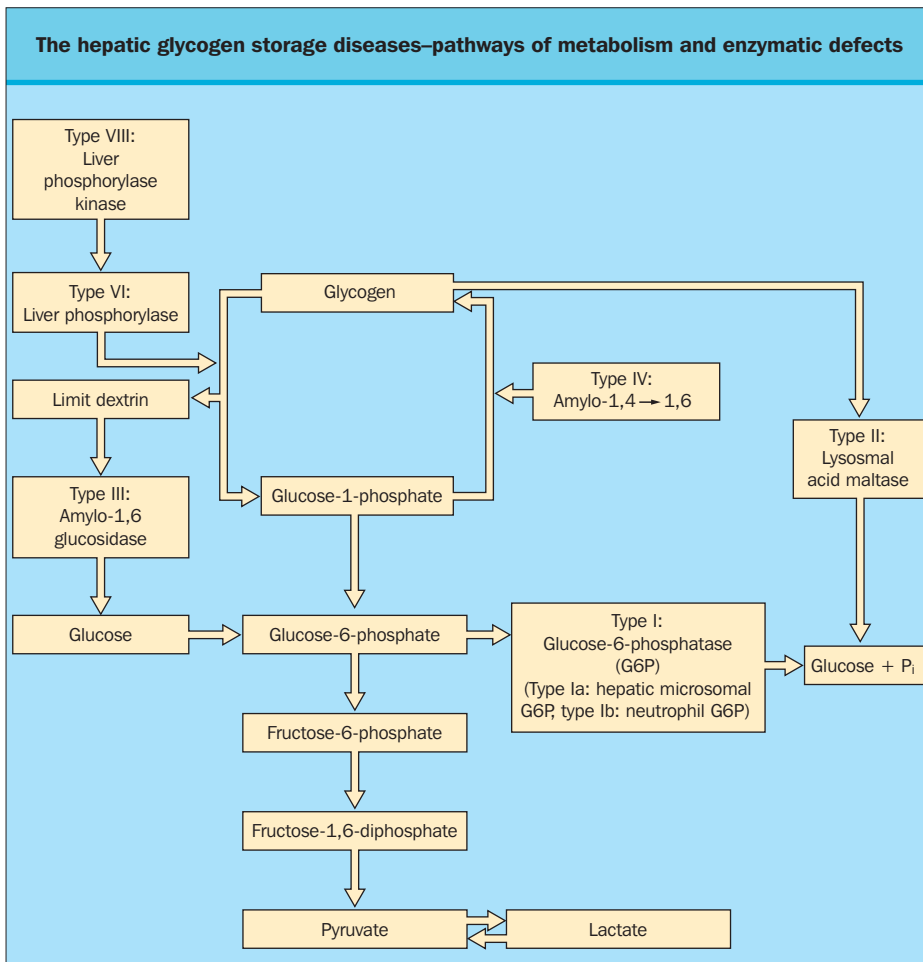
Diagnosis

Glucagon challenge and liver biopsy have traditionally been used to make the diagnosis. Administration of glucagon intramuscularly results in poor blood glucose response and elevated lactate levels. Liver biopsy shows hepatocytes filled with glycogen (see Fig. 25.15b), and special care must be taken to forward the sample to a specialized center for glycogen and specific enzyme measurement. Interestingly, Mallory bodies are found in adenomas of GSD 1 patients, in contrast with most other adenomas.

The gene for glucose-6-phosphatase, the enzyme deficient in type 1a, has been cloned. Although many different mutations have been identified, they are clustered by ethnic background. Through DNA-based analysis, type 1a may be diagnosed non-invasively, and prenatal diagnosis is being developed as well.



The hepatic glycogen storage diseases – enzyme defects, clinical features		
Glycogenosis	Enzyme defect	Clinical features
Type I (von Gierke) Type Ia Type Ib Type Ic	Glucose-6-phosphatase Translocase Phosphate/pyrophosphate translocase T-2	Hypoglycemia, acidosis, seizures, short stature, adiposity, hepatomegaly without cirrhosis and without splenomegaly. Adults with renal failure, anemia, osteopenia, hyperlipidemia, hepatic adenoma/adenocarcinoma, platelet dysfunction, gout, polycystic ovaries – type Ia, bacterial infection – type Ib
Type II (Pompe)	Lysosomal acid maltase	No hypoglycemia, macroglossia, cardiomegaly, hepatomegaly without cirrhosis and without splenomegaly
Type III (Cori)	Amylo-1,6 glucosidase	Hypoglycemia, myopathy, cardiomyopathy, hepatomegaly without cirrhosis or splenomegaly, polycystic ovaries
Type IV (Andersen)	Amylo-1,4→1,6 transglucosidase	Cirrhosis, death from liver failure
Type VI (Her)	Hepatic phosphorylase	Polycystic ovaries
Type IX	Hepatic phosphorylase- $\beta$ -kinase	Mild course, growth retardation, hepatomegaly, polycystic ovaries

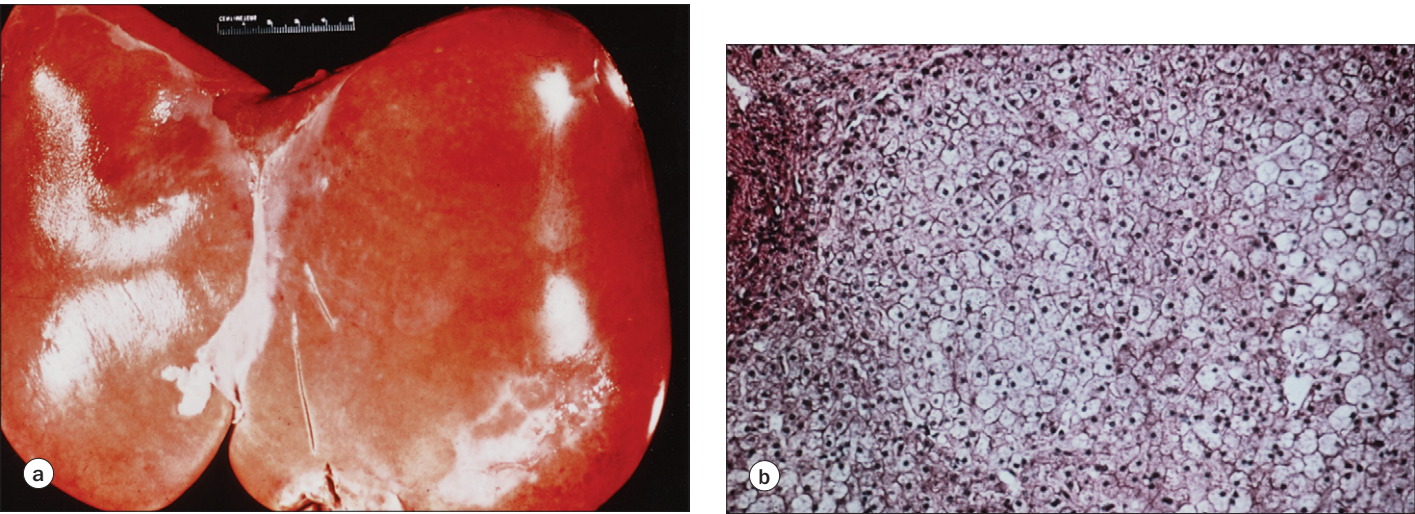
**Table 25.6** The hepatic glycogen storage diseases – enzyme defects, clinical features.**Figure 25.14** The hepatic glycogen storage diseases – pathways of metabolism and enzymatic defects.

Similar non-invasive molecular diagnosis will likely follow for the other glycogenoses.

### Treatment

Management focuses on maintaining adequate blood glucose levels (Table 25.7). Continuous nocturnal high-carbohydrate

enteral feeding and frequent daytime feedings of a diet rich in carbohydrates are the mainstays of therapy. Raw cornstarch serves as a delayed-release source of glucose and may supplement feedings. Medical treatment is given as warranted for hyperlipidemia, gout, infections and osteopenia. Granulocyte colony-stimulating factor has been used to boost neutrophil



**Figure 25.15 Glycogen storage disease Ia (von Gierke disease): liver pathology.** (a) Capsular surface of a liver from a patient with glycogen storage disease Ia (von Gierke disease). The liver is enlarged, and the capsule appears smooth and shiny, with the liver edges rounded rather than sharp. (b) Photomicrograph of a liver from a patient with glycogen storage disease type Ia. All of the hepatocytes are expanded and pale due to glycogen-laden cytoplasm. Unlike fatty liver, in which the nucleus is pushed to the side by a single large cytoplasmic vacuole, glycogen storage is more diffuse within the cytoplasm and the nucleus remains in the center of the cell. The cells appear pale because much of the glycogen dissolves during processing (hematoxylin and eosin,  $\times 40$ ). (Courtesy of Joseph Alroy, Tufts University School of Veterinary Medicine, and Barbara Banner, University of Massachusetts Medical Center.)

Hereditary fructose intolerance The hepatic glycogen storage diseases – inheritance, diagnosis, treatment	
Inheritance	Autosomal recessive, except type IV, which is sex-linked, and type IX, which has sex-linked forms
Diagnosis	Glucagon challenge $\rightarrow$ low blood glucose, high lactate Liver biopsy $\rightarrow$ glycogen-laden hepatocytes Molecular diagnosis becoming available
Treatment	Continuous nocturnal high-carbohydrate feeds, raw corn starch; supportive care of hyperlipidemia, gout, infection, osteopenia; potential gene therapy

**Table 25.7 The hepatic glycogen storage diseases – inheritance, diagnosis, treatment.**

number and function in GSD 1b. Liver transplantation may be required for treatment of adenomas, although adenomas may regress upon commencement of enteral feedings. Although most patients survive to adulthood, many die as children.

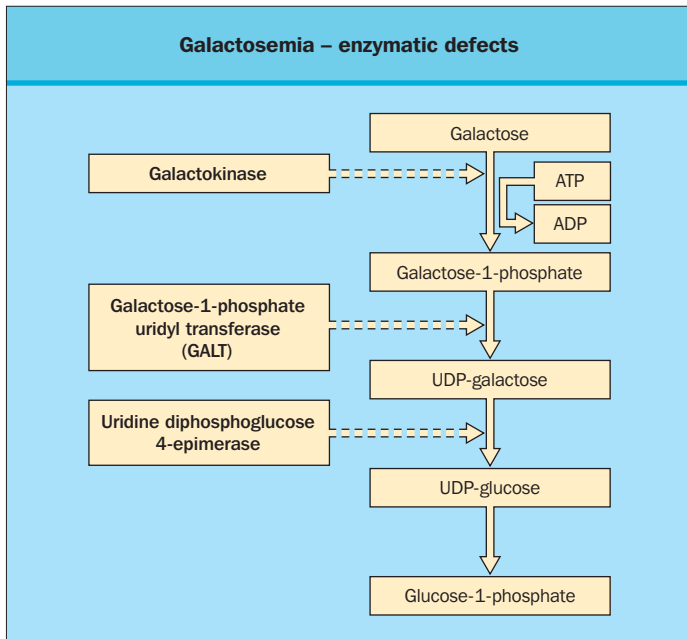
**Types II–IX GSD** (see Table 25.6, 25.7 and Fig. 25.14)  
Type II GSD (Pompe disease) results from deficiency of lysosomal acid maltase ( $\alpha$ -1,4 glucosidase). Glycogen accumulates within lysosomes of all tissues in this generalized glycogenosis, but hypoglycemia does not occur. Onset may be in infancy or adulthood, the former typically more severe. Patients have hepatomegaly without splenomegaly, macroglossia and cardiomegaly. Hyperlipidemia is evident and liver biopsy shows vacuolated lysosomes, packed with glycogen, as do all organs. The gene for lysosomal acid maltase has also been localized to

chromosome 17, and gene therapy is being investigated as a therapeutic option.

Type III GSD (Cori disease) involves a defect of amylo-1,6 glucosidase, a debranching enzyme, and is marked by myopathy, cardiomyopathy, hepatomegaly without splenomegaly, and pronounced hypoglycemia. Female patients develop polycystic ovaries. Serum aminotransferases may be elevated, and creatinine kinase may be increased secondary to myopathy. Treatment may involve enteral glucose feedings but, because gluconeogenesis is enhanced, glucose levels may be maintained with high-protein supplementation as well. The course of the disease is less virulent than GSD I or II and patients usually live to adulthood.

Type IV GSD (Andersen disease) is rare and differs from other GSD in that cirrhosis is prevalent, and, consequently, splenomegaly is as well. Deficiency of amylo-1,4 $\rightarrow$ 1,6 transglucosidase results in structurally abnormal glycogen which affects all tissues and which is found in liver biopsies as PAS-positive intracellular aggregates. The course is usually one of progressive liver failure with death or transplantation usually by 5 years of age. Neuromuscular and cardiac disease may also predominate. For unclear reasons, liver disease does not progress in some patients. Curiously, liver transplant recipients may not suffer progression of the neuromuscular or cardiac effects of extrahepatic enzyme deficiency.

Type VI GSD (Her disease) involves a sex-linked deficiency of phosphorylase and causes isolated hepatic disease marked by hepatomegaly without splenomegaly, hypoglycemia and subsequent acidosis. Polycystic ovaries are commonly found. Type VIII is caused by deficient phosphorylase activation and presents similarly. Type IX involves deficiency of phosphorylase kinase as detected in erythrocytes. Its course is typically mild, with some growth retardation, hepatomegaly and polycystic ovaries. Sex-linked forms exist.



**Figure 25.16 Galactosemia – enzymatic defects.**

## GALACTOSEMIA

There are three key enzymes in the metabolism of galactose: galactokinase, galactose-1-phosphate uridyl transferase (GALT), and uridine diphospho-glucose 4-epimerase (Fig. 25.16). Classic galactosemia involves hepatic and erythrocytic deficiency of GALT, inherited as an autosomal recessive trait. Galactose accumulation ensues and is toxic to tissues throughout the body, although the mechanism of toxicity remains unclear. The disease may be severe and result in childhood death or have a more moderate course, such that patients may survive to adulthood. This may depend on the severity of the enzyme deficiency (Table 25.8).

Accumulation of galactose is found within the fetus by 10–20 weeks' gestation, and toxic effects in utero are evidenced by early cataract development, low birth weight, and probable prenatal liver pathology. Newborns have difficulty feeding, with vomiting and diarrhea, malnutrition and growth retardation. Hepatosplenomegaly is followed by development of macro-

nodular cirrhosis, portal hypertension and ascites. Mental development is poor, with retardation and speech deficits. Ovarian failure is seen in most girls surviving to puberty. Patients with partial enzyme deficiency may survive well into adulthood, and the diagnosis should be considered in young cirrhotic patients as well as adult cirrhotic patients with other characteristic findings.

Screening tests will detect the presence of galactose as a glucose-oxidase negative urinary reducing agent; diagnosis is made definitively by measuring erythrocytic GALT activities. Prenatal diagnosis can be made by culturing cells from amniotic fluid and measuring GALT activity. Liver biopsy reveals fatty deposition in newborns and later shows regeneration, giant cells and cirrhotic changes.

Dietary intervention is the mainstay of treatment. Avoidance of galactose or lactose products can result in significant improvement and even recovery. For unclear reasons, however, dietary-independent deficits may persist and progress. These can include mental deficits, speech pathology and growth retardation. Ovarian failure almost invariably occurs in women and may be treated with exogenous estrogen and progesterone to promote the development of secondary sex characteristics and manage postmenopausal sequelae.

Deficiencies of the other key enzymes of galactose metabolism are rare. Galactokinase deficiency is mild and results in cataract formation; uridine diphosphoglucose 4-epimerase deficiency is severe and presents like classic galactosemia. Dietary intervention is the pillar of treatment.

## THE MUCOPOLYSACCHARIDOSES

### Epidemiology and pathophysiology

The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders leading to the lysosomal accumulation of mucopolysaccharides (glycosaminoglycans), resulting in a variety of mental and physical abnormalities. With one exception, their inheritance is autosomal recessive (type II is X-linked recessive). Ten known enzymatic deficiencies account for six distinct syndromes that are classified by clinical features, urinary mucopolysaccharide analysis, and are identified by historical eponyms. Enzyme deficiencies result in defective metabolism of heparan sulfate, dermatan sulfate, chondroitin sulfate, or keratan sulfate (Table 25.9).

Galactosemia			
Enzyme defect	Clinical features	Diagnosis	Treatment
Galactose-1-phosphate uridyltransferase (GALT)	<i>Prenatal:</i> cataract-like corneal changes, liver disease <i>Newborns:</i> vomiting, malnutrition, hepatosplenomegaly, macronodular cirrhosis, portal hypertension, mental and growth retardation, death <i>Adults:</i> ovarian failure, cirrhosis	<i>Screening:</i> glucose-oxidase negative urinary reducing agent <i>Confirmation:</i> erythrocyte GALT deficiency <i>Prenatal:</i> GALT levels in cultured amniotic cells	Avoidance of galactose and lactose Estrogen replacement therapy for ovarian failure
Galactokinase	Cataracts, mild course	Erythrocyte enzyme deficiency	Avoidance of galactose/lactose
Uridine diphosphoglucose 4-epimerase	Clinical picture similar to GALT deficiency (above), severe course	Erythrocyte enzyme deficiency	Avoidance of galactose/lactose

**Table 25.8 Galactosemia.**



The mucopolysaccharidoses			
Type of MPS	Enzyme defect → stored MPS	Inheritance	Clinical features
Type IH (Hurler)	$\alpha$ -L-Iduronidase → dermatan sulfate	Autosomal recessive	Hepatosplenomegaly, coarse facial features, skeletal defects (gibbus, clawhand), mental retardation, cardiac disease, corneal disease
Type IS (Scheie)	$\alpha$ -L-Iduronidase → heparan sulfate	Autosomal recessive	Same as type 1H, but no mental retardation
Type II (Hunter)	Iduronosulfate sulfatase → dermatan and heparan sulfate	Sex-linked recessive	Similar to type 1, but mental retardation less severe, retinal degeneration, no corneal disease
Type III (Sanfilippo)	(Four subtypes) → heparan sulfate	Autosomal recessive	Severe mental retardation, coarse facial features, little hepatosplenomegaly, moderate skeletal disease, eyes uninvolved
Type IV (Morquio)	N-acetylgalactosamine-6-sulfate sulfatase → keratan sulfate	Autosomal recessive	Severe skeletal deformity, corneal disease, cardiac disease, little hepatosplenomegaly, mental status unaffected
Type VI (Maroteaux-Lamy)	N-acetylhexosamine-4-sulfate sulfatase → dermatan sulfate	Autosomal recessive	Corneal disease, cardiac disease, coarse facial features, mental status unaffected
MPS, mucopolysaccharidoses			

Table 25.9 The mucopolysaccharidoses.

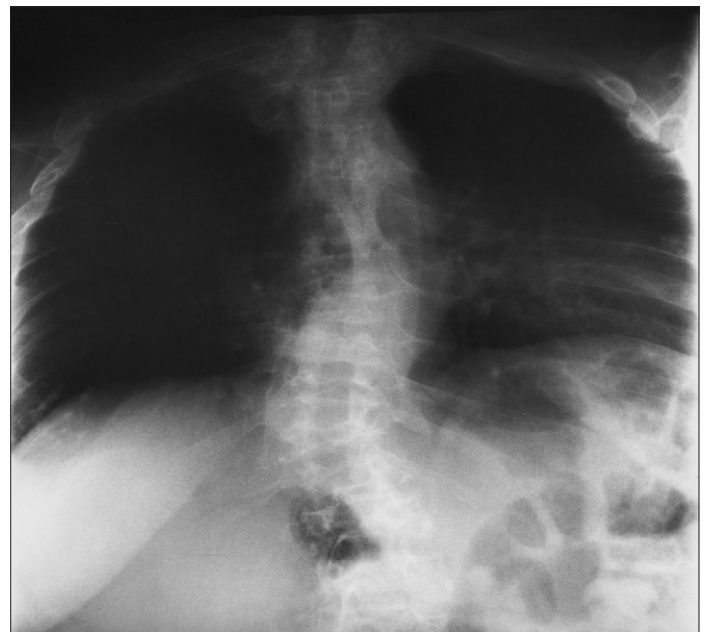
### Clinical features and diagnosis

Although specific enzyme deficiencies may produce characteristic phenotypic presentations in MPS, there are many similarities among the several forms of MPS. Hepatosplenomegaly is a prominent feature, as are coarse facial features, skeletal defects, mental retardation, corneal clouding, joint stiffness and cardiac disease.

Type IH (Hurler syndrome) is most frequently encountered and involves a deficiency of L-iduronidase, with an accumulation of dermatan sulfate and heparan sulfate. Its inheritance is autosomal recessive, and it is marked by dwarfism as well as the findings described earlier. Skeletal disease includes lumbar gibbus (hunchback) and clawhand. The adult Scheie variant, type IS, presents without mental retardation.

Type II, Hunter syndrome, involves deficiency of iduronosulfate sulfatase with an accumulation of dermatan sulfate and heparan sulfate. It is sex-linked recessive and seen only in males. Less severe than type I, patients typically survive well into adulthood. The remaining MPS have autosomal recessive inheritance, including type III (Sanfilippo syndrome). There are four enzymatic deficiencies classified as Sanfilippo variants, each resulting in accumulation of heparan sulfate. They all cause severe mental retardation and moderate skeletal deformity. Skeletal abnormalities (Fig. 25.17) are most severe in type IV, Morquio syndrome, in which keratan sulfate aggregates are found because of N-acetylgalactosamine-6-sulfate sulfatase deficiency. Corneal clouding is typical. In both types III and IV, there is less liver or spleen enlargement than types I and II. In the Maroteaux-Lamy variant, type VI, N-acetylhexosamine-4-sulfate sulfatase causes an accumulation of dermatan and presents with the characteristic MPS findings already described.

Hepatosplenomegaly is a characteristic finding on examination in all types, and liver biopsy may demonstrate fibrosis or cirrhosis. Vacuolation of hepatocytes and Kupffer cells is



**Figure 25.17 Mucopolysaccharidosis (MPS) type IV (Morquio syndrome): chest roentgenogram.** This 59-year-old woman has several findings typical of MPS IV, including spinal shortening, marked kyphoscoliosis (together contributing to dwarfism), and widening of the ribs anteriorly but narrowing posteriorly. (Courtesy of the Department of Radiology, University of Massachusetts Medical Center.)

produced by glycosaminoglycan-laden lysosomes, most evident on colloidal iron staining. Electron microscopy shows membrane-bound inclusions within hepatocytes, Kupffer cells and hepatic stellate cells.

### Treatment

Until recently, treatment has been supportive. However, animal models of enzyme replacement therapy seem promising, and bone marrow transplantation has been successfully carried out with halting of disease progression and improvement in skin, joint, heart and liver manifestations. Models for gene therapy are now being explored. Presently, life expectancy for type IH is less than 10 years, normal for type IS, and nearly normal for types II and IV. Patients with type III disease live into the second or third decade. For reasons as yet unclear, there is a great variation in the penetrance of each MPS and, therefore, in life expectancies.

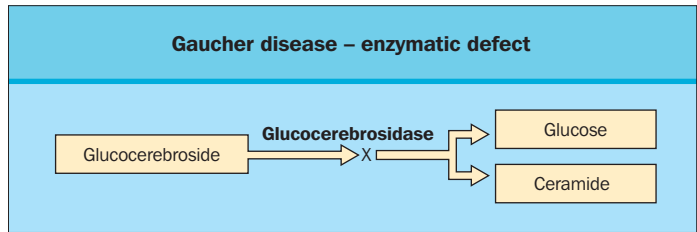
## GAUCHER DISEASE

### Epidemiology and pathophysiology

The most common lysosomal storage disease and most frequent inborn error of lipid metabolism, Gaucher disease, is a paramount example of the profound implications of genetic mutation and the tangible benefits of advances in molecular medicine. The disease is caused by a defect in  $\beta$ -glucocerebrosidase (glucosylceramidase), which catalyzes the cleavage of glucocerebroside (glucosylceramide) to glucose and ceramide (Fig. 25.18). The gene of this enzyme has been cloned and localized to chromosome 1q21. Deficiency results in the accumulation of glucocerebroside in tissue macrophages, known as Gaucher cells (Fig. 25.19b), with subsequent toxicity to these tissues. Affected macrophages are present in the liver as Kupffer cells, in bone as osteoclasts, and are present in the spleen and lung as well.

### Clinical features

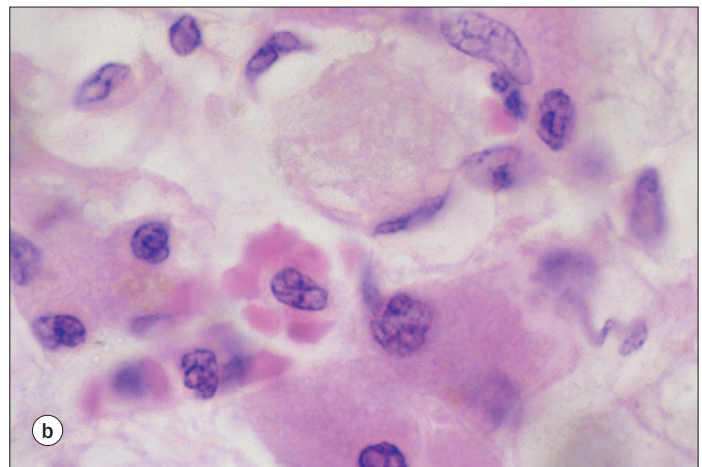
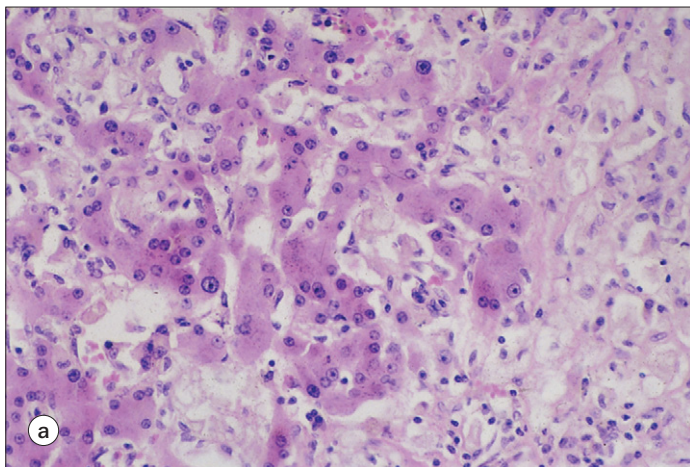
The clinical syndrome was first described by Charles Earnest Gaucher in 1882, although he erroneously believed an unidentified malignancy caused this multiorgan disease. As



**Figure 25.18** Gaucher disease – enzymatic defect.

would be predicted from the distribution of tissue macrophages, the primary syndrome is of hepatosplenomegaly, bone pain and fractures, hematopoietic disease, and pulmonary compromise. Three clinical syndromes are described (Table 25.10), classified by whether the nervous system is affected and by the age and rate of the onset of nervous system disease. Type I (non-neuronopathic) is without central nervous system (CNS) involvement. Type II is marked by early and rapid CNS affliction (acute neuronopathic), and type III (subacute neuronopathic) by CNS involvement that is delayed and more slowly progressive.

Type I is the most common. Its inheritance is autosomal recessive, with a particularly high prevalence (1 in 500–1 in 1000) among the Ashkenazi Jewish population. The incidence among all Jews is about 1 in 60 000 and is 1 in 100 000 among the general population of the USA. Onset is in childhood or adulthood, and disease severity is variable. In children, growth retardation may be subtle or severe. In advanced disease, hepatomegaly may be massive, with the sinusoidal sequestration of glucocerebroside-laden Kupffer cells (Fig. 25.19). Fibrosis is not unusual, but hepatocytes are not involved. Similarly, marked splenomegaly may develop, with Gaucher cell aggregation and fibrosis. Although spleen enlargement is usually asymptomatic, infarction may cause pain. Pulmonary disease is rare and suggests poor prognosis. The degree of bone involvement may vary widely; osteoporosis, cortical rarefaction, erosions and even avascular necrosis and collapse are all possible. Painful infarcts, known as pseudo-osteomyelitis or ‘bone crises’, are common.



**Figure 25.19** Gaucher disease: liver histopathology. (a) Photomicrograph of a liver biopsy from a patient with Gaucher disease showing infiltration of the sinusoids and compression of the hepatic cords by glucocerebroside-laden Kupffer cells, known as Gaucher cells (hematoxylin and eosin,  $\times 200$ ). (b) High-power photomicrograph of a liver biopsy from a patient with Gaucher disease. A Gaucher cell with the characteristic ‘wrinkled tissue paper’ cytoplasmic appearance is seen in a sinusoid, adjacent to hepatocytes (hematoxylin and eosin,  $\times 1000$ ). (Courtesy of Barbara Banner, University of Massachusetts Medical Center.)

Gaucher disease – inheritance, incidence, clinical features and treatment			
Disease type	Inheritance and incidence	Clinical features	Treatment
Type I	<ul style="list-style-type: none"> <li>Autosomal recessive</li> <li>1/500–1/1000 Ashkenazi Jewish</li> <li>1/60,000 general Jewish</li> <li>1/100,000 general United States</li> </ul>	<ul style="list-style-type: none"> <li>Onset as a child or an adult</li> <li>Hepatosplenomegaly</li> <li>No neurologic disease</li> <li>Growth retardation</li> <li>Bone involvement</li> <li>Pulmonary disease (poor prognosis)</li> </ul>	<ul style="list-style-type: none"> <li>Hydration, analgesia</li> <li>Enzyme replacement therapy</li> <li>Calcium, vitamin D, bisphosphonates, orthopedic repair</li> <li>Splenectomy if symptomatic</li> <li>Bone marrow transplantation</li> </ul>
Type II	<ul style="list-style-type: none"> <li>Autosomal dominant</li> <li>Less than 1/100 000</li> <li>No ethnic predisposition</li> </ul>	<ul style="list-style-type: none"> <li>Onset in infancy</li> <li>Rapidly progressive neurologic decline</li> <li>Hepatosplenomegaly</li> <li>Growth retardation</li> <li>Pulmonary involvement</li> </ul>	<ul style="list-style-type: none"> <li>As for type I disease</li> </ul>
Type III	<ul style="list-style-type: none"> <li>Autosomal dominant</li> <li>Incidence unclear, ? 1/50 000</li> <li>No ethnic predisposition</li> </ul>	<ul style="list-style-type: none"> <li>Onset in childhood</li> <li>Slowly progressive neurologic disease</li> </ul>	<ul style="list-style-type: none"> <li>As for type I disease</li> </ul>

**Table 25.10 Gaucher disease – inheritance, incidence, clinical features and treatment.**

The head and shaft of the femur are commonly affected with characteristic 'Erlenmeyer flask' deformity, and the humerus, vertebrae and pelvis may be involved (Fig. 25.20).

Thrombocytopenia and anemia secondary to splenic sequestration are common, and patients may have a history of easy bruising or bleeding. By definition, type I disease spares the CNS.

Type II is much less prevalent, likely less than 1 in 100 000. The incidence of type III may be as high as 1 in 50 000 live births, but no clear estimates are available. The inheritance of both types II and III is autosomal dominant, and there is no ethnic predilection. Rapidly progressive CNS disease in infancy is the hallmark of type II, which is also associated with poor growth, hepatosplenomegaly and pulmonary disease. Death may occur early, with an average life span of just 2 years. In type III disease, neurologic disease progresses slowly; hepatosplenomegaly is prominent. Onset is in childhood, with the expected life span varying from infancy to adulthood.

### Diagnosis

Diagnosis should be suspected in anyone with the features described above. A careful family history may further suggest the diagnosis. Marrow, liver, spleen or lung may demonstrate glucocerebroside-packed Gaucher macrophages. These are seen in other diseases, however, such as hematopoietic malignancies, and definitive diagnosis is made by demonstration of deficient  $\beta$ -glucocerebrosidase activity in leukocytes. Increased glucocerebroside levels are found but are not specific. Prenatal testing in gestations at risk can be carried out via amniocentesis or chorionic villus sampling.

With the localization and cloning of the gene for Gaucher disease, mutations have been identified which may be prognostic of the type and severity of disease. Although many mutations have been characterized, a small number constitute the majority, and a few presently have specific prognostic value. Hence, more than 95% of Ashkenazi heterozygotes can be identified by screening for just five mutations. Indeed, a single alanine to guanine point mutation at nucleotide 1226, which

codes for an asparagine to serine substitution at amino acid 370, is responsible for almost 75% of affected Ashkenazis. Homozygosity predicts moderate type I disease, but compound heterozygosity with another Gaucher allele causes type I disease with more severe enzymatic deficiency and a more severe course.

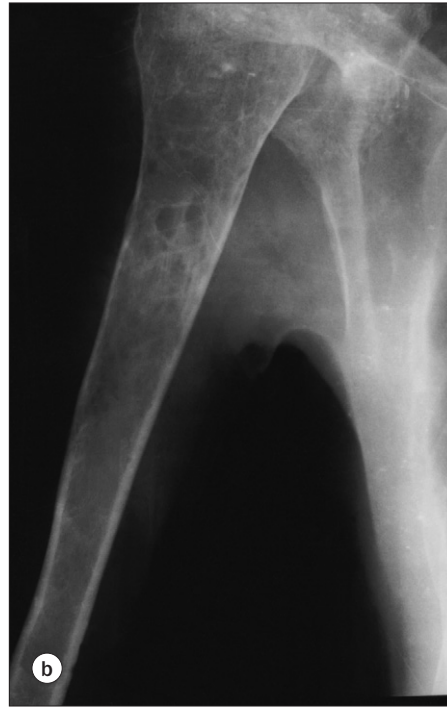
### Treatment

Traditional conservative therapy for symptomatic disease involves hydration, analgesia and surgery as warranted for bone disease. Calcium, vitamin D and bisphosphonates are helpful in delaying bone afflictions. Splenectomy is sometimes carried out but is of little benefit for the asymptomatic patient. Bone marrow transplantation has been successful but carries significant risk.

Recent advances in the therapy of Gaucher disease have, at once, begotten hope and controversy. Enzyme replacement therapy, first proposed decades ago, has become a reality in recent years and provides remarkable benefit. By targeting a mannose receptor on the plasma membrane surface of macrophages, exogenous  $\beta$ -glucocerebrosidase, which has been modified to expose mannose residues, is directed selectively to macrophages. In type I disease, this therapy has been shown to halt the progression of and actually reverse hepatosplenomegaly, hypersplenism and pulmonary disease. Marrow composition, osteoporosis and other bone lesions may improve. The therapy has not been shown effective for neurologic involvement in types II and III disease.

By targeting macrophages, replacement therapy has been made more efficient but is still very expensive. One year of therapy can cost more than \$200 000. Controversy has therefore arisen over the dose and duration of therapy necessary to evoke and maintain an adequate response, and dose-response clinical trials continue. Development of methods for mass production of the enzyme by cells containing its cDNA, to replace placenta-derived enzyme, is not expected to dramatically decrease cost. Of note, animal models of gene therapy have been promising in vivo as have been in vitro studies with human cells, and human trials of gene therapy for Gaucher disease are now underway.





**Figure 25.20 Gaucher disease: extremity roentgenograms.** (a) Femur of a 12-year-old male who presented with dull bone pain and splenomegaly and was found to have Gaucher disease type I. The distal end of the femur has lost its normal concavity, resulting in the characteristic 'Erlenmeyer flask' deformity. (b) Humerus of a 26-year-old woman with Gaucher disease type I. There is marked osteopenia with cortical rarefaction and erosions. Painful infarcts, known as pseudo-osteomyelitis, may accompany advanced disease. (Courtesy of the Department of Radiology, University of Massachusetts Medical Center.)

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 26

## Pediatric Liver Disease

Deirdre A. Kelly

### NEONATAL LIVER DISEASE

#### Jaundiced baby

Two-thirds of children with liver disease present in the neonatal period with persistent jaundice. Although physiologic jaundice is common in neonates (Table 26.1), infants who develop severe or persistent jaundice should be investigated to exclude hemolysis, sepsis or underlying liver disease. Neonatal jaundice that persists beyond 14 or 21 days should always be investigated, even in breast-fed babies.

Clinical features suggesting liver disease include pale stools and dark urine, dysmorphic features, bruising, petechiae or bleeding, hepatomegaly and/or splenomegaly, slow weight gain or failure to thrive, and previous family history or consanguinity. The differential diagnosis is between extrahepatic biliary disease, the neonatal hepatitis syndrome, and intrahepatic biliary hypoplasia.

#### Unconjugated hyperbilirubinemia

The most common causes of unconjugated hyperbilirubinemia are physiologic jaundice or breast-milk jaundice (Tables 26.1 and 26.2), although systemic disease or hemolysis from any cause must be excluded. It is important to screen for Coomb positivity, and glucose-6-phosphate dehydrogenase deficiency, and to exclude red cell membrane defects such as spherocytosis. Systemic sepsis is an important cause of unconjugated hyperbilirubinemia in the early neonatal period and requires prompt treatment with antibiotics, fluids, phototherapy and/or exchange transfusion.

#### Inherited disorders: Crigler-Najjar type I and II

This autosomal recessive disease is secondary to mutations in two genes *B-UGT1* and *B-UGT2* which lead to a deficiency of the hepatic enzyme, bilirubin diglucuronide transferase, which causes high levels of unconjugated hyperbilirubinemia in the perinatal period. In Crigler-Najjar type I peak serum bilirubin levels vary between 15 and 50 mg/dL (250–850  $\mu$ mol/L), whereas in Crigler-Najjar type II peak bilirubin levels are 12–18 mg/dL (200–300  $\mu$ mol/L).

#### Diagnosis

The diagnosis of Crigler-Najjar type I is suspected by the high level of unconjugated hyperbilirubinemia in the absence of other clinical causes. Confirmation may be obtained by aspiration of bile from the duodenum in infants over 3 months old as bilirubin diglucuronides are not present in Crigler-Najjar type I, but small amounts are detected in type II. Liver biopsy to measure enzyme levels is not necessary.

#### Physiologic neonatal jaundice

Peak 2–5 days after birth
Normal stools and urine
More severe in premature babies
Clears within 2 weeks
May persist for 4 weeks in breast-fed infants
Can rarely lead to kernicterus
Diagnosis: 80% total bilirubin is unconjugated
Treatment includes phototherapy, fluids, reassurance

Table 26.1 Physiologic neonatal jaundice.

#### Causes of unconjugated hyperbilirubinemia

Physiologic/breast-milk jaundice
Hemolysis Immune Red blood cell membrane abnormality
Metabolic disorders Crigler-Najjar types I and II Gilbert syndrome Galactosemia Fructosemia
Hypothyroidism
Sepsis
Hypoxia

Table 26.2 Causes of unconjugated hyperbilirubinemia.

#### Management

Prompt treatment is required for Crigler-Najjar type I with exchange transfusion and phototherapy to prevent kernicterus. Infants do not respond to phenobarbital. Long-term phototherapy is essential, but becomes difficult when 12–16 h per day are required for school-going children. Auxiliary liver transplantation or hepatocyte transplantation effectively reduces bilirubin levels while retaining native liver for future gene therapy. Treatment for Crigler-Najjar type II is not required except for cosmetic reasons, but bilirubin levels reduce on phenobarbital 5–15 mg/kg.



### Extrahepatic biliary disease

Biliary atresia occurs in 1:14 000 live births worldwide. There is a slight female predominance. The etiology is unknown although the association of biliary atresia with other extrahepatic anomalies suggests an abnormality of the embryologic development of the biliary tree. There is no clear genetic basis despite the association of biliary atresia with trisomy 18 and human leukocyte antigen (HLA)-B12 subtype. Although isolated cases of biliary atresia are associated with proven viral infection (cytomegalovirus), numerous studies have not indicated an association with any hepatotropic RNA viruses such as reovirus or rotavirus.

### Pathogenesis

Biliary atresia affects all parts of the biliary tree. There is gradual fibrosis and destruction of the extra- and intrahepatic biliary ducts with progressive cholestasis. The lumen of the extrahepatic duct may be obliterated at different levels leading to three main types: type I in which the common bile duct is obstructed; type II in which the common hepatic duct is obstructed; and type III in which there is fibrosis at the level of the porta hepatis – this occurs in 85% of cases.

### Clinical features

Infants who have biliary atresia are usually born at term with a normal birth weight. Jaundice is apparent on the second day of life, but is mistaken for physiologic jaundice. Biliary obstruction, as evidenced by pale stools and yellow urine, gradually develops over the next 2–4 weeks, and is associated with a gradual increase in liver size and failure to gain weight adequately despite a good appetite. Approximately 25% of babies have associated anomalies: dextracardia, ventricular or atrial septal defects, polysplenia, and the hypovascular syndrome (HVS) (Table 26.3).

The spleen is not enlarged unless there is significant hepatic fibrosis. Ascites may occasionally be present.

### Diagnosis

All children who have persistent neonatal jaundice need thorough investigation (Fig. 26.1).

A diagnosis of biliary atresia is dependent on:

- conjugated hyperbilirubinemia;
- liver biochemistry which indicates raised alkaline phosphatase (>600 IU/L) and  $\gamma$ -glutamyltranspeptidase ( $\gamma$ -GT) (>100 IU/L), and moderate elevation of alanine and aspartate aminotransferases (100–200 IU/L);
- abdominal ultrasound performed after a 4-h fast will either not demonstrate a gallbladder or indicate a small contracted gallbladder;
- radionuclide hepatobiliary imaging will not demonstrate biliary excretion from the liver into the gut after 24 h (Fig. 26.2); and
- histology that demonstrates the characteristic findings of expansion of portal tracts, proliferation of bile ducts and ductules with bile plugs, portal fibrosis, and portal edema – there may be an increase in inflammatory reaction at the porta hepatitis and variable giant cell transformation of hepatocytes (Fig. 26.3).

As none of these tests are pathognomonic for biliary atresia, the diagnosis should be confirmed by operative cholangiography with progression to a Kasai portoenterostomy if required.

Congenital abnormalities in biliary atresia	
Anomaly	Comment
<b>Cardiac</b>	
Dextrocardia	+/- situs inversus
VSD	
ASD	
Left atrial isomerism	With HVS
<b>Vascular</b>	
Predudodenal portal vein	HVS
Absent inferior vena cava	Azygous drainage
<b>Splenic</b>	
Polysplenia	+/- HVS
Double spleen	
Asplenia	
Malrotation	
Situs inversus	+/- HVS, NHP
Annular pancreas	
Immobile cilia syndrome	Bronchiectasis
ASD, atrial septal defect; HVS, hypovascular syndrome; VSD, ventricular septal defect; NHP, normal heart position.	

**Table 26.3** Congenital anomalies in biliary atresia.

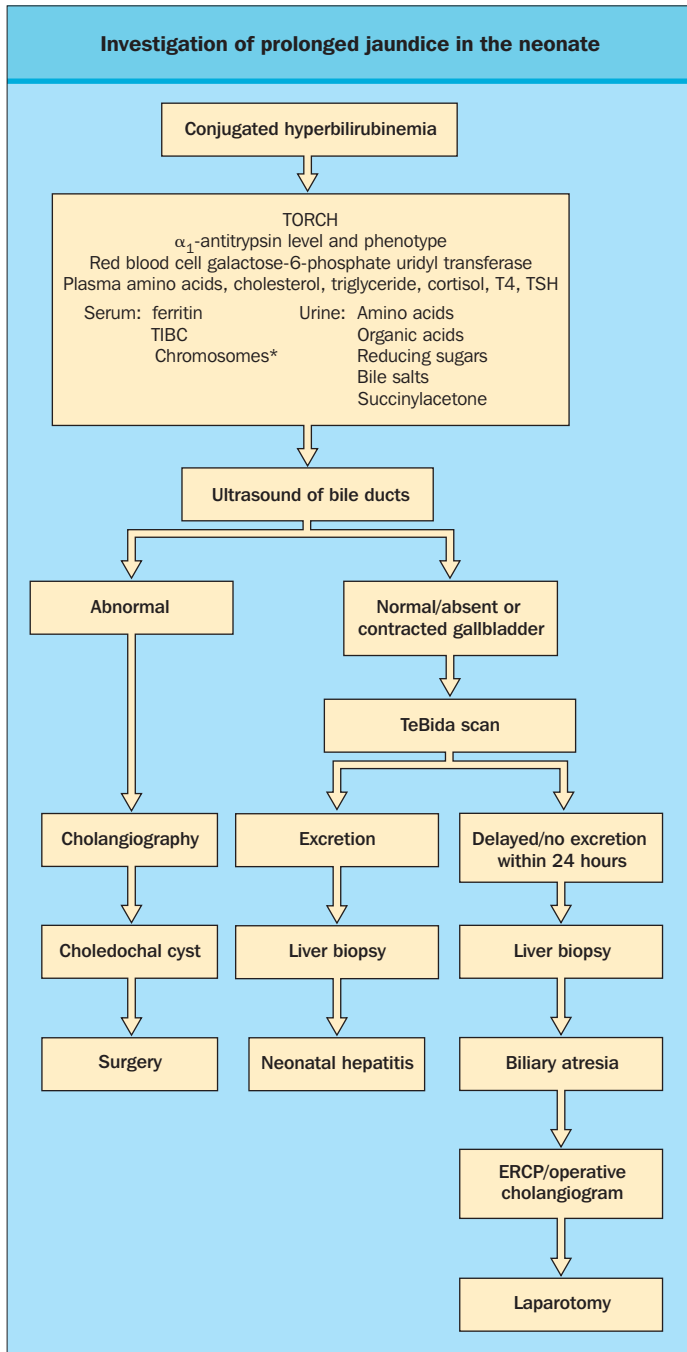
Endoscopic retrograde cholangiography (ERCP) in this age group is now possible using prototype pediatric duodenoscopes. Biliary atresia can be confidently excluded if the biliary tree is visualized, but a failure to do so may either be due to technical failure or biliary atresia and progression to operative cholangiography and laparotomy is then required.

### Management

The Kasai portoenterostomy involves resection of the obliterated biliary tract and formation of a Roux loop which is anastomosed to the porta hepatis. Between 50 and 60% of cases achieve biliary drainage which may be improved by administration of a short course of corticosteroids at the end of the first postoperative week. Revised Kasai operations are not indicated because of the poor success rate, and because of increased technical difficulties at subsequent liver transplantation.

The success of the Kasai portoenterostomy operation depends on a number of factors, which include the timing of the operation, the expertise of the surgeon, the degree of hepatic fibrosis at operation, and the presence or absence of other congenital abnormalities. Operations should be performed before 8 weeks of age, although there is no clear effect of age on survival unless the operation takes place after 100 days of age. Surgery is not recommended if there is evidence of advanced hepatic fibrosis with splenomegaly, ascites or varices.

Cholangitis is most common in the immediate postoperative period. The incidence is reduced by prophylactic antibiotics, either intravenously for 10–14 days or orally for 3–6 months.

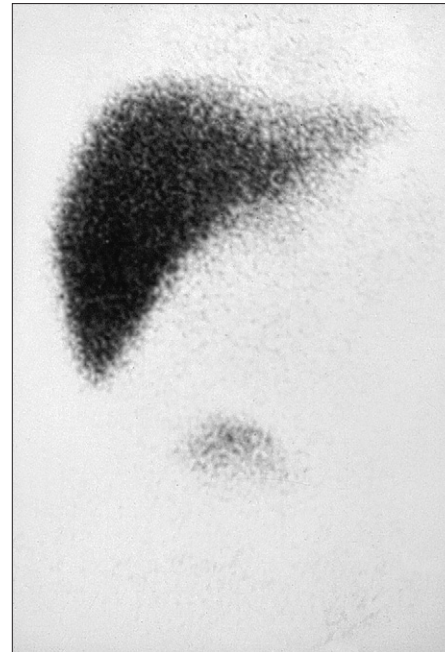


**Figure 26.1 Investigation of prolonged jaundice in the neonate.**

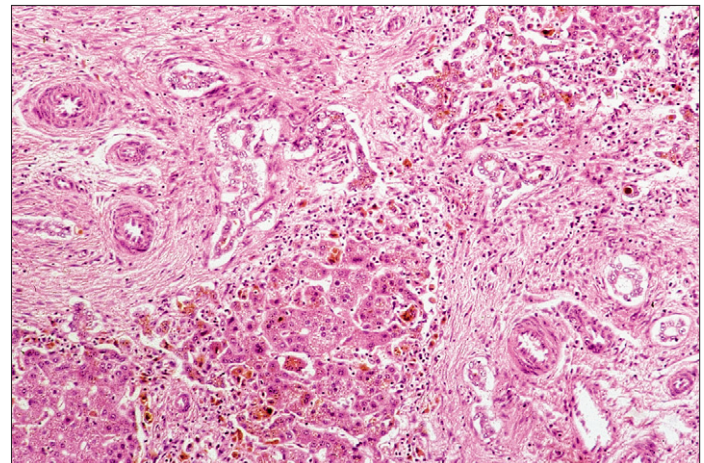
T4, thyroxine; TIBC, total iron-binding capacity; TSH, thyroid-stimulating hormone; TORCH, serology for toxoplasma, other, rubella, cytomegalovirus, and herpes simplex viruses; TeBida, 99 technetium trimethyl-1-bromoimino diacetic acid. \*Chromosomes for 18, 21, Alagille syndrome.

The clinical signs include an increase in jaundice, pyrexia, acholic stools, and tenderness over the liver. Broad spectrum antibiotics are usually effective.

Malabsorption is usually associated with a partially successful Kasai portoenterostomy and is an important factor in the development of malnutrition in neonatal liver disease. The development of cirrhosis and portal hypertension is inevitable even in those children who have had a successful Kasai



**Figure 26.2** Radioisotope scanning demonstrating excellent uptake of radioisotope but no excretion at 24 h. This represents either severe intrahepatic cholestasis, but more likely the diagnosis of extrahepatic biliary atresia.

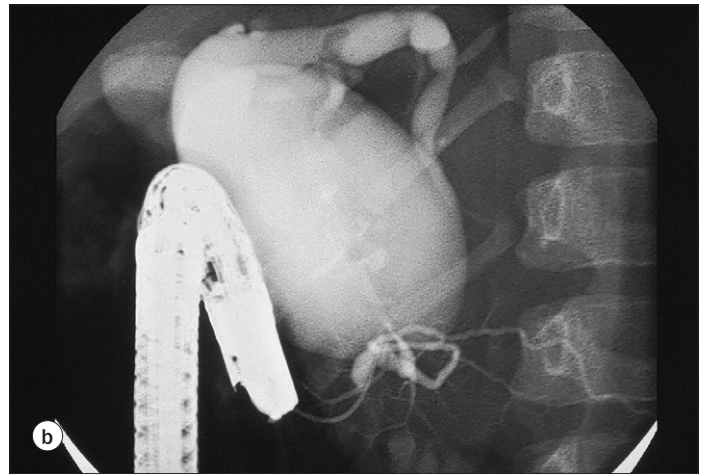
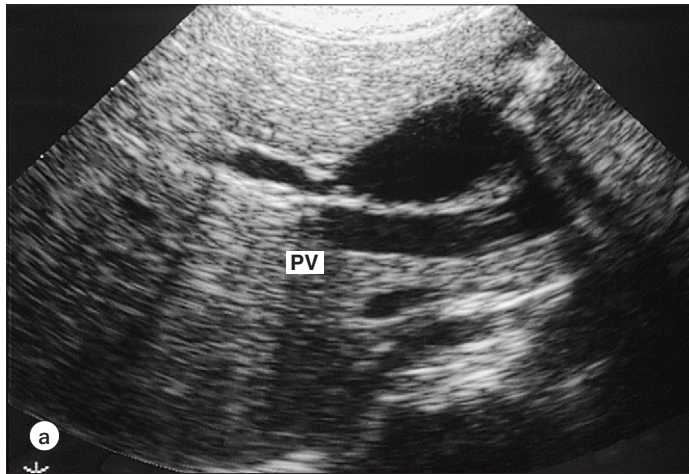


**Figure 26.3** Biopsy showing features suggestive of biliary obstruction. These are portal fibrosis, biliary ductular proliferation with bile plugs, and portal edema. There may be considerable histologic overlap between biliary atresia and the neonatal hepatitis syndrome.

portoenterostomy, although the need for transplantation varies with the rate of progression of liver disease and complications. Unsuccessful Kasai portoenterostomy is an immediate indication for liver transplantation and these children should be referred early to specialized centers for follow-up.

### **Choledochal cyst**

Choledochal cysts are localized cystic dilatation of all or part of the common bile duct. Cysts are more common in Japan (1:100 000 live births) and in females (4:1). Choledochal cysts may be congenital or acquired. There may be a structural weakness with subsequent dilatation in the wall of the biliary tree or alternatively the biliary dilatation may be secondary to pancreatitis associated with a common pancreaticobiliary channel or repeated cholangitis.



**Figure 26.4 Abdominal ultrasound demonstrating a large cystic swelling.** (a) Shows the cystic swelling, which was (b) confirmed to be a choledochal cyst on endoscopic retrograde cholangiopancreatography (ERCP).

The cyst may present in infancy with prolonged jaundice and must be differentiated from biliary atresia or the neonatal hepatitis syndrome. In older children a history of abdominal pain, jaundice and abdominal mass is classic, but is a rare presentation in childhood. Jaundice may be intermittent and associated with ascending cholangitis and recurrent pancreatitis.

#### Diagnosis

Choledochal cysts are diagnosed by abdominal ultrasound which may detect the cysts antenatal. Confirmation of the anatomy is obtained by radioisotope scan, percutaneous transhepatic cholangiogram, or endoscopically by an endoscopic retrograde cholangiopancreatography (Fig. 26.4). Histology of the liver usually indicates biliary fibrosis, cholestasis and bile plugs in the portal tract. The histologic features are completely reversible following successful surgery.

#### Management

Surgical treatment includes excision of all the affected ducts and re-establishment of biliary drainage by forming a hepaticojejunostomy. Drainage of the cyst into adjacent duodenum or jejunum is now contraindicated, because of the potential malignant transformation. The results of surgery are excellent, cholangitis is an occasional complication, and there is a 2.5% risk of malignancy in the residual biliary tree in adult life.

#### Spontaneous perforation of the bile ducts

This is a rare complication in which perforation occurs at the junction of the cystic and common hepatic ducts, perhaps due to a congenital weakness, inspissated bile, or gallstones. Infants may present at any age from 2 to 24 weeks of age with abdominal distention, ascites, jaundice and acholic stools. Biliary peritonitis, with bile in hydroceles, hernial sacs and the umbilicus may be obvious.

The diagnosis may be confirmed by abdominal ultrasound, which may show free intraperitoneal fluid and dilated intrahepatic ducts. Biochemical liver tests may be abnormal, with conjugated hyperbilirubinemia and raised alkaline phosphatase and  $\gamma$ -GT. If the biliary leak is large, liver tests may be virtually normal. Hepatobiliary scanning will demonstrate

isotope in the peritoneal cavity. Treatment includes peritoneal drainage followed by repair of the perforation.

#### Inspissated bile syndrome and cholelithiasis

Bile duct obstruction secondary to inspissated bile syndrome may be secondary to total parenteral nutrition, prolonged hemolysis and dehydration. It is more common in premature babies or those undergoing major surgery. The clinical picture is of biliary obstruction with pale stools, dark urine and abnormal liver tests. The diagnosis may be confirmed by ultrasound, which demonstrates a dilated intra- and extrahepatic duct system with biliary sludge. Percutaneous transhepatic cholangiography will outline the anatomy and may be therapeutic with lavage of the biliary tree, but laparotomy and decompression of the biliary tree may be required. The use of ursodeoxycholic acid (20 mg/kg) and cholecystikinin may prevent the need for either surgical or radiologic intervention.

Cholecystitis may occur in infants in association with gallstones from hemolysis or total parenteral nutrition (TPN), while acalculus cholecystitis may occur as part of generalized sepsis. Operative cholecystectomy (rather than laparoscopic) is the treatment of choice in this young age group for symptomatic cholecystitis in association with gallstones.

#### Neonatal hepatitis syndrome

The neonatal hepatitis syndrome includes many different causes of neonatal liver disease (Table 26.4), which may have a similar presentation (see Fig. 26.1). The commonest causes are intra-uterine infections or inherited metabolic diseases.

In contrast to children who have extrahepatic biliary disease, babies who have a neonatal hepatitis syndrome may have the following characteristics:

- small for dates or have intrauterine growth retardation (Fig. 26.5);
  - pigment is usually present in the stools although the urine will be yellow;
  - dysmorphic features characteristic of Alagille syndrome or Zellweger syndrome may be present; and
  - hepatosplenomegaly is usually present.
- Biochemical features include:



Neonatal hepatitis syndrome and diagnostic approach		
Disease	Diagnosis	Treatment
<b>Intrauterine infection</b>		
Cytomegalovirus	Urine for viral culture	Ganciclovir
Toxoplasmosis	IgM antibodies	Spiramycin
Rubella	IgM antibodies	Supportive
Herpes simplex	EM/viral culture of vesicle	Aciclovir
Syphilis	VDRL test	Penicillin
<b>Metabolic</b>		
AAT deficiency	AAT level and phenotype	Supportive
Cystic fibrosis	Sweat chloride, immunoreactive trypsin, AFO8 mutation	Supportive
Galactosemia	Galactose-1-6-phosphate uridyltransferase	Galactose-free diet
Tyrosinemia	Urine succinylacetone, serum amino acids, $\alpha$ -fetoprotein	NTBC
Hereditary fructosemia	Enzymes in liver	Fructose-free diet
Niemann-Pick type A	Sphingomyelinase	Supportive
Niemann-Pick type C	Storage cells in bone marrow aspirate and liver biopsy; fibroblast culture	Supportive
Wolman disease	Abdominal radiograph of adrenal glands	Supportive
Primary disorders of bile acid synthesis	Urinary bile acids by FAB-MS	Bile acids
Zellweger syndrome	Very-long-chain fatty acids	Supportive
<b>Endocrine</b>		
Hypopituitarism (septo-optic dysplasia)	Cortisol TSH, T4	Hormone replacement
Hypothyroidism	TSH, T4, free T4, T3	Hormone replacement

AAT,  $\alpha_1$ -antitrypsin; EM, electron microscopy; FAB-MS, fast atom bombardment ionization mass spectrometry; NTBC, 2(2-nitro-trifluoromethylbenzoyl)-1, 3-cyclononanedione; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; VDRL, venereal disease research laboratory

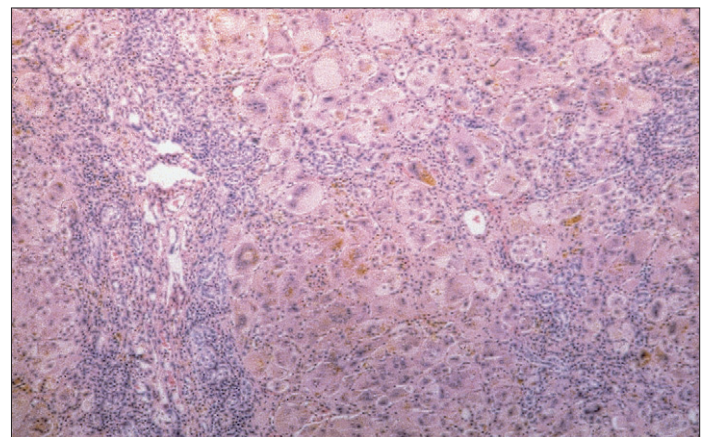
**Table 26.4 Neonatal hepatitis syndrome and diagnostic approach.**

- conjugated bilirubin 6 mg/L ( $>100 \mu\text{mol/L}$ );
- alkaline phosphatase 600–800 IU/L;
- aspartate aminotransferase (AST) and alanine aminotransferase (ALT) 200–300 IU/L; and
- hypoglycemia (depending on etiology).

Liver histology is non-specific and demonstrates giant cell hepatitis with fibrosis of the portal tracts, extramedullary



**Figure 26.5 Neonatal hepatitis.** This baby was born at 37 weeks' gestation, with obvious intrauterine retardation. He was jaundiced with hepatosplenomegaly, and malnourished with loss of fat and muscle stores. The most likely differential diagnosis is neonatal hepatitis secondary to a uterine infection or inborn error of metabolism.



**Figure 26.6 Most babies who have neonatal hepatitis have giant cell hepatitis with extramedullary hemopoiesis and a rosette formation of hepatocytes.** This is a non-specific finding and may represent many different causes of neonatal liver disease.

hemopoiesis, cholestasis and biliary ductule proliferation. There may be histologic overlap with biliary atresia (Fig. 26.6).

### Intrauterine infection

The most common cause of intrauterine infection causing neonatal hepatitis is cytomegalovirus (CMV) infection. Infants are small for dates with hepatosplenomegaly and may have thrombocytopenia, choreoretinitis, or microcephaly. A diagnosis (see Table 26.4), is based on identification of immunoglobulin (Ig)M antibodies, virus culture or a positive polymerase chain reaction (PCR) for CMV DNA. The outcome is variable. In most babies the hepatitis resolves completely within 3–6 months, but neurologic involvement with spasticity or sensorineural deafness and developmental delay may be present. Treatment with ganciclovir or oral valganciclovir is rarely necessary.

Rubella hepatitis is almost unknown now following universal vaccination, but may present with neonatal hepatitis, cataracts, congenital heart disease and deafness. Progressive liver disease has been reported.

Toxoplasmosis is prevalent in parts of France and Germany, but is unusual in the UK. It is associated with persistent neonatal jaundice, failure to thrive, hepatosplenomegaly, central nervous system involvement with choreoretinitis, hydrocephaly, microcephaly and intracranial calcification. Treatment with spiramycin may be helpful for liver disease. The long-term outcome is related to the neurologic disease, as by the time of diagnosis many children are blind or severely handicapped.

Herpes simplex in the newborn usually causes a multisystem disorder with encephalitis and acute liver failure. Antiviral treatment with acyclovir is successful if started early enough.

Congenital syphilis is now rare, but may also cause a multisystem disease with intrauterine retardation, anemia, thrombocytopenia, nephrotic syndrome, skin rash, diffuse lymphadenopathy and hepatomegaly. Diagnosis is based on serologic testing (see Table 26.4). Treatment with penicillin is usually curative.

Hepatitis A, B and C viruses and human immunodeficiency virus (HIV) are rare causes of persistent neonatal jaundice. Vertical transmission of hepatitis B virus (HBV) and hepatitis C virus (HCV) leads to an asymptomatic carrier state.

### Endocrine disorders

The neonatal hepatitis syndrome is associated with pituitary or adrenal dysfunction in approximately 30% of patients. Hypopituitarism may be due to hypothalamic dysfunction and is associated with septo-optic dysplasia, which includes absence of the septum pelucidum or malformation of the forebrain and hypoplasia of one or both optic nerves.

Hypoglycemia is a prominent symptom that is not associated with the severity of liver disease. Other signs of hypopituitarism include microgenitalia in boys, midline facial abnormalities, or nystagmus. A number of children may have severe cholestasis with acholic stools and dark urine and differentiation from biliary atresia may be difficult.

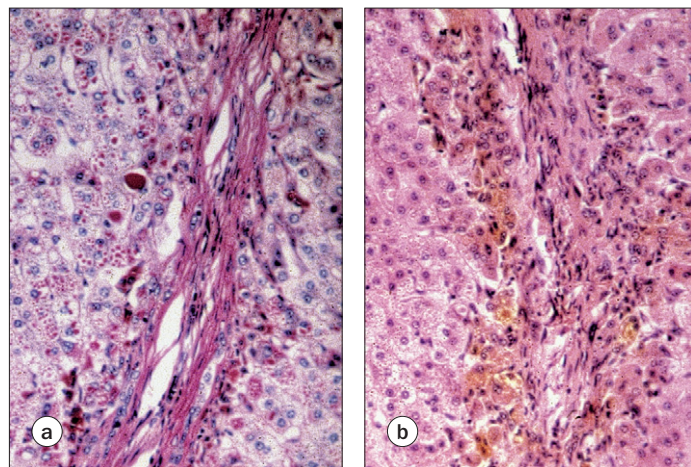
The diagnosis is established by demonstrating hypothyroidism and a low random or 9.00 am cortisol. A synacten test is rarely required for confirmation. Treatment is with hormone replacement with thyroxine, hydrocortisone and growth hormone. If diagnosed early enough, liver disease will completely resolve, but hormone replacement is required lifelong.

### Inborn errors of metabolism

The commonest inborn error of metabolism to present with persistent neonatal jaundice is  $\alpha_1$ -antitrypsin deficiency, which is an autosomal recessive disorder with an incidence of 1:7000 live births worldwide. Infants may present with intrauterine growth retardation, cholestasis, failure to thrive, hepatomegaly, or a vitamin K responsive coagulopathy which is more likely in those infants who are not given prophylactic vitamin K at birth and who are being breast-fed. The coagulopathy may be obvious with bruising and bleeding from the umbilicus, but the initial symptom may be intraventricular hemorrhage which may result in neurologic disability.

### Diagnosis

Liver biochemistry demonstrates a mixed hepatic/obstructive picture with elevated aminotransferases, alkaline phosphatase, and  $\gamma$ -GT. Radiologic investigations may indicate severe intrahepatic cholestasis with a contracted gallbladder on a fasting



**Figure 26.7  $\alpha_1$ -Antitrypsin deficiency is the commonest inherited liver disease.** The diagnosis is made on histology, demonstrating granules in hepatocytes either by a periodic acid-Schiff (PAS)-stain (**a**), or by immunoperoxidase staining (**b**), which may be detected as early as 6–8 weeks.

ultrasound, and delayed or absent excretion of radioisotope on hepatobiliary scanning. In homozygotes the diagnosis is easily confirmed by detection of a low level of  $\alpha_1$ -antitrypsin ( $<0.9$  g/L). Liver disease is usually associated with phenotype protease inhibitor P<sub>1</sub>ZZ but may occur with P<sub>1</sub>MZ heterozygotes or other variants. Liver histology demonstrates giant cell hepatitis with characteristic periodic acid-Schiff (PAS), diastase resistant, positive granules of  $\alpha_1$ -antitrypsin in hepatocytes which may be detected by 6–8 weeks of age (Fig. 26.7).

### Management

This consists of nutritional support, fat-soluble vitamin supplementation, and treatment of pruritus and cholestasis (Table 26.5).

The prognosis is varied. Jaundice disappears in most infants, of whom approximately one-third regain normal function, one-third develop an inactive fibrosis and/or cirrhosis, and one-third develop chronic liver failure requiring transplantation. Respiratory disease is rare in childhood but long-term follow-up is essential to monitor growth, development and the need for liver transplantation. Antenatal diagnosis by chorionic villus sampling is now available using synthetic oligonucleotide probes specific for the M and Z genes, or by restriction fragment length polymorphism.

### Intrahepatic biliary hypoplasia

The term intrahepatic biliary hypoplasia refers to an absence or reduction of a number of interlobular bile ducts or ductules seen in portal tracts in association with normal-sized branches of the portal vein and hepatic arteries. Biliary hypoplasia may occur in a wide spectrum of liver diseases, such as  $\alpha_1$ -antitrypsin deficiency, chromosomal abnormality such as Down syndrome, or intrauterine infection (CMV). However, this term is conventionally used with syndromic biliary hypoplasia or Alagille syndrome, or non-syndromic biliary hypoplasia which includes Byler disease and progressive familial intrahepatic cholestasis.



Management of neonatal liver disease	
Nutritional support	
Modular feed	Energy intake 150–200 cal/kg
Carbohydrate	Glucose polymer (8–10 g/kg/day)
Protein	Whey protein (2.5–3.5 g/kg/day)
Fat	50/50 MCT/LCT (8 g/kg/day)
Fat soluble vitamins	
A: 5–10 000 U/day	
E: 50–100 mg/day	
K: 1–2 mg/day	
D: 50 mg/kg/day	
Pruritus/cholestasis	
Phenobarbital	5–15 mg/kg
Ursodeoxycholic acid	20 mg/kg
Rifampin (rifampicin)	3 mg/kg
Colestyramine	1–2 g/day
Topical skin care	
MCT, medium-chain triglyceride; LCT, long-chain triglyceride	

**Table 26.5** Management of neonatal liver disease.**Alagille syndrome**

This is an autosomal dominant condition with an incidence of 1:100 000 live births worldwide. It is a multisystem disorder which is associated with cardiac, facial, renal, ocular and skeletal abnormalities. Mutations in the *Jag 1* gene on chromosome 20p have been identified in 60% of patients. Infants may present with persistent cholestasis, severe pruritus, hepatomegaly and failure to thrive. The characteristic facial features are very difficult to identify in infancy, but become more prominent later in childhood. They include a triangular face with high forehead and frontal bossing, deep widely spaced eyes, saddle-shaped nasal bridge, and pointed chin (Fig. 26.8).

Cardiac abnormalities include peripheral pulmonary stenosis, pulmonary and aortic valve stenosis and Fallot tetralogy.

Skeletal abnormalities are widespread and include abnormal thoracic vertebrae, 'butterfly' vertebrae, and curving of the proximal digits of the third and fourth finger. Posterior embryotoxin, which is detected on the inner aspects of the cornea near the junction of the iris, is demonstrated in 90% of patients by slit-lamp examination. Retinal pigmentation on fundoscopy and calcific deposits (optic drusen) in the optic nerve may be detected by ultrasound. Renal disease varies in severity from mild renal tubular acidosis to severe glomerular nephritis. One of the most difficult management problems is severe failure to thrive, which is complicated by gastrointestinal reflux and severe steatorrhea secondary to fat malabsorption or pancreatic insufficiency.



**Figure 26.8 Alagille syndrome.** This is an autosomal dominant disorder in which the typical facies may be less obvious in infants. This baby demonstrates hypertelorism, widely spaced eyes, depressed nasal bridge, and a pointed chin. He also has curving of the second metacarpal on his little finger, which is characteristic of this syndrome.

**Diagnosis**

Liver biochemistry indicates severe cholestasis with:

- conjugated bilirubin 6 mg/dL ( $>100 \mu\text{mol/L}$ );
- raised alkaline phosphatase  $>600 \text{ IU/L}$ ;
- $\gamma\text{-GT} >200 \text{ IU/L}$ ;
- raised aminotransferases; and
- plasma cholesterol  $>6 \text{ mmol/L}$  with normal triglycerides ( $0.4\text{--}2 \text{ mmol/L}$ ).

Tests of hepatic function such as albumin and coagulation are usually normal. Renal dysfunction may be identified by the presence of aminoaciduria or estimating urinary protein/creatinine ratio (normal  $<20$ ).

The skeletal abnormalities are easily identified by radiologic examination of the chest and hands. Electrocardiography may demonstrate right bundle branch block or right ventricular hypertrophy in children who have peripheral pulmonary stenosis. Echocardiography may be normal but if cardiac abnormalities are suggested clinically, then cardiac catheterization should be performed for confirmation.

Liver histology may be non-specific. The reduction in interlobular bile ducts is often difficult to identify in the neonatal period, particularly if cholestasis and giant cell hepatitis are also present. The histologic appearance differs from extrahepatic biliary atresia because of the absence of portal fibrosis and biliary ductular proliferation.

**Management**

Intensive nutritional support is essential (see Table 26.5) and pancreatic supplements may be required. Pruritus may be intractable and an indication for liver transplantation, although recent experience with a molecular reabsorbent recirculating system (MARS) has produced relief for 6–12 months. Prognosis is varied and depends on the extent of liver, cardiac or renal disease. Approximately 50% of children may regain normal liver function by adolescence while others require liver transplantation in childhood. The indications for liver transplantation are



Inherited disorders of bile acid synthesis		
Enzyme	Features	Treatment
3 $\beta$ -hydroxy- $\delta^5$ -C <sub>27</sub> -steroid dehydrogenase/isomerase	Neonatal hepatitis; normal $\gamma$ -GT; low bile acid concentration; no pruritus	CDCA or UDCA
$\delta^4$ -3-oxosteroid 5 $\beta$ -reductase	Jaundice, coagulopathy; elevated bile acid concentrations	UDCA + cholic acid
24,25-dihydroxy-cholanoic cleavage enzyme	Giant cell hepatitis; normal $\gamma$ -GT; elevated serum cholesterol; low bile acid concentrations	CDCA + cholic acid
CDCA, chenodeoxycholic acid; $\gamma$ -GT, $\gamma$ -glutamyltranspeptidase; UDCA, ursodeoxycholic acid		

Table 26.6 Inherited disorders of bile acid synthesis.

the development of cirrhosis and portal hypertension, intractable pruritus or severe growth failure. Pretransplant cardiac surgery or balloon dilatation may be indicated for severe pulmonary stenosis.

### Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is the term given to a number of conditions in which there is persistent jaundice, cholestasis, hepatomegaly, pruritus and failure to thrive. The genetic mutations underlying a number of these have been identified (PFIC-1, 2, 3) and they are now known to be disorders of bile salt transport.

#### Byler disease (PFIC-1)

This severe form of familial idiopathic cholestasis was first described in an Amish family. It is now known that it is due to mutation in the *FIC1* gene which encodes a P type ATPase which is expressed in many tissues including the liver, gut, pancreas and lung. In contrast to children who have intrahepatic cholestasis of other causes, this group of children had normal serum  $\gamma$ -GT and low or normal serum cholesterol. The clinical presentation is with persistent conjugated hyperbilirubinemia, severe pruritus, diarrhea and growth retardation. Histology shows a lack of hepatic inflammation but paucity of bile ducts and canalicular bile plugs. Cirrhosis develops in early childhood requiring liver transplantation.

The management consists of nutritional support (see Table 26.5) and treatment of pruritus which includes biliary diversion, an operative technique in which bile is diverted externally by an enterostomy. Most children have progressive fibrosis with the development of cirrhosis and portal hypertension requiring liver transplantation in childhood. The gut defect is not reversed by transplantation, and diarrhea continues.

#### PFIC-2

PFIC-2 is due to a defect on chromosome 2q24 which encodes for a human bile salt transport pump (BSEP). The clinical phenotype is milder and children do not have diarrhea.

#### PFIC-3

Mutations in the *MDR-3* gene affect the bile canalicular membrane translocator of phospholipids leading to this disorder in which there is severe pruritus, features of biliary obstruction and a high  $\gamma$ -GT. Most children require transplantation.

### Arthrogryposis, cholestasis, renal tubular dysfunction (ARC)

This rare autosomal disorder presents with cholestasis, biliary hypoplasia, renal dysfunction and a low  $\gamma$ -GT. It is usually fatal and infants die of liver and renal failure within 12 months. The genetic defect is now known to be a disorder in a soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein (SNARE) protein (Vps33b) involved in the regulation of vesicle and membrane fusion. It is the first human disorder associated with mutations in a gene involved in regulation of the SNARE-mediated mechanism of membrane tether and fusion and may lead to further understanding of the pathophysiology of biliary disorders.

### Inherited disorders of bile salt metabolism

The development of fast atom bombardment ionization mass spectrometry (FABMS) has allowed many defects in primary bile acid synthesis to be identified (Table 26.6).

Infants present with cholestasis, hepatomegaly, failure to thrive, and possible pruritus (Table 26.6). Biochemistry indicates non-specific abnormalities of aminotransferases and elevated alkaline phosphatase and/or  $\gamma$ -GT. Liver histology indicates a giant cell transformation with cholestasis and rapid development of fibrosis and cirrhosis. The diagnosis is confirmed by identifying the specific abnormal bile acid metabolites in urine. These diseases are fatal without liver transplantation, but treatment with a combination of cholic acid, chenodeoxycholic acid or ursodeoxycholic acid may prevent progression to cirrhosis and portal hypertension if started sufficiently early.

### Zellweger syndrome (cerebrohepatorenal syndrome)

This is an autosomal recessive syndrome with multisystem disease. It is associated with absent or dysfunctional peroxisome biogenesis leading to secondary defects of bile acid synthesis and abnormal  $\beta$ -oxidation of fatty acids. The incidence is 1:100 000 live births. The initial presentation is with severe hypotonia, feeding difficulties and failure to thrive. Jaundice is only present in 50% of babies, but dysmorphic features which include epicanthic folds, brush field spots and a high forehead are common in association with psychomotor retardation. There is multisystem involvement involving the brain, heart, liver and kidneys.

The diagnosis is confirmed by demonstrating abnormal bile salt metabolites using FABMS or the detection of very-long-chain fatty acids in serum. Initially hepatic pathology may appear

normal, but there is excessive hepatic iron in the first few months with the subsequent development of fibrosis and micronodular cirrhosis. Ultra-electron microscopy may indicate abnormal mitochondria and the absence of peroxisomes.

Treatment is supportive as death is inevitable. Liver transplantation is not indicated because of the progression of multi-system disease. Initial attempts to induce peroxisomes with hypolipemic drugs were unsuccessful. Primary bile acid therapy with cholic and chenodeoxycholic acid may produce some histologic improvement, but has no effect on survival.

### **Cystic fibrosis**

Cystic fibrosis is an unusual cause of neonatal cholestasis accounting for <1% of children who have persistent jaundice. It is an autosomal recessive disorder with an incidence of 1:2000 live births. The genetic defect has been localized to the long arm of chromosome 7, and more than 300 mutations have been identified in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR). The commonest mutation is Delta 508, but no mutation is specific for liver disease. The prominent clinical symptoms include persistent cholestasis, meconium ileus, hepatomegaly, failure to thrive and recurrent respiratory infections. If cholestasis is complete with acholic stools, differentiation from biliary atresia may be difficult.

### **Diagnosis**

Biochemical liver tests reveal raised aminotransferases, alkaline phosphatase, and  $\gamma$ -GT. Immunoreactive trypsin may be higher than normal for age [1300 ng/dL (>130 ng/mL)]. Serum cholesterol and triglycerides are usually normal. Although a sweat test is thought to be pathognomonic for this diagnosis, it is not usually worth doing this in babies under 4–6 weeks of age [values of 5 mg/dL (<50 mmol/L) for children <5 years]. Liver histology is varied, but usually demonstrates diffuse cholestasis with bile duct proliferation, focal biliary cirrhosis, and portal fibrosis.

### **Management**

The efficacy of ursodeoxycholic acid (20–50 mg/kg/day) in the treatment of neonatal cholestasis secondary to cystic fibrosis is unknown, but clinical studies in older children indicate that it induces a biochemical and histologic improvement. It is usual to treat children with cholestasis secondary to cystic fibrosis with ursodeoxycholic acid and fat-soluble vitamin supplementation and appropriate nutritional support (see Table 26.5). In most children who have neonatal cystic fibrosis, jaundice resolves, although persistence has been reported with the development of cirrhosis, portal hypertension and liver failure. Ongoing malnutrition and respiratory disease may contribute to death in infancy.

### **Niemann-Pick disease type C**

This is a rare autosomal recessive disorder in which there is a defect in cholesterol esterification that results in a neurovisceral lipid storage disorder with an extremely varied spectrum of clinical findings. About 60% of children will present with prolonged cholestasis and hepatosplenomegaly in infancy, some of whom may present with fetal ascites. A number of children present later (3–5 years of age) with isolated splenomegaly and/or neurologic signs and symptoms.

### **Diagnosis**

Liver histology may indicate giant cell hepatitis. The diagnosis is determined by detecting PAS diastase-resistant storage in Kupffer cells and hepatocytes. If there is active hepatitis, it is easier to detect these characteristic foamy storage cells in bone marrow aspirate. Neuronal storage indicating central nervous system involvement is present at birth and is demonstrable in the ganglion cells of a rectal biopsy. Skin fibroblast cultures will define the enzyme defect and enable antenatal diagnosis.

### **Management**

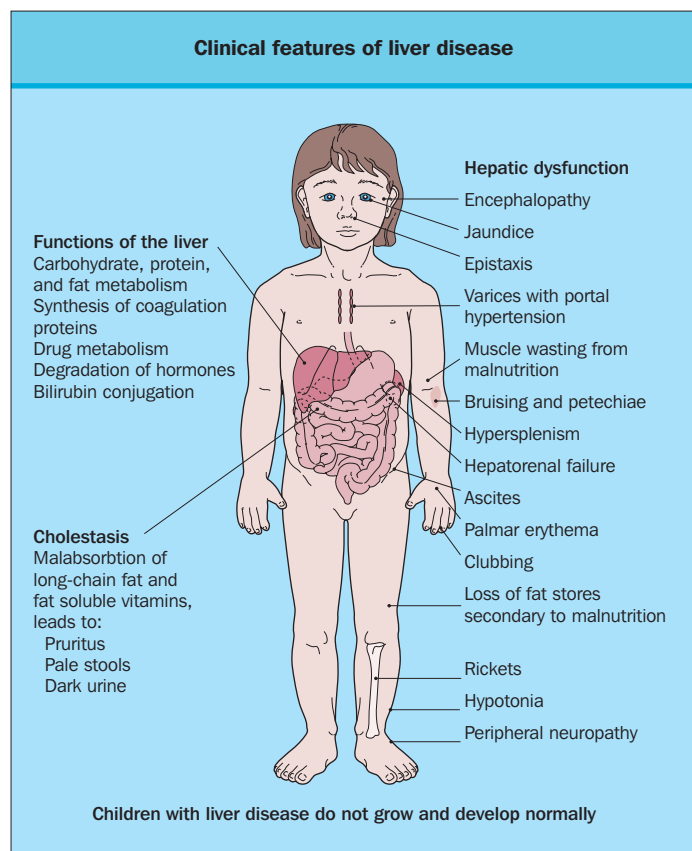
Jaundice subsides in most children although hepatosplenomegaly may persist with abnormal biochemical liver tests. Hepatic fibrosis with progression to cirrhosis and portal hypertension is a rare occurrence. Sadly, all children develop neurologic complications at a median age of 5 years, which include ataxia, convulsions, developmental delay and dementia. Supranuclear ophthalmoplegia is considered pathognomonic for this condition. Most children die in late childhood or early adolescence from respiratory infections. There is no specific treatment, although a low cholesterol diet has been suggested. Liver and bone marrow transplantation are not curative. Genetic counseling is essential, and antenatal diagnosis may be performed by chorionic villus biopsy.

## **LIVER DISEASE IN OLDER CHILDREN**

Liver disease in children older than 6 months may be acute or chronic. As in infancy, there is a predominance of inherited disorders (Table 26.7) and multisystem involvement (Fig. 26.9).

<b>Liver diseases in older children</b>	
<i>Disease</i>	<i>Diagnostic investigations</i>
Chronic hepatitis	Portal inflammatory infiltrate on biopsy
Hepatitis B, C, D, EBV, CMV	Serology
Autoimmune hepatitis	IgG >2 g/dL (>20 g/L) C3, C4, LKM, ANA, SMA
Wilson disease	Serum Cu, ceruloplasmin, urinary Cu
$\alpha_1$ -Antitrypsin deficiency	$\alpha_1$ -Antitrypsin level and phenotype
Cystic fibrosis	Sweat test, liver biopsy
Cryptogenic cirrhosis	Liver biopsy
Primary sclerosing cholangitis	ERCP and liver biopsy
Tyrosinemia type 1	Urinary succinylacetone
Hereditary fructose intolerance	Fructose 1-6-phosphate aldolase in liver
ANA, antinuclear antibodies; C3 and C4, complement; ERCP, endoscopic retrograde cholangiopancreatography; LKM, liver, kidney microsomal antibodies; SMA, smooth muscle antibodies; Cu, copper	

**Table 26.7 Liver diseases in older children.**



**Figure 26.9 Clinical features of liver disease.** Chronic liver disease affects every organ in the growing child and leads to significant growth failure and psychosocial delay, which are indications for liver transplantation.

## Acute liver disease

### Acute viral hepatitis

All forms of acute viral hepatitis occur in children and include hepatitis A virus (HAV), HBV, post-transfusion HCV, epidemic hepatitis E virus (HEV), Epstein-Barr virus (EBV), and CMV. In contrast to adults, most children are asymptomatic and anicteric, and many episodes of hepatitis are subclinical. In symptomatic cases a prodromal illness with vomiting, abdominal pain, lethargy and jaundice are common, as in adults. The diagnosis is suggested by elevations of serum aminotransferases (ALT and AST 10–100 × normal) and confirmed by specific viral serology. Liver biopsy is not required for diagnosis unless the clinical course is complicated. Centrilobular necrosis and inflammation are typical histologic changes of acute viral hepatitis.

### Management

Uncomplicated acute hepatitis is managed at home. Hospital admission is required only if the child has severe vomiting leading to dehydration, abdominal pain or lethargy, if coagulation parameters are prolonged or transaminase activity remains high. Acute liver failure is a complication in less than 5% of icteric pediatric cases. The main differential diagnoses are metabolic liver disease (e.g. Wilson disease) or drug-induced liver disease.

## Chronic hepatitis

HBV and HCV are the most common causes of chronic viral hepatitis in childhood, but are unlikely to lead to serious liver disease until adolescence or adult life.

### Hepatitis B virus infection

Children are infected in childhood by vertical transmission from a carrier mother; horizontal transmission from parents and other family members; by infected blood products; sexual abuse; or, in adolescents, drug abuse. There is an increased risk of environmental transmission in residential institutions and hemodialysis centers. Perinatal transmission occurs mainly through placental tears, trauma during delivery, or contact of the infant mucous membrane with infected maternal fluid. Intrauterine transmission has been reported, but is not a major route of transmission. HBV carrier mothers, who are HBe antigen positive, have the highest infectivity with a 70–90% risk of transmission. Those mothers who are HBe antigen negative, but HBe antibody positive may also transmit infection, and their infants are particularly at risk of developing acute liver failure, secondary to mutant HBV. About 70% of infants infected perinatally will become chronic carriers unless immunized at birth.

The natural history of chronic HBV infection in childhood is not yet established. Children are usually asymptomatic without signs of chronic liver disease. Biochemical parameters indicate mild elevation of aminotransferases (80–150 IU/L) with normal albumin, coagulation and alkaline phosphatase. Liver histology indicates a chronic hepatitis in over 90% of the carriers, which may be mild or non-specific in 40% of children. Progression to cirrhosis is likely and has been reported in Mediterranean populations, although the exact incidence is unknown.

Children with chronic HBV are not only a continuing source of infection, but are at risk of developing cirrhosis and/or primary hepatocellular carcinoma. Chronic carriers should, therefore, remain under medical supervision so that the family may be supported and counseled, as well as to screen and immunize family members. In addition, the patient should be monitored for evidence of seroconversion or progressive liver disease and/or hepatocellular carcinoma.

Annual review should include HBV serology and viral markers of HBV DNA, standard liver tests,  $\alpha$ -fetoprotein and abdominal ultrasound.

### Management

The indications for treatment in childhood are persistently raised serum aminotransferases, presence of HBe antigen with detectable HBV DNA in serum, and features of chronic hepatitis on liver biopsy. Interferon- $\alpha$  (5–10 MU/m<sup>2</sup> thrice weekly) by subcutaneous injection for 6 months has a sustained clearance rate of 40–50% of those treated. Children who have active histology, low HBV DNA levels (<1000 pg/mL), high serum aminotransferase enzymes, and horizontal transmission are more likely to respond to interferon. Pretreatment with corticosteroids remains unproven in the short term, but may have a long-term effect on seroconversion over 3–4 years. Lamivudine has similar results in children as in adults with 26% seroconversion after 12 months treatment. The incidence of YMDD mutants is approximately 18% increasing with duration of therapy. Adefovir dipivoxil is currently under evaluation.



The most important strategy to prevent HBV transmission in childhood includes routine antenatal screening of all women during pregnancy, with immunization of at-risk infants, or universal immunization of all infants. The implementation of universal immunization continues to be controversial, although a number of countries in Europe and North America have adopted this policy.

### Hepatitis C virus infection

The importance of hepatitis C virus infection in children lies in its propensity to develop chronic liver disease. Children infected with HCV form three main groups: those who were parenterally infected prior to blood product and donor organ screening in 1990; children who have been vertically infected; and a group of children who have been sporadically infected but the route of acquisition remains obscure. In contrast to HBV infection, vertical transmission of HCV is unusual, ranging from 2 to 10% of offspring born to HCV-RNA-positive mothers. The risk of transmission is increased up to 48% by coexisting maternal HIV infection and in those who have high HCV RNA titers. Breast feeding is not contraindicated.

### Diagnosis

The diagnosis is made by screening children at risk. Serum aminotransferases are typically normal or very slightly elevated, HCV serology will indicate anti-HCV antibodies and the presence of HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR). Histology reveals classic features of chronic hepatitis C, which include mild portal tract inflammation, lymphoid aggregates, and mild periportal piecemeal necrosis with steatosis and apoptosis. Giant cell hepatitis in association with HCV infection has been described in neonates.

### Management

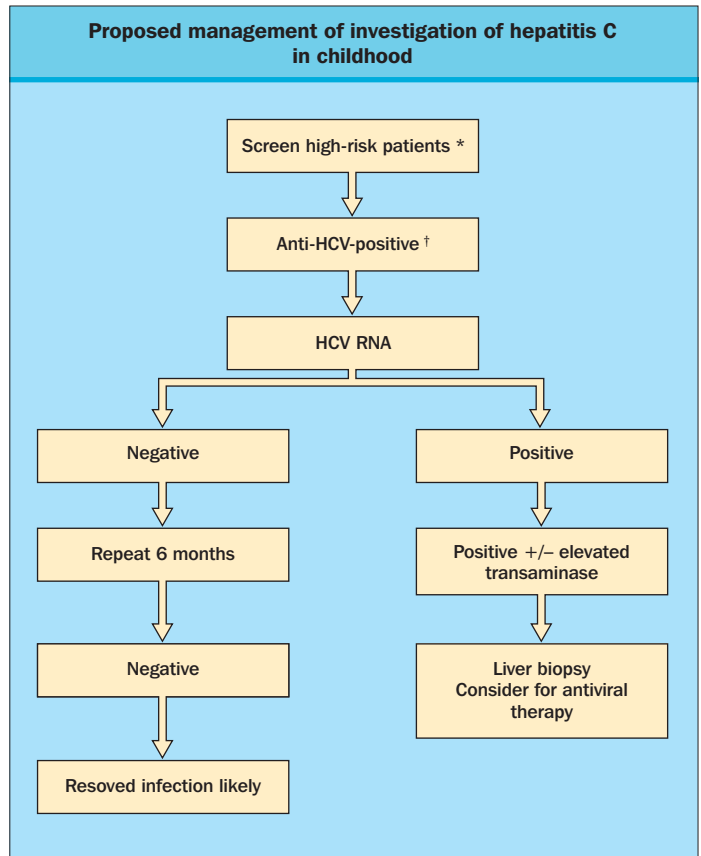
The first step is to establish the diagnosis by measuring not only anti-HCV antibody, but confirming that active infection is present by detecting HCV RNA by RT-PCR (Fig. 26.10).

The range of genotypes identified in childhood resembles that in adult populations and does not help in diagnosis, but may provide long-term data on epidemiology and prognosis. The natural history of chronic HCV in childhood is not known, but the spontaneous seroconversion rate is 20% in children who have received blood products, and slightly less in perinatally infected children.

Children who have persistent positivity of HCV RNA and evidence of liver disease should be selected for therapy. Meta-analysis of interferon- $\alpha$  therapy in adults has indicated that monotherapy with interferon is unlikely to produce sustained clearance of HCV RNA or biochemical remission in more than 25% of patients. A number of studies have confirmed similar results in children. The combination of interferon and ribavirin given for 12 months has an overall sustained viral response rate of 45%, with higher response rates (70%) in children with genotypes 2 and 3. Clinical trials with pegylated (PEG) interferon and ribavirin are in progress.

### Autoimmune hepatitis

Autoimmune hepatitis is a chronic inflammatory disorder affecting the liver, which is usually responsive to immunosuppressive drugs. It may affect children of any age from 6 months onwards,



**Figure 26.10 Prolonged management of investigation of hepatitis C in childhood.** \*High risk patients include recipients of multiple transfusions/pooled blood products, and infants of hepatitis C virus (HCV) positive mothers. †Anti-HCV by third generation assay. May be positive in infants of HCV positive mothers up to 9 months by passive transfer.

and there is a 3:1 female preponderance. Both forms of autoimmune hepatitis type I [antinuclear antibodies (ANA), and smooth muscle antibodies (SMA)] and type II [liver, kidney microsomal antibodies (LKM)] present in childhood.

### Clinical features

In type I autoimmune hepatitis, the median age of onset is 10 years and the clinical presentation varies from acute hepatitis with autoimmune features to the insidious development of cirrhosis, portal hypertension and malnutrition. The association of multiorgan disease is higher in type I hepatitis with autoimmune thyroiditis, celiac disease, inflammatory bowel disease, hemolytic anemia and glomerulonephritis being the most common (Fig. 26.11).

In type II autoimmune hepatitis, the age of onset is younger (median age 7.4 years); the clinical presentation is more likely to be severe with acute liver failure in 11%; and multiorgan disease is less common. The form LKM II may develop in association with hepatitis C or secondary to drugs such as antiepileptic drugs.

The diagnosis is made in the same way as in adults by the identification of:

- elevated serum aminotransferases;
- increased total globulin or IgG concentrations greater than 1.5 times above the normal limit;



**Figure 26.11 Boy with autoimmune hemolytic anemia.** Autoimmune liver disease has an insidious onset in childhood and may present with cirrhosis and portal hypertension. Type I autoimmune hepatitis may also present with multisystem involvement, as in this patient.

- seropositivity for ANA, SMA or LKM I antibodies with titers greater than 1:40 (up to 25% of children may not have detectable autoantibodies on presentation);
- characteristic histologic features of chronic hepatitis (Fig. 26.12); and
- exclusion of hepatitis C and Wilson disease.

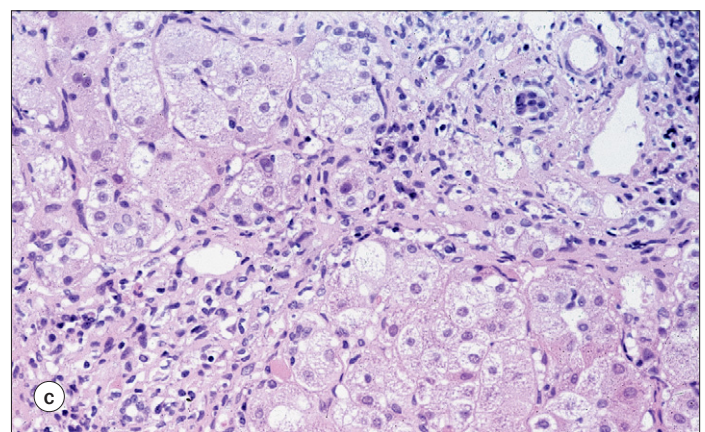
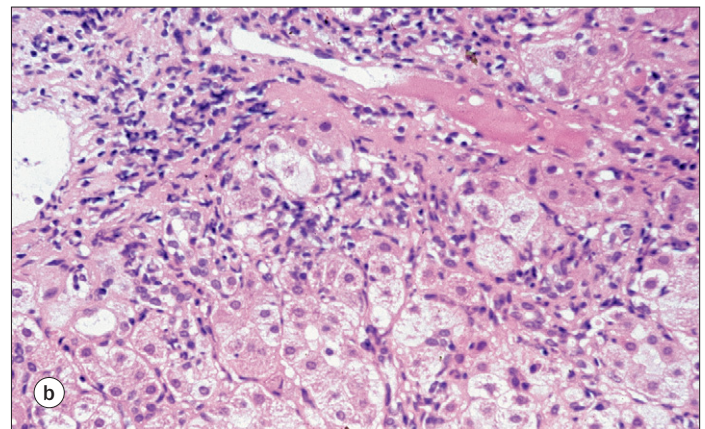
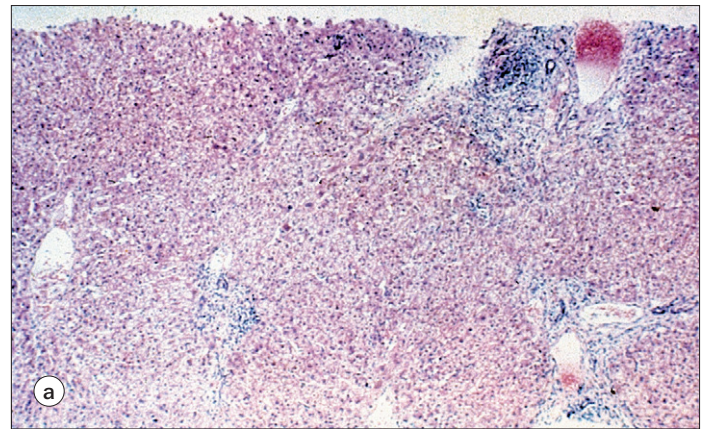
#### Management

Both forms of autoimmune hepatitis respond to immunosuppression with prednisolone 2 mg/kg/day (maximum 60 mg) in association with azathioprine 0.5–2 mg/kg/day. About 90% of children will respond to the above regimen, but cyclosporine (2–4 mg/kg/day), tacrolimus (1–2 mg/day) or mycophenolate mofetil (20 mg/kg/day) may be helpful in inducing or maintaining remission. There has been no established optimal duration of treatment. Discontinuation of corticosteroids and/or azathioprine may be considered if liver tests have been normal for at least 1 year, but up to 80% of children will relapse following discontinuation of treatment.

Despite a biochemical response to immunosuppression, histologic progression may develop over many years. Failure of medical treatment is more likely in patients presenting at an early age, those who have type II autoimmune hepatitis, coagulopathy, high bilirubin, and established cirrhosis at presentation. Indications for liver transplantation include a presentation with acute liver failure, progression to end-stage chronic liver disease, or intolerable side-effects or failure of medical treatment. Transplantation may not provide a complete cure as the recurrence rate is approximately 25%, although this is relatively easy to control with corticosteroids.

#### Sclerosing cholangitis

The spectrum of sclerosing cholangitis in childhood includes neonatal sclerosing cholangitis and autoimmune sclerosing cholangitis in association with type I autoimmune hepatitis or immunodeficiency (Table 26.8).



**Figure 26.12 Histology of chronic hepatitis.** The features of chronic hepatitis in childhood are very similar to those in adults, with an increase in inflammatory infiltrate in the portal tracts (a). This may extend beyond the limiting plate [piecemeal necrosis (b)] leading to bridging fibrosis (c). Differential diagnosis is between an autoimmune hepatitis, chronic viral hepatitis, or Wilson disease.

Clinical features include abdominal pain, weight loss, intermittent jaundice resembling autoimmune hepatitis; and cholestasis with cholangitis and pruritus or cirrhosis with portal hypertension. Laboratory investigation will indicate elevated alkaline phosphatase ( $>16 \times$  normal) and  $\gamma$ -GT ( $50\text{--}100 \times$  normal). Bilirubin levels may be normal or intermittently elevated in at least 50% of patients; serum aminotransferases are moderately elevated ( $3\text{--}5 \times$  normal); and prothrombin times and albumin levels are usually normal early in the disease.



<b>Diseases associated with sclerosing cholangitis in childhood</b>	
<i>Disease</i>	<i>(%)</i>
Autoimmune hepatitis 1	? 50
Inflammatory bowel disease	33
Histocytosis X	19
Immunodeficiency	12
Other	3
Neonatal onset	18
No associated disease	22

**Table 26.8 Diseases associated with sclerosing cholangitis in childhood.**

Elevated prothrombin time may be due to fat-soluble vitamin deficiency and is responsive to vitamin K. The diagnosis is confirmed by cholangiography [either operative, percutaneous, transhepatic, endoscopic or magnetic resonance imaging (MRI)], which reveals typical lesions of irregular intrahepatic ducts, focal saccular dilatations, short annular strictures, an abnormally large gallbladder, or extrahepatic ductular irregularities. Histology may demonstrate pathognomonic fibrous obliterative cholangitis with periductular fibrosis, but more often indicates features of chronic hepatitis with cholestasis.

### Management

Immunosuppression is only of benefit in sclerosing cholangitis associated with autoimmune hepatitis. Treatment of inflammatory bowel disease does not prevent progression of sclerosing cholangitis. Medical therapy is directed towards treatment of cholestasis with fat-soluble vitamin supplementation, nutritional support, and management of pruritus. Treatment with ursodeoxycholic acid (20 mg/kg/day) may be of benefit, although there are no control data available in children. Isolated biliary strictures may require radiologic or surgical intervention but surgical drainage procedures are not generally indicated and may increase the risk of ascending cholangitis and progression of liver disease.

The majority of children will progress to liver failure and develop the complications of portal hypertension. Median survival or time to transplantation is 10 years from the onset of disease. Indications for liver transplantation include progressive cholestasis and intractable pruritus or the development of cirrhosis and portal hypertension. Extrahepatic disease, such as colitis may become more severe following liver transplantation.

### Non-alcoholic steatohepatitis (NASH)

The increase in childhood obesity and the recognition of insulin resistance in a number of inherited disorders has led to the diagnosis of this disorder in childhood. As in adults, children may have simple steatosis or steatohepatitis which may progress to cirrhosis in childhood. The main difference is the frequent association with inherited syndromes associated with insulin resistance. NASH has also been reported after hypothalamic surgery, perhaps as a result of hyperphagia. The long-term outcome is not determined, but there may be a response to weight

reduction and exercise. Therapeutic trials with metformin and other drugs have not been evaluated in childhood.

### Drug-induced liver disease

The mechanisms leading to drug-induced liver damage are similar in adults and children, although there is less risk of adverse drug reactions in younger children compared with adults, which may reflect polypharmacy or concurrent disease. However, there is a specific increased risk of valproate hepatotoxicity in children less than 3 years of age (see page 416).

### Acetaminophen (paracetamol) poisoning

Acetaminophen toxicity is the most common cause of drug-induced acute liver failure. It leads to a direct dose-dependent hepatotoxic effect. Children have a lower incidence of liver failure with acetaminophen overdose than adults (unless taken with alcohol), perhaps because the rate of glutathione resynthesis is higher.

### Aspirin

Aspirin gives rise to dose-dependent hepatotoxicity that is mild, asymptomatic and reversible. Adverse effects are associated with levels exceeding 15 mg/dL in 90%, which may occur in children treated for juvenile chronic arthritis. Hepatic features include an asymptomatic elevation in aminotransferases with a normal bilirubin level, which usually occurs 6 days after initiation of treatment. In less than 5% of children, severe hepatocellular injury may ensue with prompt recovery on withdrawal of treatment.

Reye syndrome may be defined as an acute, non-inflammatory encephalopathy with hepatic injury occurring without a recognized cause. It has been associated in children with ingestion of aspirin in 90% of cases, especially in children who have intercurrent illnesses such as chickenpox or influenza. The pathogenesis is multifactorial and may reflect a genetic predisposition with a mitochondrial enzyme abnormality in which the viral infection or aspirin ingestion may have induced the hepatocellular insult. There is no relationship between salicylate level and severity of hepatic dysfunction. Reye syndrome in association with aspirin ingestion is now almost unknown due to the recommendation that aspirin should not be prescribed in children under the age of 12 years.

### Metabolic disease in older children

Metabolic disease in older children presents with hepatomegaly often in the absence of jaundice and with or without splenomegaly or neurologic involvement. The main causes of metabolic liver disease in older children include  $\alpha_1$ -antitrypsin deficiency, cystic fibrosis, Gaucher disease, tyrosinemia type I, glycogen storage diseases, hereditary fructose intolerance and Wilson disease.

### Glycogen storage disease

The hepatic glycogen storage disorders (GSD) are a group of inherited disorders affecting the metabolism of glycogen to glucose. Characteristic findings include hepatomegaly, growth failure and hypoglycemia. The diagnosis is based on demonstrating the respective enzyme deficiency. All are autosomal recessive, except for phosphorylase kinase deficiency which is X-linked.



**Glycogen storage disease type 1a**

Glucose-6-phosphatase is a microsomal enzyme found in hepatocytes, renal tubular epithelium, pancreatic and intestinal mucosa, and is essential for hepatic glucose export. Deficiency of the enzyme results in complete dependency on exogenous carbohydrate. The clinical and biochemical effects of the disease result from hypoglycemia and the body's response to hypoglycemia.

Infants usually present with hypoglycemic seizures, hepatomegaly and failure to thrive. Biochemical investigations reveal fasting hypoglycemia ( $<1.5$  g/L) with lactic acidosis ( $>5$  mmol/L), hyperlipidemia (cholesterol  $>6$  mmol/L and triglycerides  $>3$  mmol/L), and hyperuricemia. Hepatic aminotransferases are usually normal or mildly elevated. Liver histology reveals steatosis and glycogen storage with no fibrosis. Histochemical stains for glucose-6-phosphatase are negative and the enzyme will not be detected in the liver.

The initial aim of dietary treatment is to provide a continuous supply of exogenous glucose in order to maintain normal blood sugars and suppress counter-regulatory responses. This is best achieved in infants by frequent day-time feeding, use of oral uncooked corn starch which is hydrolyzed in the gut to release glucose slowly over hours, and continuous nocturnal enteral glucose feeds. If dietary control is strict in infancy, normal growth and development will take place, although hepatomegaly and hyperlipidemia persist. Long-term complications include osteoporosis, renal dysfunction and calculi, and hepatic adenomata which have the potential for malignant transformation. Liver transplantation will correct the hepatic metabolic defect, but is not indicated for metabolic control. The gene for glucose-6-phosphatase has been isolated and many mutations described. Antenatal diagnosis by chorionic villus sampling is possible if a known mutation has already been identified within the family.

**Glycogen storage disease type I non-a (previously known as 1b, 1c, 1d)**

In this disorder glucose-6-phosphatase is present but dysfunctional due to a defect in glucose-6-phosphate transport into the microsome. The clinical and biochemical features are similar to GSD type 1a. In GSD type 1b, neutropenia with recurrent infections from oral ulcers and inflammatory bowel disease has been reported. The gene defect in GSD I non-a has been characterized and antenatal diagnosis is possible.

**Glycogen storage disease type 3**

In this disorder there is deficiency in the debrancher enzyme or amylo-1-6-glucosidase deficiency. The metabolic defect is mild as other routes of gluconeogenesis are intact and there is no renal involvement. The defect is expressed in muscle in 85% of cases (type 3a). The clinical presentation is similar to GSD type 1, without renal involvement. In time a peripheral myopathy and cardiomyopathy may develop. As the abnormally structured residual glycogen is fibrogenic, hepatic fibrosis and cirrhosis are complicating features. Diagnosis is by identifying the deficient enzyme in leukocytes or liver tissue.

Dietary treatment is similar to that of GSD type I, but a higher protein intake is recommended due to the demand of gluconeogenic amino acids. Most metabolic abnormalities diminish at puberty and long-term outcome is determined by

the development of myopathy, cardiomyopathy or cirrhosis. Antenatal diagnosis is possible by enzyme measurement or mutation analysis on chorionic villi samples.

**Glycogen storage disease type 4**

This rare disease is due to a deficiency of the branching enzyme. It usually presents with evidence of severe liver disease in late infancy but there may be cardiac, muscle and neurologic involvement. Hepatic histology demonstrates cirrhosis and accumulation of abnormally shaped glycogen that is diastase resistant. Dietary treatment is as for other forms of GSD. There is rapid development of cirrhosis necessitating liver transplantation in the first 5 years of life. Progression of extrahepatic disease has been reported post-transplantation.

**Glycogen storage disease types 6 and 9**

These variants are due to defects in hepatic phosphorylase and phosphorylase kinase, respectively. The phenotype of both GSD types 6 and 9 is milder than in other forms of GSD. Children present with hepatomegaly and growth failure, but hypoglycemia is rare. Hyperlipidemia and ketosis may occur. Hepatic aminotransferases are often slightly raised, but progression to cirrhosis is unusual. Dietary treatment other than nocturnal corn starch is rarely necessary and spontaneous catch-up growth occurs before puberty. Neither cardiomyopathy nor myopathy has been recognized, and the long-term outlook is excellent.

**Hereditary fructose intolerance**

This autosomal recessive disorder is due to the absence or reduction of fructose-1-phosphate aldolase B in liver, kidneys and small intestine. The incidence has been estimated at 1:20 000 live births. The genetic mutation has been identified and is on chromosome 9. Clinical presentation is related to the introduction of fructose or sucrose in the diet. Vomiting is a prominent feature with failure to thrive, hepatomegaly and coagulopathy. Occasionally infants may present with acute liver failure with jaundice, encephalopathy and renal failure. Renal tubular acidosis and hypophosphatemic rickets occur (Fig. 26.13). Older children demonstrate aversion to fructose-containing food.



**Figure 26.13** Chest radiograph demonstrates an enlarged heart secondary to fluid overload, and severe rickets secondary to renal tubular acidosis. The most likely diagnosis is between tyrosinemia type I and hereditary fructosemia.

Biochemical liver tests indicate raised hepatic aminotransferases, hypoalbuminemia, and hyperbilirubinemia. Plasma amino acids may be elevated secondary to liver dysfunction and there may be hyperuricacidemia and hypoglycemia. Hematologic abnormalities such as anemia, acanthocytosis and thrombocytosis are associated. Urinary investigations will indicate fructosuria, proteinuria, amino aciduria, and organic aciduria in association with a reduction in the tubular re-absorption of phosphate.

Diagnosis is suggested by reducing substances in the urine and confirmed by a reduction or absence of enzymatic activity in liver or intestinal mucosal biopsy or by mutation analysis. Hepatic pathology varies from complete hepatic necrosis to diffuse steatosis and periportal intralobular fibrosis which may progress to cirrhosis if fructose is continued. Fructose elimination reverses hepatic and renal dysfunction. Antenatal diagnosis is possible by chorionic villi sampling.

### **Gaucher disease**

This autosomal recessive disorder is secondary to a deficiency of glucosylceramide- $\beta$ -glucosidase which is deficient in leukocytes, hepatocytes and amniocytes. It may present in infancy with acute liver failure, but is more usual in late childhood with hepatosplenomegaly, and respiratory, neurologic and bone disease. The diagnosis is suggested by the identification of large multinucleated Gaucher cells in bone marrow aspirate and liver and confirmed by enzyme assay. Hepatic fibrosis may be severe leading to cirrhosis. Recent therapy for Gaucher disease includes enzyme replacement, substrate deprivation for the non-neuronopathic form, bone marrow or liver transplantation.

### **Wilson disease**

Wilson disease is an autosomal recessive disorder with an incidence of 1:30 000 live births. The Wilson disease gene is on chromosome 13 and encodes a copper-binding ATPase.

### **Clinical features**

Clinical features in childhood include hepatic dysfunction (40%) and psychiatric symptoms (35%). Children under the age of 10 years usually present with hepatic symptoms. The hepatic presentation of Wilson disease resembles that of adults who have hepatomegaly, vague gastrointestinal symptoms, acute or subacute liver failure and chronic hepatitis or cirrhosis. Children may present with deteriorating school performance, abnormal behavior, lack of coordination and dysarthria. Renal tubular abnormalities, renal calculi, and acute hemolytic anemia are associated features. The characteristic Kayser-Fleischer rings are not usually detected before the age of 7 years and may be absent in up to 80% of older children.

### **Diagnosis**

Biochemical liver tests indicate chronic liver disease with low albumin [ $<3.5$  g/dL ( $<35$  g/L)], minimally elevated aminotransferases, and a low alkaline phosphatase ( $<200$  IU/L). There may be evidence of hemolysis on blood film. The diagnosis is established by detecting a low serum copper [ $<1$  mmol/dL ( $<10$  mmol/L)], a low serum ceruloplasmin [ $<20$  mg/dL ( $<200$  mg/L)], excess urine copper ( $>1$  mmol/24 h), particularly after penicillamine treatment (20 mg/kg/day), and an elevated hepatic copper ( $>250$  mg/g dry weight of liver). Approximately 25% of children may have a normal or borderline

ceruloplasmin as it is an acute phase protein. Radioactive copper studies are only indicated in children who have equivocal copper and/or ceruloplasmin values in whom liver biopsy is contraindicated.

Histologic features of Wilson disease depend on the clinical presentation. There may be microvesicular fatty infiltration of hepatocytes, chronic hepatitis (see Fig. 26.12), hepatocellular necrosis, multinucleated hepatocytes and Mallory hyaline, hepatic fibrosis and cirrhosis. In children who have acute liver failure the histologic features are those of severe hepatocellular necrosis with underlying cirrhosis.

### **Management**

Current management includes a low copper diet and penicillamine (20 mg/kg/day), which is effective if started before the development of significant hepatic fibrosis. If penicillamine toxicity is unacceptable, alternative therapy includes trientine (triethylene tetramine) 25 mg/kg/day, in addition to oral zinc. In asymptomatic children or in those who have minimal hepatic dysfunction, the outlook is excellent, although acute liver failure with hemolysis may occur if treatment is discontinued. Liver transplantation is essential for children who present with acute liver failure and in children with advanced cirrhosis and portal hypertension.

It is essential for the family to be screened in order to treat asymptomatic patients and to detect heterozygotes. Mutation analysis is now more reliable than measurement of serum copper and ceruloplasmin, although the basal 24-h urinary copper is still a sensitive test.

### **Cystic fibrosis liver disease**

The incidence of liver disease in children who have cystic fibrosis varies from 4.5 to 20%, depending on age and the definition of significant liver disease.

### **Pathophysiology**

The etiology of cystic fibrosis liver disease has only been partially explained. Despite major advances in the understanding of the genetic defects in cystic fibrosis, no definite genetic mutation has been associated with the development of liver disease. A low familial concordance of the development of liver disease within siblings suggests that environmental factors may be important. There is an increased frequency of the HLA antigens A2, C7, DR2 (DRW15) and DQW6, suggesting that genetic factors controlling the lymphocyte-mediated immune response might be implicated. The discovery of the cystic fibrosis transmembrane receptor in the apical membrane of biliary epithelial cells has been a major step in the understanding of the etiology.

Bile acid malabsorption is a constant finding in untreated children who have cystic fibrosis. In the duodenum the total concentration of bile salts is decreased, but increases in the glycine/taurine ratio, and the percentage of potentially toxic dihydroxy bile salts have been noted. The abnormally low duodenal pH secondary to pancreatic insufficiency further exacerbates bile acid malabsorption and impairs micellar formation. Supplementation with taurine improves the glycine/taurine ratio of bile salts in the serum but not in the duodenum. It is possible that the abnormalities in bile salt concentration and the increase in hydrophobic and toxic bile acids may play an important role in the production of viscous bile and/or biliary

sludge, which may lead to partial biliary obstruction and focal biliary fibrosis.

### Clinical features

Most children who have cystic fibrosis and liver disease are asymptomatic in the early stages. In infants, cholestatic neonatal hepatitis may be a presenting feature (see above), but more commonly the presentation is associated with asymptomatic hepatosplenomegaly or the complications of portal hypertension.

### Diagnosis

Early detection of liver disease using standard liver tests is unsatisfactory. In general, there will be transient abnormalities of alkaline phosphatase in up to 50% of patients and increases in  $\gamma$ -GT in 30% of males and 60% of females. Serum bilirubin levels and coagulation times remain normal until late in the disease. Ultrasonography detects increased echogenicity (41%) (but does not differentiate fatty infiltration from fibrosis) microgallbladder (10–40%) and gallstones (20–25%). Hepatobiliary scanning demonstrates pooling in intrahepatic bile ducts, which may be a normal finding, or the presence of biliary strictures.

Liver histology may indicate fatty infiltration, focal biliary cirrhosis and multilobular cirrhosis. Steatosis is the commonest finding at biopsy and includes a mixture of micro- and macro-vesicular fatty infiltration. Non-specific mild inflammation around the portal tracts is commonly found in association with chemical cholangitis (granular eosinophilic secretions in bile ducts in association with ductal proliferation of bile ducts). Fibrosis develops initially around the portal tracts and gradually extends between portal tracts until cirrhosis has developed. Cholestasis and bile plugs are rarely identified. Liver biopsy should be performed to establish the extent and severity of liver disease and is indicated when there is persistent transaminitis, hepatic echogenicity on ultrasound, hepatomegaly and/or splenomegaly, or evidence of hepatic dysfunction.

### Management

Treatment consists of nutritional support and the prevention and management of hepatic complications. Nutritional support is critically important in children who have cystic fibrosis, regardless of whether they have liver disease. If cystic fibrosis is complicated by clinically significant liver disease, then the following is recommended:

- increasing energy intake to 150% of average requirements by carbohydrate supplements, such as glucose polymer, or by increasing the percentage of fat;
- increasing the proportion of medium chain triglycerides to 50% of the fat content; and
- supplementation with fat soluble vitamins, including vitamin A (5–15 000 IU/day), vitamin E (100–500 mg/day), vitamin D (50 ng/kg), and vitamin K (1–10 mg/day).

The use of ursodeoxycholic acid in the management of cystic fibrosis liver disease is now accepted despite the absence of good clinical trials. There is clear evidence that treatment with ursodeoxycholic acid improves the biochemical indices of liver function, and is effective when prescribed before the development of significant liver disease.

The main hepatic complication of cystic fibrosis liver disease is the development of portal hypertension and the development

of esophageal varices, which should be diagnosed by endoscopy. Prophylactic sclerotherapy is not recommended, but injection sclerotherapy or banding is usually effective once variceal hemorrhage develops. In order to avoid repeated anesthetics for sclerotherapy or banding, inserting a transjugular intrahepatic portal systemic shunt (TIPSS), which can be performed in quite young children, may be preferable.

Current data indicate that cystic fibrosis liver disease is a progressive disease leading eventually to cirrhosis and portal hypertension in all cases. Indications for liver transplantation include the development of end-stage liver failure with jaundice, ascites and coagulopathy, which are late features in this disease. Liver transplantation is indicated in children who have cystic fibrosis liver disease before the development of significant pulmonary complication (<50% of normal function) in order to prevent the necessity for heart, lung and liver transplantation. The use of pulmonary DNase preoperatively is recommended. Perioperative antibiotics should be based on the sensitivity of colonized pulmonary bacteria. The outcome following liver transplantation is similar to that in children transplanted for other causes of liver disease and lung function may improve after transplantation.

### Hepatic tumors

Liver tumors are relatively rare in childhood and occur in the region of 0.5–2.5 per million population. Hepatocellular carcinoma in childhood occurs at an older age than hepatoblastoma in association with underlying cirrhosis secondary to hepatitis B, hepatitis C, or  $\alpha_1$ -antitrypsin deficiency. A rare fibrolamellar hepatocellular tumor is occasionally seen in childhood and occurs in non-cirrhotic livers. Hepatoblastoma is most commonly seen in children under the age of 18 months and is rare after the age of 5 years. There is a male predominance of 3 to 2. The commonest presenting feature is of an abdominal mass and distention, and rarely anorexia, weight loss, pain, vomiting and jaundice. Hepatoblastoma is sometimes associated with sexual precocity due to the release of human chorionic gonadotropic hormones ( $\beta$ -HCG). Osteoporosis may occur in up to 20% of cases leading to bone fractures and vertebral compression. Hepatoblastoma occurs with certain well-recognized associations, in particular familial adenomatous polyposis coli.

### Diagnosis

Laboratory investigations will demonstrate:

- a normocytic normochromic anemia in 50% of children;
- thrombocytosis (greater than  $1000 \times 10^9/L$ ) in 30% of children;
- normal liver tests in hepatoblastoma, but these may be abnormal in hepatocellular carcinoma due to underlying cirrhosis;
- $\alpha$ -fetoprotein may be a useful diagnostic and prognostic marker and is elevated in 90% of hepatoblastoma patients and 60% of hepatocellular carcinoma patients; and
- transcobalamin-I may be a useful marker in fibrolamellar hepatocellular carcinoma.

Abdominal ultrasound, computed tomography (CT) scanning or MRI imaging will determine the site and extent of the lesion, and establish the presence of any metastases while providing information as regards suitability for surgical resection. Vascular structures are best identified on angiography or MRI imaging and are an essential investigation before surgery. Chest radio-



graph and CT scanning are important baseline investigations to define the presence of pulmonary metastases. Liver biopsy is necessary for histologic confirmation and selection of chemotherapy, despite the risk of disseminating tumor.

Hepatoblastoma may be classified into four groups: fetal, embryonal, macrotrabecular and small-cell undifferentiated tumors. Microscopic features distinguishing hepatocellular carcinoma from hepatoblastoma are the presence of tumor cells that are larger than normal hepatocytes with frequent tumor giant cells, broad cellular trabeculi, nuclear pleomorphism, and the absence of hematopoiesis. The fibrolamellar variant of hepatocellular carcinoma consists of plump tumor cells with deeply eosinophilic cytoplasm and a marked fibrous stroma separating epithelial cells into trabeculi.

It is important to differentiate hepatoblastoma and hepatocellular carcinoma from other undifferentiated embryonal sarcoma and this is only possible on histologic criteria. Benign vascular tumors such as hemangioma or hemangioendotheliomas are usually differentiated by the radiologic appearance on CT scanning and histology. Focal nodular hyperplasia is a rare benign tumor of childhood which may be single or multiple.

The malignant tumors must also be differentiated from hepatic adenomas which present with a pattern similar to that seen in adults. They may occur at any age, including in neonates and have even been reported in the fetus in utero. There is no link between maternal contraceptive use and childhood adenoma. Adenomas have been reported in patients receiving anabolic steroids, GSD type I, familial diabetes mellitus, hemosiderosis, and galactosemia. Histologically, the tumors may be partially encapsulated, and consist of thick cords of benign hepatocytes lacking portal structures and bile duct. It may be particularly difficult to distinguish between adenoma and well-differentiated hepatocellular carcinoma.

### Management

About 90% of hepatoblastomas will respond to chemotherapy and surgery. A minority will require liver transplantation for unresectable tumors. Current chemotherapy includes a combination of cisplatin and doxorubicin (PLADO), which will reduce the tumor in the majority of patients. Of the total, 71% will be amenable to surgical resection with an 82% 5-year survival rate. Tumors which are multifocal, central in situation or involving the portal vein are not amenable to resection and these children should be considered for liver replacement. An increasing number of patients have been successfully transplanted with a 5-year survival rate of 80%.

Hepatocellular carcinomas are less responsive to treatment and only 50% will respond to PLADO chemotherapy. Surgical resection is also less successful. The recent addition of carboplatin to PLADO may improve the response rate in these tumors. Response to therapy may be monitored by serial measurement of  $\alpha$ -fetoprotein levels. Patients who have good responses to chemotherapy will have a rapid fall of serum  $\alpha$ -fetoprotein, but persistent elevation suggests residual disease or metastases.

## ACUTE LIVER FAILURE IN INFANCY AND CHILDHOOD

The standard definition of acute liver failure used in adults is hard to apply in pediatric practice for a number of reasons.

Acute liver failure in children	
<i>Etiology</i>	<i>Diagnostic investigations</i>
<b>Infection</b>	
Viral hepatitis A, B, C, undefined, EBV, CMV	Viral serology
<b>Poison/drugs</b>	
Acetaminophen (paracetamol)	Acetaminophen levels
Isoniazid, halothane	Halothane antibodies
Amanita phalloides	
<b>Autoimmune hepatitis</b>	
	Autoimmune screen
<b>Metabolic</b>	
Wilson disease	Serum copper, ceruloplasmin
Tyrosinemia	Urinary succinylacetone
Reye syndrome	Microvesicular fat in liver Urinary dicarboxylic acids

**Table 26.9 Acute liver failure in children.**

Firstly, many infants present with acute liver failure from an inborn error of metabolism implying pre-existing disease. Secondly, encephalopathy may be difficult to detect in infants and small children and be less severe than coagulopathy in the early stages. Thus, caution is required when defining acute liver failure in infancy. The etiology of acute liver failure includes many causes common in adult practice, in addition to the high incidence of acute liver failure associated with metabolic liver disease (Table 26.9).

### Acute liver failure in infancy

Acute liver failure in infancy usually presents with multisystem involvement. The diagnosis may initially be difficult as jaundice may be a late feature. Infants are usually small for gestational dates, with hypotonia, severe coagulopathy and encephalopathy. Neurologic problems such as nystagmus and convulsions may be secondary to cerebral disease or encephalopathy. Renal tubular acidosis is common. Investigations include a search for multi-organ disease.

### Galactosemia

This rare autosomal disorder is secondary to a deficiency of galactose-1-phosphase uridylyltransferase. Acute illness results from the accumulation of the substrate galactose-1-phosphate (gal-1-P) following the introduction of milk feeds. Infants present with collapse with sepsis, hypoglycemia and encephalopathy in the first few days of life or with progressive jaundice and liver failure. Cataracts are present. The disease may be complicated by Gram-negative sepsis, which stimulates a life-threatening severe bleeding diathesis.

The diagnosis is established by the detection of urinary reducing substances in the absence of glycosuria, and confirmed by reduced enzyme activity in erythrocytes. Hepatic pathology demonstrates fatty change, periportal bile duct proliferation,

and iron deposition with extra medullary hematopoiesis. If galactose ingestion persists, hepatic fibrosis and cirrhosis may develop or may even be present at birth.

Liver function improves following exclusion of galactose from the diet unless liver failure or cirrhosis has developed. Galactose elimination is life long, but efficacy may be limited by endogenous synthesis of gal-1-P. The long-term outcome is disappointing. Learning difficulties and growth disturbance are described and are more common in girls, 75% of whom also develop ovarian failure. Detection of galactosemia in a neonatal screening program will lead to early detection, except for infants who present with acute liver failure. Antenatal diagnosis is possible by chorionic villi sampling.

### **Neonatal hemochromatosis**

This presumed autosomal recessive disorder is the most common cause of acute liver failure in the neonate. It is characterized by the prenatal accumulation of intrahepatic iron, due either to a primary disorder of fetoplacental iron handling, or a secondary manifestation of fetal liver disease. Intrauterine growth retardation and premature delivery is common. Clinical features include hypoglycemia, jaundice and coagulopathy within the first 2 weeks, with a fatal outcome without treatment.

Biochemical liver tests demonstrate elevated bilirubin, and reduced aminotransferases and albumin. Serum iron binding capacity is low and hypersaturated (90–100%), with a grossly elevated ferritin level ( $>1000$  ng/L). Diagnostic liver biopsy is not feasible because of the coagulopathy, but extrahepatic siderosis is found in minor salivary glands obtained by lip biopsy. MRI may confirm excess hepatic or extrahepatic iron. Liver histology at autopsy demonstrates pericellular fibrosis, giant cell transformation, ductular proliferation and regenerative nodules. The distribution of siderosis is similar to adult hereditary hemochromatosis, with hepatocellular and extrahepatic parenchymal deposition, and sparing of the reticuloendothelial systems.

Medical management includes supportive therapy for acute liver failure and an 'antioxidant cocktail', which combines *N*-acetylcysteine (150 mg/kg/day), vitamin E (25 mg/kg/day), selenium (2–3 mg/kg/day), prostaglandin  $E_1$  (0.4–0.6 mg/kg/h), and desferrioxamine (30 mg/kg/day). Some children have responded to this regimen, but the majority require liver transplantation. Extrahepatic iron is mobilized following successful liver transplantation.

Currently early antenatal diagnosis is not possible, but the diagnosis may be suspected by the detection of non-specific abnormalities such as hydrops fetalis or intrauterine growth retardation. Prenatal iron accumulation may be detectable by MRI, but the sensitivity is unknown. A recent report of maternal autoimmune disease preceding this syndrome (presence of anti-Ro and anti-La antibodies) may be relevant to etiology. Administration of immunoglobulin to the mother from 20 weeks' gestation may reduce the severity of this disease in an affected infant.

### **Disorders of mitochondrial energy metabolism**

This group of disorders includes a wide range of clinical phenotypes with any mode of inheritance: autosomal recessive; autosomal dominant; or transmission through maternal DNA. A number of different defects involving the electron transport

chain have been described. The clinical features develop secondary to electron transport chain dysfunction, which results in cellular ATP deficiency and the generation of toxic free radicals. Clinical symptoms vary, depending on the nature of the primary defect, the tissue or organ distribution and abundance, and the importance of aerobic metabolism in the affected tissue. The constituent proteins of the electron transport chain are encoded in two genomes, either nuclear DNA or mitochondrial DNA (mtDNA) which is maternally inherited. In the context of liver failure, two entities are relevant: isolated deficiencies of the electron chain enzymes and mtDNA depletion syndromes.

### **Deficiencies of the electron transport chain enzyme**

The most common isolated defects are complexes 4 and 1, although multiple deficiencies have been reported. Infants present with multisystem involvement manifesting hypotonia, cardiomyopathy and proximal renal tubulopathy and a severe metabolic acidosis. Relevant diagnostic investigations include elevated blood lactate, lactate:pyruvate ratio  $>20$ , increased 3-OH-butyrate:acetoacetate ratio  $>2$ , or an increase in lactate, possible ketone bodies and, following a glucose load (2 g/kg  $\times$  50 g), the detection of specific organic acids such as urinary 3-methyl-glutaconic acid or other Krebs cycle intermediates. Coagulopathy is usually extreme, and may prevent liver or muscle biopsy, or cerebrospinal fluid (CSF) examination. The definitive diagnosis is based on demonstrating biochemical dysfunction of electron chain function in liver or muscle by histochemistry or enzyme analysis in fresh tissue. Demonstration of an elevated CSF lactate compared with plasma lactate indicates neurologic involvement.

Supportive management is usually the only option. Liver transplantation is only successful if the defect is confined to the liver, but is contraindicated if multisystem involvement is obvious as neurologic deterioration persists or may develop post-transplant. Antenatal diagnosis is rarely possible as the underlying gene defects are unknown.

### **Mitochondrial DNA depletion syndromes**

Mitochondria normally contain more than one copy of mtDNA and replication is regulated by a number of factors encoded by nuclear genes. Mutations in these nuclear genes lead to a reduction in copy numbers of mtDNA resulting in mitochondrial depletion. The clinical presentation and biochemical findings are similar to those of infants presenting with isolated electron transport chain deficiencies. In most patients tissue measurement of electron chain activities show deficiencies in complexes 1, 3 and 4, although activity may be within the normal range. The diagnosis is confirmed by demonstrating an abnormally low ratio for mtDNA:nuclear DNA in affected tissue. Treatment is supportive as liver transplantation is contraindicated. Antenatal diagnosis is not currently possible.

### **Tyrosinemia type I**

Tyrosinemia type I is an autosomal recessive disorder due to a defect of fumarylacetoacetase (FAA), which is the terminal enzyme in tyrosine degradation. The gene for FAA is on the short arm of chromosome 15 and many mutations have been described. Intermediate metabolites such as maleyl- and fumarylacetoacetate are highly reactive compounds that are locally toxic within the liver. The secondary metabolite



succinylacetone has local and systemic effects, including inhibition of porphobilinogen synthase, and is thought to be responsible for cardiac, renal and neurologic disease.

### Clinical features

This is heterogeneous, even within the same family. Acute liver failure is a common presentation in infants between 1 and 6 months of age who present with mild jaundice, coagulopathy, encephalopathy and ascites. Hypoglycemia is common, either due to liver dysfunction or hyperinsulinism from pancreatic islet cell hyperplasia. In older infants, failure to thrive, coagulopathy, hepatosplenomegaly, hypotonia and rickets are common (Fig. 26.13). Older children may present with chronic liver disease, a hypertrophic cardiomyopathy, renal failure or a porphyria-like syndrome with self-mutilation. Renal tubular dysfunction and hypophosphatemic rickets may occur at any age. There is a high risk of hepatocellular carcinoma.

### Diagnosis

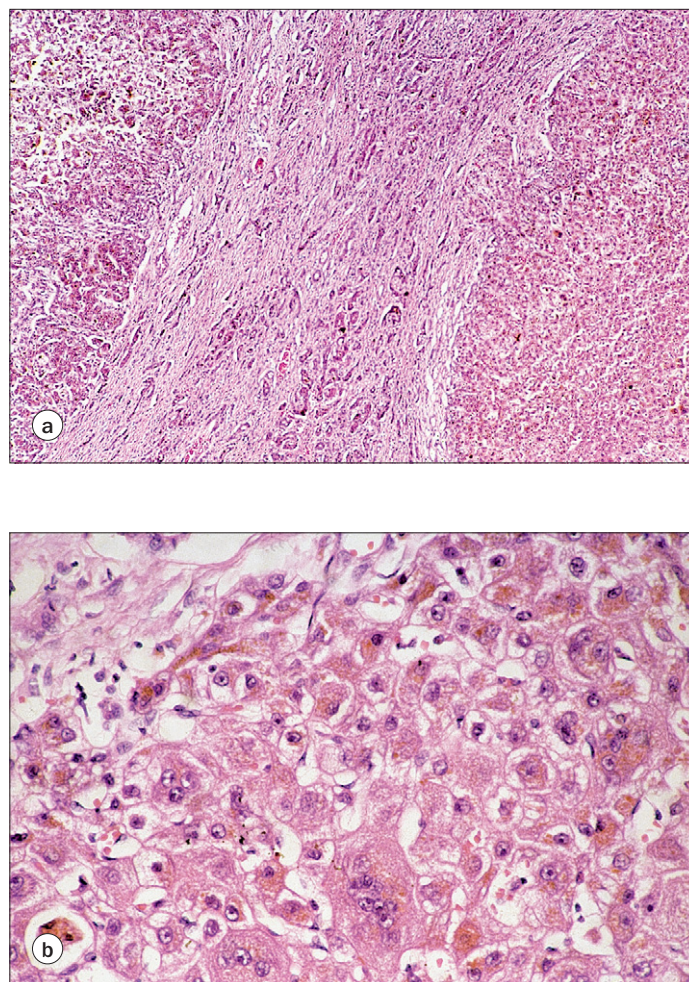
Biochemical liver tests reveal an elevated bilirubin, aminotransferases, alkaline phosphatase and a reduced albumin. Plasma amino acids indicate a three-fold increase in plasma tyrosine, phenylalanine and methionine with grossly elevated  $\alpha$ -fetoprotein levels. Urinary succinyl acetone is a pathognomonic but not an invariable finding. The diagnosis is confirmed by measuring FAA activity in fibroblasts or lymphocytes. Proximal tubular dysfunction may be suspected if there is phosphaturia and aminoaciduria, and confirmed by a reduction in renal tubular absorption of phosphate (<80%).

Echocardiography may reveal a hypertrophic cardiomyopathy, while radiologic examination may indicate severe hypophosphatemic rickets. Hepatic histology is non-specific with steatosis, siderosis and cirrhosis, which may be present in infancy (Fig. 26.14). Hepatocyte dysplasia is common and is associated with a risk of hepatocellular carcinoma.

### Management

Initial management is with a phenylalanine and tyrosine-restricted diet which may improve overall nutritional status and renal tubular function, but does not affect progression of liver disease. The recent discovery of 2(2-nitro-trifluoromethylbenzoyl)-1,3-cyclohexenedione (NTBC), which prevents the formation of toxic metabolites, has altered the natural history of this disease in childhood. Worldwide more than 100 children have been treated with NTBC. There is rapid reduction of toxic metabolites, normalization of tubular function, prevention of porphyria-like crises, and improvement in both nutritional status and liver function, particularly in those who have acute liver failure.

The long-term outcome of children who have tyrosinemia type I treated with NTBC is unknown. These children require long-term monitoring and follow-up with 6-monthly abdominal ultrasounds and CT scans, or MRI and  $\alpha$ -fetoprotein estimation for early detection of hepatocellular carcinoma (Fig. 26.15). The current indications for liver transplantation for this condition include the development of acute or chronic liver failure unresponsive to NTBC, or suspicion of development of hepatocellular carcinoma. Antenatal diagnosis is possible either by chorionic villi sampling which measures FAA directly, or from mutation analysis, or by measurement of succinyl acetone



**Figure 26.14 Histology of a liver biopsy.** (a) Indicating non-specific steatosis and fibrosis. (b) There is early hepatic dysplasia, which is a frequent finding in tyrosinemia type I. A focus of hepatocellular carcinoma was found elsewhere in the liver at transplantation.



**Figure 26.15 Computed tomography (CT) scan indicating multiple nodules in a patient who has tyrosinemia type I.** These nodules may represent regenerative nodules or foci of hepatocellular carcinoma.



in the amniotic fluid. Prospective affected siblings may benefit from early NTBC therapy.

#### **Familial hemophagocytic lymphohistocytosis**

This rare disorder may be inherited as an autosomal recessive condition, or be secondary to a viral illness. There is progressive visceral, neurologic and bone marrow infiltration with lymphocytes and large erythrophagocytic histiocytes. Children present with fever, hepatosplenomegaly, jaundice, skin rash, edema and encephalopathy in the first year of life. There is a pancytopenia, coagulopathy, and biochemical features of acute liver failure, hypofibrinogenemia, and hypotriglyceridemia. Diagnosis is established by identifying the characteristic erythrophagocytic histiocytes in bone marrow, liver and CSF. The disease is usually fatal, although treatment with antimetabolites and corticosteroids, or bone marrow transplantation may be helpful. Liver transplantation is contraindicated if there is extensive bone marrow or neurologic involvement.

#### **Acute liver failure in older children**

##### **Acute liver failure**

All forms of acute viral hepatitis may present in childhood and the presentation will be similar to that of acute liver failure in adults, except where specified below. In infants, infection with herpes simplex virus (HSV-1, HSV-2, HSV-6, varicella-zoster virus, or CMV) may cause particularly severe forms of hepatic failure, often in immunocompromised hosts. In older children, hepatitis A is the most common defined viral cause of acute liver failure. However, the commonest cause of acute liver failure, as in adults, is sporadic non-A-E or seronegative hepatitis, which accounts for almost two thirds of children with acute liver failure. As in adults, this carries a particularly poor prognosis.

#### **Drugs and toxins**

Liver injury due to drugs and toxins is the second most common cause of acute liver failure in older children. Sodium valproate has been associated with more than 150 cases of fatal hepatotoxicity worldwide, although the mechanism is unclear. Valproate has a complex metabolic fate undergoing partial mitochondrial  $\beta$ -oxidation, forming acyl compounds with coenzyme A and carnitine and inhibiting cellular carnitine uptake. Hepatotoxicity has occurred in patients who have abnormalities of fatty acid oxidation, mitochondrial energy metabolism, the urea cycle, and those who have presumed metabolic disorders such as Alpers syndrome, suggesting that the normal response to valproate may precipitate hepatotoxicity in those who have abnormal intermediary metabolism.

Hepatotoxicity may occur at any age, but is more likely in children aged <2 years, those on multiple epileptic therapy, and those who have previous neurologic abnormalities or developmental delay. Clinical features usually develop within 6 months of starting therapy and include nausea, vomiting, increasing seizure frequency, jaundice, edema, and hypoglycemia leading to drowsiness and coma. Biochemical investigations reveal moderate increases in hepatic aminotransferases and bilirubin, and severe coagulopathy. Hepatic histology demonstrates severe microvesicular fatty change with hepatocellular necrosis and occasionally cirrhosis.

Once liver disease is established, the outlook is poor unless valproate has been promptly discontinued. Carnitine is not effec-

tive in preventing or treating hepatotoxicity, but *N*-acetylcysteine may have a hepatoprotective role. Liver transplantation may be contraindicated as neurologic disease may progress.

#### **Progressive neuronal degeneration of childhood (Alpers disease)**

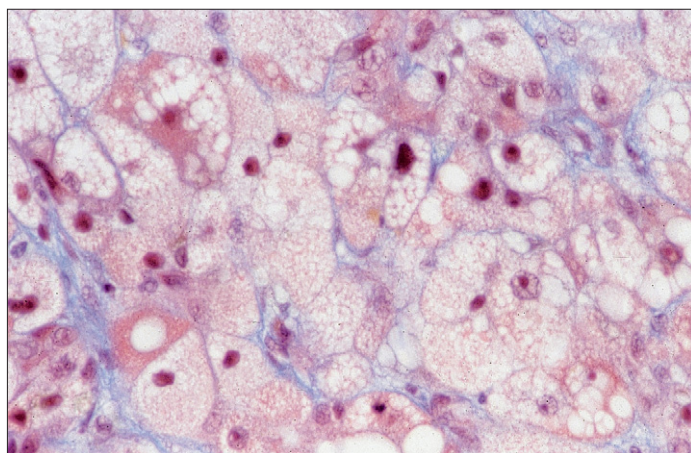
The etiology of this familial disorder is unknown. It is thought to be autosomal recessive, and in some cases an electron chain transport defect has been identified. Despite a normal neonatal period, there may be both physical and developmental delay followed by the sudden onset of intractable seizures between the ages of 1 and 3 years. Although biochemical evidence of liver dysfunction is often present at this stage, clinical liver involvement is a preterminal event. Hepatic disease presents as jaundice, hepatomegaly, and coagulopathy with rapidly progressive liver failure.

There are no specific biochemical features. Electroencephalogram demonstrates high amplitude polyspikes and CT or MRI scans show areas of low density in the occipital and posterior temporal areas. There is a gradual extinction of visual evoked responses. Liver histology characteristically shows microvesicular fatty change, bile duct proliferation, focal necrosis leading to bridging fibrosis and cirrhosis (Fig. 26.16). Neuropathology reveals cortical involvement with neuronal cell loss and astrocyte replacement.

The condition is uniformly fatal, with most children dying before the age of 3 years and within a few months of developing overt liver disease. It is important to avoid the use of valproate as it is likely to accelerate the development of liver disease. Liver transplantation is contraindicated, as neurologic progression continues after transplant. Antenatal diagnosis is currently impossible.

#### **Immune and metabolic mechanisms**

Autoimmune hepatitis type II [associated with liver, kidney and microsomal antibodies (LKM)] is a relatively common cause of acute liver failure in childhood. The diagnosis is established by identifying elevated serum immunoglobulins and LKM antibodies (see Table 26.7). A minority of patients will respond to immunosuppressant therapy in this situation but most will require liver transplantation. Acute liver failure has also been



**Figure 26.16 Histology of liver biopsy taken at autopsy.** It indicates microvesicular fatty infiltration in hepatocytes, which is characteristic of Reye syndrome, Alpers disease or mitochondrial disorders.

recorded in association with juvenile rheumatoid arthritis, despite immunosuppressive agents being more effective in this setting. Although most inborn errors of metabolism present early in infancy, Wilson disease, tyrosinemia type I or Alpers disease present in older children.

### **Clinical manifestations of acute liver failure**

The onset of liver disease varies according to etiology. There may be a prodromal illness with lethargy, fatigue, malaise, vomiting, diarrhea and jaundice with the subsequent development of coagulopathy and encephalopathy. Encephalopathy is difficult to detect in infants and may present with drowsiness, irritability or day/night reversal of sleep rhythm. Older children become aggressive, which is misinterpreted as antisocial behavior. The later stages of encephalopathy and hepatic coma are similar in older children and adults. A poor prognosis is indicated if any of the following features are present:

- rise in bilirubin [ $>18$  mg/dl ( $>300$   $\mu$ mol/L)];
- a fall in aminotransferases without clinical improvement and increasing coagulopathy;
- prothrombin time  $>60$  s;
- metabolic acidosis (pH  $<7.3$ );
- hypoglycemia (glucose less than 4 mmol/L);
- a decrease in liver size (not usual in metabolic liver disease); and
- increasing hepatic coma grade to II or III.

Supportive management for acute liver failure in childhood includes:

- maintaining blood glucose levels greater than 4 mmol/L with 10–50% dextrose;
- fluid restriction (50–75% of standard maintenance) using colloid to maintain circulating volume;
- prevention of gastrointestinal hemorrhage from stress erosions using  $H_2$  receptor antagonists;
- prevention of sepsis with broad-spectrum antibiotics;
- prophylactic antifungal therapy; and
- treatment of coagulopathy if required with fresh frozen plasma and intravenous vitamin K (2–10 mg).

Hepatic encephalopathy is managed in the early stages with reduction of protein intake to 1–2 g/kg and provision of high calorie feeds using glucose polymer (8–10 g/kg) and oral lactulose. Increasing encephalopathy unresponsive to conservative management requires elective ventilation. The development of cerebral edema, detected by clinical signs or on CT scan, is an ominous sign. Conservative management includes fluid restriction ( $<50\%$  of maintenance), mannitol, and elective hyperventilation. The role of intracranial pressure monitoring is controversial. Electrodes may be difficult to insert in children who have severe coagulopathy, particularly as the blood product replacement may exacerbate cerebral edema. Intracranial pressure monitoring may improve selection for transplantation but does not affect overall survival.

Liver transplantation should be carefully considered in all children who have acute liver failure, but difficulties arise in the selection of infants who have inborn errors of metabolism, because of the difficulty in excluding multisystem disease. Liver transplantation is indicated in those children who have poor prognostic factors, namely seronegative hepatitis, rapid onset of coma grade III or IV, or severe coagulopathy (may be more severe than coagulopathy in metabolic disease). There should be

no evidence of irreversible brain damage on CT scans or evidence of multisystem disease (normal muscle biopsy, normal electrocardiogram, etc).

The mortality rate is greater than 70% for children who are in grade III or IV coma, or who have persistently abnormal coagulation (prothrombin time  $>60$  s). Prognosis may be better in children who have hepatitis A or who have taken an acetaminophen overdose. The causes of death in children not transplanted were sepsis (15%), hemorrhage (50%), renal failure (30%), and cerebral edema (56%). There is a 50–70% 1-year survival rate following liver transplantation, which is less than transplantation for children who have other indications. Survivors with liver transplantation face the psychologic sequelae of such a procedure and the long-term complications of this operation.

Of the hepatic support systems under evaluation, only exchange blood transfusion, plasmapheresis and possibly MARS, have had any value in pediatric practice in reducing coagulopathy and prolonging life until a donor liver was obtained.

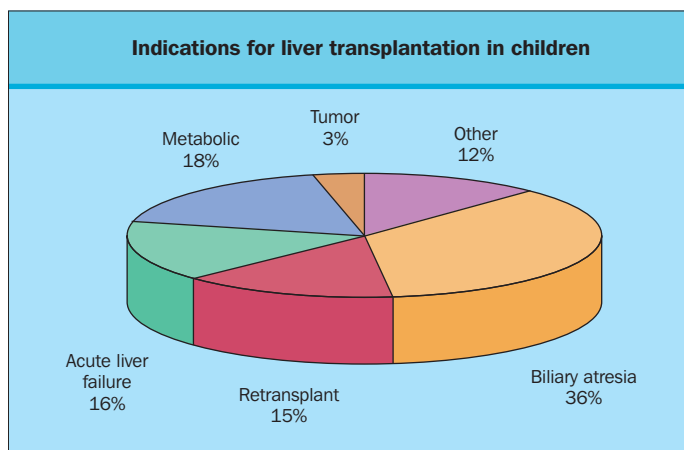
## **LIVER TRANSPLANTATION**

The success of pediatric liver transplantation has revolutionized the prognosis for many infants and children who would otherwise die of liver failure. The main factors in improving survival in this age group include advances in preoperative management such as the treatment of hepatic complications, nutritional support and selection for transplantation. The rapid developments in innovative surgical techniques to expand the donor pool have extended liver transplantation to the neonatal age group, while improvements in postoperative management including immunosuppression have led not only to increased survival, but also improved quality of life. The range of indications for liver transplantation in children now includes semi-elective liver replacement and transplantation for inborn errors of metabolism with extrahepatic disease and unresectable hepatic tumors.

### **Indications**

As in adults, most children are transplanted for chronic liver failure. Biliary atresia is the most common indication for children transplanted under the age of 2 years (Fig. 26.17).

As many children who have cirrhosis and portal hypertension have well-compensated hepatic function, the timing of liver



**Figure 26.17** Indications for liver transplantation in children. Birmingham Program, 1988–1998.

transplantation may be difficult to decide. In general, the most useful guide is a serial estimation of hepatic function, such as:

- persistent rise in total bilirubin [6 mg/dL ( $>100$  mmol/L)];
- prolongation of prothrombin time [international normalized ratio (INR)  $>1.4$ ];
- progressive fall in serum albumin [ $<3.5$  g/dL ( $<35$  g/L)];
- the development of malnutrition resistant to nutritional management (deterioration in measurements of triceps skin-folds, midarm muscle area, negative growth velocity); and
- hepatic complications such as chronic hepatic encephalopathy, refractory ascites, intractable pruritus, or recurrent variceal bleeding unresponsive to optimal medical management.

Children who have chronic liver disease have a significant reduction in developmental motor skills which may be reversed following liver transplantation. Thus, these children should be transplanted before the complications of their liver disease impair the quality of their lives and before growth and development are irreversibly delayed.

### Acute liver failure

The indications for liver transplantation for children who have acute liver failure depend on the etiology and the extent of the multisystem disease. In general children who have acute liver failure should be referred early to a specialist unit in the field of transplantation in order to provide time for stabilization and consideration for liver transplantation.

Children who have a poor prognosis and, therefore should have early listing for liver transplantation, include those who have:

- seronegative hepatitis;
- rapid onset of coma with progression to grade III or IV hepatic coma;
- diminishing liver size; and
- a fall in serum aminotransferases associated with increasing bilirubin [ $>18$  mg/dL ( $>300$   $\mu$ mol/L)] and persistent coagulopathy ( $>50$  s).

Liver transplantation is contraindicated for children who have evidence of multisystem involvement (e.g. mitochondrial disease) or irreversible brain damage from cerebral edema or hypoglycemia.

### Inborn errors of metabolism

Liver transplantation is indicated for inborn errors of metabolism if the hepatic enzyme deficiency leads to irreversible liver disease or liver failure and/or hepatoma (e.g. tyrosinemia type I, Wilson disease) or severe extrahepatic disease. Selection of patients who have severe extrahepatic disease is difficult, as it is necessary to evaluate the quality of life of the child on medical management and to compare the potential mortality and morbidity of their original disease with the risk of complications following liver transplantation. The timing of transplantation in these disorders depends on the rate of progression of the disease, the quality of life of the affected child on conservative management, and the development of severe reversible extrahepatic disease.

### Malignant disease

Indications for transplantation for liver tumors include either unresectable benign tumors causing hepatic dysfunction or unresectable malignant tumors refractory to chemotherapy without evidence of extrahepatic metastases.

### Evaluation

The aim of the evaluation process is to:

- assess the severity of liver disease and the extent of hepatic complications;
  - consider the technical aspects of the operation with regard to vascular anatomy and size;
  - exclude any significant contraindications to successful transplantation; and
  - prepare the child and family psychologically.
- The histologic diagnosis of the original disease should be reviewed and the severity and extent of hepatic function determined by evaluating:
- albumin [3.5 g/dL ( $<35$  g/L)];
  - coagulation time (INR  $>1.4$ );
  - bilirubin – rise in bilirubin [9 mg/dL ( $>150$   $\mu$ mol/L)] is usual in cholestatic patients, but may be a late feature in other diseases such as cystic fibrosis; and
  - the extent of portal hypertension - this should be estimated by visualizing esophageal and gastric varices by gastrointestinal endoscopy.

It is normal to establish baseline renal function, hematologic parameters, and background serology, which includes CMV status. The most important technical information required is the vascular anatomy and patency of the hepatic vessels. Most of this information may be obtained by color-fluid Doppler ultrasound, and examination of the liver and spleen. Occasionally MRI or angiography may be required to clarify abnormal anatomy in the hypovascular syndrome or to determine the extent of portal vein thrombosis. Liver transplantation causes important hemodynamic changes during the operative and anhepatic phases, and thus baseline information on cardiac and respiratory function is essential and may be obtained from an electrocardiogram, echocardiogram or oxygen saturation study.

One of the main aims of liver transplantation is to improve quality of life post-transplant, and thus it is essential to exclude any neurologic or psychologic defects that may be irreversible after transplantation. The psychologic and developmental assessment of children may be performed using the standard tests, such as the Griffith developmental scale (children under the age of 5 years), the Bailey developmental scales, or Stanford B and A intelligence scales for children of all ages.

Chronic liver disease has an adverse effect on the dentition of young children, which includes hypoplasia, staining of the teeth and gingival hyperplasia. As gingival hyperplasia may be a significant problem post-transplant, good methods of dental hygiene should be established before transplantation.

### Contraindications to transplantation

As medical and surgical expertise is improved, there are fewer contraindications to pediatric liver transplantation based on technical restrictions of age and size. Increased experience has indicated that certain medical conditions are not curable by transplantation and these include:

- the presence of severe systemic sepsis, particularly fungal sepsis;
- malignant hepatic tumors with extrahepatic spread;
- severe extrahepatic disease which is not reversible post-transplant, including severe cardiopulmonary disease (not amenable to corrective surgery), or severe structural brain damage; and
- mitochondrial disease with multisystem involvement.



### Preparation for transplantation

Most live vaccines are contraindicated post-transplant, and thus it is essential to ensure that routine immunizations are complete, namely diphtheria, pertussis, tetanus and polio, pneumovax as protection from streptococcal pneumonia, and *Hemophilus influenzae* type b vaccine. In children older than 6–9 months, measles, mumps, rubella and varicella vaccination should be offered. Hepatitis A and B vaccinations may also be prescribed pretransplant.

The treatment of specific hepatic complications is an important part of preoperative management. Acute variceal bleeding should be managed in a standard way with resuscitation, endoscopic sclerotherapy or esophageal banding, vasopressin or octreotide infusions. Esophageal banding is preferable to injection of sclerotherapy, if technically possible, as the inevitable development of postsclerotherapy variceal ulcers may be further adversely affected by post-transplant immunosuppression. In children who have uncontrolled variceal bleeding, the insertion of a transjugular intrahepatic portal systemic (TIPS) shunt has proved an effective management strategy, even in quite small children. Sepsis, particularly cholangitis and spontaneous bacterial peritonitis, should be appropriately treated with broad-spectrum antibiotics. In children who have acute liver failure, antifungal therapy should be started while awaiting liver transplantation.

Fluid retention leading to ascites and cardiac failure is inevitable in most children who have liver failure. It is managed with a combination of salt and fluid restriction (to two-thirds of maintenance fluids), diuretics and albumin infusions. Hemodialysis or hemofiltration are rarely required for children who have chronic liver failure, but may be essential in managing fluid retention and cerebral edema in children who have acute liver failure.

The development of effective nutritional strategies has been an important advance in the preoperative management of children who have chronic liver failure and may have improved morbidity and mortality post-transplant. A high calorie protein feed should provide between 150 and 200% of average energy requirements. Problems arise providing these high energy feeds in fluid-restricted patients and therefore modular feeds provided by a nocturnal nasogastric enteral feeding or continuous feeding may be useful. Parenteral nutrition may be required if enteral feeding is not tolerated due to ascites and variceal bleeding.

### Psychologic preparation

One of the most important aspects of the transplant assessment is the psychologic counseling and preparation of both child and family. A skilled multidisciplinary team, which includes a play therapist and psychologist, is essential to the success of this preparation and may be successfully achieved through innovative play therapy and toys and books suitable for children. Particularly careful counseling is required for parents of children referred for liver transplantation because of an inborn error of metabolism, which has not led to liver failure. These parents may find it more difficult to accept the risks and complications of the operation, the potential mortality, and the necessity for long-term immunosuppression. Parents of children who require transplantation for acute liver failure may be too distressed to appreciate fully the significance and implications of liver transplantation and may require ongoing counseling and education postoperatively.

### Innovative surgical techniques

The traditional form of operation, whole-graft orthotopic liver transplantation, is now a rare occurrence in pediatric liver transplantation due to the shortage of size-matched organs for young children. This scarcity of size-matched donors led to a high waiting list mortality but the development of reduction hepatectomy, in which left lateral segments of a larger liver are cut down to fit a child, have reduced the waiting list deaths from 15% to less than 5%, and extended the range of liver transplantation to young infants under the age of 1 year. Although morbidity may be higher in children receiving reduction hepatectomy, there is no long-term difference in outcome or survival compared with orthotopic liver transplantation. Another innovation has been the development of split-liver transplantation in which a single donor liver is offered to two recipients. Recent results, which involve in situ liver splitting has improved both graft and patient survival. Of particular relevance for pediatric liver transplantation is the development of living related transplantation. There are a number of ethical problems associated with this procedure that are related to the donor morbidity and potential mortality. The main advantages are related to the greater flexibility in timing the transplant, the use of 'good quality grafts', and the reduction in pressure on the organ donor pool.

Recent results demonstrate that there is 91% survival in children receiving living related grafts transplanted electively, but survival in children who have acute liver failure is only 57%, which is comparable with the results using cadaver donors. The choice of living related transplantation in the management of children who have acute liver failure remains controversial, as not only is graft survival less likely, but it is difficult for parents to make clear decisions in this emotional situation.

Auxiliary liver transplantation is a particularly attractive technique in which part of the donor liver is inserted beside or in continuity with the native liver, and it has been used in metabolic disease and in acute liver failure. In the former, it is retained in case of graft failure or for future gene therapy, and it is particularly appropriate for children who have a functionally normal liver but severe extrahepatic disease. Auxiliary liver transplantation is now the operation of choice for children who have Crigler-Najjar type I and has proved successful in reducing the levels of unconjugated bilirubin and improving quality of life. Its role in other metabolic diseases, such as organic acidemias, has yet to be established. In the context of acute liver failure, auxiliary transplantation is targeted at patients who have the capacity for recovery in the native liver (see Ch. 33).

Hepatocyte transplantation with hepatocyte transfusion through the portal vein may be of value in metabolic disease or acute liver failure, but is still at an early stage of development.

### Postoperative management

The immediate postoperative period is concerned with monitoring graft function, initiating immunosuppression and preventing or managing complications. The majority of children remain in the intensive therapy unit for 24–48 h, unless there are complications of graft function or cardiac difficulties. Graft function is evaluated in the standard way by measuring acid–base status, blood glucose levels, coagulation times, serum bilirubin, amino-transferases and alkaline phosphatase. There is considerable variation in the details of the immunosuppression regimens in

use, but, as in adults, calcineurin inhibitor and corticosteroid-based regimens dominate with anti-interleukin-2 antibodies, mycophenolate and sirolimus being used in selected cases.

Prophylactic antibiotics may be prescribed for 48 h unless there is continuing infection. The incidence of stress ulcers and excess gastric acid secretion is particularly high in children recovering from liver transplantation and warrants appropriate prophylaxis. Antiplatelet drugs, such as aspirin and dipyridamole, are often prescribed to prevent vascular thrombosis that is particularly high in children, but these are typically discontinued at 3 months.

### Early complications after liver transplantation

- Early graft failure is a fairly rare occurrence but may be secondary to primary non-function (within 48 h), hyperacute rejection (up to 4 days), or hepatic artery thrombosis (0–10 days). The only successful treatment is retransplantation, but the mortality of retransplantation is up to 50%.
- Hepatic artery thrombosis is more common in children than in adults because of the small size of the vessels and occurs in 10% of children. The incidence has fallen following the introduction of reduction hepatectomy with the use of larger donor blood vessels.
- Oliguria may develop in association with poor graft function, hypovolemia, or immunosuppressive therapy. Most children improve with conservative management and less than 10% require dialysis for renal failure unless transplanted for acute liver failure.
- Hypertension from cyclosporine, tacrolimus or prednisolone therapy is common and responds to fluid restriction and standard antihypertensive therapy.
- Acute cellular rejection responsive to increased immunosuppression occurs in 50–80% of children at 7–10 days. It is less common in infants (20%), possibly as a result of immune tolerance. Most acute rejection episodes respond to corticosteroids. Tacrolimus is more effective in preventing steroid resistant rejection without an increase in toxicity as compared to cyclosporine in children.
- Chronic rejection is less common, but may occur at any time post-transplant. There may be a response to an increase in immunosuppression, addition of other drugs (such as mycophenolate mofetil or sirolimus) but many children require retransplantation.
- Biliary complications occur in 18–20% of children and are more common in those receiving reduction hepatectomies than in those receiving full liver grafts. Biliary strictures may be secondary to anastomotic stricture, edema of the bile ducts or hepatic artery ischemia, whereas biliary leaks may be secondary to leakage from the cut surface of the liver in reduction hepatectomy or more commonly from hepatic artery ischemia. Most biliary leaks will settle with conservative management, but large leaks leading to biliary peritonitis, biliary abscess or sepsis will require surgical drainage and reconstruction. Many biliary strictures are amenable to endoscopic or percutaneous dilatation and placement of a biliary stent. Long-term ursodeoxycholic acid may be useful in these patients.
- Bacterial sepsis remains the commonest complication following liver transplantation and may be related to central line insertion.

- Fungal infections (*Candida albicans* or aspergillosis) have been documented in up to 20% of patients, particularly those receiving liver transplantation for acute liver failure.

### Late complications after liver transplant

#### Cytomegalovirus and Epstein-Barr virus infections

CMV and EBV infections are common as the majority of children undergoing liver transplantation are negative for both CMV and EBV, while the majority of donor livers (particularly reduction hepatectomies from adults) are likely to be CMV and/or EBV positive. The risk of CMV disease is directly related to receiving a CMV-positive donor, but may be treated effectively with ganciclovir, valganciclovir or hyperimmune CMV globulin.

The development of primary EBV infections is a significant long-term problem. As many as 65% of children undergoing liver transplantation will be EBV-negative, and 75% of this group will have a primary infection within 6 months of transplantation. It is important to diagnose primary EBV infection as early as possible [EBV early capsid antigen and polymerase chain reaction (PCR)], so that immunosuppression may be reduced to prevent further progression to lymphoproliferative disease.

#### Lymphoproliferative disease

There is a close relationship between primary EBV infection and the development of lymphoproliferative disease, ranging from benign hyperplasia to malignant lymphoma. In the majority of children the clinical features mimic infectious mononucleosis, but a minority develops isolated lymphoid involvement or malignant lymphoma. The diagnosis is based on histology from the affected tissue, which may also demonstrate polymorphic B-cell proliferation or lymphomatous features. It may be necessary to differentiate monoclonal from polyclonal infiltrates using immunofluorescence staining for heavy and light chain immunoglobulins. Although almost every organ in the body may be affected, the liver and gut are most usually involved. It is clear that lymphoproliferative disease is more likely in the presence of intense immunosuppression, particularly with OKT3 or multiple agents. Treatment includes reduction of immunosuppression, ganciclovir or the infusion of cytotoxic T cells programmed against EBV. Chemotherapy with standard lymphoma regimens is used when the lymphoproliferative disease becomes overtly malignant.

#### Side-effects of immunosuppression

These are numerous and in the main are similar to those seen in adults. The major difference in pediatric practice is the effect of corticosteroids on growth and ultimate height. Hirsutism and gingival hyperplasia, which are well-known side-effects of cyclosporine, have an important effect on quality of life, particularly in adolescents. The prevention of nephrotoxicity, which is common to both cyclosporine and tacrolimus, needs careful monitoring of immunosuppressive levels to minimize this long-term effect.

#### Late technical problems

These include biliary strictures and hepatic artery or portal vein thrombosis; management is the same as in adult patients.

**Growth failure**

Growth failure affects approximately 20% of children after liver transplantation. The most important factors related to growth failure are:

- excessive corticosteroid dosage;
- recurrent hepatic complications and cholestasis;
- intercurrent illnesses, such as EBV and CMV; and
- behavioral problems which interfere with calorie intake.

Successful management of growth failure involves a skilled multidisciplinary team, including a dietitian and psychologist, ensuring adequate calorie intake (by nasogastric tube, if necessary) and early reduction or discontinuation of corticosteroid therapy.

**Recurrence of disease**

This is an increasingly important problem. As with adults, hepatitis B and C can recur, but the scale of the problem is much less. Recent data indicates that up to 25% of children transplanted for autoimmune hepatitis will have a recurrence, both immunologically and histologically. Giant cell hepatitis with autoimmune hemolytic anemia is a rare disorder that has been shown to recur post-transplant and is now considered a contraindication to transplantation. The outcome for children transplanted for malignant hepatic tumors is related to the rate of recurrence, which is low if there are no extrahepatic metastases present at the time of surgery.

**De novo autoimmune hepatitis**

A number of recent studies have documented the development of autoantibodies (ANA, SMA, LKM) after transplantation in both children and adults in recipients who did not have autoimmune hepatitis pretransplant. The incidence is in the order of 2–3%. The histology is typical of autoimmune hepatitis and it can progress to fibrosis. The etiology is unknown, but there is usually a good response to steroid therapy, with or without azathioprine.

**Long-term renal function**

The development of nephrotoxicity with both cyclosporine and tacrolimus is common although only 4–5% of patients develop severe chronic renal failure requiring renal support or transplantation. The use of low dose calcineurin inhibitors or renal sparing drugs such as mycophenolate mofetil or sirolimus for maintenance immunosuppression reduces renal dysfunction. Acute postoperative hypertension is seen in 65% of children, but only persists in 28%.

**Hyperlipidemia**

Cyclosporine and sirolimus both increase serum lipids, particularly cholesterol, which resolves on transfer to tacrolimus or mycophenolate.

**Non-compliance with therapy**

Non-compliance with immunosuppressive therapy is probably underestimated in liver transplant recipients. Although it is possible that children who were grafted at a young age are more likely to accept medication through their adolescence than those transplanted later in life, this has yet to be proven.

**Survival**

Current results indicate that 1-year survival after pediatric liver transplantation may be as high as 90%, while long-term survival

rates (5–8 years) range from 60 to 80%. Patients receiving elective living related transplantation initially had a higher 1-year survival (94%) compared with those receiving cadaveric grafts (78%), but recent figures are now in excess of 95% irrespective of graft origin. Nutritional status pretransplant is a significant risk factor for morbidity and mortality, as data indicating that children who had malnutrition had a 60% chance of surviving the first year compared with 95% in better-nourished children, highlighting the necessity for intensive preoperative nutritional support. It is clear that short-term survival is better in children transplanted electively compared with those transplanted for acute liver failure who continue to have 1-year survival rates of 60–70%, which is related not only to the severity of the liver disease, but is also due to multiorgan failure.

**Outcome after metabolic liver transplantation**

In  $\alpha_1$ -antitrypsin deficiency, Byler disease and Wilson disease, there are both phenotypic and functional cures of the original disease. In tyrosinemia type I, liver transplantation corrects hepatic enzyme deficiency and prevents the development of liver cancer, although the kidney continues to produce toxic metabolites. Long-term nephrotoxicity from immunosuppressive agents is, therefore, a problem in this group of children. In Crigler-Najjar type I, urea cycle defects and primary oxalosis, the metabolic defect is completely corrected, and rehabilitation depends on the extent of extrahepatic disease pretransplant.

In organic acidemia, such as propionic acidemia and methylmalonic acidemia, the metabolic defect is widespread throughout body tissue, but liver replacement provides sufficient hepatic enzyme to prevent metabolic acidosis, under normal conditions. The majority of these children is able to take a normal protein intake, but will be at risk of mild metabolic acidosis during intercurrent infections.

**Quality of life after transplantation**

Children who survive the initial 3-month post-transplant period without major complications should achieve a normal lifestyle, despite the necessity for continuous immunosuppressive monitoring. Prospective studies have indicated a rapid return to normal nutritional status in over 80% of children within 1-year post-transplant. Linear growth may be delayed between 6 and 24 months post-transplant, which is directly related to corticosteroid dosage and to malnutrition and preoperative stunting.

Early studies of neuropsychologic development pre- and post-transplant demonstrated that the rate of improvement post-transplant is related to the extent of motor or psychologic developmental delay pretransplant, thus highlighting the necessity for early transplantation, particularly for infants who have chronic liver disease. Prospective studies have shown that there is an initial deterioration in psychosocial development post-transplant, which may be related to the prolonged hospitalization, and to stress of the transplant operation. Following the resumption of normal life there is a return to pretransplant psychosocial scores within 1–2 years. Most children return to nursery or normal school within 3 months of transplantation.

Long-term studies have shown that children surviving liver transplantation enter puberty normally, girls will develop menarche, and both boys and girls will have pubertal growth spurts. Successful pregnancies have been reported.



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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 27

## Benign Tumors of the Liver

John Karani

### INTRODUCTION

There are very few issues in clinical hepatology that evoke as much debate as the pathogenesis, diagnosis and management of benign liver tumors. The advances in radiologic techniques have led to an improvement in lesion conspicuity that allows the demonstration of small liver tumors that would not have been detected a decade ago. The number of pathologic entities that are now diagnosed, both independently and in association with chronic liver diseases or multisystem disorders, continues to increase. Although didactic algorithms defining lines of investigation and management are of value, there will always be a variance in observation and expertise in radiologic and pathologic interpretation that may adversely affect or advance the diagnostic pathway. In addition, the natural history of many of these tumors, particularly the rarer types, is not yet clearly established and therefore predicting prognosis may be erroneous. However, any individual clinical practice should establish the basic principles of management recognizing the key radiologic and pathologic characteristics and the spectrum of their clinical presentations.

### PRINCIPLES OF INVESTIGATION AND MANAGEMENT

A common clinical scenario is that a focal lesion has been detected on imaging. Often this will be an incidental finding unrelated to the patient's symptoms. There is a differential diagnosis that will include tumors of a wide pathologic spectrum. Each of these tumors will require different treatment and carry a different prognosis. The role of clinical and radiologic investigations is to predict the pathology of these tumors and reserve surgical or image guided biopsy for lesions that demonstrate indeterminate characteristics. It is important in the clinical assessment to ascertain whether the tumor has arisen within normal hepatic parenchyma or if there is clinical and biochemical evidence of diffuse parenchymal liver disease. There is an association of certain benign lesions with multisystem disorders and therefore this possibility should be evaluated in the clinical examination and serologic investigations. Liver function tests will be normal in the majority of patients with benign tumors. As a general rule any asymptomatic lesion in a patient with normal liver function that exhibits the pathognomonic radiologic characteristics of an individual benign tumor should be managed conservatively. Surveillance imaging can be used to confirm that the size and morphology of the lesion does not change.

The indications for surgery are difficult to define and are often not dependent upon the type of tumor. Hepatic resection and embolization carry a significant morbidity but are indicated and may be life-saving in those patients presenting with severe

spontaneous hemorrhage. If pain is the presenting feature and is attributable to the tumor and also cannot be controlled by medical management, then surgery is indicated. This has to be preceded by a thorough risk versus benefit analysis for each individual patient for any surgical resection will carry short-and long-term morbidity, irrespective of whether the tumor is benign or malignant.

### HISTOLOGIC CLASSIFICATION

Benign tumors can arise from all of the cellular components of the liver. Hepatocytes can give rise to hepatocellular adenomas; cystadenomas can arise from biliary epithelium and hemangiomas from mesenchymal tissue and a combination of cells may be found in the tumor-like lesions such as focal nodular hyperplasia. Table 27.1 represents an abbreviated version of the pathologic classification that is now accepted worldwide. The specific pathologic characteristics of the more important of these lesions are described in the following text.

### RADIOLOGY

There are now a number of techniques to detect and characterize benign liver lesions, including ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), nuclear

Histological classification	
Benign epithelial tumors	Liver cell adenoma Bile duct adenoma Bile duct cystadenoma Biliary papillomatosis
Benign non-epithelial tumors	Hemangioma Infantile hemangioendothelioma Lymphangioma Angiomyolipoma Pseudolipoma Fibroma (fibrous mesothelioma) Leiomyoma
Tumor-like lesions	Cysts Fibropolycystic disease Focal nodular hyperplasia Nodular regenerative hyperplasia Mesenchymal hamartoma Biliary hamartoma (von Meyenburg complex) Inflammatory pseudotumor

Table 27.1 Histological classification.

scintigraphy and arteriography. Each of these has their own merit but in practice none is pre-eminent. The correct diagnosis is usually made by a correlative imaging strategy interpreted by an experienced radiologist who has knowledge of the clinical spectrum and associations of these lesions.

The following text outlines the simple questions that these techniques have to answer either singularly or in combination.

- Is the lesion cystic or solid or a combination of both these features? This question is reliably answered by US, supplemented by CT or MRI to determine the relative proportion of the cystic and solid elements.
- Is this a single lesion or are other tumors present and do they have similar characteristics indicating the same pathology? It is important to remember that lesions of >5 mm can be detected by all these techniques.
- Is the lesion vascular or avascular? This can be determined by contrast enhanced CT, US or MRI and the pattern of enhancement through the arterial, portal venous and delayed phases of these studies will be a differentiating characteristic between lesions. For example if there is centripetal enhancement with delayed 'filling in' then this is characteristic, but not pathognomonic, of a cavernous hemangioma. Arteriography can confirm the pattern of vascularity but in addition the arterial pattern may be characteristic of an individual tumor. Focal nodular hyperplasia and cavernous hemangioma are such examples.
- Are Kupffer cells present in the lesion?  $^{99m}\text{Tc}$  sulfur colloid scintigraphy or MRI with intravenous supermagnetic iron oxide particles (SPIO) will define the distribution of Kupffer cells in the lesion.
- Are hepatocytes present in the lesion?  $^{99m}\text{Tc}$ -iminodiacetic acid (IDA) derivative scintigraphy or MRI with the paramagnetic chelate manganese dipyridoxyl diphosphonate (Mn-DPDP) will define the distribution of hepatocytes in the lesion.
- Is there another specific tissue characteristic present by which the tumor can be reliably diagnosed? The presence of a capsule, calcification or hemorrhage is a recognized diagnostic feature of certain types of tumors.
- Is there evidence of chronic parenchymal liver disease or portal hypertension?
- Is the lesion resectable? The segmental anatomy and vascular relationships of the lesion have to be accurately assessed by CT or MRI.
- At what stage can the investigations stop? It may be possible to stop the radiologic pathway after a single investigation. For example, cavernous hemangiomas of <3 cm generally have pathognomonic features on US. They are echogenic with posterior acoustic enhancement and lie peripherally but close to a major hepatic vein. If these features are observed in an asymptomatic patient then the investigative sequence should stop.
- Is a biopsy required and can this be safely performed? As a general rule, all lesions that have indeterminate radiologic characteristics should be biopsied. If the lesion is large and symptomatic and will be resected independent of its pathologic characteristics then preoperative image guided biopsy is not necessary.

The following text outlines pathogenesis, pathology, radiology and basic principles of management of the more common benign lesions.

## BENIGN EPITHELIAL TUMORS

### Liver cell (hepatocellular) adenoma

This is the most studied benign tumor of the liver with a spectrum of lesions now defined. The majority of liver cell adenomas are related to the use of the oral contraceptive pill. The estimated annual incidence has been put at 3.4/100 000 contraceptive users equivalent to approximately 300 cases per year in the USA. Case control studies and epidemiologic evidence have shown that the development of liver cell adenoma in a woman relates to the dose, duration of usage and increasing age. Low dose oral contraceptives in current use carry little or no risk of tumor development.

Although 'spontaneous' liver cell adenoma occurs in both men, women and children, it is more commonly a sequela of therapy with androgenic anabolic steroid therapy for impotence, or to enhance muscle development in body builders and athletes. Children of either sex treated with the same drugs for Fanconi syndrome, refractory anemias and bone marrow aplasia carry an equivalent risk of liver cell adenoma development. All the compounds incriminated have been 17-alkyl ( $\alpha$ -ethinyl) substituted derivatives of the basic steroid structure with methyltestosterone, oxymethalone and norethandrolone the most commonly implicated drugs.

Rarer etiologic factors are glycogen storage disease type 1a, familial diabetes mellitus and Klinefelter syndrome and therapy with clomifene, danazol and norethisterone.

The simultaneous occurrence of liver cell adenoma and focal nodular hyperplasia has been observed but this is uncommon. Peliosis hepatitis is often present and may be extensive if the tumor is due to anabolic androgenic steroids. Reports of spontaneous regression after drug withdrawal and progression or malignant change are rare. Most gonadal steroid related liver tumors do not produce  $\alpha$ -fetoprotein and do not metastasize despite a frequently equivocal histologic appearance which makes it difficult to distinguish from hepatocellular carcinoma.

### Pathology

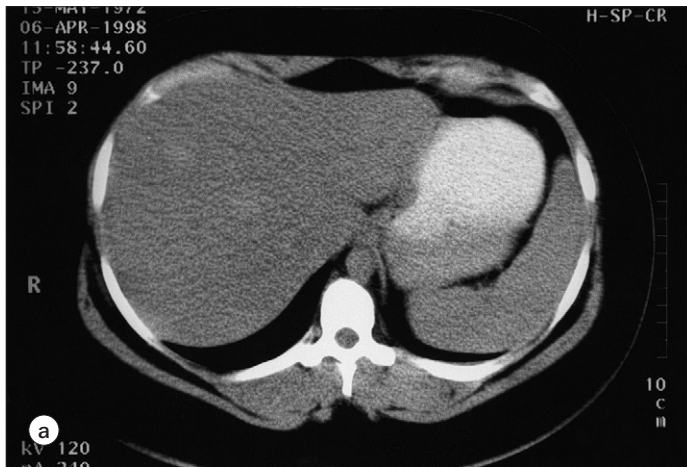
Macroscopically the tumors arise in an otherwise normal liver and are typically large, often measuring up to 10 cm at presentation. Pedunculation is present in approximately 10% of cases. The majority of these lesions are solitary and well demarcated but are seldom encapsulated on sectioning. The color varies from yellow to tan and they are highly vascular with areas of hemorrhage, with infarction being a characteristic feature. Rarer instances of multiple tumors occur. This disorder is termed liver cell adenomatosis and probably represents a distinct pathologic entity of differing etiology.

Microscopically the tumor is composed of liver cells arranged in plates that are two or three cells thick, separated by compressed sinusoidal spaces lined by endothelium. Enzymatically active Kupffer cells may be demonstrated by periodic acid-Schiff (PAS) diastase or Perle stains. Mitoses are absent or few with uniform cell nuclei. Excess glycogen or fat may present in larger than normal hepatocytes producing pale and eosinophilic cytoplasm. Bile ducts are absent but bile may be present as droplets within the cytoplasm or as plugs in distended canaliculi.

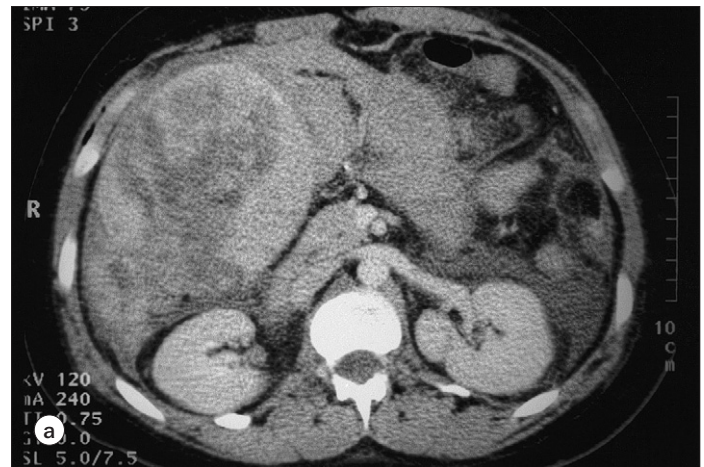
### Radiology

With all the imaging techniques the characteristics of a liver cell adenoma depend upon its size, the degree of hemorrhage or





**Figure 27.1 Liver cell adenoma.** Computed tomography demonstrates multiple liver cell adenoma appearing as hyperdense nodules within a fatty liver on the unenhanced scan (a). Following intravenous contrast the lesions become more conspicuous due to their enhancement in the arterial phase of the scan (b).



**Figure 27.2 Ruptured liver cell adenoma.** Computed tomography demonstrates a hypervascular adenoma that has undergone spontaneous rupture with development of a hemoperitoneum (a). Selective hepatic arteriography demonstrates the abnormal vessels on the periphery of the lesion with the central avascular area representing the line of rupture with contained hemorrhage (b).

infarction complicating the tumor or the amount of fat or glycogen present. Supportive diagnostic features may be present such as diffuse fatty replacement of the liver supporting a pre-existing storage disorder or the presence of free intraperitoneal hemorrhage.

Sonography typically demonstrates a large hyperechoic mass with central anechoic areas corresponding to zones of internal hemorrhage. Severe necrosis may develop following infarction resulting in a complex cystic sonographic appearance. Color flow and spectral scanning demonstrate the enhanced peripheral vascularity of the tumor.

Unenhanced CT usually demonstrates a hypodense or isodense lesion that, in the presence of severe fatty replacement, the tumor may appear of higher density than the surrounding abnormal hypodense parenchyma. Hyperdense areas of fresh hemorrhage may be present. Typically, the non-necrotic and non-hemorrhagic regions of the lesion will show transient enhancement during the arterial phase with a centripetal pattern of enhancement developing through the portal and late venous phases (Fig. 27.1) Unlike hemangiomas, this pattern of peripheral enhancement does not persist with the lesion becoming

isodense or hypodense by the portal venous phase because of arteriovenous shunting.

On MRI studies, variable characteristics are present but adenomas may show increased signal intensity on T<sub>1</sub>-weighted images resulting from the presence of fat or glycogen. The presence of an increased signal on T<sub>2</sub>-weighted images and enhancement following gadolinium are supportive as diagnostic features. No adenomas have been reported with uptake by ferrite reflecting a lack of Kupffer cells.

Characteristic angiographic features are of a hypervascular mass with centripetal flow and abnormal peripheral arteries. A more generalized abnormality of the liver may be present with an abnormal hepatogram phase characteristic of peliosis.

### **Clinical features**

Symptomatic patients present with episodic or acute abdominal pain. The latter is invariably secondary to hemorrhage within the tumor. This may be further complicated by rupture into the peritoneum (Fig. 27.2). If not recognized and appropriately treated this sequela carries a mortality of approximately 10%. The factors that adversely influence the risk of

spontaneous rupture are the size of the tumor, pregnancy and menstruation.

### Management

Current opinion is that adenomas >10 cm carry sufficient risk of hemorrhage to warrant surgical excision. In a patient presenting with acute abdominal pain, severe active hemorrhage and shock, emergency radiologic intervention with arterial embolization is indicated as a prelude to resection. At the other end of the clinical spectrum is the patient on oral contraceptives where the tumor has been detected as an incidental finding on imaging. In this clinical scenario a conservative approach can be followed, as the natural history is not so well defined that the prognosis can be predicted. Complete resolution and progression on withdrawal of estrogen are both documented. A policy of close observation by imaging can be adopted. Should the tumor enlarge or the patient develops symptoms attributable to the lesion, then these are clear indications for surgery. With successful surgical resection, the long-term prognosis is good.

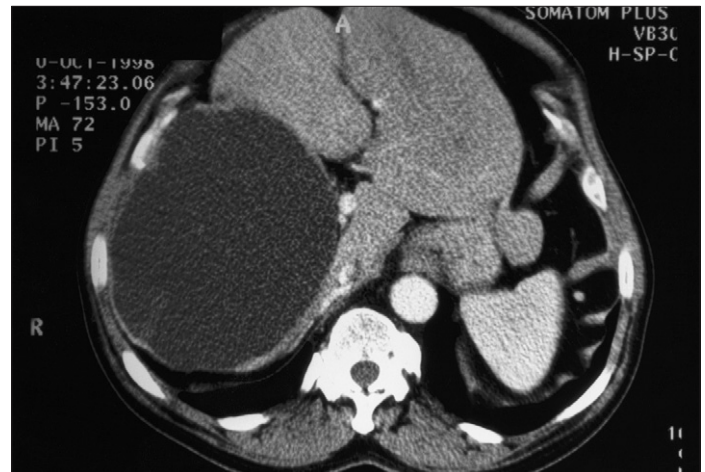
An elective radiologic approach using selective arterial embolization with coils and particulate material to ablate the vascularity of the tumor can provide a therapeutic alternative particularly if the lesions are multiple and their anatomy mitigates against low risk hepatic resection. As with many uncommon liver tumors of variable presentation, management and biological activity, establishing patients and then recruiting them onto a long-term case controlled study to investigate the role of radiologic intervention as alternatives to surgery and conservative management is difficult. However, individual experience and reports indicate that this approach merits consideration.

### Bile duct cystadenoma

This is a rare tumor that is not unique to the liver as tumors with similar characteristics are reported in the pancreas and ovary. They constitute less than 5% of cysts of biliary origin with 85% arising from the intrahepatic biliary tree with the remainder arising from the extrahepatic bile ducts or gallbladder. With regard to pancreatic neoplasms, this similarity is explainable on the similar development of the pancreas and the biliary system from the endodermal diverticula of the gut. The primordial germ cells normally migrate from the posterior wall of the yolk sac along the wall of the hindgut through the yolk stalk, a structure that is intimately related with the gut from which the primordia of the hepatobiliary system and pancreas arise. This embryologic association may explain the ovarian-like stroma visible in these tumors, which is reported as a feature carrying a better prognosis.

### Pathology

The cystadenomas are usually large multiloculate cystic tumors lined by columnar, mucin-producing epithelium. Categorization of the tumor into benign or a malignant cystadenocarcinoma is largely dependent upon the histologic assessment of the epithelium which may show internal papillary structures or cystic invaginations. Foci of cellular atypia, particularly nuclear enlargement and hyperchromasia, multilayering or solid epithelial masses indicate 'borderline' change with capsular invasion an indicator of malignant change.



**Figure 27.3 Biliary cystadenoma.** This appears on computed tomography as a large hypodense lesion with mural nodularity. There is atrophy of the posterior segments of the right lobe of the liver.

### Radiology

The tumors are slow growing and by the time of presentation usually measure over 10 cm. They are therefore easily detected by imaging (Fig. 27.3). Specific radiologic features are the complex cystic appearance of the tumor with multiple loculi containing mucinous fluid that may be detected by all techniques. The tumors are characteristically avascular on angiography. There are no specific radiologic criteria to differentiate cystadenocarcinoma and cystadenoma but an increased mural nodularity favors malignant change. Therefore the role of imaging is three-fold: firstly to give an indication as to the potential diagnosis, secondly to define the segmental anatomy and vascular relationships prior to liver resection, and finally to exclude a pancreatic or ovarian primary cystadenocarcinoma metastatic to the liver.

### Clinical features

The majority of these tumors occur in women with a peak incidence in the fifth decade. Patients present with pain or discomfort and a palpable mass. Biliary cystadenomas do not communicate with the biliary tree although compression or projection into major bile ducts may occur and result in obstructive jaundice or recurrent cholangitis.

### Management

If anatomically possible, surgical resection is the treatment of choice and carries a good prognosis even if there is histologic evidence of malignant transformation.

### Bile duct adenomas and microhamartomas

Bile duct adenomas are rare tumors, with hamartomatous features formed by numerous small non-malignant ducts separated by mature connective tissue. They are usually an incidental finding at laparotomy or autopsy appearing as single, pale well-circumscribed nodules. Over 90% are subcapsular in position and therefore are easily resected.

Microhamartomas or von Meyenburg complexes represent as not uncommon finding in wedge biopsies or at autopsy. They are tiny, usually multiple lesions composed of small dilated, angulated bile ducts in a stroma of fibrous tissue. They lie within



or adjacent to portal tracts. They are usually asymptomatic but have been reported in association with portal hypertension akin to congenital hepatic fibrosis and, rarely, the development of cholangiocarcinoma within the complexes.

## BENIGN NON-EPITHELIAL TUMORS

### Hemangioma

This is the most common benign tumor of the liver with a reported incidence from 1 to 20%. This latter figure is a result of a prospective autopsy study in which a dedicated search for this lesion was performed. Most reports suggest a predominant prevalence in women. Although this tumor may occur in any age group, it is most commonly found in the third, fourth and fifth decades. Serial longitudinal growth studies have shown that most hemangiomas remain stable, although they may occasionally increase or decrease in size. Hormonal influences in growth have also been documented with enlargement of some tumors during pregnancy and regression of others after steroid therapy.

### Pathology

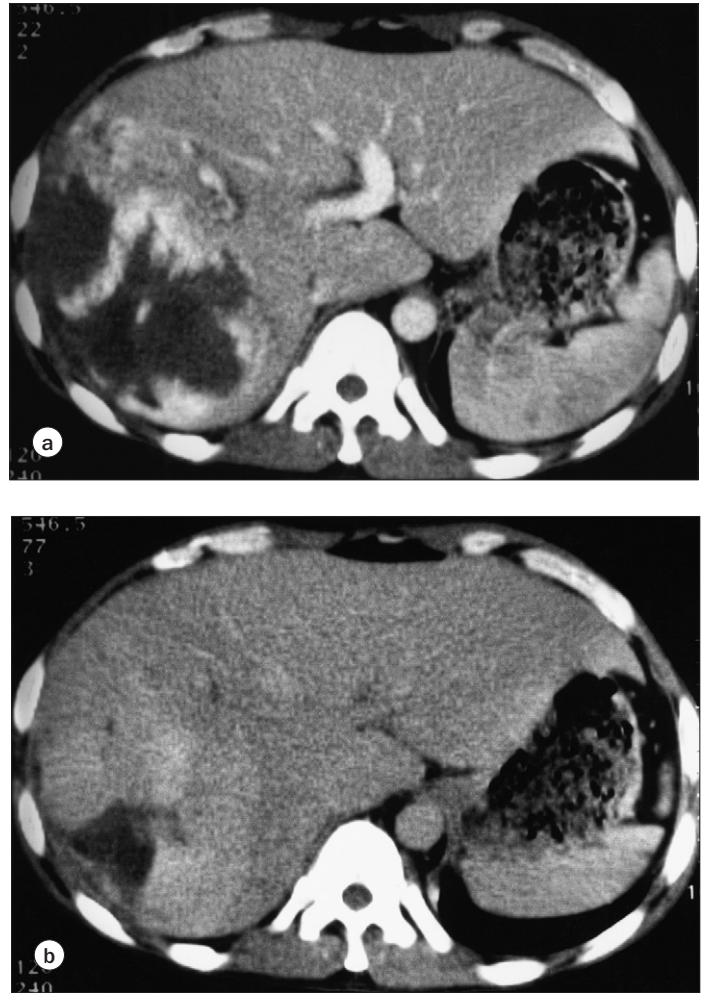
Hemangiomas may be single or multiple, intraparenchymal or pedunculated, with a size varying from a few millimeters to greater than 20 cm. However, most hemangiomas are solitary, of less than 5 cm, and are found in a subcapsular site in the right lobe adjacent to peripheral divisions of the hepatic vein. Histologically, they are derived from mesodermal elements and are composed of cavernous vascular spaces lined by a single layer of flat epithelium and filled with blood. Thin fibrous septae separate the vascular channels. They have a predisposition to thrombosis with subsequent development of fibrosis, dystrophic calcification or ossification. Larger hemangiomas have a propensity to involute, developing a central fibrocollagenous scar as vascular occlusion and organization of central thrombi occurs, with central necrosis a potential sequela. Rarely, vascular occlusion may lead to total sclerosis of the hemangioma and formation of a fibrous nodule.

### Radiology

Each radiologic technique has characteristic features by which hemangiomas may be reliably diagnosed but there is a significant minority that will be atypical. These require a combination of radiologic techniques to establish the diagnosis. The appearances will reflect the varying representation of the histologic elements within the tumor. Larger tumors often have mixed or atypical features and the differentiation from other benign or malignant tumors, of differing prognosis and management, may be difficult.

Characteristic appearances on US are of a well-circumscribed hyperechoic lesion of <3 cm, exhibiting posterior acoustic enhancement. They lie in a subcapsular position adjacent to a hepatic vein. If fatty infiltration of the liver is present then the tumors may be hypoechoic. Demonstration of blood flow on color Doppler flow imaging is a variable feature occurring in between 10 and 50% of hemangiomas in published series. Larger (>3 cm) or giant (>10 cm) will have varying degrees of hemorrhage, calcification or fibrosis modifying their US appearance.

On CT hemangiomas are characteristically of low density although in the presence of fatty infiltration they may appear hyperdense relative to the surrounding hepatic parenchyma. After administration of intravenous contrast agent, there is nodular peripheral enhancement of the tumor by the large



**Figure 27.4 Hemangioma.** Computed tomography demonstrates a giant cavernous hemangioma with centripetal enhancement in the early vascular phase (a) and 'filling in' in the delayed phase (b).

feeding vascular nidus of the tumor. Centripetal enhancement then occurs with 'filling in' of the tumor (Fig. 27.4). Although small lesions 'fill in' completely, large hemangiomas may show central avascular zones corresponding to the central fibrotic scar (Fig. 27.5). The rate of 'filling in' of the tumor is variable and is dependent on the flow dynamics of the hemangioma and on the timing of the acquisition of the scan relative to the bolus of contrast, and may be delayed for up to 20 min after the injection. It is important to emphasize that only approximately 54% of large hemangiomas have this characteristic perfusion pattern. However, only 2% of other primary and malignant tumors exhibit these features so their presence typically should allow a confident diagnosis of a hemangioma to be made. The converse argument is that up to 46% of hemangiomas exhibit atypical features of either absent, mixed or central enhancement or incomplete 'filling in' on delayed scans so clear differentiation from malignant tumors may not be possible in these cases. Therefore, it is an important rule that in the CT evaluation of a patient with a documented extrahepatic malignancy, strict adherence to these criteria must be made to ensure a metastasis is not misinterpreted as an atypical hemangioma.



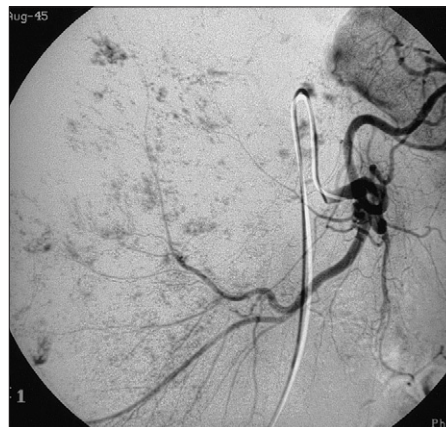


**Figure 27.5 Hemangioma.** Computed tomography demonstrates a central scar within a cavernous hemangioma of the right lobe of the liver. There is a small hemangioma in the left lobe that also exhibits centripetal enhancement.

With MRI hemangiomas characteristically demonstrate marked hyperintensity on  $T_2$ -weighted images that may contain low intensity areas correlating with the pathologic zones of fibrosis. After administration of the vascular phase contrast agent gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) there is early peripheral nodular enhancement with 'filling in' on delayed scans paralleling the characteristics of CT. Smaller hemangiomas more often demonstrate uniform enhancement, potentially indistinguishable from the appearances of hypervascular metastases or hepatocellular carcinomas (Fig. 27.6). Increasing the  $T_2$ -weighting of the acquisition may improve the discrimination, with hemangiomas usually being of higher signal intensity than malignant nodules. As hemangiomas do not contain Kupffer cells or normal hepatocytes they do not enhance with either superparamagnetic iron oxide particles or Mn-DPDP.



**Figure 27.6 Hemangioma.**  $T_2$ -weighted magnetic resonance imaging scans of same case as Figure 27.3 demonstrate the characteristic hyperintensity of the hemangioma with the central hypointense scar. The smaller lesion in the left lobe appears uniformly hyperintense.



**Figure 27.7 Hemangioma.** Selective hepatic arteriography demonstrates the characteristic features of a hemangioma with pools of contrast and absent venous shunting.

Hemangiomas cause a focal defect on both hepatobiliary excretion and sulfur colloid scintigraphy that is accompanied by normal uptake in the surrounding liver. Tagged red blood pool scans may be diagnostic with again the features of centripetal uptake with 'filling in' on delayed scans.

Angiography is usually reserved for the atypical tumors where assessment by non-invasive techniques singularly or in combination has not resulted in a definitive diagnosis. The appearances are pathognomonic with small pools of contrast, which persist beyond the late venous phase without evidence of tumor neovascularity or arteriovenous shunting (Fig. 27.7).

None of these techniques is pre-eminent and it is often a correlative imaging analysis by an experienced hepatobiliary radiologist that allows the diagnosis to be made.

#### **Clinical presentation and management**

The vast majority of hemangiomas will remain asymptomatic and are discovered as an incidental finding on imaging investigations for an unrelated condition. In most instances knowledge of the characteristic features should allow a definitive diagnosis to be made and obviate the need for any further investigations. Abdominal pain may occur with larger lesions with hemorrhage, infarction or compression of adjacent viscera complicating the tumor. Thrombocytopenia from platelet sequestration, hypofibrinogenemia from deposition of an intravascular fibrin clot, and spontaneous rupture are all rare complications. It is the severity of the clinical presentation rather than any specific morphologic characteristic of the hemangioma that directs management. Rupture and hemorrhage are at the extreme of the clinical spectrum, and the requirement for surgical intervention is without question. In the patients with abdominal pain, the clinical analysis is frequently more difficult. If it is clear that the patient's symptoms are attributable to a large hemangioma and if radiologic assessment confirms that the lesion is resectable then surgery is indicated. Radiologic intervention with particulate embolization of the arterial supply is a therapeutic alternative but as yet there is no controlled trial to confirm its efficacy. Neither of these interventions, with their inherent morbidity, should be undertaken unless an objective risk benefit analysis of the patient's symptoms has been undertaken.

#### **Infantile hemangioendothelioma**

This is the commonest mesenchymal tumor of the liver and may represent part of a generalized mesenchymal disorder with

cutaneous hemangiomas present in over 50% of cases. Less commonly accompanying lesions in the gastrointestinal tract, trachea, pulmonary parenchyma or central nervous system may also occur. More than 80% are diagnosed in the first 6 months of life with an approximately equal sex incidence. Complications include congestive cardiac failure secondary to arteriovenous shunting, rupture, and consumptive coagulopathy with anemia, thrombocytopenia and hemolytic jaundice as presenting features. Occasionally these lesions present as an asymptomatic mass that resolves spontaneously without complication, although there is an increased risk of hepatic hemorrhage from trauma.

### Pathology

The tumors can be solitary or multicentric, varying in size from a few millimeters to >15 cm. They are well demarcated but non-encapsulated with the cut surface exhibiting a reddish-brown appearance with a spongy consistency which may show central areas of focal hemorrhage, scarring or calcification. Histologically the tumor is composed of anastomosing vascular channels lined by a solitary (type 1) or multiple layers (type 2) of plump endothelial cells. The infiltrative growth pattern between the liver cell plates is the feature that distinguishes the tumors from cavernous hemangiomas. The tumor cells are benign and mitoses are rare. Extra medullary hemopoiesis is a frequent finding. Low grade angiosarcomas, which may also present in infancy, and which carry differing management and prognostic implications, are the most important histologic diagnostic differential of the type 2 lesions.

### Radiology

The radiologic features reflect the degree of arterialization and arteriovenous shunting within the tumor. High volume shunts are associated with a hyperdynamic arterial flow, dilatation of the draining hepatic veins and features of cardiac failure with cardiomegaly and pulmonary plethora. The infants who present within the first few days of life with these features are often misdiagnosed as having congenital heart disease with an intra-cardiac shunt. The role of radiology is to examine the extent of replacement of the normal liver parenchyma by the hemangioendothelioma, assess the degree of vascularity and pathologic shunting and to determine whether any other organs are involved.

The US features of these tumors vary. Many of the tumors are well circumscribed, but their internal structure may differ in characteristics. Diffuse alteration in the parenchymal pattern with complete replacement of the normal liver pattern may be present, even in the presence of normal synthetic liver function. Doppler US characteristics are diagnostic in the majority of infants with a high volume systolic and end diastolic arterial flow and dilatation of anatomically normal main hepatic veins.

CT demonstrates the focal vascular spaces as enhancing masses on both the arterial and portal venous phases of contrast enhancement. The degree of persistence of tumor enhancement on the delayed phase will be dependent on the degree of arteriovenous shunting. The tumors with high volume shunts will be characterized by a rapid clearance of contrast and dilated hepatic veins (Fig. 27.8). Those without a major arteriovenous shunt will show persistent and increasing enhancement with 'filling in' resembling adult cavernous hemangiomas (Fig. 27.9).

MRI will demonstrate the tumors as hyperintense vascular spaces on T<sub>2</sub>-weighted images. The enlarged hepatic arteries and



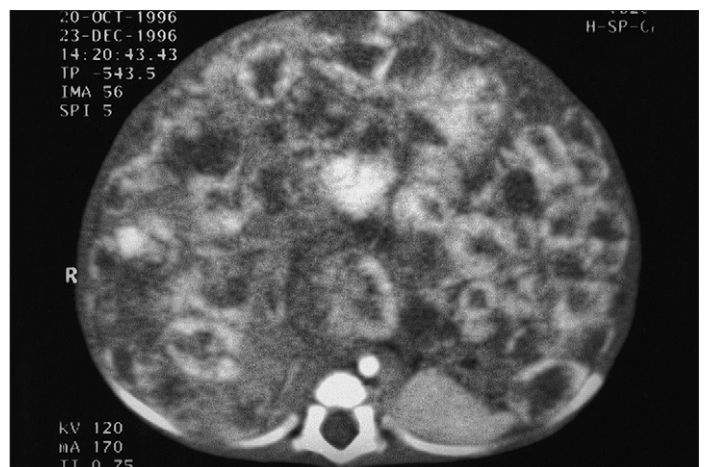
**Figure 27.8 Hemangioendothelioma.** Segmental hemangioendothelioma presenting in an infant appearing as a hypervascular mass on computed tomography.

decompressing hepatic veins are demonstrable on conventional multiplanar imaging or dedicated vascular image acquisition techniques (magnetic resonance angiography). Areas of focal calcification, hemorrhage and necrosis, which have variable representation within the tumors, will all have their characteristic appearances with the individual techniques.

Angiography provides a conclusive diagnosis, detailed anatomy of the tumor and its feeding arteries. Hypervascular tumor vessels are present with varying degrees of arteriovenous shunting and enlargement of the hepatic veins and hepatic artery. There is often diminution in the size of the abdominal aorta distal to the origin of the enlarged hepatic artery, as a consequence of the steal of blood through the liver.

### Management

The management of these tumors is dependent on the severity of the clinical presentation. This is a rare benign tumor and yet there are many treatments advocated in published series and



**Figure 27.9 Hemangioendothelioma.** Diffuse hepatic hemangioendothelioma of infancy with multiple enhancing nodules within an enlarged liver.

individual case reports. Mild degrees of cardiac failure may be treated with digitalis and diuretics, but the response to steroids and interferon seems to vary from center to center. Radiotherapy has been used but all these non-surgical management lines have to be judged by the natural history of these tumors that may undergo spontaneous regression and involution in the first few months of life without any intervention. Surgery is mandatory for intraperitoneal hemorrhage, but treatment in the presence of severe cardiac failure, unresponsive to medical treatment, is more contentious. Hepatic resection, hepatic artery ligation or embolization and transplantation all have a potential role. Hepatic artery ligation is a well-established technique with numerous published reports of its efficacy. It remains to be seen whether it is totally replaced by embolization as a method of occluding the arterial inflow to the hemangioendothelioma. Occluding the arterial supply with a metallic spring embolus delivered following percutaneous catheterization of the hepatic artery should result in the same alteration in hemodynamics as with surgical ligation but without the operative morbidity.

Resection is a surgical option when the tumor is confined to a lobe or segment but this occurs in the minority of cases. The greatest risk from resection lies in intraoperative hemorrhage. Liver transplantation may be the only option if arterial ligation fails with development of early arterial collateralization of the tumor and recurrence of severe cardiac failure.

### Lymphangioma

Hepatic lymphangiomas commonly occur as multiple masses of dilated lymphatic channels containing proteinaceous fluid or blood. Multiple organ involvement, including the spleen, peritoneum, kidneys, lungs, gastrointestinal tract and skeleton may be present, especially in children.

### Lipomatous tumors

Hepatic lipomas are uncommon benign tumors that are generally asymptomatic incidental findings on imaging. They may be composed solely of fat cells, or may contain varying proportions of adenomatous, angiomatous and myomatous tissue resulting in adenolipomas, myelolipomas and angiomyolipomas. Approximately 10% of patients with tuberous sclerosis and renal angioliipomas will have hepatic fatty tumors, either lipoma or angiomyolipoma. There is no sex predilection and they have been reported in a broad adult age range (24–70 years).

### Pathology

The tumors are composed of vessels, smooth muscle, fat and hemopoietic tissues in various combinations. The unusual histologic appearances may cause diagnostic difficulty especially in the leiomyomatous element which may be either spindle or epithelioid. Electron microscopy and immunocytochemistry readily identify the various components of the tumor.

### Radiology

The radiologic appearance of these fatty lesions is dependent on their internal composition. Fat has specific tissue characteristics. On US it is of increased echogenicity with acoustic enhancement posterior to the lesion. It has a specific density on CT of –20 to –115 Hounsfield units (HU) and on magnetic resonance it is hyperintense on both T<sub>1</sub>- and T<sub>2</sub>-weighted acquisitions. The presence of angiomatous or myomatous tissue will necessarily

alter the homogeneity of these characteristic features. Although pure lipomas do not enhance after vascular phase contrast agents, fatty hepatic tumors with angiomatous or adenomatous elements may show variable enhancement and make diagnosis by imaging more difficult and a tissue diagnosis by image guided biopsy may be necessary.

### Management

If a definitive histologic or radiologic diagnosis is made in an asymptomatic patient then no treatment is indicated. The tumors that are characterized by the presence of angiomatous features may present with hemorrhage and abdominal pain. In these rare instances surgical resection or embolization will have to be considered.

## TUMOR-LIKE LESIONS

### Focal nodular hyperplasia

Although rare focal nodular hyperplasia (FNH) is the second most common tumor, constituting approximately 8% of primary hepatic tumors found at autopsy. It is found most commonly in females during their reproductive years. Fewer than 20% are found in children and these account for 2% of childhood tumors. The etiology of the lesion is unknown. The preponderance in women, as with adenomas, suggests an association with estrogens, but the prevalence of oral contraceptive use in these cases is similar to that of a matched control population. Patients with FNH frequently have other vascular and neuroendocrine anomalies including cavernous hemangiomas, glioblastomas, astrocytomas, pheochromocytomas and multiple endocrine neoplasias. Lesions in children have been described in association with glycogen storage disease type 1, sickle cell disease and cyanotic congenital heart disease.

The pathogenesis of FNH has been related to neoplastic change, hamartomatous malformation or focal liver injury. The current view is that it represents a hyperplastic response to an abnormal blood flow caused by a pre-existing vascular malformation within the liver or abnormal or absent portal venous flow. It has been suggested that increased and turbulent blood flow results in platelet disruption with arterial thrombosis and release of platelet derived growth factors that may then stimulate liver cell hyperplasia.

### Pathology

This lesion is typically solitary and forms a well-circumscribed fibrous lobular mass. The majority of tumors are smaller than 5 cm, with a mean diameter of 3 cm at the time of diagnosis. They are rarely encapsulated and 20% are pedunculated. The lesion tends to bulge on its cut surface and displays multiple yellow–brown nodules of liver parenchyma separated by fibrous septa. Histologically it resembles cirrhosis but is focal and surrounded by normal liver parenchyma. A central stellate scar is often seen and it may contain thick-walled vessels. The frequent finding of bile duct proliferation around the liver cell nodules may be in response to cholestasis. The nodules consist of normal liver cells arranged in plates two or three cells thick and contain increased amounts of glycogen or fat. FNH is distinguished from liver cell adenoma by its multinodularity, and the presence of septa and proliferating bile ductules.





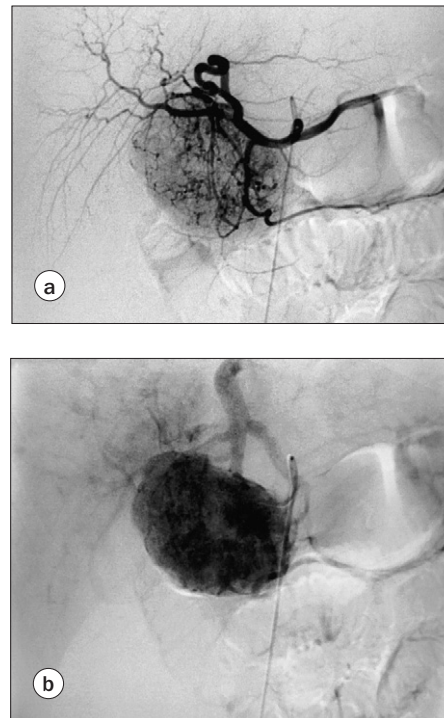
**Figure 27.10 Focal nodular hyperplasia.** Characteristic features of increased density, with enlarged peripheral arteries and stellate scars exhibited in the arterial phase computed tomography (a). The lesion becomes isodense in the portal venous phase (b).

### Radiology

Up to 60% of FNH lesions contain Kupffer cells with reticulo-endothelial cellular function and these lesions may therefore demonstrate normal or increased uptake on sulfur colloid scintigraphy. The remainder may be seen as a photon defect. If uptake is present this may provide a differentiating feature from an adenoma. Hepatobiliary excretion scans show tracer uptake in the majority of cases, and isotope excretion can be observed in 50% of delayed scans. In practice, nuclear scintigraphy has a very limited role both in the detection and tissue characterization of focal liver lesions such as FNH with greater reliance placed upon other imaging techniques.

Sonographically, FNH may have a variable appearance, but the majority of these lesions are well-demarcated lesions that are hyperechoic or isoechoic relative to the surrounding liver. Although a central scar is a common feature, it is not specific to FNH and also it may not be visible on US. The color flow Doppler characteristics mimic the angiographic and macroscopic feature of increased blood flow with a pattern of blood vessels radiating peripherally from a central feeding artery.

On unenhanced CT studies, FNH is typically hypodense or isodense to the liver parenchyma. Its presence can often be inferred by an alteration in the surface contour of the liver and



**Figure 27.11 Focal nodular hyperplasia.** Arteriography demonstrates the characteristic signs of focal nodular hyperplasia with a centrifugal 'spoke-wheel' arterial pattern (a) and an intense blush with draining veins (b).

a hypodense central scar. The degree of enhancement following iodinated contrast medium is variable, but they generally rapidly enhance in the arterial and early portal venous phase becoming isodense in the late portal phase (Fig. 27.10). Apart from a possible central scar, which will maintain its hypodensity, enhancement of the tumor is uniform throughout these phases. It is this feature and the rapidity at which they occur which may distinguish FNH from cavernous hemangioma where the centripetal pattern of enhancement and 'filling in' is considerably slower to develop. Spontaneous intratumoral hemorrhage and a capsule are not features and if present favor the diagnosis of an adenoma.

On MRI most lesions are isointense or hypointense on T<sub>1</sub>-weighted images and show increased signal on T<sub>2</sub>. However, some lesions are isointense on both sequences and, as with the isodense tumors on CT, may only be detected by the expansion of the liver surface they create by their mass effect and a central scar if present. Following intravenous gadolinium a rapid intense blush may occur that rapidly fades leaving the tumor close to isointensity in the equilibrium delayed phase of the examination. Delayed and increasing enhancement of the central scar on delayed scans is a characteristic of FNH. It is proposed that this feature is due to the presence of vascularized myxoid tissue in the scar. Fibrolamellar hepatocellular carcinomas and malignant cholangiocellular tumors contain scars but these do not demonstrate this radiologic sign.

Angiographically, FNH is a hypervascular tumor with a centrifugal arterial supply creating a 'spoke-wheel' pattern in the majority of lesions. During the capillary hepatogram phase an intense blush develops with large draining veins visible (Fig. 27.11).

### Clinical presentation and management

The majority of lesions are discovered incidentally and only approximately 15% result in symptoms. These are often of non-

specific abdominal discomfort. Rupture and hemoperitoneum are rare. In the asymptomatic patient it is important to establish the diagnosis by confirming the presence of characteristic imaging features. Image guided biopsy for a histologic diagnosis is reserved for lesions that demonstrate atypical or indeterminate radiologic features. In those rare patients presenting with spontaneous hemorrhage, surgical resection or embolization is indicated. In those patients with non-specific abdominal pain this decision is far more difficult. It must be clear that the patient's symptoms are attributable to this lesion by excluding any other cause and conducting a careful analysis of the risks of morbidity from surgery versus the potential symptomatic benefit to every individual patient.

### **Nodular regenerative hyperplasia**

This is a rare entity that is known by numerous synonyms, including nodular transformation of the liver, non-cirrhotic nodulation, miliary hepatocellular adenomatosis and adenomatous among others, but the current term of nodular regenerative hyperplasia (NRH) is now universally accepted. Autopsy series have shown prevalence as high as 0.6% but only one-third of these cases had clinical features in life potentially attributable to this diagnosis. It has been reported in all age groups with equal sex incidence. NRH is the major cause of non-cirrhotic portal hypertension in the Western world.

Various systemic diseases are associated with NRH including rheumatoid arthritis, Felty syndrome, polyarteritis nodosa, CREST syndrome (calcinosis cutis, Raynaud phenomenon, esophageal hypomobility, sclerodactyly, telangiectasia), subacute bacterial endocarditis, tuberculosis, diabetes, myeloproliferative disorders, lymphomas and after steroid or cytotoxic therapy and in recipients of bone marrow, renal and liver transplants where therapy with azathioprine has been implicated.

Common to all these disorders is a circulatory or vascular disorder and it has been postulated that NRH is a secondary and non-specific adaptation of the liver parenchyma to an alteration in total liver blood flow. This condition may not involve the whole liver and may be accentuated near the porta hepatis and therefore termed partial nodular transformation.

### **Pathology**

Confirming the diagnosis by liver biopsy is difficult and many cases are only diagnosed at surgery or autopsy. The liver is divided into nodules that vary in size between 0.1 and 1 cm, although rarely nodules measuring up to 10 cm have been reported. Histologically the nodules are composed of cytologically normal hepatocytes arranged in abnormal cell plates that are two or more cells thick. The features are of pure liver cell nodularity, enlarging and distorting acini or lobules but not replacing them. Histologically, this disorder can be distinguished from micronodular cirrhosis by the absence of fibrous septa between the nodules. Obliterative vascular changes may also be present.

### **Radiology**

The radiologic features are those of portal hypertension with splenomegaly, varices, ascites and variable alteration in the hepatic parenchyma. Nodules may be present on all imaging techniques but the liver parenchyma may remain uniform. Differentiation from cirrhotic portal hypertension may be difficult on imaging appearances alone but venous pressure studies in

confirming the hemodynamic profile of presinusoidal portal hypertension with a patent portal vein are important diagnostic criteria.

### **Clinical presentation and management**

Clinical manifestations may be non-specific, including malaise, fatigue and abdominal pain, particularly if it has developed in association with a multisystem disorder. Alternatively it may be related to complications of portal hypertension with ascites and variceal hemorrhage. Synthetic liver function is usually preserved with minimal elevation of the serum aminotransferases, bilirubin and prothrombin time.

Management is dependent on the degree of portal hypertension commencing with medical therapy and local management of the varices, proceeding to shunting and portal decompression in those patients with recurrent variceal hemorrhage if these first-line therapeutic measures fail.

### **Mesenchymal hamartoma**

This lesion probably represents a failure of normal development in utero of the ductal plate. Histologically, there is a mixture of mesodermal and endodermal structures in a loose connective tissue stroma and fluid filled spaces without an endothelial lining. Bile duct, liver cell and angiomatous elements are present and the appearance is of well-differentiated ductal structures surrounded by loose mesenchyme containing fibroblasts. The characteristic natural history of progressive enlargement of the lesion is secondary to degeneration and fluid accumulation within the cystic component. The prominent mesenchymal and cystic components that are visible on histology and radiologic investigations will allow differentiation from FNH and liver cell adenoma.

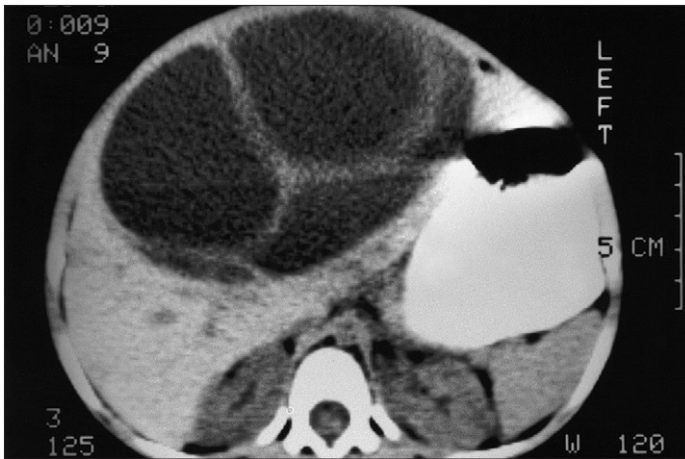
These lesions are benign and account for approximately 6% of primary tumors presenting in childhood. The majority will present in childhood with two-thirds of patients presenting in the first year of life. Almost all patients will present before the age of 5 years but rarely this may be delayed until adolescence or early adult life. There is a slight male preponderance.

The commonest presentation is with symptoms of progressive abdominal enlargement. Less commonly, infants may present with respiratory depression or peripheral edema from the mass effect of the lesion.

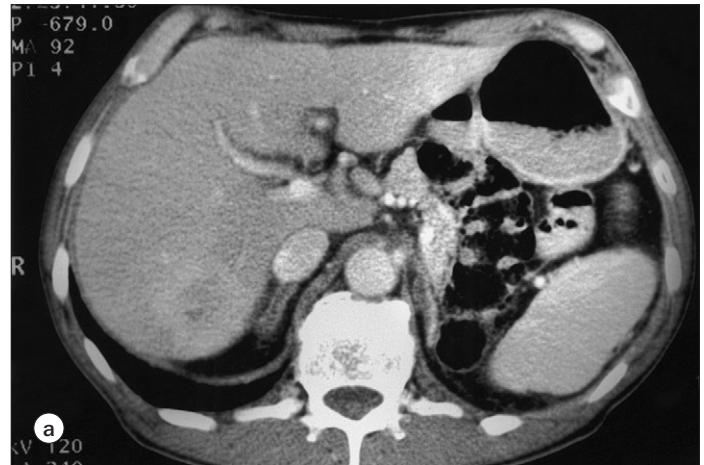
US and CT assessment will demonstrate a well-circumscribed complex cystic lesion with internal septation (Fig. 27.12). With contrast enhancement or angiography, the lesion is predominantly avascular but neovascularity is seen in the more solid elements. The MRI appearances depend on whether an individual lesion is predominantly stromal or cystic. The lesions with a fibrotic stroma predominating will appear hypointense relative to the surrounding liver on T<sub>1</sub>-weighted images. Alternatively, if the lesion is mainly cystic its major feature will be its hyperintensity on the T<sub>2</sub>-weighted images. The fibrous stroma and internal septation are the features that will distinguish this tumor from a simple cyst.

As the natural history of these lesions is of progressive enlargement, treatment is removal by a combination of resection and enucleation. Marsupialization into the peritoneal cavity or transplantation are alternative treatments for unresectable lesions.





**Figure 27.12 Mesenchymal hamartoma.** The scan demonstrates the characteristic computed tomography features of a mesenchymal hamartoma. This tumor presented in infancy with the appearances of a multiseptate cyst.



**Figure 27.13 Inflammatory pseudotumor.** The computed tomography (a) demonstrates a lesion in the right lobe posteriorly with peripheral enhancement and central low density. T<sub>2</sub>-weighted magnetic resonance imaging scan following manganese dipyridoxyl diphosphonate; (b) demonstrates the tumor as a hypointense lesion with manganese enhancement of the periphery of the lesion.

### Inflammatory pseudotumor

Synonyms for this very rare lesion include 'pseudolymphoma', 'histiocytoma' and 'plasma cell granuloma'. It is known to occur at other sites including the lung and orbits. Only about 50 cases have been reported to arise within the liver. Most patients are young with a significant male preponderance. The clinical presentation is of an acute infection with relapsing fever, abdominal pain, vomiting, diarrhea and jaundice. No causative organism or definite predisposing factor has been confirmed. Leukocytosis, a raised erythrocyte sedimentation rate (ESR) and polyclonal hyperglobulinemia are present in about 50% of cases.

Macroscopically, the lesion is tumor-like. It may be either solitary or multiple and measure up to 25 cm. Microscopically, the lesion contains chronic inflammatory cells in which polyclonal plasma cells predominate. There is a lamellar pattern of sclerosis. The lesions commonly resolve slowly with involution but biliary strictures and sepsis are rare sequelae of those arising at and involving the porta hepatis (Fig. 27.13).

### Miscellaneous tumor-like lesions

This group of conditions is of marginal importance in clinical practice but includes those due to infections such as hydatid disease, tuberculosis and larval infection that may produce a solitary necrotic nodule. Pseudolipoma represents the attachment of omental or pericolic fat lobules to the liver. Focal fat deposition occurs with obesity, alcoholic liver disease, diabetes and hyperlipidemia. This produces a characteristic radiologic pattern on US CT of 'geographical liver' where fat replaces normal liver parenchyma but without distortion of the portal vasculature.

Compensatory lobar hyperplasia may follow segmental intrahepatic or extrahepatic portal vein or bile duct occlusion. Endometriosis of the liver is recorded, arising in either a subcapsular or intrahepatic site. Adrenal nest tumors that arise from ectopic adrenal tissue which is identical to that in adrenocortical tumors and ectopic pancreas are both documented causes of tumor-like lesions of the liver.

### Cysts

#### Simple hepatic cyst

This is defined as a single unilocular cyst lined by a single layer of cuboidal, bile duct epithelium. The wall is a thin layer of fibrous tissue of <1 mm thick. They may be single or multiple and typically arise in a subcapsular site. The adjacent hepatic parenchyma is normal, distinguishing these cysts from the simple cysts occurring in polycystic disease in which the parenchyma shows regions of fibrosis with von-Meyenburg complexes. These cysts vary in size from <1 cm to >20 cm. They are much more common in females, with a ratio of females to males of 4 to 5:1. They have an overall incidence of 2.5% in the general population but are generally found in less than 1% of patients <60 years of age but rising to approximately 7% in those patients who are older than 80 years of age. Autopsy series have reported an incidence as high as 14%.

Patients are usually asymptomatic, but symptoms can develop secondary to their mass effect with abdominal pain, rupture, infection or intracystic hemorrhage as presenting features. The



most effective treatment for symptomatic cysts is wide excision and drainage. Guided percutaneous drainage is an option, particularly to assess whether this cures the presenting pain, but has a high recurrence with reaccumulation of fluid within the cyst. Sclerosing the cyst with alcohol following percutaneous decompression reduces this rate of recurrence.

### Radiology

With US imaging, they appear as well-defined, echo-free lesions that transmit sound with posterior acoustic enhancement. Thin septations can be present but thicker septa or eccentric mural thickening of the cyst are indications of a complicated cyst or

neoplasm. With CT, simple cysts appear as homogeneous, well-circumscribed, hypodense lesions with HU measurements in the range of simple fluid (<20 HU). Small lesions may show artificially high density because of partial volume averaging of the adjacent hepatic parenchyma. They do not enhance following intravenous contrast. On MRI they characteristically show uniform low signal on T<sub>1</sub>-weighted images, and high signal on T<sub>2</sub>-weighted images. Small central cysts may be indistinguishable from both vessels and small cavernous hemangiomas on T<sub>2</sub>-weighted acquisitions. Post-intravenous contrast studies with gadolinium may allow differentiation by demonstrating enhancement of both the hepatic vessels and hemangiomas.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 28

## Biliary Disease, Gallstones and Cystic Disease

Gregory T. Everson and Fernando E. Membreno

This chapter discusses disorders of the biliary tract including cholelithiasis, cholecystitis and cystic diseases of the liver. The specific aims are to describe the epidemiology and pathogenesis of these disorders, define the key clinical syndromes, provide methods for diagnosis and elucidate the natural history and impact of therapy.

### GALLSTONES AND BILIARY TRACT DISEASE

#### Cholelithiasis

##### *Epidemiology and risk factors*

Epidemiologic studies suggest that more than 20 000 000 Americans have gallstones and that nearly 750 000 cholecystectomies are performed annually in the USA, at an estimated total cost of over 7 billion dollars per year. The majority of gallstones are composed predominantly of cholesterol, and risk factors for gallstones include: age, female gender, use of estrogen replacement therapy or contraceptive steroids, pregnancy, obesity and rapid weight loss in obese subjects. Gallstones are highly prevalent in Hispanic populations and in native American populations of the southwestern USA. In one cholecystographic survey of Pima women residing in Arizona, more than 70% of women age 25 or older had a history of cholecystectomy, gallstones or non-visualization of the gallbladder. Other investigators have also reported high rates of gallstones or gallbladder disease in other North American indigenous groups, including the Chippewas, Micmacs and Cree-Ojibwas. Other risk factors for gallstones include: use of fibric acid derivatives (clofibrate), prolonged total parenteral nutrition (TPN), ileal resection or jejunoileal bypass, ileal disease (e.g. Crohn disease), celiac disease, vagotomy, spinal cord injury, diabetes mellitus, chronic hemolytic disease and cirrhosis.

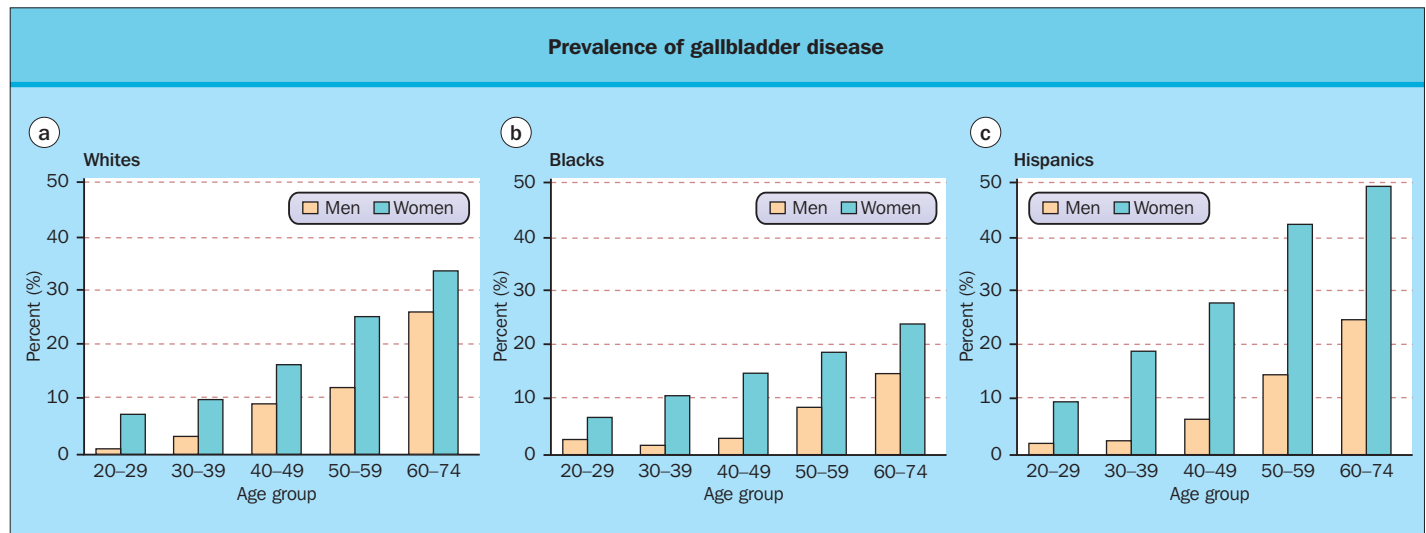
In the Third National Health and Nutrition Examination Survey (NHANES III) gallbladder ultrasonography was used to define the prevalence and risk factors for gallbladder disease in adults (age range 20–74 years) in the USA. Screening ultrasonography was performed in over 14 000 participants in NHANES III and gallbladder disease was defined by either the presence of cholelithiasis or prior gallbladder surgery. Extrapolation of the results of NHANES III to the general US population indicated that the overall prevalence of gallbladder disease was 20.5 million, and 8.7 million had had prior cholecystectomy. As demonstrated in nearly every population study previously conducted in other parts of the world, the prevalence of gallbladder disease in the USA was greater in women and increased with age in both men and women (Fig. 28.1). The difference in prevalence between men and women was greatest in younger age groups. Alcohol consumption was associated with lower risk and diabetes mellitus and obesity were associated with higher risk of gallbladder

disease in both sexes. In women, risk of gallbladder disease correlated with number of completed pregnancies, use of cigarettes and reduced physical activity. The design of NHANES III allowed comparison of differences in prevalence of gallbladder disease between ethnic groups. Non-Hispanic black men had a lower prevalence of gallbladder disease than either non-Hispanic white men or Hispanic men (Fig. 28.2). The latter two groups had similar prevalences. Hispanic women had the highest prevalence of gallbladder disease, which persisted even after controlling for comorbid conditions and other risk factors. In addition, non-Hispanic black women had a lower risk for gallbladder disease than either Hispanic or non-Hispanic white women, which was particularly pronounced at older age and with increasing body mass index (BMI). Pathophysiologic mechanisms to explain these differences have not been defined.

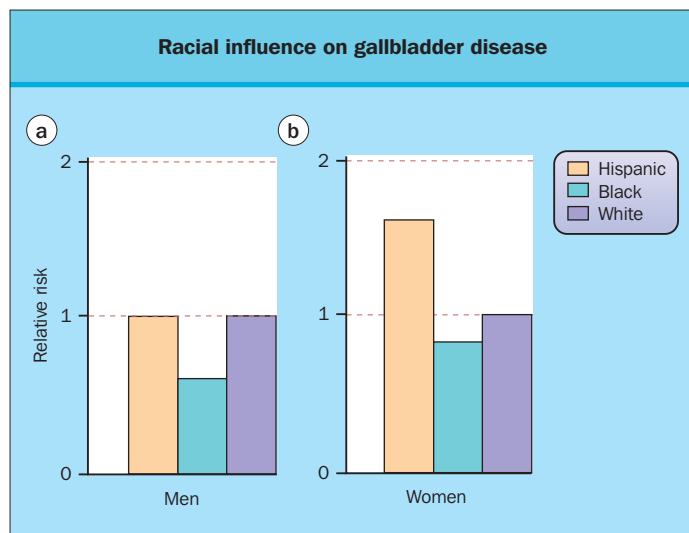
One theory to explain the higher rates of gallbladder disease in native Americans and Mexican Americans is genetic adaptation due to evolutionary forces from harsh nutritional conditions that promoted the efficient utilization and storage of nutrients by females. A postulated consequence of this genetic adaptation is rapid formation of gallstones during periods of nutritional excess. However, the risk for gallbladder disease varies among Hispanic subgroups. The 1982–1984 Hispanic Health and Nutrition Examination Survey (n = 2299) used screening ultrasonography and found that Mexican American men and women had a 1.5–1.8 times higher risk for gallstone disease than Cuban Americans and mainland Puerto Ricans. The risk was three times higher in women compared to men, and Mexican American women in the age group of 60–74 years had a prevalence of gallstones of 44.1%. Mixed genetic heritage best explains these differences. Mexican Americans are the descendants of native Americans originating from Mexico who admixed with European conquerors. Variable degrees of genetic admixture may explain the differences in prevalence of gallstone disease among different Hispanic subgroups.

#### *Pathogenesis*

Gallstones are typically classified by their chemical composition: cholesterol, black pigment and brown pigment. Cholesterol gallstones are defined as gallstones that are more than 50% cholesterol by weight. Cholesterol gallstones are not made of pure cholesterol; salts of calcium, pigments and glycoprotein may be found in the center, in radiating spikes, or in concentric rings within the stone. Pigment gallstones are composed of less than 20% cholesterol and contain carbonate, phosphate and calcium salts of bilirubin and palmitate distributed throughout the stone material. Black pigment stones are composed almost entirely of salts of bilirubin, occur in the setting of chronic hemolytic disease, and are formed in the gallbladder under sterile conditions. Brown pigment stones



**Figure 28.1 Prevalence of gallbladder disease.** (a) Non-Hispanic whites; (b) non-Hispanic blacks; (c) Hispanics. The prevalence of gallbladder disease (gallstones on screening ultrasonography or prior cholecystectomy verified by absence of gallbladder on screening ultrasonography) in the USA for men and women as determined from the Third National Health and Nutrition Examination Survey (NHANES III) is shown. In all age groups, women have a greater prevalence of gallbladder disease than men; the difference is most dramatic at younger ages.



**Figure 28.2 Racial influence on gallbladder disease.** (a) The relative risk of gallbladder disease based upon ethnicity is shown for men. Non-Hispanic black men had a lower risk of gallbladder disease compared with both non-Hispanic white men and Hispanic men. Relative risk was adjusted for age and body mass index (BMI). (b) The relative risk of gallbladder disease based upon ethnicity is shown for women. Compared with non-Hispanic white women, Hispanic women had a higher risk and non-Hispanic black women had a lower risk of gallbladder disease. Relative risk was adjusted for age and BMI.

are soft, amorphous and contain bilirubin salts plus a number of other components. They are the main type of primary bile duct stones, and tend to form in the setting of infection.

Our current understanding of the pathogenesis of cholesterol gallstones suggests that at least three independent processes may be required: secretion of 'lithogenic' bile by the liver, nucleation of cholesterol molecules to initiate crystal formation, and stasis of bile in the gallbladder to allow crystals to elongate and agglomerate to form 'sludge' or stones. Excess formation of

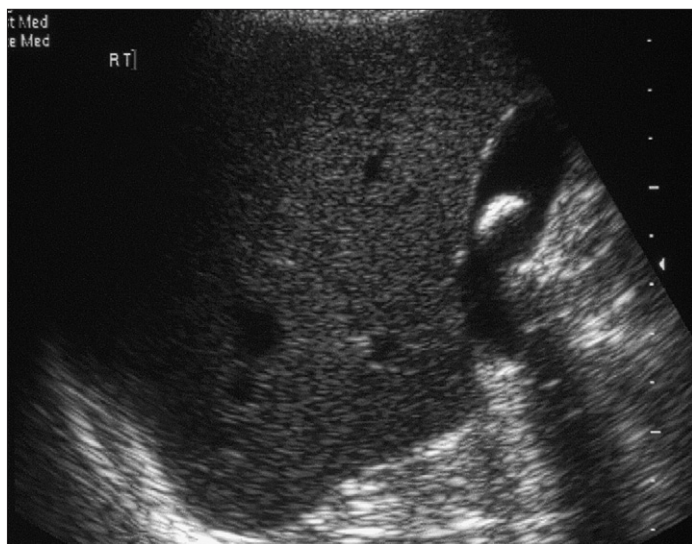
deoxycholate due to prolonged intestinal transit may contribute to these pathogenetic mechanisms.

The secretion of bile that is supersaturated with cholesterol is a prerequisite for cholesterol gallstone formation. Supersaturation may result from either enhanced cholesterol secretion (obesity, pregnancy, contraceptive steroids and estrogens) or reduced bile acid secretion (fasting, ileal disease or resection, rapid weight loss). The initial secretion of cholesterol by hepatocytes into bile is tightly coupled to phospholipid secretion in the form of unilamellar vesicles. When bile is unsaturated, bile acids enhance the disappearance of vesicles as cholesterol is transferred to cholesterol/bile acid/phospholipid micelles. When bile is saturated, the unilamellar vesicles aggregate into large, cholesterol-rich, multilamellar vesicles, which encourages nucleation of cholesterol molecules to form crystals and, ultimately, gallstones.

Gallbladder mucins and other glycoproteins secreted by the liver and, possibly, gallbladder mucosa have been proposed as pronucleation factors. These glycoproteins have been shown to promote the formation of cholesterol crystals from saturated bile and often are found in the core matrix of both cholesterol and pigment gallstones. In contrast, antinucleation factors (apoproteins A-1 and A-2) found in non-lithogenic bile may prevent the formation of cholesterol crystals from vesicular aggregates.

The healthy gallbladder prevents formation of gallstones by acidifying and concentrating bile, and by vigorously expelling crystals and sludge during contractions. Cholesterol nucleation takes place over days. If the gallbladder empties vigorously several times daily, removing mucus as well as bile from the gallbladder, then crystal formation and development of gallstones may not occur. Gallbladder hypomotility may be a factor in the formation of gallstones during TPN, rapid weight loss, prolonged fasting, pregnancy, celiac disease, massive small bowel resection and biliopancreatic bypass procedure for treatment of obesity. Diminished gallbladder contractility and delayed intestinal transit prolongs exposure of hepatic bile to intestinal anaerobic bacteria enhancing the transformation of cholate to deoxycholate. This





**Figure 28.3 Ultrasound detection of gallstones.** This ultrasonograph demonstrates a gallstone within the gallbladder lumen. Criteria for diagnosis include: echogenic concretions in the lumen, shadowing of the ultrasonographic beam, and mobility of the concretions with positioning of the patient. (Courtesy of Dr H. Irving.)

secondary bile acid is more lithogenic than its respective primary bile acid, cholate, promoting formation of gallstones and further inhibiting the motility of the gallbladder.

### **Clinical manifestations of gallstones**

#### **Asymptomatic gallstones**

Most patients with gallstones are asymptomatic and will remain asymptomatic for several years. Such patients are regularly detected on routine ultrasonography (Fig. 28.3). One study of the natural history of truly asymptomatic gallstones indicated that only 20% of these patients will develop symptoms when followed up to 25 years. It has been suggested that the asymptomatic gallstone population is really comprised of two populations: those that will ultimately become symptomatic (usually within the first 5 years of follow-up) and those that never will develop symptoms. The risk of developing symptoms is 1–3%/year for the first 5–10 years, but then drops to 0.1–0.3% with prolonged observation. The risk of developing serious complications from gallstones (acute cholecystitis, cholangitis, pancreatitis, sepsis, gangrenous gallbladder) is low, only about 0.1%/year. In the most comprehensive long-term study of natural history, biliary colic always preceded any serious complication by several months. Given the costs associated with cholecystectomy, the relatively benign natural history, and the fact that biliary colic nearly always precedes more serious complications, prophylactic cholecystectomy is not recommended for asymptomatic patients. However, prophylactic cholecystectomy may be considered in the following circumstances: patients with large ileal resections, patients with known gallstones who will be traveling or working in remote regions of the world, and certain populations or medical conditions that are associated with high rates of complications or cancer (Pima Indians, calcified gallbladder, porcelain gallbladder and gallstones associated with sickle cell disease). One

decision analysis suggested that patients with diabetes mellitus do not benefit from prophylactic cholecystectomy.

In general, most gallstones do not spontaneously dissolve or exit the gallbladder asymptotically. In the National Cooperative Gallstone Study, 1% of patients experienced spontaneous clearance of gallbladder stones over a period of 2 years while taking placebo. Even this low rate of clearance was questioned as the technique used to detect gallstones was oral cholecystography, which is much less sensitive than the currently recommended method of real-time ultrasonography (98% sensitivity and specificity). In contrast, two recent ultrasonographic studies suggested that one-third of cholesterol gallstones that develop during pregnancy dissolve. The type of stone that is more likely to dissolve is small (<0.5 cm diameter) and composed predominantly of cholesterol. In general, calcified stones, pigment stones and stones that have persisted over several years are not likely to dissolve spontaneously. Gallstones that are symptomatic are not likely to disappear spontaneously.

#### **Symptomatic gallstones**

Biliary colic is the most specific symptom of cholelithiasis and is defined as pain that is localized to either the right upper quadrant of the abdomen or epigastrium. It is characterized by a short crescendo period of increasing pain (5–15 min), a plateau of steady pain (15 min to several hours), and a decrescendo period of decreasing pain and resolution of pain (15 min to 2 h). Patients are asymptomatic between attacks of colic. Non-specific symptoms that do not correlate well with gallstones include the following: nausea, vomiting, dyspepsia, diarrhea, weight loss or gain and heartburn. Elderly, diabetic and immunocompromised patients may develop serious complications of gallbladder disease (gangrenous gallbladder, empyema of the gallbladder, perforation of the gallbladder, suppurative cholangitis) and exhibit few or no localizing abdominal complaints or findings on physical examination. In these patients gallbladder disease may present as fever, altered mental status, loss of appetite and weight loss, or sepsis.

The best data that address the natural history after an episode of biliary colic are from the National Cooperative Gallstone Study. Patients entering the trial had a preceding history of biliary colic and were then randomized to receive one of two doses of chenodeoxycholate or placebo. A total of 305 patients were enrolled in the placebo arm and were followed clinically. Patients with frequent attacks of biliary-type pain 12 months before entry into the trial continued to experience frequent attacks of pain. Some developed complicated biliary disease, and many required cholecystectomy. In contrast, those who had infrequent attacks of pain continued to have few attacks, experienced a lower complication rate and had lower rates of cholecystectomy. In general, patients with biliary colic can be expected to continue to experience biliary colic during follow-up. The severity of the attacks is highly variable but may be relatively consistent within a given individual. Patients with frequent, severe episodes may be at greatest risk for complicated gallbladder disease.

Rarely, a large gallstone in the gallbladder will erode through the gallbladder wall into an adjacent viscus (usually duodenum), traverse the bowel, impact at a point of narrowing (usually terminal ileum), and cause bowel obstruction or gastric outlet obstruction (Bouveret syndrome).

### Therapeutic options

#### Medical treatment

Medical treatment for gallstones include oral bile acid therapy with either chenodeoxycholic acid or ursodeoxycholic acid, contact dissolution with methyl-tert-butyl ether (MTBE) or extracorporeal shock-wave lithotripsy. Generally, these forms of treatment are of historical interest only and are seldom, if ever, used today. Small cholesterol gallstones that are newly formed after rapid weight reduction or pregnancy may be particularly amenable to dissolution with oral bile acids. However, high recurrence rates (10%/year for first 5 years) limit long-term effectiveness. The great success and high patient acceptance of laparoscopic cholecystectomy have virtually eliminated the clinical application of oral bile acid dissolution therapy, shock-wave lithotripsy and percutaneous puncture of the gallbladder for MTBE treatment.

#### Endoscopic sphincterotomy

Endoscopic sphincterotomy is recommended for the treatment of choledocholithiasis and its complications of obstructive jaundice, cholangitis or pancreatitis. Numerous studies of endoscopic sphincterotomy for choledocholithiasis have verified its efficacy at nearly eliminating the risk of subsequent complications of passage of additional bile duct stones. Patients who are reasonable surgical candidates should undergo cholecystectomy, because the risk of acute cholecystitis in patients who have had endoscopic sphincterotomy but whose gallbladder has been left in place is 5–20% during a 3–5 year follow-up. Preoperative endoscopic retrograde cholangiopancreatography (ERCP) and sphincterotomy should be restricted to patients with a high probability of choledocholithiasis such as those with jaundice, abnormal liver tests and ductal dilatation on abdominal ultrasonography. The common duct of patients suspected of having choledocholithiasis can be adequately evaluated by intraoperative cholangiogram at the time of laparoscopic cholecystectomy. Either postoperative ERCP with sphincterotomy or laparoscopic common bile duct (CBD) exploration (when surgical proficiency in this technique is available) is equally safe and efficacious in achieving stone clearance.

#### Surgery for symptomatic gallstones

A conservative non-interventional approach is only warranted for patients with asymptomatic gallstones. Once gallstones become symptomatic the gallbladder should be removed. Chronic use of analgesics in the treatment of biliary pain is discouraged as it merely masks symptoms and may increase the likelihood that the patient will only present later with more serious complications of the disease. Most clinicians recommend cholecystectomy for symptomatic disease based upon the high likelihood of recurrent symptoms and the risk of developing serious complications once biliary colic has occurred. Diabetic patients may be at particularly high risk for complications as compared with non-diabetics. Complications include severe cholecystitis, gangrenous gallbladder, empyema of the gallbladder and perforated gallbladder. The operative mortality of emergency surgery for complicated gallstone disease in diabetic patients has been reported to be as high as 15–20%, or four to five times higher than that for matched groups of non-diabetic patients.

Elective cholecystectomy is the preferred treatment for symptomatic cholelithiasis. The indications for cholecystectomy

include biliary colic, cholecystitis, symptomatic gallbladder polyps, gallstone pancreatitis, symptomatic biliary dyskinesia, calcified gallbladder wall, large gallstones (>2 cm), non-functioning gallbladder and being a chronic typhoid carrier. There are two basic techniques: open laparotomy or laparoscopy. Until the late 1980s, open laparotomy and cholecystectomy were the standard surgical methods against which all other therapies were judged. In 1988, it was shown that the gallbladder could be safely and effectively removed by laparoscopic technique. Since then most surgeons performing cholecystectomy have switched from the open to the laparoscopic technique and several studies have compared results of the two methods. Advantages of laparoscopic cholecystectomy include: short duration of hospital stay, lower hospital cost, early return to home and job, high patient acceptance, and low rate of complications, particularly in certain populations such as morbidly obese patients (Table 28.1). Disadvantages include: higher risk of major morbidity when the surgery is done by technically inexperienced surgeons, higher rate of serious bile duct injuries, and potential need to convert an elective cholecystectomy to an urgent cholecystectomy if complications occur. The risk of conversion to open cholecystectomy is increased in patients with acute disease, pancreatitis, bleeding disorders, unusual anatomy and prior abdominal surgeries. Open cholecystectomy may still be the preferred approach for patients with complex gallbladder disease [empyema, infarction, perforation causing generalized peritonitis or subphrenic abscess, biliary-enteric fistula leading to bowel obstruction (gallstone ileus) and bacterial cholangitis]; although at the present time no specific contraindications exist for laparoscopic cholecystectomy other than poor surgical risk factors.

The reported operative mortality rates for diabetics and non-diabetics are shown in Table 28.2. Stable diabetics who undergo elective cholecystectomy have no major increase in expected operative mortality. In contrast, diabetics who are poorly controlled and who undergo cholecystectomy for complicated biliary tract disease do experience increased morbidity and mortality. For these reasons, the development of biliary colic in a diabetic patient with gallstones is an indication for early elective cholecystectomy.

#### Postcholecystectomy syndrome

Approximately 5% of patients will experience persistent or recurrent non-specific symptoms after cholecystectomy. Most commonly patients complain of dyspepsia or upper abdominal pain. The differential diagnosis is extensive and includes recurrent or retained calculi in the cystic or CBD, pancreatitis, cholangitis, papillary stenosis or operative injury to the biliary tree. Once anatomic or mechanical factors have been eliminated it is necessary to consider the possibility of disordered biliary motility (biliary dyskinesia) as the basis for pain. Biliary dyskinesia implies a disorder of the normal contraction of the gallbladder or altered function of the sphincter of Oddi. After removal of the gallbladder, the source of biliary dyskinesia is limited to the sphincter of Oddi. The latter may be responsible for postcholecystectomy syndrome in 5–15% of patients. The diagnosis of sphincter of Oddi dysfunction is dependent upon accurate measurement of the basal pressure of the sphincter via biliary manometry at ERCP. Patients with basal sphincter pressures greater than 40 mmHg can experience relief of their pain after endoscopic sphincterotomy (Table 28.3). Additional

Surgical series of laparoscopic cholecystectomy						
Author	Year of study	Patients	Percentage to open	Mortality	Major complications	Bile duct injury
Larson	1992	1963	4.5	0.1	2.1	0.3
Southern Surg Club	1991	1518	4.7	0.07	1.5	0.5
Cuschieri	1991	1236	3.6	0	1.6	0.3
Soper	1992	618	2.9	0	1.6	0.2
Spaw	1991	500	1.8	0	1.0	0
Lillemoe	1992	400	4.0	0	5.0	0.5
Wolfe	1991	381	3.0	0.9	3.4	0
Deziel	1993	77 604	1.2	0.04	0.19	0.59
Wherry	1994	5607	8.08	0.04	0.5	0.57
Russell	1996	15 221				0.25
MacFadyen	1998	114 005	2.16	0.06		0.5
Hannan	1999	24 385		0.23	1.17	
Modified from Soper et al (94) by permission of N Engl J Med.						

**Table 28.1 Surgical series of laparoscopic cholecystectomy.** Large series confirm low complication rates after laparoscopic cholecystectomy with conversion rates to open cholecystectomy under 5%.

Mortality rates from acute complications of biliary tract disease: effect of diabetes mellitus						
Period	Surgery	Diabetes mellitus		Mortality rates (%)		Total
		No surgery	Total	Surgery	No diabetes No surgery	
1953–1958	31	13	20	10	3	4
1960–1981	7	0	7	2	8	3

**Table 28.2 Mortality rates from acute complications of biliary tract disease: effect of diabetes mellitus.** Mortality rates from biliary tract disease are consistently higher in patients with diabetes mellitus.

features that support the diagnosis and a response to sphincterotomy are: dilated CBD, abnormal liver tests and prolonged retention of contrast in the biliary system at the time of ERCP. Some patients have postcholecystectomy pain but lack any objective evidence for biliary disease ('type III' sphincter of Oddi dysfunction). In most cases, these patients do not respond to sphincterotomy, suggesting that sphincter function is not related to their symptoms. One group examined these patients for underlying visceral hyperalgesia in response to balloon distention of rectum and duodenum and selectively reproduced symptoms with duodenal distention. They also found that these patients were characterized by somatization, depression, obsessive-compulsive behavior and anxiety.

### Acute cholecystitis

Cholecystitis may or may not be associated with the presence of gallstones. Calculous cholecystitis occurs in over 90% of cases

Effectiveness of endoscopic sphincterotomy for sphincter of Oddi dysfunction								
Patient group	SO pressure <40 mmHg				Outcome (%)	SO pressure >40 mmHg		
	n	Good	Fair	Poor		Good	Fair	Poor
Sham	12	31	18	67	12	15	10	75
ES								
ES	12	7	12	58	11	80	11	9
(P = NS)					(P <0.05)*			

**Table 28.3 Effectiveness of endoscopic sphincterotomy for sphincter of Oddi dysfunction.** Sphincter of Oddi (SO) pressures >40 mmHg identify the patients most likely to benefit from endoscopic (ES) sphincterotomy.



and implies gallbladder inflammation in the presence of gallstones. The presence of gallstones may cause persistent obstruction of the gallbladder outlet because of impaction in the neck of the gallbladder, Hartmann's pouch, or cystic duct. These events lead to the release of a number of potential inflammatory mediators such as prostaglandins, cholesterol-supersaturated bile, lysolecithin and phospholipase A. The increase in intraluminal pressure also causes gallbladder distention that leads to wall edema and may compromise venous and lymphatic drainage resulting in ischemia and necrosis of the gallbladder. Although bile is sterile, acute cholecystitis may lead to secondary infection with positive bile cultures in 20–75% of patients. The most common organisms isolated from cultures of gallbladder bile are *Escherichia coli*, *Klebsiella* and *Enterococcus*. Clinical features of calculous cholecystitis include fever, right upper quadrant abdominal pain with tenderness to palpation that may radiate to the back, right scapula or right clavicular area, leukocytosis and ultrasonographic images that demonstrate cholelithiasis. Additional ultrasonographic features of cholecystitis may include thickening of the wall of the gallbladder and pericholecystic fluid.

Gallbladder sludge refers to gallbladder mucin with entrapment of particulate matter nucleated from bile (Fig. 28.4). Sludge formation occurs within the gallbladder when gallbladder emptying is slow and incomplete. It is often associated with prolonged fasting or lack of stimulation of intestinal release of cholecystokinin. Although sludge is a reversible stage in gallstone pathogenesis, gallstones may develop, leading to symptomatic biliary tract disease. Sludge is a risk factor for gallstones, but it may be evacuated from the gallbladder and gallstone formation may be prevented.

Acalculous cholecystitis is inflammation of the gallbladder in the absence of gallstones and represents 2–12% of cases of proven acute cholecystitis. Although the pathogenesis is unknown, multiple factors such as gallbladder stasis, paresis and ischemia may play a role. Acalculous cholecystitis is more likely to occur in critically ill patients and these patients tend to have higher morbidity and mortality rates. Risk factors for acute acalculous cholecystitis include: prior surgical procedure (non-biliary), male

gender, severe trauma, burns, TPN, multiorgan failure, diabetes mellitus, acute renal failure, immunocompromised host (AIDS, cancer chemotherapy, leukemia/lymphoma, uremia) and old age.

### Diagnosis

Murphy's sign, which is also known as 'inspiratory arrest', is a valuable sign of acute cholecystitis. The patient is asked to take a deep inspiration while the examining fingers are held under the liver border, usually around the tip of ninth rib where the gallbladder may descend upon them. Inspiration is arrested in midcycle by painful contact with the fingers. Carcinoma of the gallbladder may produce a similar sign when it has invaded through the gallbladder wall involving the serosa or visceral peritoneum overlying the gallbladder.

One may be nearly certain of the diagnosis in the appropriate clinical situation when the following are present: leukocytosis, fever and right upper quadrant tenderness to palpation. In the absence of the latter findings, one must rely upon imperfect confirmatory radiologic studies [ultrasonography, dimethyl iminodiacetic acid (HIDA)-scintigraphy]. Findings on ultrasonography that support the diagnosis of cholecystitis are: a thickened edematous gallbladder wall and a pericholecystic fluid collection. Although patients with acalculous cholecystitis may have sludge within the gallbladder lumen, sludge alone may not be pathologic. Sludge may be present in the absence of cholecystitis and absent in the presence of cholecystitis. A positive scan by HIDA-scintigraphy for acute cholecystitis is characterized by normal uptake and clearance of HIDA by the liver, rapid excretion into the biliary system, visualization of the extrahepatic bile ducts, and appearance of HIDA in the intestine, but no visualization of the gallbladder. This finding implies cystic duct obstruction from either inflammation or an impacted stone. False-positive scans may occur in patients on TPN, alcoholics, or those who have had a prolonged fast or who have just eaten. In addition, in acalculous cholecystitis, the cystic duct may be patent and allow entry of radionuclide into the gallbladder lumen, resulting in a false-negative scan. Positive findings on ultrasonography or HIDA-scintigraphy need to be assessed carefully before proceeding to cholecystectomy.

### Clinical management

The immediate management of acute cholecystitis consists of fasting, fluid and electrolyte correction and pain management. Definitive therapy is cholecystectomy although certain peri-operative medical complications increase morbidity and mortality (Table 28.4). Empiric antibiotic coverage is prescribed for patients presenting with systemic signs such as high fever, leukocytosis, tachycardia or hypotension; or if there is no clinical improvement after 12 h of conservative management. About 20% of patients will require emergent surgery for complications such as peritonitis or perforation. For the remaining patients, early laparoscopic surgery (within 48–72 h of presentation) has been shown to be superior to delayed surgery (6–12 weeks after the attack) due to an easier dissection, reduced hospital stay and reduced rates of complications of recurrent gallstone disease. However, there may be a slightly increased risk of gallstone and bile spillage, with the subsequent risk of abscess formation with early laparoscopic surgery.

Mirizzi syndrome is a clinical syndrome of cholecystitis and jaundice that develops when a stone impacts in Hartmann's



**Figure 28.4 Biliary sludge.** This ultrasonograph demonstrates 'sludge' within the gallbladder lumen. In contrast to the features of cholelithiasis, sludge is echogenic and mobile but fails to cast an ultrasonographic shadow. (Courtesy of Dr H Irving.)

Factors independently associated with operative mortality after cholecystectomy			
Characteristic	Number of patients (Total = 29 271)	Number of deaths (Total = 180)	Adjusted odds ratio (95% Conf. interval)
Emergent surgery	9023	141	3.2 (2.0–5.1)
Acute myocardial infarction	63	15	14.1 (7.0–28.0)
Cardiac arrhythmia	1636	59	7.4 (1.5–3.7)
Pneumonia	252	15	5.2 (3.2–8.3)
Renal failure	190	24	8.8 (5.2–15.0)
Cancer	420	29	5.2 (3.2–8.3)
Laparoscopic surgery (versus open surgery)	14 131	21	0.22 (0.13–0.37)

**Table 28.4** Factors independently associated with operative mortality after cholecystectomy.

pouch. The resulting inflammation compresses and obstructs the common hepatic duct or CBD. Obstruction of the bile duct further promotes the development of cholangitis. The stenosis of the bile duct can mimic a malignant stricture. Acute cholecystitis is often associated with mildly abnormal liver tests, but a serum bilirubin greater than 85  $\mu\text{mol/L}$  (5 mg/dL) strongly suggests CBD obstruction. If untreated, the stone may erode into the CBD and create a biliobiliary fistula. Management involves drainage of the biliary system, antibiotic therapy, intravenous fluids and cholecystectomy.

### Acute cholangitis

Acute cholangitis results from impaction of a gallstone in the bile duct and ascending infection behind the obstruction. Right upper quadrant pain, jaundice and fever (with chills and rigors) occurring together constitute the Charcot triad, which indicates acute cholangitis. The triad occurs in 70% of cases of acute cholangitis and is the most common complication of choledocholithiasis. Hypotension and mental confusion secondary to sepsis in a patient with Charcot triad constitutes the Reynold pentad. Only 10% of patients with acute suppurative cholangitis present with all five of these features. The most common cause of cholangitis in the USA is choledocholithiasis secondary to cholelithiasis. Primary bile duct stones are more common in oriental countries where oriental cholangiohepatitis is endemic. Other causes of cholangitis include: obstructing primary tumors of the ampulla, bile ducts or pancreas; primary sclerosing cholangitis, and post procedural effects (e.g. after ERCP). Relatively rare causes include: hemobilia, parasites and hereditary abnormalities of the biliary tree.

### Diagnosis

The incidence of bile duct stones increases with increasing age and is more likely to occur in patients with multiple gallstones. The diagnosis of CBD stones is suggested by biliary colic, abnormal liver tests, pale stools, dark urine and dilatation of the bile duct on ultrasonography or CT scanning. However, CBD stones may be present without dilatation of the CBD or abnormal liver tests. The diagnosis is established by cholangiography. Magnetic resonance cholangiopancreatography (MRCP) is accurate for detecting choledocholithiasis and delineating biliary anatomy, but the use of this test is probably of limited value

in acute cholangitis. The preferred diagnostic test is ERCP, because the retained stone may also be easily treated with a sphincterotomy.

### Clinical management

Therapy for cholangitis should be individualized because of the wide spectrum of severity of illness. Antibiotic therapy and intravenous hydration should be initiated promptly on an empiric basis. Antibiotics are tailored to the types of organisms associated with acute cholangitis: *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Bacteroides fragilis*. In the elderly or immunocompromised patient, anaerobic coverage is necessary since these patients are at increased risk for developing suppurative cholangitis. Effective antibiotic regimens include: ampicillin (or amoxicillin) plus an aminoglycoside, piperacillin alone, third generation cephalosporin or fluoroquinolones. In cases where bacteroides is suspected, the addition of metronidazole is recommended. Sulfonamides are not recommended for acute cholangitis. It is important to remember that antibiotics are viewed as adjuncts to the primary therapy, CBD drainage.

About 70% of patients will respond with a stabilization of their clinical course. Patients failing to respond to empiric therapy within 24 h should be considered for immediate biliary decompression since mortality rates approach 100% in this subgroup of patients without biliary drainage. The latter may be accomplished by endoscopic sphincterotomy with removal of the impacted stone, endoscopic placement of a biliary stent or nasobiliary drainage tube, percutaneous transhepatic drainage (radiology), or cholecystectomy plus CBD exploration or placement of T-tube. The choice of each of these modalities depends upon the expertise of the available specialists, the condition of the patient and estimated operative risk. In general, either endoscopic or surgical treatment is recommended. Radiologic placement of drainage catheters is usually restricted to the circumstance where endoscopy is unsuccessful. Several trials have examined the role of urgent endoscopy versus early surgery and have demonstrated that ERCP done early in the course of acute cholangitis is safe and effective in controlling biliary tract sepsis (Tables 28.5 and 28.6). It has not yet been determined whether the outcome is superior with the combination of early ERCP combined with sphincterotomy, CBD stone extraction

Severe acute cholangitis: mortality			
Cause	Surgery group (n = 41)	Endoscopic drainage group (n = 41)	
Heart failure (n)	2	0	
Bronchopneumonia (n)	2	2	
Renal failure (n)	1	0	
Multiorgan failure (n)	4	2	
Sepsis (n)	3	0	
Cerebellar hemorrhage (n)	1	0	
Total	13	4	<i>P</i> <0.03

**Table 28.5 Severe acute cholangitis: mortality.** Mortality in patients with severe acute cholangitis: a randomized trial comparing urgent biliary tract surgery to endoscopic cholangiography and drainage. This randomized study confirmed lower mortality rates with endoscopic therapy.

and drainage of the biliary system followed by elective laparoscopic cholecystectomy as compared with urgent cholecystectomy, CBD exploration and T-tube placement. There also have been no critical evaluations of costs associated with each of these two approaches.

### Pancreatitis and gallbladder carcinoma

Additional complications of CBD stones include obstructive jaundice and gallstone pancreatitis. Endoscopic retrograde cholangiopancreatography with sphincterotomy is recommended for removal of CBD stones, even in the setting of active gallstone pancreatitis. If complete stone extraction is not possible due to medical, technical or anatomic reasons, then palliative placement of biliary and/or pancreatic stents is indicated to relieve the intraductal pressure and prevent recurrent obstruction. Elective cholecystectomy, either open or laparoscopic, is indicated after the first episode of gallstone pancreatitis to prevent future recurrence of pancreatitis.

Carcinoma of the gallbladder is the most common malignant lesion of the biliary tract and the fifth most common among malignant neoplasms of the digestive tract. Approximately 0.1–3% of gallbladders removed for symptomatic cholelithiasis or cholecystitis, especially in the elderly, contain adenocarcinoma. Pima Indians, who have an extremely high prevalence of gallstones, also have the highest rate of gallbladder cancer and mortality in the USA. Calcified (porcelain) gallbladders pose an extreme risk for development of carcinoma, as high as 25%. Gallbladder polyps are another predisposing factor for gallbladder neoplasm. In a retrospective series of 100 patients, 88% of polyps larger than 10 mm were malignant polyps. Therefore patients with polyps >10 mm should undergo elective cholecystectomy even in the absence of symptoms.

### CYSTIC DISEASES OF THE LIVER

The fibrocystic diseases of the liver are autosomal-dominant polycystic disease, solitary hepatic cysts, congenital hepatic fibrosis, choledochal cysts and Caroli disease. Histopathologically they are characterized by ectasia of intrahepatic bile ducts, cysts of biliary epithelial cell origin, portal fibrosis and persistence or

Severe acute cholangitis: morbidity			
Complication	Surgery group (n = 41)	Endoscopic drainage group (n = 41)	
Heart failure (n)	1	3	
Bronchopneumonia (n)	15	7	
Wound dehiscence (n)	1	0	
Wound infection (n)	7	1	
Gastrointestinal bleeding (n)	2	0	
Renal dysfunction (n)	11	7	
Bleeding after papillotomy (n)	0	1	
Disseminated intravascular coagulopathy (n)	2	0	
Total	39	19	<i>P</i> <0.05

**Table 28.6 Severe acute cholangitis: morbidity.** Morbid complications in patients with severe acute cholangitis: a randomized trial comparing urgent biliary tract surgery to endoscopic cholangiography and drainage. This randomized study confirmed lower complication rates with endoscopic therapy.

lack of remodeling of the embryonic ductal plate (ductal plate malformation). The clinical spectrum ranges from an asymptomatic state to cholangitis or manifestations of liver failure. Polycystic liver disease is highlighted since there have been many recent advances in its natural history, genetics and treatment.

### Polycystic liver disease

#### Classification

Human polycystic liver disease is due to mutations in four distinct genes and is one of the most common inherited disorders involving the liver (Table 28.7). Mutations in two of these genes, *PKD1* and *PKD2*, are associated with autosomal dominant polycystic kidney disease (AD-PKD). In AD-PKD, renal cystic disease tends to dominate the clinical picture. Hepatic cysts are the most common extrarenal manifestation. *PKD1* mutations account for 85–90% of cases of AD-PKD; nearly all of the remaining 10–15% are due to mutations in *PKD2*. The possibility of a '*PKD3*' gene has been proposed since families with AD-PKD, unlinked to *PKD1* or *PKD2*, have been occasionally reported in the literature. Patients with mutations in *PKD2* tend to have later-onset disease and greater life expectancy compared to patients with mutations in *PKD1*. Mutations in a third gene, *PRKCSH*, and a recently identified fourth gene, *SEC63*, cause the comparatively rare, isolated form of autosomal dominant polycystic liver disease (PCLD). The latter patients with PCLD are distinguished from AD-PKD patients by lack of renal cystic disease.

#### Natural history

The natural history of polycystic liver disease, regardless of etiologic mutation, is strikingly similar. The prevalence and number of hepatic cysts in patients with AD-PKD increase with



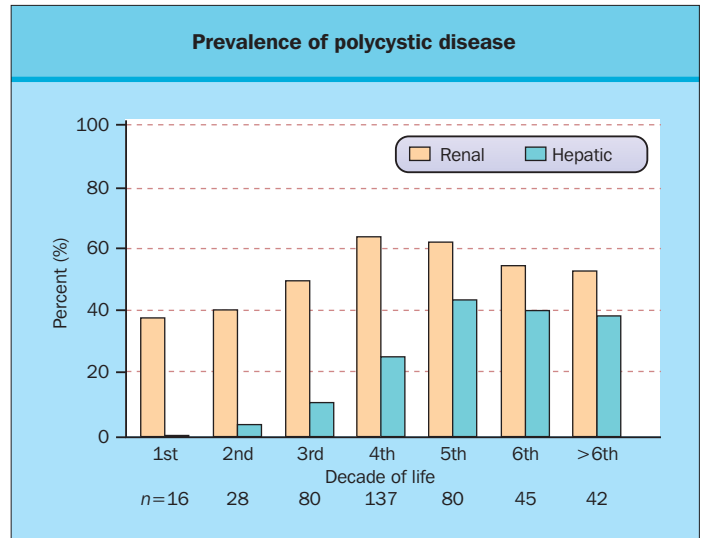
Genetic and phenotypic classification of polycystic disease			
Inheritance	Gene	Protein	Disease expression
Autosomal dominant (adults) <sup>1</sup>			
AD-PKD 1	<i>PKD1</i>	polycystin-1	Liver and kidney
AD-PKD 2	<i>PKD2</i>	polycystin-2	Liver and kidney
PCLD 1 <sup>2</sup>	<i>PRKCSH</i>	hepatocystin	Liver
PCLD 2 <sup>2</sup>	<i>SEC63</i>	sec63	Liver
Autosomal recessive (pediatrics)			
AR-PKD	<i>PKHD1</i>	fibrocystin	Kidney <sup>3</sup>
AD-PKD, autosomal dominant polycystic kidney disease; PCLD, isolated polycystic liver disease; AR-PKD, autosomal recessive polycystic disease.			
<sup>1</sup> Autosomal dominant forms of polycystic disease tend to present clinically in late adolescence or adult life. In contrast, autosomal recessive polycystic kidney disease is a serious renal disease in pediatric populations.			
<sup>2</sup> The <i>SEC63</i> gene mutations for PCLD have only recently been described and the designations of PCLD 1 and PCLD 2 not yet established.			
<sup>3</sup> The most common hepatic association of AR-PKD is congenital hepatic fibrosis.			

**Table 28.7 Genetic and phenotypic classification of polycystic disease.**

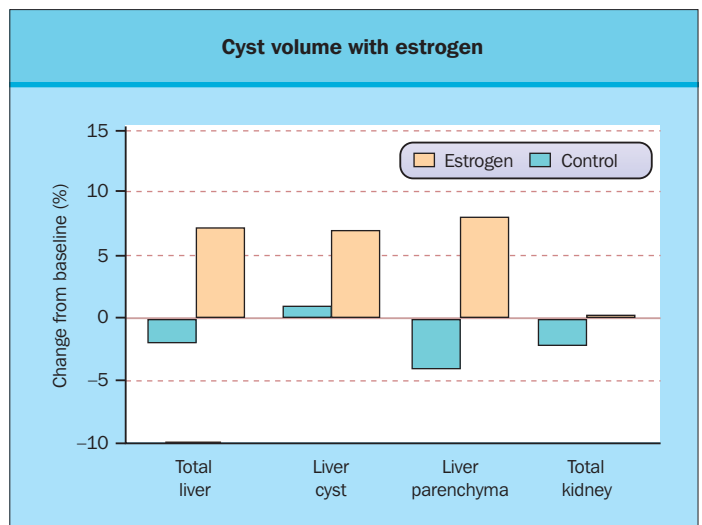
increasing age, female gender, severity of renal cystic disease and severity of renal dysfunction (Fig. 28.5). By age 60 nearly 80% of AD-PKD patients have hepatic cysts. While men and women have equal lifetime risk to develop hepatic cysts, women experience greater numbers and larger cysts. Severe hepatic cystic disease correlates with both the numbers of pregnancies and the use of exogenous female steroid hormones. One longitudinal study of anovulatory women with AD-PKD treated with hormone replacement suggested that estrogens selectively increase the severity of hepatic cystic disease (Fig. 28.6). Female tendency toward more extensive hepatic cystic disease has also been noted in PCLD.

### Genetics (Table 28.7)

The 14.1 kb *PKD1* gene has been cloned and sequenced and the resultant protein of 4304 amino acids, polycystin-1 (PC-1), has been characterized. There are over 230 mutations of the *PKD1* gene associated with disease which are evenly dispersed without evidence for clustering. However, some studies have suggested that the type or position of mutation may predict intracranial aneurysms. Furthermore, a study of 80 families with AD-PKD due to mutations in *PKD1* indicated that the location of the mutation in the 5' end of the gene predicted more rapid progression to, and greater severity of, end-stage renal disease, but more severe disease has also been attributed to mutations within the N-terminus. Any correlation between the type or position of the mutation and variation in hepatic cystic disease has not been demonstrated.



**Figure 28.5 Prevalence of polycystic disease.** The frequency of renal and hepatic cysts is displayed by age in a population at risk for autosomal dominant polycystic kidney disease (AD-PKD). Cysts were detected by real-time ultrasonography. The population at risk included 239 patients with AD-PKD and 189 unaffected family members. The number of subjects in each decade is indicated at the bottom.



**Figure 28.6 Cyst volume: postmenopausal estrogen increases liver volume.** Percentage of volume change in liver and kidney at the end of 1 year of follow-up is shown. Estrogen-treated patients are depicted by the green bar and non-treated controls as an orange bar.

The *PKD2* gene is smaller than *PKD1* (only 5.3 kb) and codes for a 968 amino acid protein, polycystin-2 (PC-2). There are over 60 identified mutations of *PKD2* and, like the mutations of *PKD1*, they are evenly dispersed throughout the gene without clustering at any one position. The severity of disease may vary with site of mutation in *PKD2* and the functional consequence on the resultant PC-2 protein. The relationship of the site of mutation of *PKD2* to phenotypic expression of polycystic disease is under study but no clear relationships have yet been reproducibly defined.

The first demonstration that PCLD was genetically distinct from *PDK1* and *PKD2* was from phenotypic and genetic studies

of three generations of a family affected by polycystic liver disease without renal cysts. It was later determined that PCLD was due to mutations in protein kinase C substrate 80K-H gene (*PRKCSH*) on chromosome 19p13. *PRKCSH* encodes for hepatocystin. Mutations that affect mRNA splicing or truncate hepatocystin have been reported in PCLD families. However, the distribution of mutations within the gene and the genotype–phenotype relationships remain speculative. Further studies indicated that the *PRKCSH* locus was probably not responsible for all cases of PCLD, and suggested the presence of a second PCLD gene. Davila studied 10 families by linkage analysis and identified a locus on chromosome 6q as containing a second PCLD gene. An analysis of genes in that region identified mutations in *SEC63* as causing PCLD in eight families. *SEC63* encodes a protein in the endoplasmic reticulum. The authors hypothesized that the protein could interact with the polycystin proteins from *PKD1* and *PKD2* and suggested that disruption of this interaction could be relevant for the development of cystic disease.

### Characteristics of liver cyst epithelium

Liver cysts arise from intrahepatic bile duct epithelial cells. The bile duct epithelium normally comprises 2–4% of the liver cell mass and serves to dilute and alkalinize bile in a hormone-dependent fashion. Secretin stimulates cyclic adenosine monophosphate (cAMP)-dependent  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion by biliary epithelia. Liver cyst epithelial cells retain differentiated secretory function as AD-PKD liver cysts secrete fluid and generate a positive luminal pressure after intravenous secretin administration.

### Characteristics of polycystin-1 and polycystin-2

PC-1 is a large (around 460 kDa) integral membrane protein with a prominent extracellular NH<sub>2</sub>-terminal domain, 11 putative transmembrane domains, and a small intracellular COOH-terminal domain. PC-1 might regulate  $\text{Ca}^{2+}$  or G-protein signaling and may interact with PC-2. PC-2 is a 110 kDa integral membrane protein with intracellular NH<sub>2</sub>- and COOH-terminal tails. The transmembrane domains share marked homology with voltage-gated cation channels and the COOH terminus has an EF-hand domain, a motif often expressed in voltage-gated calcium channels. Biophysical studies have confirmed that PC-2 functions as a cation channel. Together, the PC-1 and PC-2 complex is predicted to form a signal transduction unit that translates extracellular cues into intracellular information. Additional studies demonstrated that PC-1 and PC-2 form a mechanotransduction complex co-localized to the primary cilium of epithelial cells. Interestingly, two other proteins that give rise to epithelial cysts, polaris and cystin, also localize to the primary cilium of epithelial cells. Furthermore, fibrocystin, the protein linked to autosomal recessive PKD (ARPKD), is localized to the primary cilium of biliary epithelia and reduction of fibrocystin expression leads to loss of ciliary structure.

### Contributory events in cyst expansion

Proliferating epithelial cells form nascent cysts that detach from the original duct to form autonomous structures that no longer communicate directly with the duct. Expansion of these autonomous cysts can be promoted by luminal fluid secretion, remodeling of the underlying matrix and neovascularization.

Normal intrahepatic bile duct cells function as both absorptive and secretory epithelium where net absorption is favored under basal conditions. Secretion is triggered by hormonal signaling, primarily via secretin. Retaining characteristics of intrahepatic bile duct epithelial cells, human hepatic cysts generate a positive intraluminal pressure under basal conditions and have increased rates of fluid secretion following intravenous administration of secretin. For cyst epithelium to invade the surrounding tissues, the extracellular matrix must be remodeled. Matrix remodeling occurs through the secretion of metalloproteases into the surrounding matrix, vascularization for metabolic support, and production and secretion of a rich cocktail of cytokines and growth factors.

### Molecular diagnostics

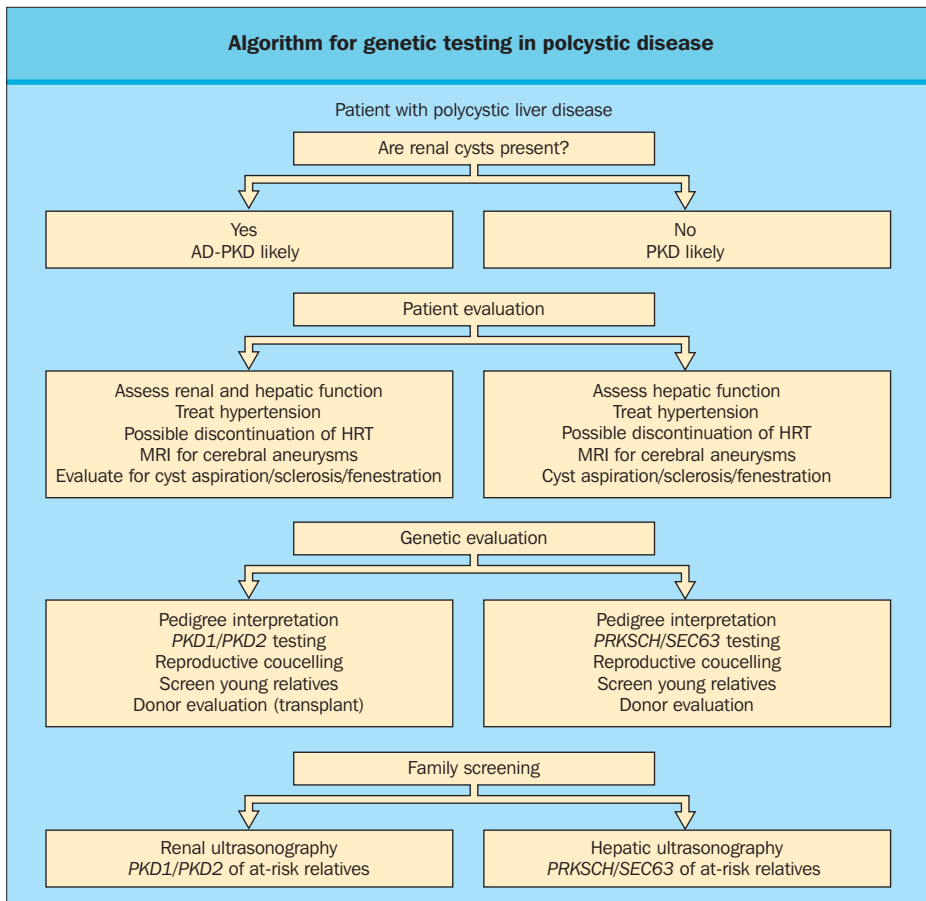
The molecular diagnostic approaches to AD-PKD have advanced considerably with the availability of direct gene sequencing (Table 28.8). Using commercially-available methods, pathologic mutations are detected in 44–76% of families with mutations of *PKD1* and 75% of families with mutations of *PKD2*.

Molecular genetic testing is relatively new and consensus guidelines are lacking. Ultrasonography is a reasonable screening tool in adults (Fig. 28.7). In young (age < 30 years), presymptomatic individuals at risk of AD-PKD, molecular genetic testing may offer a number of advantages over other approaches. In this group, ultrasonography may lack sensitivity and linkage analysis is impractical. The identification of a *PKD1* or *PKD2* mutations could affect family planning or choice of future diagnostic studies. Discovery of AD-PKD mutations may also encourage regular blood pressure monitoring and screening for associated conditions such as cerebral aneurysms or mitral valve prolapse. Genetic testing may also have a role in the evaluation of young family members being considered as living donors for renal transplantation to another family member with renal failure from AD-PKD. Clarifying the mutation status as negative in such potential donors would reduce future risks to both the donor and the recipient.

Clinical genetic testing for PCLD is also available in Europe and includes genetic sequencing of *PRKCSH*. Clinical testing for *SEC63* mutations will likely be available soon. Because the sensitivity of ultrasonography in PCLD is undefined, use of *PRKCSH* testing (and ultimately *SEC63* testing also) to identify presymptomatic patients at risk of the disease, may have even more relevance than in AD-PKD. While genetic testing results may not immediately alter the management of a PCLD-affected patient, the ability to screen other asymptomatic, at-risk family members (and potential transplant donors) for mutations is important for patient care. As patients are frequently concerned about the risks to their offspring, formal genetic counseling is recommended even when the mutation status is unknown.

### Clinical features

Patients with small (<2 cm) or few hepatic cysts tend to be clinically asymptomatic. In contrast, patients who develop massive hepatic cystic disease, based upon a definition for massive as a total liver cyst:parenchymal volume ratio >1, become symptomatic with abdominal pain or discomfort, early postprandial fullness, or shortness of breath. Rarely, patients can experience complications of advanced liver disease such as portal hypertension with variceal hemorrhage. The consequences of



**Figure 28.7** Algorithm for genetic testing in polycystic disease.



**Figure 28.8** Cysts on CT. Multiple cysts are seen throughout the liver in a patient with polycystic liver disease. (Courtesy of Dr H. Irving.)

progressive AD-PKD include renal failure, requirement for hemodialysis and renal transplantation. Dialysis, in particular, is thought to increase the risk for hepatic complications. A single center reported that 21% of polycystic patients on dialysis experienced hepatic complications, mainly hepatic cyst infection, hemorrhage or cyst adenocarcinoma, a finding that was not confirmed in a subsequent report by a different center.

Typically, liver parenchymal volume is preserved despite extensive hepatic cystic disease and extraordinary distortion of the hepatic architecture. The only blood test abnormality is a modest elevation of  $\gamma$ -glutamyltransferase, which correlates with hepatic cyst burden. Rarely, a patient with polycystic liver disease will experience hepatic decompensation and variceal hemorrhage, ascites or encephalopathy.

The most common, clinically-relevant complications arising in hepatic cysts are intracystic hemorrhage, infection or post-traumatic rupture (Table 28.9). Cyst adenocarcinoma, biliary obstruction, Budd-Chiari syndrome or hepatic failure is rarely reported. Associated conditions include mitral valve prolapse, diverticulosis, inguinal hernia and cerebral aneurysm.

### Therapy

#### Medical treatment

There are no effective medical therapies for polycystic liver disease. Somatostatin analogs, which block the effects of secretin, failed to demonstrate any significant effect on hepatic cyst growth or size. As noted above, hepatic cystic disease, but not renal cystic disease, worsens under the influence of female gender, pregnancy or use of exogenous female steroid hormones. These observations suggest that women with polycystic livers should avoid estrogen replacement therapy. However, proof that avoidance of estrogen effectively alters the course of hepatic cystic disease is lacking. The clinician must individualize hormonal replacement therapy in polycystic patients by weighing the



Complications of polycystic liver disease			
Classification	Specific type	Diagnostic tests	Treatment
Arising within cyst			
	Infection	MRI, In <sup>111</sup> WBC scan	Fluoroquinolones Drainage
	Hemorrhage	CT or MRI	Pain control Drainage (rare)
	Carcinoma	CT or MRI Aspiration cytology	Surgery
Compression by cyst			
	Biliary obstruction	ERCP	Stent placement Cyst decompression
	Venous obstruction		
	– Hepatic	Hepatic venography	Cyst decompression Resection Transplantation
	– Portal	MRI/MRA Mesenteric angiography	Cyst decompression Resection Transplantation
Hepatic dysfunction	Portal hypertension	Endoscopy (varices) US/CT/MRI	Band ligation Cyst decompression Resection Transplantation
	Hepatic failure	Exceedingly rare Look for other cause	Transplantation
CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; In-WBC scan, Indium-labeled white blood cell scan; MRI, magnetic resonance imaging; US, ultrasonography.			

Table 28.8 Complications of polycystic liver disease.

potentially deleterious effect on hepatic cystic disease against other potential benefits and risks.

#### Radiologic cyst aspiration and sclerosis

Symptomatic patients with one or a few dominant cysts may be considered for cyst aspiration and sclerosis. Most patients with polycystic disease have too many cysts or the cysts are of insufficient size to warrant this approach. Cyst sclerotherapy requires ultrasonographic or computed tomography (CT) guided percutaneous puncture of the targeted cyst and placement of an intracystic drainage catheter. Success in obliterating individual cysts in polycystic patients is approximately 70–90%.

#### Cyst fenestration

Cyst fenestration is a common surgical approach to symptomatic, massive hepatic cystic disease. Two approaches have been used: open laparotomy and, more recently, laparoscopy. Several series of open laparotomy, encompassing large numbers of patients, indicate that this approach results in general amelioration of symptoms. However, open laparotomy is associated with prolonged hospitalizations, morbidity i.e. bleeding, infection, bile leak, ascites (0–50%), and even mortality (<1%). Because it is less invasive, laparoscopic cyst fenestration is gaining increasing acceptance as an alternative surgical technique (Fig. 28.8). The advantages of laparoscopic surgery include: less morbidity,

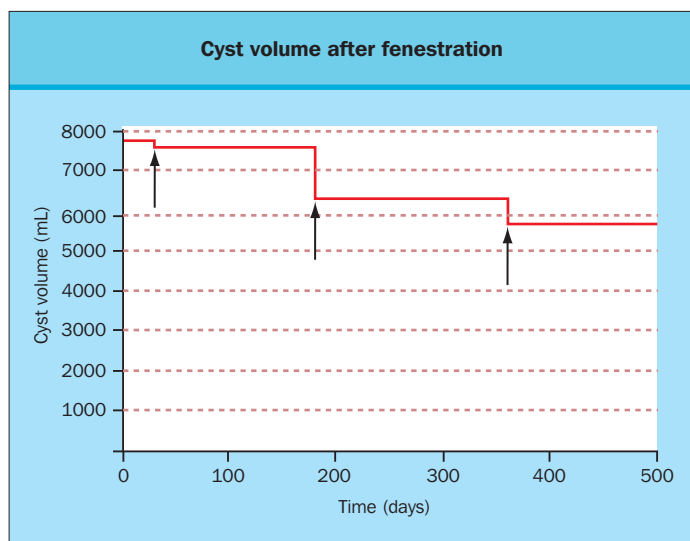
reduced hospital stay and the potential for outpatient surgical management. One recent review, totaling 40 cases, indicated that symptoms recurred in about half necessitating repeat laparoscopic cyst fenestration. Although there were no deaths, surgical conversion to open surgical cyst fenestration occurred in 10% of cases and 35% of patients experienced complications.

#### Liver resection

One center reported their experience with partial liver resection in the management of 31 patients with highly symptomatic, massive hepatic polycystic disease. Patients ages ranged from 34 to 69, the gender ratio (M:F) was 3:28, and renal function varied from normal to dialysis-dependent. Nearly all patients experienced significant relief from symptoms and sustained reduction in symptoms was common (>95%). However, over 50% of the patients experienced significant perioperative morbidity and there was one perioperative death (due to rupture of an intracranial aneurysm). Therefore, most surgeons reserve hepatic resection for those cases that are refractory to cyst decompression.

#### Liver transplantation

Polycystic liver patients with symptoms (e.g. severe abdominal pain or severe weight loss due to early satiety) refractory to other treatments or symptomatic hepatic cystic disease associated with end-stage renal cystic disease may be considered for



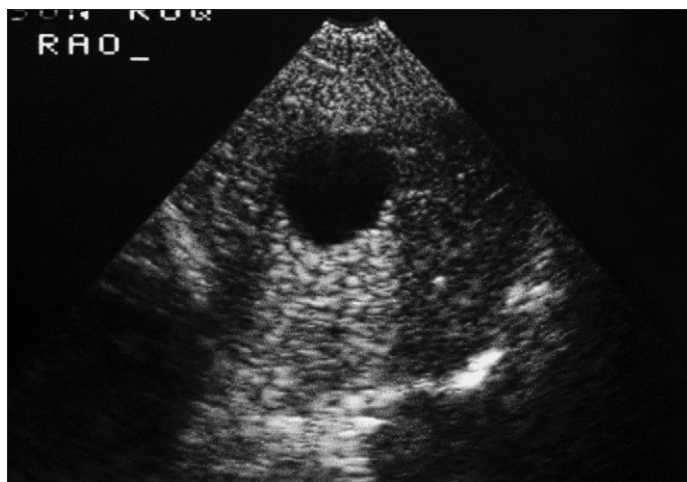
**Figure 28.9 Cyst volume after fenestration.** The reduction in liver volume achieved by sequential laparoscopic cyst fenestration in a single patient is shown. Initial liver volume was 7710 mL, and the final volume after three procedures (arrows indicates the days of laparoscopic cyst decompression) was 5677 mL.

liver or combined liver-kidney transplantation. Rare indications for hepatic transplantation include variceal hemorrhage, ascites, obstruction of hepatic venous outflow (Budd-Chiari equivalent), or biliary tract obstruction by extensive cystic disease not amenable to other interventions. Survival at 1, 3 and 5 years for patients undergoing isolated hepatic transplantation ( $n = 128$ ) are 78.1, 71.7 and 68.7%, respectively. Survival at 1, 3 and 5 years for patients undergoing combined liver-kidney transplantation ( $n = 78$ ) are 79.5, 75.5 and 75.5%, respectively. The genetic basis of polycystic liver disease warrants caution with respect to living donor liver transplantation.

### Solitary hepatic cyst

#### Characteristics

Solitary hepatic cysts are relatively common, usually asymptomatic, and most often are discovered incidentally during the evaluation of a wide variety of abdominal symptoms or disorders. The exact prevalence of solitary hepatic cysts for the US population is unknown, but the female:male ratio is approximately 4:1. A recent Taiwanese study used ultrasonography in a large-scale community-based screening program for simple hepatic cysts to explore the age- and sex-specific prevalence, and in a hospital-based study to record the size of simple hepatic cysts. A total of 3600 subjects in eight communities underwent screening ultrasonography, and 156 simple hepatic cysts were detected in 132 study subjects. The overall prevalence was, therefore, 3.6%. The prevalence increased with age, ranging from 0.8% below age 40 to 7.8% in subjects over 60 years of age. The size of 219 hepatic cysts in 167 hospitalized patients were as follows: 53% had diameters between 1 and 3 cm, and only 7% were larger than 5 cm. Cysts occurred more commonly in the right lobe and were twice as common in women. All of these cysts were asymptomatic and none of the patients suffered clinical consequences (Fig. 28.9).



**Figure 28.10 Solitary hepatic cyst.** Ultrasonography of a solitary cyst in the right lobe of the liver. (Courtesy of Dr H. Irving.)

#### Treatment

Asymptomatic solitary hepatic cysts are best managed conservatively. The preferred treatment of symptomatic cysts is ultrasound- or CT-guided percutaneous cyst aspiration followed by alcohol (or doxycycline) sclerotherapy. This approach is more than 90% effective in controlling symptoms and ablating the cyst cavity. The recurrence rate after successful ablation is only 5–15%. If the radiologically guided, percutaneous approach is ineffective or unavailable, treatment may include either laparoscopic or open surgical cyst fenestration. The laparoscopic approach is increasingly utilized for anatomically accessible cysts and greater than 90% efficacy is reported.

### Congenital hepatic fibrosis

#### Characteristics

Congenital hepatic fibrosis is an uncommon, autosomal-recessive, inherited disorder that is most often associated with autosomal recessive polycystic kidney disease (ARPKD). Mutations responsible for ARPKD, and presumably congenital hepatic fibrosis, arise in the *PKDH 1* gene which encodes the protein fibrocystin (Table 28.7). Other clinical associations of congenital hepatic fibrosis include renal dysplasia, nephronophthisis, Meckel-Gruber syndrome, Ivemark syndrome, Jeune syndrome, vaginal atresia and tuberous sclerosis. Congenital hepatic fibrosis can also coexist with other fibrocystic liver diseases such as Caroli disease and choledochal cyst. The histopathologic features may vary, but in nearly all cases there is fibrous enlargement of the portal tracts, which contain abnormally shaped bile ducts. Congenital hepatic fibrosis typically involves all lobes of the liver equally, but on occasion one lobe of the liver may be preferentially affected. Von Meyenburg complexes (VMCs), also referred to as bile duct microhamartomas, are dilated, ectatic, intra- and interlobular bile ducts embedded in a fibrous stroma, and occur in nearly all patients with congenital hepatic fibrosis (VMCs are also commonly found in both ADPKD and Caroli disease).

#### Clinical features

Congenital hepatic fibrosis presents in three clinical forms: portal hypertension, recurrent cholangitis and asymptomatic or

latent disease. The first two forms are usually diagnosed in early childhood in patients who present with variceal hemorrhage or unexplained biliary sepsis, respectively. In contrast, some patients will be detected later, during their adult years, when they are evaluated for unexplained hepatomegaly or portal hypertension. Rarely, patients present with evidence of both portal hypertension and cholestasis, the latter due to either associated biliary anomalies (Caroli disease) or to intrinsic destructive cholangiopathy. In general, hepatic function is well preserved, despite portal hypertension or bouts of cholangitis, although some patients experience progressive hepatic failure in long-term follow-up.

### Treatment

The first-line treatment of variceal hemorrhage is endoscopic variceal eradication (either sclerotherapy or ligation treatment), followed by institution of  $\beta$ -adrenergic blockade. In most cases, varices may be successfully obliterated by the endoscopic approach, thereby controlling this potentially life-threatening complication. Surgical shunts or transjugular intrahepatic portosystemic shunt (TIPS) are reserved for patients who fail endoscopic therapy, bleed from gastric varices or who have portal hypertensive gastropathy. Occasionally patients will experience progressive hepatic fibrosis and hepatic dysfunction after long-standing portosystemic shunt surgery, and development of this complication may necessitate consideration for liver transplantation.

In patients with cholangitis, radiologic imaging [ultrasonography, biliary radionuclide scintigraphy, CT or magnetic resonance imaging (MRI)] may be required to determine whether the patient with congenital hepatic fibrosis has concomitant biliary cystic disease. If the latter is present, the treatment of cholangitis is centered on provision of adequate biliary drainage, relief of obstruction (papillotomy with stone extraction or stricture dilatation), and control of infection with antibiotics. In the absence of biliary cystic disease or cholangiocarcinoma (said to occur in as many as 6% of cases of congenital hepatic fibrosis), cholestasis may be related to idiopathic inflammatory destructive cholangiopathy and respond to ursodeoxycholic acid therapy. Indications for hepatic transplantation include variceal hemorrhage or hemorrhage from portal hypertensive gastropathy that is not responsive to endoscopic treatment or amenable to portosystemic shunt surgery or TIPS, recurrent cholangitis that is not amenable to medical, endoscopic, radiologic or surgical therapy; and hepatic failure (development of coagulopathy, biochemical deterioration, ascites or portosystemic encephalopathy).

### Choledochal cyst

#### Characteristics

Choledochal cysts are usually present since childhood but do not cause sufficient symptoms to lead to their detection until adulthood in at least 20% of patients. Choledochal cysts are cystic dilatations that may occur throughout the macroscopic intra- and extrahepatic biliary tree. Although the term choledochal cyst has been used for any cystic dilatation of the biliary tree, isolated choledochal cysts are usually restricted to only the common hepatic or bile duct. Despite the uncommon occurrence of choledochal cysts, there are hundreds of reports in the literature encompassing over 3000 cases. Choledochal cyst is a rare condition in the Western hemisphere, but it is relatively more common among Japanese and other Asian popu-

lations. Several classifications of choledochal cysts have been proposed but the most commonly cited in the medical literature by Todani (77) is outlined in **Figure 28.10**. In this system, the most common cyst is a type I cyst, representing more than 80% of cases. The pattern of inheritance is unclear and there have been no definitive studies of either the genetic markers or molecular biology of choledochal cysts.

The pathogenesis of choledochal cysts is unknown. Histologically, the cyst walls are made up of fibrous tissue representing a chronic inflammatory process and the epithelial lining is either partially or completely absent.

### Clinical features

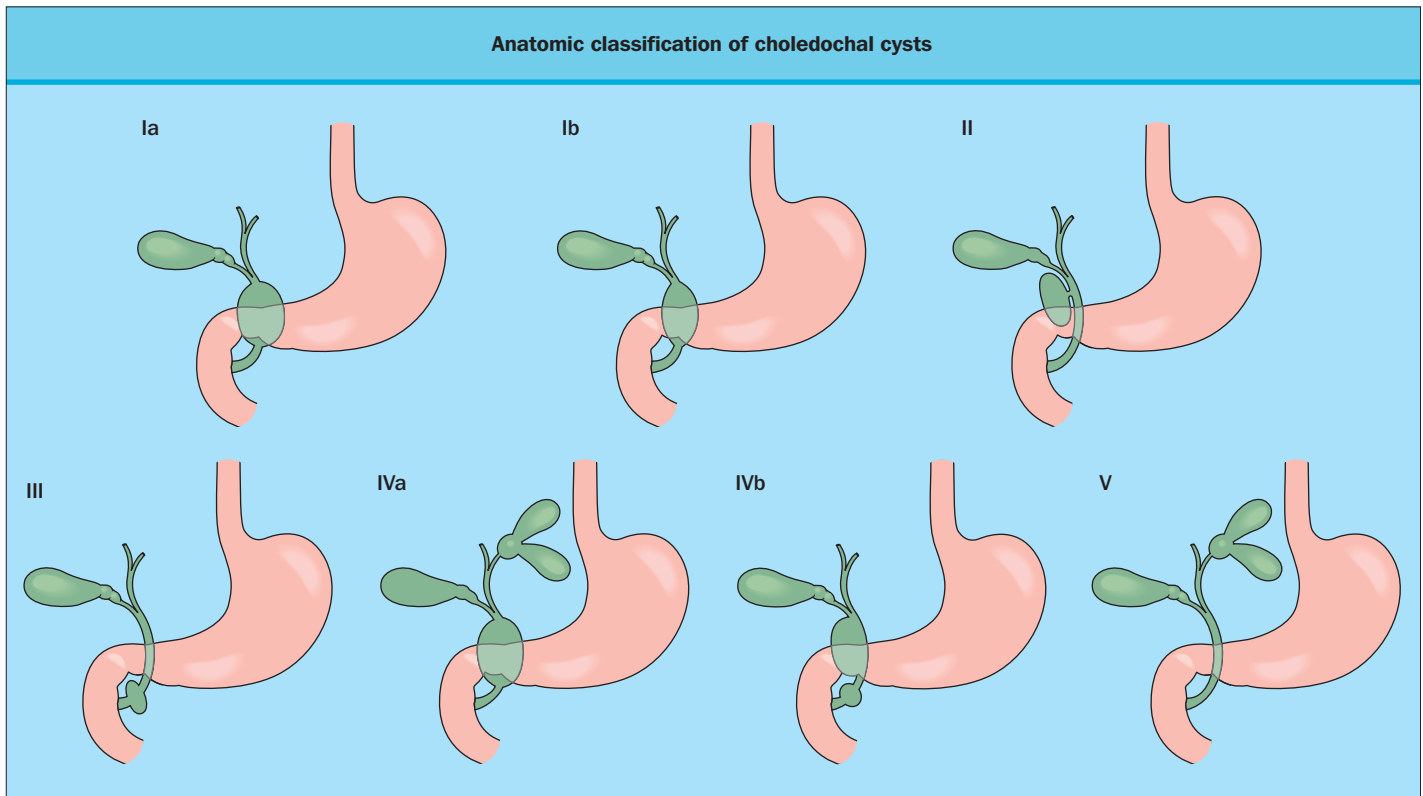
The most common clinical presentation of choledochal cyst is a relatively young patient (child or adolescent) with pain, a mass in the right upper quadrant or epigastrium and jaundice. In one series of 740 cases, jaundice was the most common and consistent presenting feature. In infants, jaundice is often the only sign and the disorder may be difficult to distinguish from biliary atresia. The majority of the patients are diagnosed before 30 years of age and the male:female ratio in most series is about 1:4.

Reported complications include spontaneous or traumatic rupture, rupture during pregnancy, liver abscess, cirrhosis and complications related to portal hypertension including gastroesophageal varices and development of cholangiocarcinoma. The incidence of cholangiocarcinoma ranges from 2.5 to 17.5%, which is 5–35 times greater than the population at large. The incidence of carcinoma increases throughout life and approaches 50% by age 50 years. Internal drainage procedures without cyst excision appear to accelerate the development of cholangiocarcinoma. Bacterial overgrowth and increased levels of secondary bile acids may contribute to cyst metaplasia and carcinoma. The tumors may originate in different parts of the pancreatobiliary system, including the liver, gallbladder, intrahepatic ducts, pancreatic ducts or pancreas.

### Diagnosis

The diagnosis of a choledochal cyst should always be suspected in a child presenting with recurrent abdominal pain, jaundice and raised serum amylase. Initial imaging of the biliary tree by ultrasonography or radionuclide scintigraphy (HIDA scans) is usually diagnostic. Confirmation and anatomic definition may require CT, ERCP or a percutaneous transhepatic cholangiogram (PTC). Patients with extrahepatic choledochal cysts have an increased incidence of anomalous pancreaticobiliary junction, which requires ERCP when planning for excision of the cyst. In recent years, MRCP has shown to be equivalent to ERCP in detecting and defining not only choledochal cysts but also the presence of an anomalous union of the pancreatic and bile ducts. MRCP can also define the length of extrahepatic bile duct involved with the cyst which is important when planning a surgical approach. Particularly in the pediatric population, MRCP offers an attractive non-invasive diagnostic alternative with the lack of ionizing radiation. Endoscopic ultrasonography has also been a useful imaging method for patients with suspected anomalous pancreaticobiliary junction. Prenatal ultrasonography can detect choledochal cysts in utero, which may help antenatal counseling since early neonatal cyst excision and duct revision may be required.





**Figure 28.11 Anatomic classification of choledochal cysts.** The various anatomic types of choledochal cysts are shown. Type Ia, choledochal cyst; type Ib, segmental choledochal dilatation; type II, extrahepatic duct diverticulum; type III, choledochoceles; type IVa, multiple intra- and extrahepatic duct cyst; type IVb, multiple extrahepatic duct cyst; type V, intrahepatic duct cyst (Caroli disease). Reproduced from Todani et al (77) by permission of Excerpta Medica.

### Treatment

It is generally agreed that choledochal cysts require surgical treatment. The preferred surgical treatment is complete cyst excision with a Roux-en-Y hepaticojejunostomy. This eliminates any opportunity for stasis, infection, stone formation, and risk of cholangiocarcinoma. The procedure provides excellent long-term results with low morbidity and mortality, but lifelong follow-up may be necessary to avoid potential problems, such as biliary cirrhosis. Internal cyst drainage procedures (cystoduodenostomy, cystojejunostomy) have often been unsatisfactory with a complication rate as high as 50%, and this procedure may actually accelerate the development of cholangiocarcinoma. Unilobar intrahepatic cystic disease is usually treated by resection of the affected lobe of the liver. Radiologic and endoscopic drainage procedures are used to stabilize patients with acute or recurrent cholangitis. However, recurrent symptoms are common and the risk of development of cholangiocarcinoma is not eliminated. When features of end-stage liver disease develop, due to long-standing, progressive biliary obstruction with secondary biliary cirrhosis, or when endoscopic, radiologic or surgical therapy have failed to resolve the biliary infectious complications, orthotopic liver transplantation may be considered.

### Caroli disease

#### Characteristics

In 1958, Caroli described a syndrome of congenital malformation of intrahepatic bile ducts characterized by segmental cystic dilatation of the intrahepatic ducts, increased incidence of

biliary lithiasis, cholangitis and liver abscesses, absence of cirrhosis and yet portal hypertension, and an association of renal cystic disease. Subsequent to Caroli's initial reports, two distinct disease entities associated with Caroli disease have been recognized. The simple type, which is associated with medullary sponge kidney in 60–80% of cases; and the periportal fibrosis type, which is associated with congenital hepatic fibrosis, cirrhosis, portal hypertension and esophageal varices.

### Clinical features

The most common presenting symptoms of Caroli disease are recurrent episodes of fever, chills and abdominal pain due to cholangitis, with a peak incidence in early adult life. Males and females are equally affected, in contrast to the female predominance of polycystic disease, simple hepatic cysts and choledochal cysts. More than 80% of affected patients present with symptoms before the age of 30 years. Rarely, the disease presents later in life with evidence of portal hypertension and its complications, most commonly bleeding esophageal varices. The risk of development of cholangiocarcinoma in Caroli disease is about 7%. Biliary lithiasis is found in one-third of patients and predisposes patients to recurrent episodes of cholangitis due to obstruction and ascending infections. Occasional patients also experience multiple liver abscesses. The genetics are unclear, with both autosomal-dominant and autosomal-recessive patterns of inheritance proposed. There have been no molecular biologic studies.

### Diagnosis

Caroli disease is typically discovered by ultrasonography performed during evaluation of biliary obstruction or cholangitis. Communication of the intrahepatic cysts with the biliary tree is usually confirmed by scintigraphy, CT scan after biliary contrast, ERCP or PTC. Some studies have suggested that CT is superior to ultrasonography in the initial evaluation of adult patients. Ultrasonography is preferred in children. PTC and ERCP provide detailed examination of the biliary tree and may aid in therapy (Fig. 28.11).

### Treatment

Adequate biliary drainage is the primary approach to the management of Caroli disease. Endoscopic therapy with ERCP is effective in removing sludge or stones from the CBD but is of limited utility in providing adequate drainage of intrahepatic cysts. In contrast, PTC is more effective in draining these cysts and avoids recurrent episodes of cholangitis, especially if patients comply with periodic flushing and changing of drainage catheters. Rarely the cystic disease is confined to one hepatic lobe, and, in this circumstance, hepatic lobectomy is often curative. Although some have advocated hepaticojunctionostomy after partial hepatectomy as primary therapy, the long-term efficacy of this procedure is uncertain, and the extensive surgery could compromise the outcome of liver transplantation. There



**Figure 28.12 Caroli disease.** This endoscopic retrograde cholangiopancreatography demonstrates the typical intrahepatic biliary cysts of Caroli disease. (Courtesy of Dr H. Irving.)

are two main indications for hepatic transplantation in Caroli disease: hepatic decompensation and recurrent cholangitis that is unresponsive to endoscopic or radiologic interventions.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 29

## Malignant Tumors of the Liver

Philip J. Johnson

Tumors of the liver enter into the differential diagnosis of many patients presenting to the hepatologist and tend to be managed by hepatologists and hepatobiliary surgeons rather than by oncologists. A sound knowledge of these tumors is therefore required by all involved in the practice of clinical hepatology. In many parts of the world primary liver cell cancer (PLCC) or 'hepatocellular carcinoma' (HCC) is a common malignant tumor and represents a major public health problem. The liver is also the most frequent, and often the clinically predominant, organ site of metastatic malignant disease. This chapter deals firstly with the most common primary malignant tumor of the liver, HCC, then some other primary liver tumors, and finally secondary liver tumors.

### HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma, which accounts for 70–80% of all primary liver tumors, is the sixth most common cancer in the world (fifth in men, ninth in women), and the 500 000 to 1 million cases that occur each year account for at least 5% of all new cancers. It tends to present late in its natural history because small tumors cause few or no symptoms and the large size of the liver precludes easy palpation of the tumor until it has gained considerable dimensions. The large functional reserve of the liver also delays symptomatic presentation with disturbances such as jaundice or ascites. Patients who are carriers of the hepatitis B virus (HBV), hepatitis C virus (HCV), or those who have cirrhosis of any other etiology, are at a very high risk of HCC development. Physicians managing such patients need to maintain a high index of suspicion (Table 29.1).

### Epidemiology and pathogenesis

Elucidation of the epidemiology of HCC has led to one of the great medical success stories of the 20th century. Not only have many of the most important risk factors been identified and their impact quantified, but also preventive measures based on these results are now being implemented.

Age-adjusted incidence rates for HCC vary from around 2–3/100 000 in Northern Europe, the USA and Australia, up to more than 100/100 000 in parts of sub-Saharan Africa and the Far East (Table 29.2). These figures may hide important information. For example, within the USA, where the overall incidence rate is low, there is marked variation in relation to ethnicity with rates per 100 000 of 18, 4 and 2 for Chinese, black Americans and white Americans respectively. Furthermore, within individual countries there may be very striking geographic differences in incidence, even among ethnically homogenous peoples. Thus, in some parts of China incidence rates are below 10/100 000 whereas in others, rates exceed 100/100 000

### Key features of hepatocellular carcinoma (HCC) and secondary liver tumors

HCC	Secondary liver tumors
HCC usually presents late, when the tumor is already large	Liver metastases are present in 40% of patients who have extrahepatic malignancy who come to autopsy
The non-tumorous liver is usually cirrhotic	Survival is directly related to the size of the tumor
Resection offers the only hope of long-term survival, but is only an option for a minority of patients because: the tumor has already metastasized, or liver function is too poor, or the tumor is too large	All solitary lesions should be investigated for the possibility of surgical resection
Recurrence after resection or transplantation is common particularly when the tumor is large or there is histologic evidence of vascular invasion	In patients who have good performance status cytotoxic chemotherapy should be considered, either given as a standard intravenous regimen or as a chronic infusion
HBV vaccination should ultimately decrease the incidence of HCC in many high incidence areas	Postoperative adjuvant treatment improves survival and decreases the risk of hepatic metastases in patients who have Duke stage C colorectal cancer
HBV, hepatitis B virus	

**Table 29.1** Key features of hepatocellular carcinoma (HCC) and secondary liver tumors.

(Fig. 29.1). Rates are consistently higher for men than for women. The incidence of HCC rises with age in all areas of the world, and peaks between 35 and 75 years, the peak being earlier in higher incidence areas (Fig. 29.2). Around 70–90% of cases of HCC are associated with cirrhosis and among patients with cirrhosis the annual rate of HCC development is in the order of 1–5% per annum.

All factors linked to the development of HCC, including chronic HBV, HCV infection and excessive alcohol consumption, also cause cirrhosis. Once cirrhosis is established, even if the causative factor is withdrawn (for example, alcohol in alcoholic cirrhosis, or iron in the case of hemochromatosis), the increased risk of HCC remains. The incidence in the West appears to be rising, perhaps in relation to the increased

Age adjusted incidence rates of hepatocellular carcinoma (HCC) (per 100 000 of population) in various countries			
	Country	Male	Female
Low incidence areas	UK	1.6	0.8
	USA (white)	2	1
	Australia	1.1	0.5
	Germany	4	1.2
	Denmark	3.6	2.3
Intermediate incidence areas	Italy	7.5	3.5
	Spain	7.5	4
	Romania	11.8	7.9
	Argentina	8	5
High incidence areas	Japan	20	5
	Hong Kong	32	7
	Mozambique	113	31
	Zimbabwe	65	25
	Senegal	25	9
	Taiwan	85	—

Where figures for different areas of the same country are available, typical values have been given. Most registries do not distinguish between HCC and other primary tumors, so the figures should, at best, be considered as a rough guide.

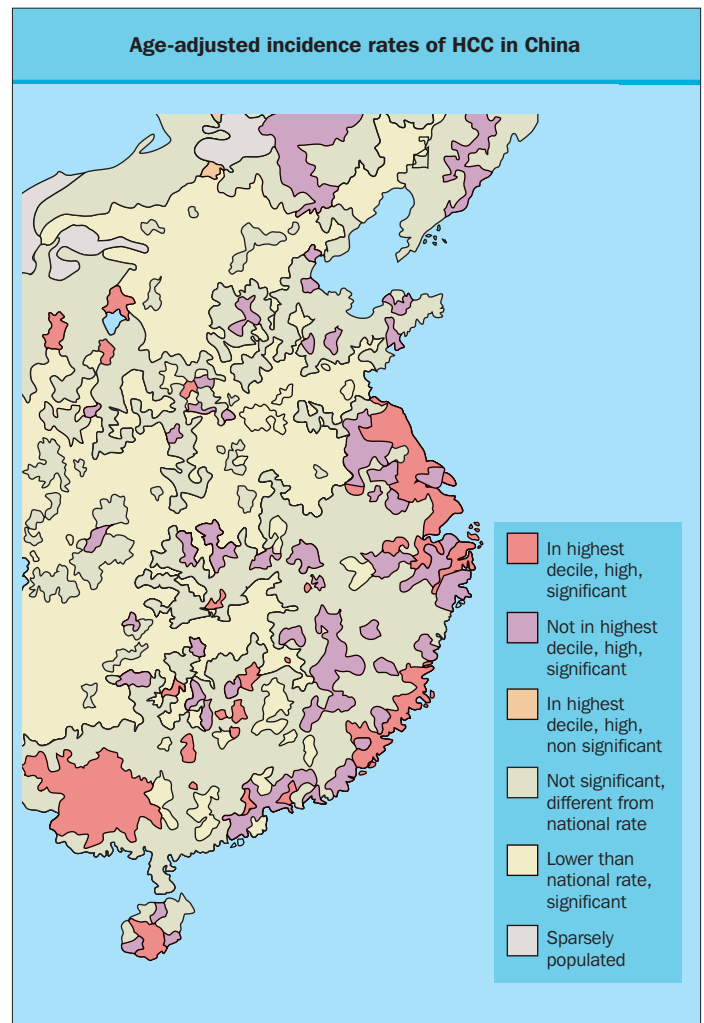
**Table 29.2** Age adjusted incidence rates of hepatocellular carcinoma (HCC) (per 100 000 of population) in various countries.

prevalence of chronic HCV infection (see Ch. 16), but caution should be exercised. Over the last 30 years diagnostic tools have become much more sensitive, and lesions which would not have been detected in the 1970s are now routinely picked up by new imaging techniques.

### Hepatitis B virus infection

From a worldwide perspective, HBV infection is the major etiology of HCC accounting for perhaps 75% of all cases. Areas of the world with the highest HCC incidence have carriage rates for hepatitis B surface antigen (HBsAg) of greater than 10% (Fig. 29.3). A landmark prospective study of 22 707 male Chinese civil servants, about 15% of whom were HBsAg seropositive, showed conclusively that chronic HBV infection precedes HCC development and the relative risk for a male HBsAg carrier for HCC was about 100. In other areas of the world the relative risk for HBV carriers is much lower and this probably reflects variable impact of additional risk factors such as aflatoxin exposure and the age at which the infection was contracted. Other cancers do not develop with undue frequency among HBV carriers. The association of HBV and HCC is confined to *chronic* HBV infection, particularly when acquired at birth or during childhood. The period from acquisition of the virus to tumor development can be as short as 4 years and as long as 80 years.

Although the weight of epidemiologic evidence linking chronic HBV infection to the subsequent development of HCC is now overwhelming, elucidation of the mechanism has proved elusive. Hepatitis B virus DNA, integrated into the host genome, can be detected in most cases, even in the absence of serologic evidence of HBV infection. However, there does not appear to be a consistent integration site and HBV does not

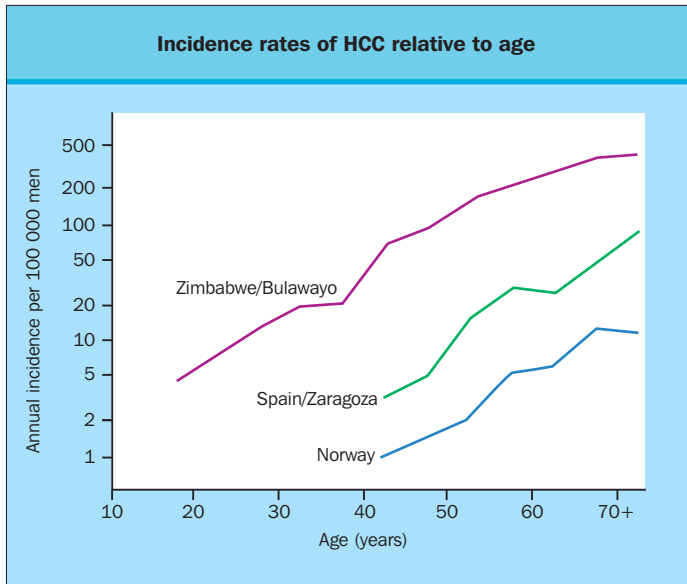


**Figure 29.1** Age-adjusted incidence rates of hepatocellular carcinoma (HCC) in China in relation to the national average. Note that, although the prevalence of hepatitis B carriage is fairly constant throughout China, there is a great deal of geographic variation, with cases tending to cluster (red and purple areas) strongly around the coastal strip.

contain recognized oncogenes. Preliminary evidence from Taiwan where mass vaccination against HBV was introduced in 1984 for children of mothers who were HBsAg carriers, and universally in 1986, shows that a steady decrease in the number of children who develop HCC, aged 6–14 years, is already evident. The incidence rate has fallen from 0.7/100 000 (1981–1986) to 0.57 (1986–1990) and to 0.36 (1990–1994).

### Hepatitis C virus infection

With the development of tests for antibodies to HCV in 1989 it became apparent, at least in Japan and the West, that HCV was as important as HBV in the pathogenesis of HCC. In Europe, HCV positivity in patients who have HCC ranges from 20 to 76%, with a tendency to increase from north to south (Table 29.3) and in the USA it accounts for about 50% of cases. There appears to be a very long incubation period as assessed by following up patients who have hemophilia and in whom the time of acquisition could be accurately documented. Since HCV has no reverse transcriptase activity and is not a retrovirus, it should, in theory, have no direct oncogenic potential. Most



**Figure 29.2** The change in incidence rates of hepatocellular carcinoma (HCC) according to age in three different geographic areas.

Reproduced from Munoz & Bosch in Okuda & Ishak (87) by permission of Springer Science and Business Media.

authors have attributed the association with HCC to the chronic liver disease that HCV may cause.

There is now good evidence that effective antiviral treatment for both hepatitis C and hepatitis B is decreasing the risk of HCC development particularly if disease control is established before the onset of cirrhosis. Co-infection with HBV and HCV is associated with a very high risk of HCC development. Chronic carriers of HCV, particularly in areas of the world where the infection is common, are at a significantly increased risk of B-cell non-Hodgkin lymphoma and this should be borne in mind when investigating focal hepatic lesions in such patients.

**Rate of positivity for anti-hepatitis C virus (anti-HCV) among patients who have hepatocellular carcinoma (HCC) in various countries compared with a control population from the same country**

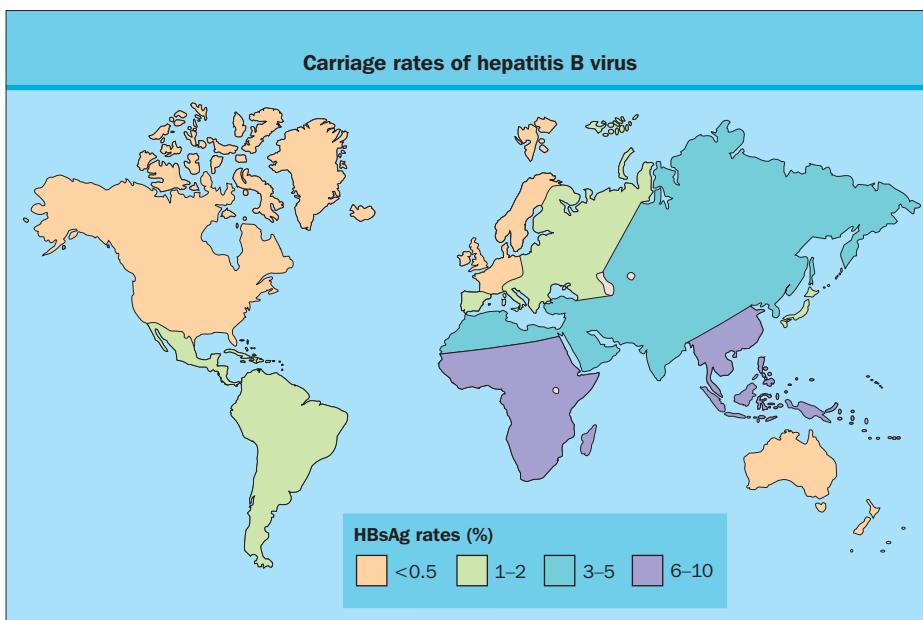
Country	Anti-HCV positivity	
	Percentage of HCC patients	Percentage of control population
Italy	76	<1
France	58	<1
Spain	75	7.5
Greece	40	6
UK	10	<1
USA	20	1
Japan	68	7

There is considerable variation within country and figures may even depend on the particular assay used. Figures should therefore only be taken as rough estimates.

**Table 29.3** Rate of positivity for anti-hepatitis C virus (anti-HCV) among patients who have hepatocellular carcinoma (HCC) in various countries compared with a control population from the same country.

#### **Aflatoxin exposure and other possible etiologic factors**

Aflatoxins are mycotoxins generated by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These mycotoxins are among the most potent naturally occurring carcinogens known. Humans are exposed following ingestion of nuts and meal that are stored under hot humid conditions where these molds grow. The relative risk of HCC for individuals exposed to aflatoxin is very high, similar to that for HBV carriage, but for individuals who have both risk factors the relative risk is increased in a multiplicative manner. These are likely to be major etiologic factors in humid



**Figure 29.3** Worldwide carriage rates of the hepatitis B virus. Note that the areas with the highest carriage rates coincide with the areas that have the highest incidence of hepatocellular carcinoma (HCC).



parts of China and Africa, and may account for the wide variation of incidence in these continents.

There is a strong association between the use of contraceptive preparations and benign hepatic adenomas (see Ch. 27). Although there may be an increased risk of HCC development after prolonged usage, the absolute risk remains extremely small.

### Clinical features and disease associations

The most common mode of presentation of HCC is with the triad of abdominal pain in the right upper quadrant, weight loss and hepatomegaly. Patients who present in this manner usually have a tumor larger than 6 cm in diameter and diameters of over 15 cm are not uncommon. The pain is usually a dull ache, sometimes referred to the shoulder. Sudden attacks of more severe pain may be caused by spontaneous bleeding into the tumor. Hepatomegaly, often massive, is an invariable feature of symptomatic malignant liver tumors. The liver feels firm or stony hard. Usually, the symptoms will have been present for only a few weeks in high incidence areas or for a few months in lower incidence areas. Delay in seeking medical advice is seldom a reason for late diagnosis or a factor that can be held responsible for delayed intervention. Less common presentations are listed below.

#### Hepatic decompensation

In a male who has previously well-controlled cirrhosis and who develops ascites, recurrent variceal hemorrhage or encephalopathy, HCC must always enter the differential diagnosis. The ascites becomes difficult to control with standard diuretic therapy and may be bloodstained. Jaundice of the hepatocellular type becomes steadily progressive.

#### Gastrointestinal hemorrhage

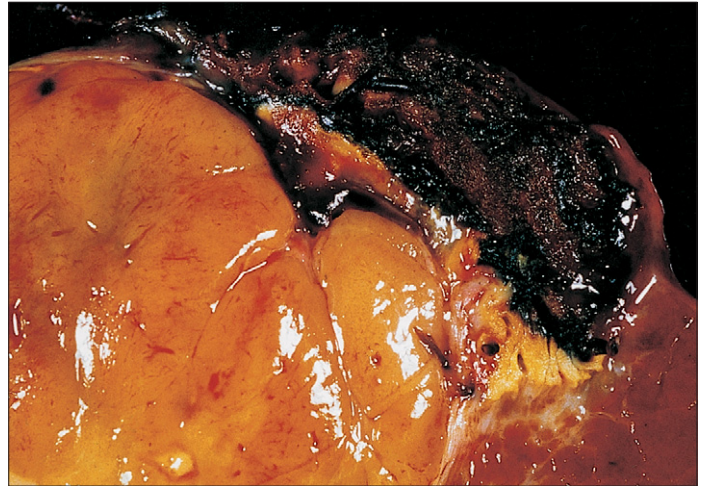
About 10% of cases will present with gastrointestinal bleeding. In 40% of the cases the bleed will have been from esophageal varices; this is much more frequent if the patient has portal vein invasion by the tumor. Bleeding from duodenal ulceration and other benign causes accounts for the remaining 60% of cases. The patient may have cutaneous stigmata of chronic liver disease, but this is by no means always the case. The majority of patients who present with HCC, as well as having no history of chronic liver disease, have no clinical signs thereof. The failure to detect clinical signs of chronic liver disease does not imply its absence.

#### Tumor rupture – ‘hemoperitoneum’

Spontaneous rupture is a particularly dramatic presentation of the tumor (Fig. 29.4). There is a sudden onset of abdominal pain and swelling. Although hemoperitoneum is a frequent event late in the course of the disease, it may occasionally be a presenting feature. The patient presents with shock and a rigid, silent abdomen. The diagnosis is established by paracentesis, which reveals bloodstained fluid or frank blood.

#### Asymptomatic presentation

Increasingly, because of surveillance programs, tumors are being detected before any symptoms develop (see below). With current imaging procedures, tumors as small as 0.5–1 cm can be detected. The frequency of asymptomatic diagnosis is entirely dependent on the intensity of the screening program. In most countries those cases detected at this stage remain a small



**Figure 29.4 Macroscopic picture of ruptured hepatocellular carcinoma (HCC).** Note the large blood clot on the surface of the liver. The vessels responsible for the hemorrhage are visible. (Courtesy of Dr CT Liew.)

fortunate minority. Not surprisingly, the natural history of these very early lesions is different from symptomatic tumors and apparent survival is much better. Surgical resection or liver transplantation may be curative in this group.

#### Endocrine, paraneoplastic syndromes, other rare modes of presentation

HCC has a reputation for paraneoplastic presentations, but this is largely due to highly selective reporting. Overall less than 1% of all cases will present with any of these syndromes, although asymptomatic cases will be detected more frequently if sought assiduously. Among the paraneoplastic syndromes, a high red cell count (erythrocytosis) is well recognized, and is presumed to be related to ectopic production of erythropoietin or an erythropoietin-like substance. Two types of hypoglycemia are recognized. The first ('type A') occurs during the terminal stage of a rapidly growing tumor and is seldom symptomatic. A much greater clinical problem is type B disease, in which severe symptomatic hypoglycemia occurs relatively early in the disease course. The hypoglycemia does not respond consistently to any therapeutic approach and the physician usually resorts to long-term enteral glucose administration. Other even rarer presentations include gynecomastia, hypercalcemia and hyperthyroidism. Spread to involve the hepatic vein is a cause of the Budd-Chiari syndrome with massive tense ascites. Obstructive jaundice may be due to compression of the bile ducts by tumor rather than hepatocellular failure. In approximately half the cases the site of obstruction is extrahepatic. Other rare causes of jaundice include direct growth of the tumor into the bile duct or bleeding from the tumor into the biliary system (hematobilia). HCC has occasionally been reported as a cause of 'pyrexia of unknown origin' (PUO). The PUO occurs late in the disease and leads to diagnostic difficulties during management. Diarrhea of uncertain pathogenesis is also a frequent symptom.

#### Clinical features of specific histologic subtypes

Two rare, histologically defined, primary liver cancers deserve special consideration since they have specific clinical correlates. The fibrolamellar variant of HCC has several distinctive clinical

features, including a young age at presentation (median age 20 years), normal  $\alpha$ -fetoprotein (AFP) levels, and a lack of any association with cirrhosis. Patients are invariably HBV-negative. The histologic picture is one of central dense fibrotic bands and eosinophilic tumor cells. The tumor, which is very rare in high incidence areas, carries a rather better prognosis than the normal type of HCC. Another histologic variant with which fibrolamellar carcinoma may be confused has very marked sclerosis (the sclerosing variant), but does not carry a better prognosis. It is often associated with hypercalcemia and is sometimes mistakenly classified as a cholangiocarcinoma.

#### **Hepatocellular carcinoma in the non-cirrhotic liver**

About 10–20% of HCC cases arise within a histologically normal liver and these too have distinctive clinical features. Compared with HCC complicating cirrhosis, the incidence in females is higher, the typical age of presentation is lower, the tumor is less often AFP-positive, and survival is slightly better. The characteristic clinical features are detailed in **Table 29.4**, where they are compared with those of HCC associated with cirrhosis. The distinction is important since, as the regenerative

capacity of the normal liver is greater than that of the cirrhotic liver, the options for more aggressive surgical management are greater. However, these tumors have longer presymptomatic stages than HCC complicating cirrhosis, and consequently tend to present with greater tumor size and bulk.

#### **Diagnosis**

One of the symptom complexes described above, with appropriate clinical examination, will raise the suspicion of HCC. The initial investigations comprise three processes:

- imaging procedures (including plain chest radiograph);
- laboratory investigations (serum AFP estimation, routine liver function tests, and serology for chronic viral hepatitis); and
- histologic confirmation of the diagnosis.

The natural desire to confirm the diagnosis histologically, particularly in view of the gravity of the prognosis, needs to be tempered with a consideration of benefit to risk ratio for the patient. For example, in a patient who has severely decompensated cirrhosis, and in whom liver transplantation is not an option, it is unlikely that establishing the diagnosis of HCC will have any impact on further management and will certainly risk hemorrhage, which may lead to further decompensation.

#### **Imaging**

Imaging techniques play a key role in all aspects of HCC management, from surveillance of the high risk patients through diagnosis and staging in the symptomatic patient, to providing therapeutic options such as transarterial chemoembolization (TACE) and monitoring response to therapy.

The approach to characterization of a focal occupying lesion within the liver will depend on local availability and expertise. Ultrasound examination, which is usually the initial procedure, shows the tumor as hypoechoic while small, becoming progressively hyperechoic with ill-defined margins as it enlarges. This investigative approach is particularly appropriate for regular screening of cirrhotic patients for the development of HCC, since lesions as small as 1.0 cm can be detected. Ultrasound scanning (US) can also assess the patency of the portal and hepatic veins, particularly when Doppler flow studies are undertaken (**Fig. 29.5**). It permits differentiation between solid and cystic space occupying lesions, and can allow accurate measurement of the main tumor size and daughter nodules, if present. Tumor and cirrhotic nodules can often be differentiated, but the entire examination is very operator-dependent and of limited specificity. US is also invaluable for guiding liver biopsy of focal lesions, directing and assessing the efficacy of ablative therapies and is widely used intraoperatively. New ultrasound-based technology is rapidly entering routine clinical practice. Tissue harmonic imaging may enhance conventional gray-scale ultrasound scanning by improving the effective contrast resolution and the use of Doppler sonography and power Doppler, with or without intravascular contrast agents, can detect and characterize the vascularity of small tumors.

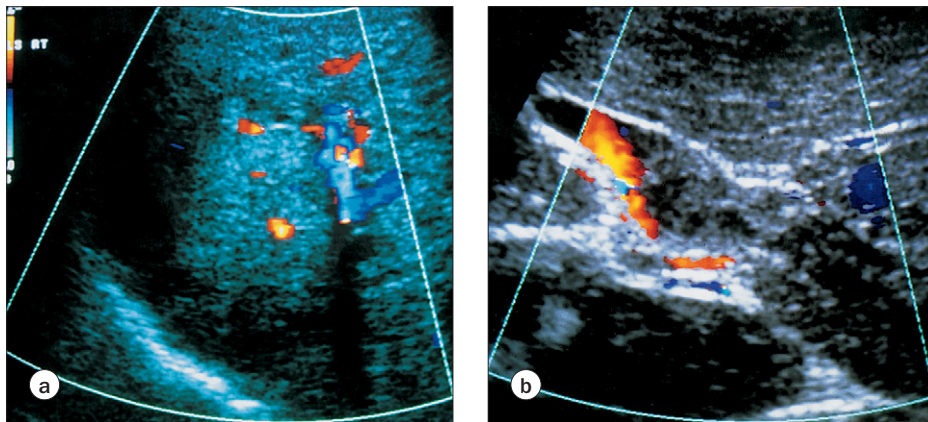
Multislice helical computed tomography (CT) is currently the imaging modality of choice for diagnosis and staging of focal hepatic lesions and planning for surgical resection. The abdomen can be scanned in less than 10 s, and partial volume averaging and respiratory misregistration are markedly decreased compared to conventional CT scanning. In most units multiple phase hepatic CT is possible. Phase I, the arterial phase starts between 10 and 30 s after injection of contrast and predominantly

**Clinical and laboratory features of a group of 50 patients who have non-cirrhotic hepatocellular carcinoma (HCC) compared with 100 patients with cirrhotic HCC**

	No cirrhosis	Cirrhosis
<b>Demographics</b>		
Age (years)	36 $\pm$ 14	56 $\pm$ 13*
Male	56	98
Female	44	2*
<b>Presentation</b>		
Abdominal pain	50	44
Awareness of a mass	36	7*
Weight loss	32	36
Hemoperitoneum	2	2
Hepatic decompensation		
Jaundice	10	24*
Fluid retention	8	28*
Upper gastrointestinal hemorrhage	0	11*
Encephalopathy	0	3*
<b>Laboratory features</b>		
Elevated SAP	90	96
Elevated AST	58	93*
Elevated bilirubin	34	65*
Low serum albumin	40	73*
Prolonged PT	30	50*
AFP positive	46	87*
Median log AFP (ng/mL)	7000	7000
All figures quoted are percentages except where stated otherwise. AST: aspartate aminotransferase activity; PT, prothrombin time; SAP, serum alkaline phosphatase activity. The series was predominantly based on a series from a low-incidence Western area.		
*Indicates that the difference is statistically significant at $p > 0.05$ level. Data from Melia et al (84).		

**Table 29.4 Clinical and laboratory features of a group of 50 patients who have non-cirrhotic hepatocellular carcinoma (HCC) compared with 100 patients with cirrhotic HCC.**





**Figure 29.5 Color Doppler studies.** (a) Large right hepatocellular carcinoma (HCC) compressing the portal veins and (b) portal vein thrombosis secondary to HCC. (Courtesy of Dr Wei Tse Yang.)

hypervascular lesions such as HCC are best visualized during this phase; around 10% of cases can *only* be visualized during this phase. Phase II, the portal venous phase begins 60–70 s after injection. The normal liver now enhances more than the tumor and hypovascular lesions such as metastases are best seen during this phase. Precontrast and delayed phases are sometimes taken but significantly increase radiation exposure and probably add little additional information. Three-dimensional reconstruction

can be used to provide images that may be useful in planning surgery and in determining the volume of the left lobe, a factor that may predict whether or not a patient will have sufficient hepatic reserve to undergo a right hepatectomy. The presence or absence of cirrhosis is important information when surgical resection is being planned and CT may provide clues; splenomegaly, an irregular surface outline and varices all suggest the presence of cirrhosis (Fig. 29.6).



**Figure 29.6 Comparison of HCC on computed tomography (CT) findings in (a) a cirrhotic liver and (b) a non-cirrhotic liver.** In the former ascites tends to develop early, there is splenomegaly, and the liver surface is uneven. In the latter the tumor tends to be larger at presentation. Note that the tumors are best appreciated in the arterial phase of the examination. (Courtesy of Dr Simon Oliff.)



Magnetic resonance imaging (MRI) is becoming more widely available and has similar sensitivity and specificity to helical CT and provides excellent soft tissue contrast resolution without radiation exposure or the risk of iodinated contrast reactions. However, the sensitivity of dual phase CT, MRI and US decreases markedly in lesions less than 2 cm in diameter. Between one-third and one-half of lesions that are detectable macroscopically in explanted liver (removed at liver transplantation, the 'gold standard') are missed. Both CT and MRI have the advantages over US in that a detailed search for primary or secondary lesions outside the liver is possible. The plain chest radiograph should not be forgotten and occasionally lung metastases are seen at presentation; this clearly has implications for the extent to which further investigation would be appropriate. In addition, it may be possible to see a bulge on the left diaphragm, a finding that is very characteristic of HCC.

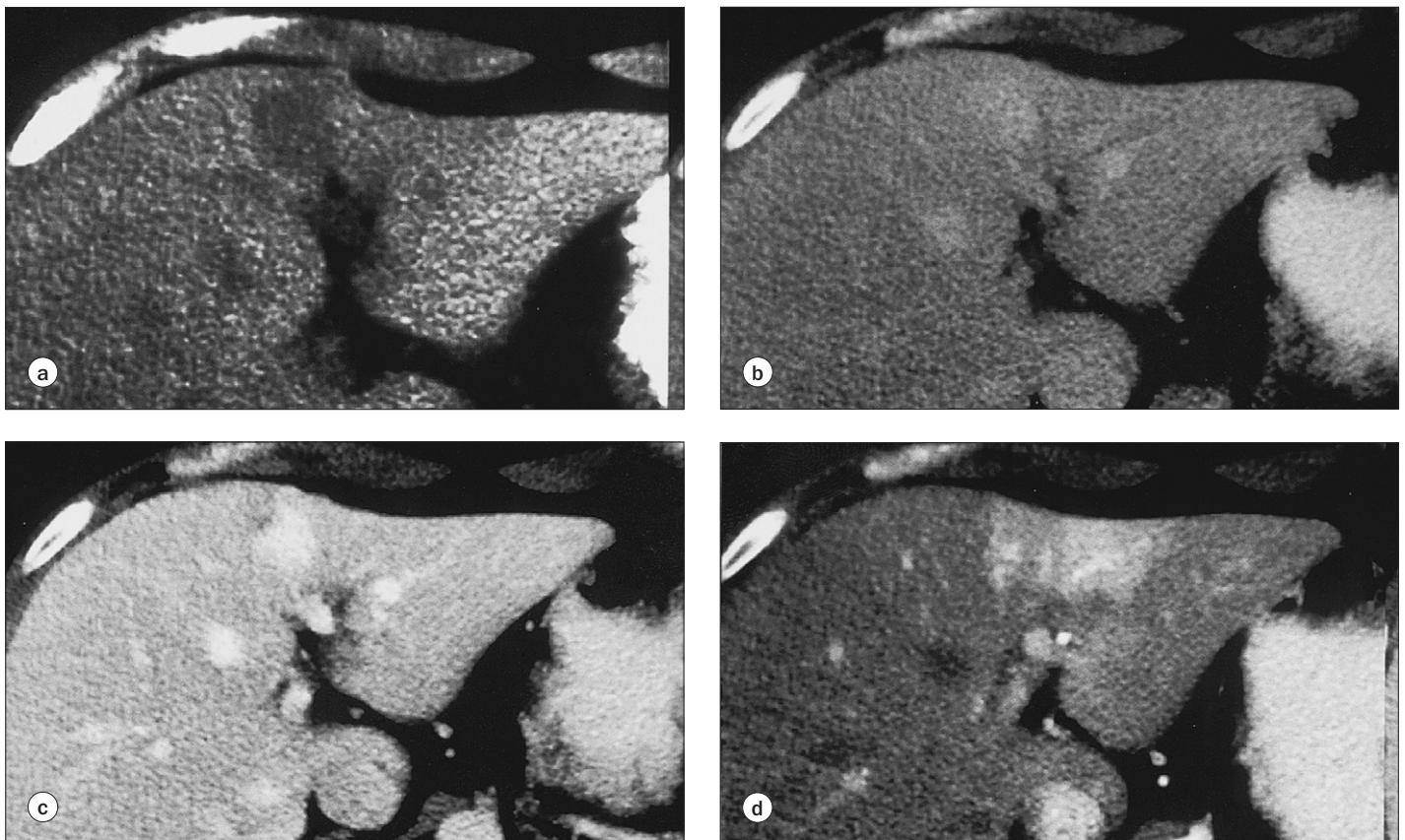
Distinction between hemangiomas and HCC is a frequent problem, particularly when screening the asymptomatic patient, and dynamic CT scanning or MRI are the most widely used approaches for discrimination (**Fig. 29.7**). In cases of doubt, hepatic angiography is used to differentiate the blush that is characteristic of tumor neovascularization from the venous phase filling of a hemangiomatous lesion. A second dilemma that is frequently faced is the distinction between a small HCC and a dysplastic nodule. Whilst those nodules that do not show early arterial enhancement on CT are probably benign there is considerable overlap and current imaging techniques cannot make

the distinction with confidence, although new MRI contrast agents show promise of helping in this difficult situation.

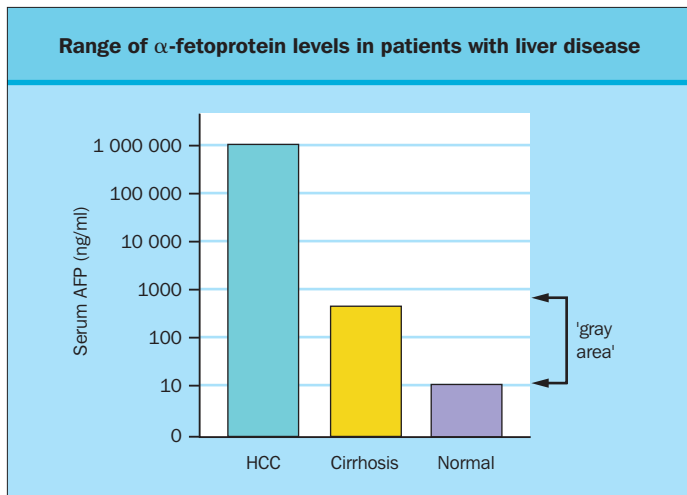
Imaging technology is advancing rapidly and it is certain that more sensitive and specific techniques such as gadolinium enhanced MRI angiography will soon become widely available. Nonetheless it should not be forgotten that the usefulness of all these imaging techniques can be enhanced by the provision of clearly defined clinical questions and accurate clinical information to the radiologist undertaking the examination.

### **Laboratory investigations**

AFP is the major protein component of fetal serum during early fetal life. It becomes virtually undetectable shortly after birth when it is replaced by albumin, and only rises again in concentration when certain pathologic conditions develop. At the time of presentation of HCC, serum AFP levels are elevated above the reference range of 0–10 ng/mL in 50–75% of patients who have HCC (**Fig. 29.8**). The median value is in the order of 1000 ng/mL in low incidence areas and 10 000 ng/mL in high incidence areas, where levels may reach 10 000 000 ng/mL (10 g/L). The test is primarily of value in the diagnosis of HCC in patients who have cirrhosis where a level above 500 ng/mL is, in the presence of a liver mass, virtually diagnostic. Levels above 100 ng/mL should raise the possibility of HCC, but levels as high as 1000 ng/mL may occasionally occur in other non-malignant liver diseases, particularly severe untreated chronic hepatitis and acute liver failure. However, trends are as important as



**Figure 29.7 Computed tomography (CT) scans of hemangioma in the left lobe of the liver.** The progressive filling in of the lesion on a dynamic contrast CT scan (a>b>c>d) is characteristic of a hemangioma rather than hepatocellular carcinoma (HCC). (Courtesy of Dr Wei Tse Yang.)



**Figure 29.8 Range of  $\alpha$ -fetoprotein (AFP) levels in patients with liver disease.** Note the wide range of levels that can be seen in hepatocellular carcinoma (HCC) patients, and the 'gray area' of overlap between patients who have chronic liver disease (but no HCC) and those who have HCC. AFP levels will be raised in 75% of HCC patients, but only about 5% of those who have chronic liver disease.

absolute values and a steadily rising value over a 1–2 month period is very strongly suggestive of HCC. Levels of AFP correlate with changes in tumor bulk in individual patients, but isolated values are not reliable general indicators of the extent of the tumor. AFP is less useful in distinguishing between primary and secondary tumors in the non-cirrhotic liver. Only 50% of such cases of HCC arising in normal livers have elevated levels, and up to 10% of patients who have hepatic metastases will have elevated levels. Other primary tumors such as non-seminomatous germ cell tumors and hepatoblastomas also consistently express a high concentration of AFP, but there is seldom any clinical difficulty in differentiating such tumors from HCC. Other tumor markers are of value in the diagnosis of the fibrolamellar variant of HCC, and these include the vitamin B<sub>12</sub>-binding protein and neurotensin.

#### **Histologic confirmation of the diagnosis**

There are two distinct aims. The first is to decide whether the lesion detected by an imaging procedure is malignant, and the second is to determine the histogenesis of the lesion, or at least to distinguish between a primary and a secondary tumor. The first aim is usually relatively easy, although to distinguish between a very-well-differentiated HCC and a benign adenoma may, on occasion, prove difficult. However, the pathologist will often have more difficulty in confidently distinguishing between primary and secondary tumors when the tumor is poorly differentiated. This distinction may be important in clinical management when, for example, deciding whether to proceed to further investigation with a view to resection. Under these circumstances the serum AFP may be helpful, a grossly raised value supporting the diagnosis of HCC.

An alternative strategy is to biopsy the non-tumorous part of the liver. If this is cirrhotic, then a primary lesion becomes the more likely diagnosis. Where doubt remains about the histological diagnosis a panel of markers for immunostaining of biopsies, including hepatocyte specific antigen, polyclonal carcinoembryonic antigen (CEA) (which gives a characteristic pericanalicular pattern) and thyroid transcription factor 1 (TTF1) (which gives

a cytoplasmic staining pattern) usually serve to confirm the diagnosis. Differentiation from cholangiocarcinoma is based on positive staining for the bile duct specific cytokeratin 7 and cytoplasmic staining with polyclonal CEA. Both tend to be negative in HCC although overlap cases are increasingly recognized.

The stage at which biopsy should be undertaken requires careful consideration because of the small risk of tumor dissemination along the needle track. Provided that normal criteria for safe liver biopsy are met, the conventional percutaneous approaches using Menghini, Trucut, or fine-needle (the latter depending on the availability of expert cytology) are appropriate. It is now routine practice to obtain tissue by using ultrasound to guide the operator or by combining biopsy with laparoscopy. When there is reason to believe that the lesion may be resectable, many surgeons prefer to avoid preoperative biopsy, and the possible risks of tumor dissemination, and await frozen section confirmation at the time of operation.

Following careful physical examination, and with the above investigations to hand, the physician is in a position to make an initial assessment. In many instances a confident diagnosis can be established at this stage, particularly in high incidence areas. In the presence of a hepatic mass that is radiologically consistent with HCC, a known history of chronic liver disease or a positive test for chronic HBV or HCV infection, and an AFP level of greater than 500 ng/mL, the diagnosis of HCC is almost certain, even in the absence of histologic confirmation. Under other circumstances the diagnosis should be confirmed histologically either by percutaneous biopsy or at laparotomy.

#### **Natural history and prognosis**

In high incidence areas the median survival from the time of diagnosis is, in the absence of resection, measured in terms of weeks to a few months. In low incidence areas the figure is in terms of 6–12 months. Whether this reflects later diagnosis or differing etiologies remains unclear. In general, those with larger tumors, vascular invasion (either microscopic invasion or portal vein invasion), poor liver function, or evidence of metastases do very poorly with a prognosis measured in months. On the other hand, those with small tumors (<3 cm), which develop in a patient with good liver function (Child grade A cirrhosis), and who are detected in the presymptomatic stage, may survive over 3 years without treatment. In practice, such lesions are invariably subjected to some form of therapy so that accurate estimation of the natural history is impossible. As surveillance programs become routine, and imaging sensitivity increases, even smaller tumors, down to 1 cm, are being detected and their optimal management is an area of intense research at present.

Whilst there is broad agreement regarding such prognostic factors, there is no consensus as to how these should be integrated into a staging system. Over the past decade there have been at least five new staging systems, reflecting dissatisfaction with the TNM system that is used in most aspects of oncology. Some of these systems are concerned with prognosis in those with advanced disease, others the prognosis after, or suitability for, surgical resection and yet others aim to guide treatment options. A recent modification of the AJC/TNM system that takes the degree of cirrhosis/fibrosis into account may become more widely applied for those who come to surgical resection (Table 29.5). The American Hepato-Pancreato-Biliary Association

New UICC/AJCC staging system for hepatocellular carcinoma (HCC)			
Primary tumor (T)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
T1	Solitary tumor without vascular invasion		
T2	Solitary tumor with vascular invasion or multiple tumors ≤5 cm		
T3	Multiple tumors more than 5 cm or tumor involving a major branch of the portal or hepatic vein		
T4	Tumor(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum.		
Regional lymph nodes (N)			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastases		
N1	Regional lymph node metastases		
Distant metastases (M)			
MX	Distant metastases cannot be assessed		
M0	No distant metastases		
M1	Distant metastases		
Stage grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T4	N0	M0
Stage IIIC	Any T	N1	M0
Stage IV	Any T	Any N	M1
Histologic grade (G)			
Gx	Grade cannot be assessed		
G1	Well differentiated		
G2	Moderately differentiated		
G3	Poorly differentiated		
G4	Undifferentiated		
Fibrosis score (F)			
F0	Fibrosis score (no fibrosis to moderate fibrosis)		
F1	Fibrosis score (severe fibrosis to cirrhosis)		

**Table 29.5 New UICC/AJCC staging system for hepatocellular carcinoma (HCC).**

(AHPBA) recently endorsed the use of the CLIP (Cancer of the Liver Italian Program) score for staging of HCC. This score takes into account liver function (Child-Pugh class), tumor morphology, serum AFP and portal vein invasion and appears to accurately predict patient survival.

### Management

Physicians searching the literature for effective treatment of HCC should be aware that clinical trials are often undertaken on patients who have characteristics that suggest a better prognosis within the above mentioned spectrum of prognoses. The results are then compared (usually favorably) with figures for the overall group, leading to an overstatement of the therapeutic potential in an unselected population of patients who have HCC. At the time of writing the only potentially curative treatment is surgical resection including transplantation. Unfortunately, however, most cases will fall within the category of being unresectable. The major reasons why patients cannot undergo conventional resectional surgery with a view to cure are the extent of the tumor (including metastases), the implications of the associated cirrhosis, extrahepatic metastases, or unrelated co-morbidity. The presence of cirrhosis does not preclude resection, but the results are best in patients who have Child class A cirrhosis. Liver transplantation is feasible in patients who have small tumors, irrespective of the Child category. Extensive, bilobar disease and/or invasion of the major vessels including the inferior vena cava, main portal vein, and common hepatic artery generally preclude surgical resection.

### Surgical approaches

Liver resection is a major operation that carries an operative mortality of up to 5% in non-cirrhotic, and up to 10% in cirrhotic patients. No single liver function test can predict the survival of an individual patient undergoing liver resection, but once the liver disease decompensates, patients almost always develop liver failure after resection. Child grade C cirrhosis is therefore usually considered to be a contraindication to surgery, but liver failure can still occur after resection in those who have Child grades A or B. It has been proposed that the presence of portal hypertension, as judged by a hepatic venous pressure gradient of  $>10$  mmHg, is the best single test predicting post-operative liver failure. However, the amount of intraoperative blood loss, the duration and degree of hypotension and size of the remaining liver remnant also influence survival.

Although cirrhosis is no longer considered an absolute contraindication, the operative mortality is significant for several reasons. Many cirrhotic patients have a coagulopathy and a low platelet count because of hypersplenism. Portal hypertension increases the risks of bleeding because of the opening up of portosystemic collaterals in the retroperitoneum. The cirrhotic liver is firm, difficult to manipulate, and does not readily take suture material. Most of these problems are now surmountable with technical advances, but surgical successes are often negated by postoperative complications. Estimates of the functional capacity of the residual tissue are difficult to determine pre-operatively and this may prove to be inadequate after surgery. Initial adequate function may deteriorate secondary to infection and cirrhotic patients are intrinsically immunocompromised and at high risk of sepsis. A patient who has a normal liver can tolerate resection of about 75% of the liver, but in a cirrhotic



liver a right lobectomy (55% resection) is the upper limit of surgical resectability.

### **Surgical resection**

CT is the usual preoperative investigation. This shows the site and extent of the disease, but may miss up to one-third of nodules detected at surgery. Angiography has the potential to delineate the vascular anatomy, confirm patency of the portal vein, and may detect tumor not seen on CT (**Fig. 29.9**). The most reliable way of detecting macroscopic abdominal spread and assessing the extent of liver involvement is during laparotomy. Intraoperative ultrasound locates otherwise undetected small primary and secondary lesions and tumor thrombus in portal and hepatic veins. It also helps to determine the resection margins and the plane of liver transection in relation to the hepatic vasculature, thereby helping to preserve as much functioning liver tissue as possible.

The liver can be divided into eight segments, each receiving its hepatic arterial, portal venous and biliary duct branches. The hepatic venous branches are distributed between, rather than within, the individual segments. Accurate delineation of the segments to be excised has been facilitated by intraoperative ultrasound, and by the injection of dyes into the portal or arterial blood supply. The five commonly used lobar and/or segmental resections are shown in **Figure 29.10**. In a right trisegmentectomy, the right lobe of the liver and medial segment of the left lobe are removed. In a left trisegmentectomy, the left lobe of the liver and the anterior segment of the right lobe are removed. An extended right hepatectomy usually refers to a right trisegmentectomy, but may also refer to a slight modification of a right lobectomy in which the hepatic parenchyma is transected well to the right of the falciform ligament rather than at this ligament, thus sparing most of the medial segment. An extended left lobectomy should not be confused with the left trisegmentectomy, since it does not result in removal of the full anterior segment of the right lobe. Non-anatomic resection refers to resection of the liver along non-anatomic planes. In

general, this results in more bleeding and can leave behind parts of the liver remnants deprived of their blood supply and subject to necrosis.

Improvements in perioperative support have radically decreased the morbidity associated with surgical resection. Nonetheless after major resection cholestasis and fluid retention and impaired synthetic function may combine to increase hospital stay. A major risk factor for postoperative complications is the 'future liver volume'. Percutaneous portal vein remobilization has recently been advocated as a method for increasing liver cell mass – by inducing selective hypertrophy of the non-diseased liver (usually of the left lobe). A key aspect to the planning for this procedure is calculation, on the basis of CT scan, of the future liver volume.

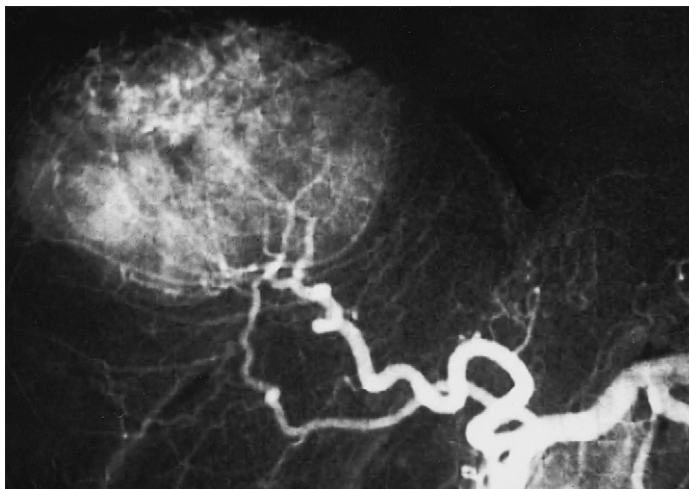
About 10–15% of patients who have HCC will come to resection and about one-third to one-half of these will survive for 5 years. Postoperative disease recurrence, usually intrahepatic, is the main cause of death.

### **Orthotopic liver transplantation**

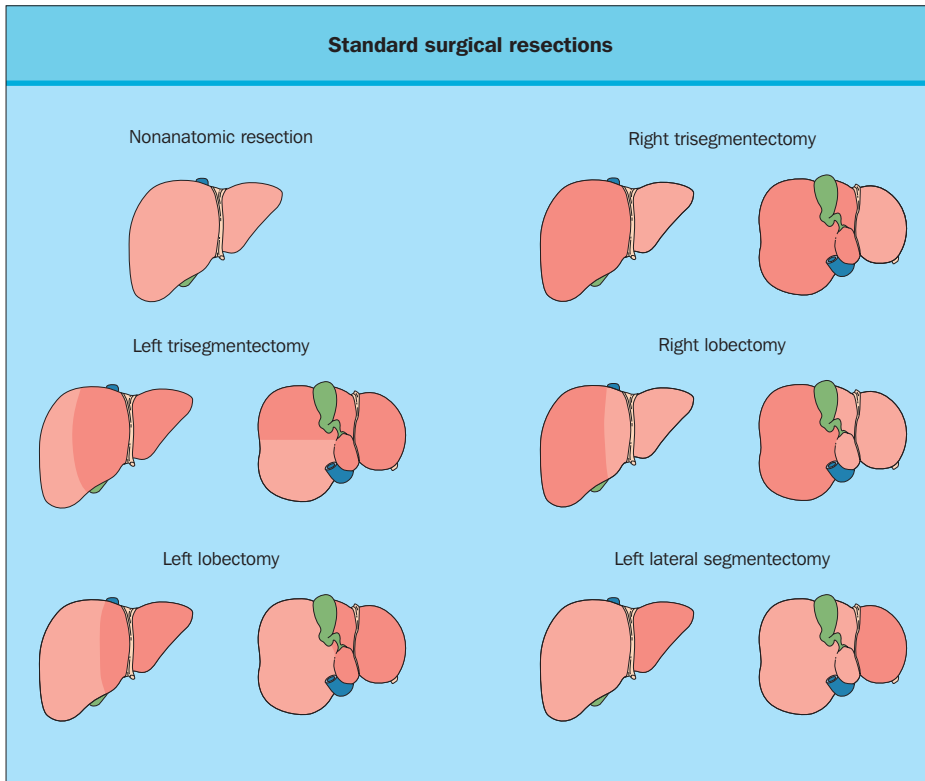
Orthotopic liver transplantation potentially overcomes the problems of intrahepatic tumor distribution, clears micro-metastatic liver tumors and relieves hepatic insufficiency all of which limit the application of conventional resection. Many of the first patients to achieve long-term survival in the 1970s after transplantation had malignant liver disease. Many tolerated the operation well and recovered rapidly but, despite careful pre-operative assessment that attempted to exclude those who had extrahepatic spread, tumor recurrence was common. By the early 1990s a multicenter registry showed only an 18% survival at 5 years with only 9% disease free at 2 years. Presumably this stemmed from undetectable extrahepatic micrometastases, perhaps promoted by the requisite immunosuppression. This experience led many groups to abandon transplantation for malignant disease other than when the tumor was found incidentally in a patient undergoing transplantation for advanced cirrhosis.

More recently it has become apparent that the number of nodules, maximum tumor size and/or the degree of vascular invasion (either microvascular invasion or portal vein invasion) are the major adverse factors influencing recurrence rates (**Fig. 29.11**). Guidelines proposed by Mazzaferro et al (1996) limit transplantation to those with a single tumor less than 4 cm in diameter or up to three nodules with the largest being less than 3 cm in diameter. With these criteria, 5-year survival of around 75% can be expected. Small tumors not numbering more than two have a prognosis similar to those cirrhotic patients without HCC who undergo liver transplantation.

Despite these excellent results, there is a growing discrepancy between the number of patients awaiting liver transplantation and the number of donor livers that become available (see Ch. 37). This means that units tend to be strict in confining transplants to those who meet the above criteria. Live donor transplantation may increase the supply but this approach is not widely used at present. Several groups are investigating the possibility of 'bridging' the gap between diagnosis and going on the transplant list, and the time at which a donor liver becomes available. TACE, percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA) have all been used but there is no consensus about their benefit.

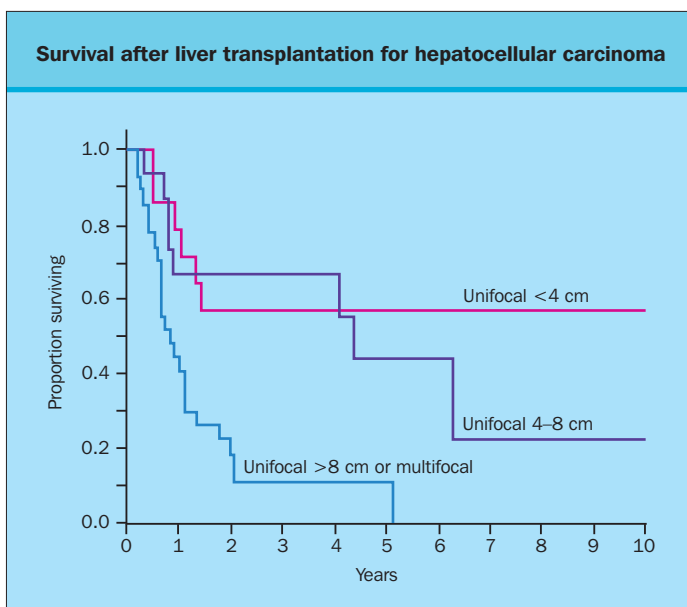


**Figure 29.9** Hepatic angiography of a large hypervascular hepatocellular carcinoma (HCC) in the dome of the liver. (Courtesy of Dr Wei Tse Yang.)

**Figure 29.10** Standard surgical resections.

The question whether, in a particular patient, resection or transplantation is to be preferred is controversial. When the patient has decompensated liver disease the answer is clearly transplantation, but in other circumstances, and while the results with liver transplantation are still improving, referral for an opinion to a center where both options can be considered should be undertaken. In addition to the surgical considerations,

the potential for further de novo malignant change in the cirrhotic tissue left in situ can influence the decision. Trials assessing the extent to which ablative therapies such as RFA may be as effective as surgical resection is currently underway. Pre- and postoperative adjuvant treatments are often given but are not, as yet, of proven benefit.

**Figure 29.11** Survival of patients who have HCC after liver transplantation in relation to tumor size and number. Reproduced from McPeake et al (95) by permission of Elsevier.

### Management of the patient who has inoperable disease

The physician must clearly define the aims and limitations of any proposed treatment. These aims should be set out before the patient in a manner that, while realistic, avoids destroying all hope. There are now so many options for the management of patients who have inoperable disease that it is easy to lose sight of certain basic principles. For the vast majority of cases the aim is good palliation; no treatment has yet been convincingly and consistently proven to increase survival, other than perhaps in a subset of patients treated with TACE.

Non-surgical treatment can be classified as locoregional, including intra-arterial or percutaneous local ablative approaches, a combination of the two, or systemic. When regional lymph nodes are involved or there are extrahepatic metastases, locoregional treatment is seldom indicated. Intra-arterial treatment is also contraindicated when there is involvement of the main portal venous system. It is among the patients who are unsuitable for any of the above treatments, that systemic chemotherapy is usually considered.

'Liver failure' as indicated by overt jaundice, recurrent or diuretic resistant ascites, recurrent gastrointestinal hemorrhage or encephalopathy unexplained by other factors will, in the view of most authorities, preclude any form of active treatment other than liver transplantation. In such patients prognosis is primarily

defined by the underlying liver function rather than the tumor; effective antitumor therapy may not necessarily improve overall survival. Figures will vary from center to center, and around the world, but as a very broad generalization 15% of patients will be considered for surgical resection, 50% for non-surgical therapies and 35% will be unsuitable for any active treatment, and will receive best supportive care. These figures will change as more patients are detected in the asymptomatic stage by screening programs.

#### ***Intra-arterial therapy – the theoretical basis***

With the disappointing results seen with systemic therapy, several approaches that aim to target the tumor specifically have been developed. There are two ways in which targeting may be achieved. The first approach is based on the observation that primary and secondary liver tumors derive the bulk of their blood supply from the hepatic artery. This approach to selectivity may be further enhanced by new arteriographic procedures that permit 'super selective' catheterization of the tumor feeding artery. Direct infusion of cytotoxic agents may increase the drug exposure (the time/concentration interval) of the tumor to the drug by up to 400-fold. The dose-limiting toxicity then becomes regional (i.e. hepatic), rather than systemic. The extent to which regional advantage is obtained depends on both the rate of drug elimination and the blood perfusion rate. Thus, drugs with a short half-life are particularly appropriate for this route of administration and measures to decrease liver blood flow may be expected to enhance their activity. Nonetheless, if the tumor is maximally sensitive at drug levels achieved by systemic administration there will be no advantage with this approach.

On pharmacologic grounds, the most appropriate drugs are 5-fluorouracil (5-FU) and 5-fluorodeoxyuridine (FUDR), both of which have half-lives of only a few minutes. Unfortunately, 5-FU, at least as a single agent, falls into the category of ineffective therapy for patients who have HCC.

A second source of selectivity is the use of lipiodol as a vehicle for cytotoxic chemotherapy. This oily based contrast medium, when injected into the hepatic artery at the time of arteriography, is cleared from normal hepatic tissues but accumulates in malignant tumors, probably because of the leaky character of neovascular tissue, combined with the lack of lymphatic clearance from tumor tissue. The lipiodol forms an emulsion with the cytotoxic agent and then acts as a reservoir for the prolonged delivery of the agent to the tumor, and perhaps enhances uptake by the tumor cells. The extent to which the lipiodol acts as an embolizing agent in itself remains controversial.

#### ***Regional drug delivery and transcatheter oily chemoembolization (TACE)***

There seems no doubt that, compared with systemic administration, drugs given intra-arterially are more effective, although it must not be forgotten that patients treated in this manner invariably have a better performance status than those treated with systemic therapy. For this reason, better results would be expected regardless of any inherent increased efficacy of the treatment.

Following hepatic angiography to identify the arterial anatomy and the blood supply of the tumor a catheter is placed in the appropriate vessel. Not infrequently angiography

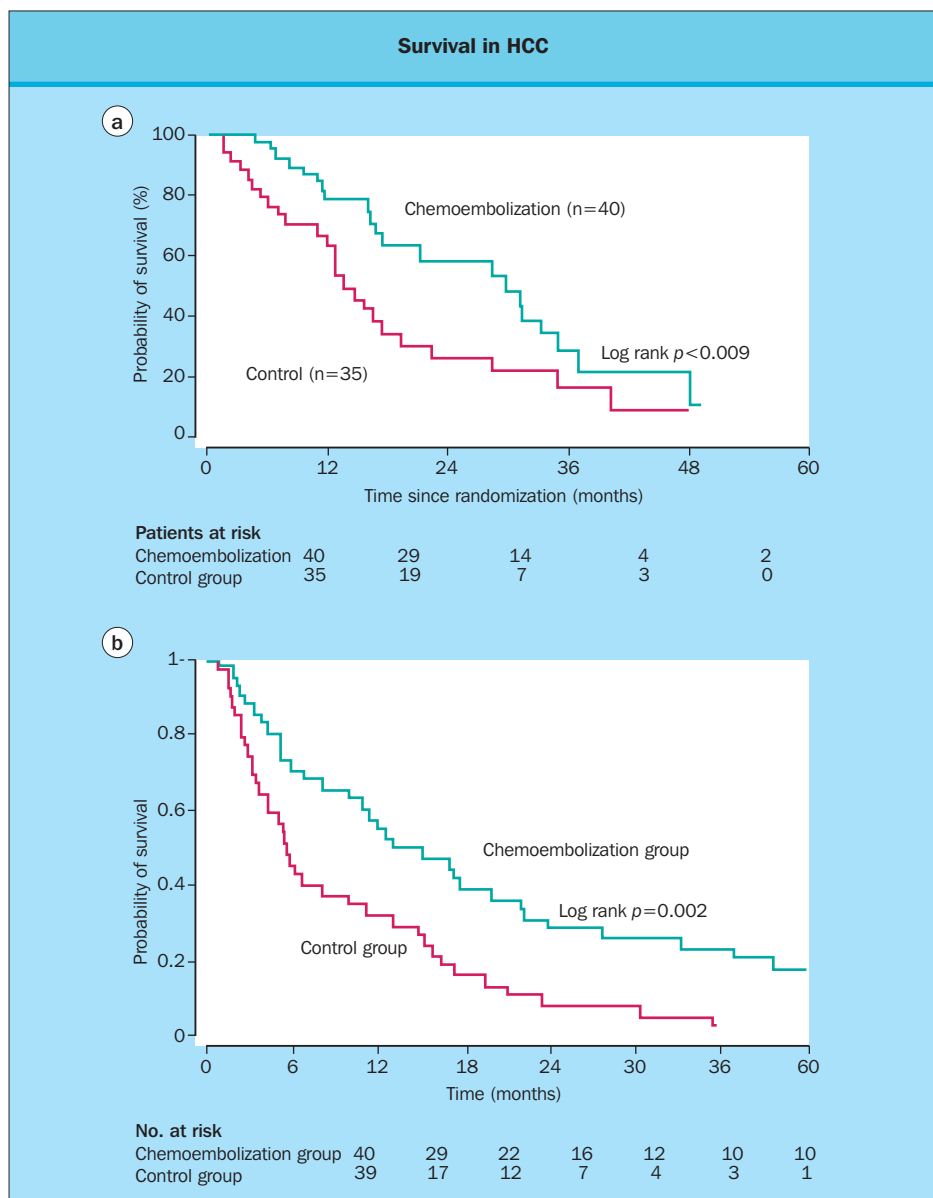
identifies tumor not detected by CT scanning. In the past the entire liver has been covered by placement of the catheter in the proper hepatic artery but nowadays it is more common to use the left or right hepatic artery when the whole of one lobe is involved, or, where feasible, to selectively catheterize just the tumor feeding arteries and the procedure becomes 'segmental'. The cytotoxic drug (usually doxorubicin or cisplatin) is mixed with lipiodol and the emulsion is injected slowly. Finally, embolization with 0.5–1 mm of gelatin cubes or a similar material is undertaken.

The presence of Child grade C cirrhosis is usually considered to be a contraindication, as is thrombosis of the portal vein, because the cirrhotic liver is crucially dependent on the hepatic artery in this situation, and any further interruption thereof may lead to liver failure. Thrombosis of the portal vein is also an indication of particularly bad prognosis and is associated with the development of extrahepatic disease. If the procedure is undertaken by an experienced interventional radiologist the mortality should be well below 5% and significant side-effects are rare (1%) apart from occasional gall bladder infarction. Effective embolization is often associated with the so-called 'post embolization syndrome' of fever, pain and vomiting for up to a week after which it subsides spontaneously. Significant deterioration in liver function may occur but usually only when Child grade C patients are treated. Although widely regarded as standard treatment for almost 15 years, and clear evidence that tumor necrosis was indeed caused, early controlled trials did not show an increase in survival and the consensus was that although the 'primary effect' (i.e. causing tumor volume reduction) is good, there is little effect on long-term survival for which other factors such as the tumor type, degree of spread and serum AFP level are more significant than the treatment.

However, more recently, two trials and a systematic review have, for the first time, provided evidence that TACE may indeed, in selected cases, improve survival. In the first of these, randomized 80 subjects, to either TACE (with cisplatin in lipiodol followed by gelatin sponge embolization) or best supportive therapy (Fig. 29.13b). The survival figures at 1, 2 and 3 years were 57, 31 and 26% compared to 32, 11 and 3%, respectively ( $p=0.006$ ). In the second study, from Spain, 112 patients were randomized to TACE with doxorubicin again followed by gelfoam embolization, or best supportive therapy. Survival figures at 1 and 2 years were 82 and 63% in the TACE group compared to 75 and 50% for embolization alone and 63 and 27% for the groups receiving best supportive therapy (Fig. 29.12a). In both studies the procedure was repeated if there was no evidence of progressive disease. A systematic review suggested that chemoembolization with either doxorubicin or cisplatin, but not embolization alone showed a significant benefit (2-year probability of survival, compared with control, odds ratio 0.53 with 95% confidence limits of 0.32–0.89). The systematic review again suggested that benefits were mainly in those with well-preserved liver function (Child grade A) and without vascular invasion.

Whilst these two studies have, according to many authorities, established TACE as the standard care for patients with larger HCC, caution is still appropriate. Both trials were small, and some criticisms about how well the treatment and control groups were balanced have been raised. Furthermore, and of particular importance in designing further comparative studies,





**Figure 29.12 Survival curves from two prospective randomized trials of arterial chemoembolization for the treatment of hepatocellular carcinoma (HCC).** Survival curves from two prospective randomized trials of arterial chemoembolization for the treatment of HCC. (For references see Llovet and Bruix 2003) (a) From Spain. (b) From Hong Kong.

there remains considerable controversy as to what is actually meant by the term 'chemoembolization' and the relative importance of the 'embolization' and the 'chemotherapy' aspects of the treatment. It is notable that different cytotoxic agents were used in the two trials. Some in the field aim to develop extensive tumor necrosis by the embolization whilst others use the embolic material to slow down the blood flow to the tumor and not to permanently occlude the vessels and thereby permit repeat procedures.

#### **Percutaneous ethanol (alcohol) injection**

Ultrasound or CT-guided percutaneous injection of sterile absolute alcohol into liver tumors, via a 19–22 gauge needle, under local anesthetic consistently induces vascular thrombosis and coagulative necrosis. A total of between 2 and 100 ml of alcohol is injected during the course of several sessions (depending on the tumor size) and distributed as uniformly as possible throughout the lesion. The most suitable patients are

those with small tumors (<3 cm), with good underlying liver function (Child grade A). In such patients, 'complete response' is obtained in around 80% with survival figures at 3 and 5 years in the order of 75 and 50% respectively. In such patients it seems likely that results may be as good as surgical resection.

The benefits of PEI decrease markedly in larger lesions; the procedure becomes more tedious and it is more difficult to generate complete necrosis, in part because of the presence of septa within the lesion but also because of 'run off' of the alcohol into the vasculature; most centers will not consider PEI for lesions larger than 5 cm.

The procedure is cheap, simple to perform, does not require a general anesthetic and is virtually free of any associated mortality. The only complications are intense pain if the alcohol is allowed to leak out into the peritoneal cavity, transient pyrexia, occasional episodes of hemoperitoneum (<5% of cases) and, rarely, tumor seeding along the needle tract. Nonetheless, as with surgical resection, in 50–70% of cases there will be intra-

hepatic recurrence. This is particularly so in larger lesions and those that are multifocal to begin with. Most will occur within 2 years of the initial procedure.

How should one decide between surgical resection and PEI in a patient with a solitary small liver tumor before prospective randomized clinical trials directly comparing the procedures are available? There are no hard and fast rules and it is probably fair to say that there is a trade-off between more early morbidity and mortality with surgical resection and more late deaths with PEI. If the patient is young, there are no coexisting medical conditions and liver function is good, most would still favor surgical resection. This has the added theoretical benefit of removing surrounding tissue that may be the site of micrometastatic disease. If there is any factor indicating significant operative risk, co-morbidity, or the patient is elderly and frail, then PEI is probably preferable. In the future it is likely that the current position of PEI will be challenged by RFA largely on the grounds that the lesions can be dealt with in a fewer number of sessions.

### **Thermal and laser ablation**

Both heating and cooling locally administered under ultrasound control, have been used to induce tumor necrosis. 'Cryotherapy', which relies on a 'freeze-thaw' process is undoubtedly effective in delivering local tumor control even in larger lesions (up to 8 cm) but the probe needs to be large (up to 10 mm in diameter) and the treatment needs to be delivered under general anesthetic at the time of laparotomy. RFA, in which heat is developed from an alternating electric current in the radiofrequency range, can result in complete necrosis of a 3 cm tumor in less than 1 h and in one session. RFA is considered to be 'minimally invasive', the needle electrodes being only 15-gauge. Depending on tumor size and site it may be administered percutaneously, intraoperatively or laparoscopically. Some lesions, particularly those near to large vessels, may be technically difficult to access.

Overall the complication rate is under 10%, rather lower than that reported for cryotherapy as is the mortality. Such opinions should be taken cautiously as the size of tumor treated with RFA tends to be smaller than with cryoablation and thus the inherent risks of the procedure are also smaller. Nonetheless there is a general trend toward RFA and away from cryotherapy. Moreover, the more rapid achievement of complete tumor necrosis and easier access to tumor margins has also led to a general trend towards RFA over PEI. Indeed such is the enthusiasm for RFA that prospective randomized trials comparing RFA with surgical resection are currently underway.

Other technologies are being developed to achieve similar ends to RFA and PEI and these include photodynamic therapy and laser thermal ablation. The latter has yielded encouraging results, and has the advantage that it can be carried out under magnetic resonance guidance, the whole procedure being monitored with near real time thermal imaging to assess the efficacy of tissue necrosis.

### **Radiotherapy and internal irradiation with intra-arterial radioisotopes**

The application of external beam irradiation for the treatment of liver tumors has been severely limited by the radiosensitivity of normal hepatocytes. Maximum tolerance of normal liver

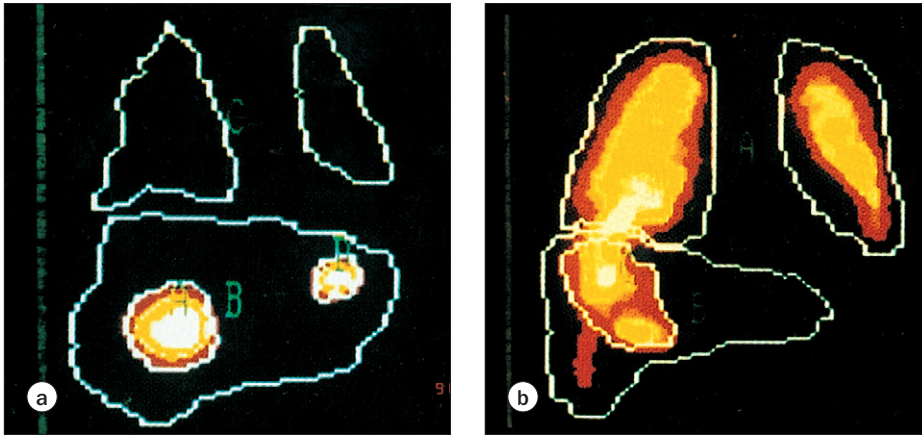
to radiation is generally accepted to be between 2500 and 3000 cGy; above this level the risk of radiation hepatitis (veno-occlusive disease with perivenular congestion and fibrosis) increases rapidly.

Therapeutic doses of radioisotopes can be administered into the hepatic artery using  $^{90}\text{Y}$  tagged to resin-based or glass microspheres or  $^{131}\text{I}$  in conjunction with lipiodol. Lipiodol- $^{131}\text{I}$  emits mainly  $\gamma$ -radiation. The volume of radioactive lipiodol administered is limited by the size and vascularity of the tumor; thus in practice, radioactive lipiodol is used only in patients with tumors less than 5 cm in diameter. About 40% of patients will gain objective remissions with minimal toxicities while keeping the radiation dose to a normal liver below 2000 cGy.  $^{90}\text{Y}$ , a pure  $\beta$ -emitter, is more powerful than  $^{131}\text{I}$  with a mean tissue penetration of about 2.5 mm. Optimal tumor regression and reduction of serum AFP level are seen when the average radiation dose to the tumor is above 12 000 cGy. The partial response rate is more than 50%. Despite the presence of cirrhosis, there is little evidence of radiation hepatitis, even when the non-tumorous liver receives up to 7000 cGy. Leakage of the microspheres into the right gastric artery or gastroduodenal artery may occasionally cause radiation gastritis or duodenitis. Systemic leakage of the microspheres to involve the lungs, which are also sensitive to irradiation, may occur if there is extensive arteriovenous shunting within the tumor. For this reason, the degree of lung shunting must be determined before administration of the radioisotope by using a  $^{99\text{m}}\text{Tc}$  macroaggregated albumin ( $^{99\text{m}}\text{Tc}$ -MAA) scan (Fig. 29.13).

From the above it is clear that all these locoregional therapies, and indeed surgical resection, the definitive locoregional treatment, are all capable of delivering complete local control in a percentage of cases, the percentage decreasing as the tumor size increases. It seems likely that all these would, in patients without underlying liver disease or with well compensated liver disease, result in improvement in survival but this has, to date, only been demonstrated in two small series of patients receiving TACE. The treatment of choice will depend on the ease and cost of the therapy, its complication and acceptability rate, and benefit that can be shown in prospective randomized clinical trials. The problem for all these remains that recurrence is the rule. What is needed therefore is effective chemotherapy to supplement local control. To date this has not been demonstrated but remains the long-term goal.

### **Systemic therapy**

Almost all the cytotoxic agents used in oncologic practice have been evaluated, and none has been shown (as a single agent or in combination with other agents) consistently to improve survival or to achieve a response rate of greater than 20%. The most widely used agent has been Adriamycin (doxorubicin). In a review of several published trials involving more than 600 patients, the objective response rate was 19% with a median survival of 4 months. A reasonable approach is to administer three courses and to reassess at 2 months. If there is evidence of a response, in terms of a greater than 50% fall in serum AFP or a decrease in liver or tumor size as determined by ultrasound or CT scanning, then treatment should be continued to a maximum dose of 550 mg/m<sup>2</sup>. Above this cumulative dose, cardiotoxicity becomes increasingly frequent. In the absence of response, active treatment should be abandoned or changed. Recently a



**Figure 29.13**  $\gamma$  scan of the liver after injection of technetium-labeled macroaggregated albumin. This serves as a simulation to examine the distribution of yttrium-labeled microspheres that will occur when these are given as treatment for hepatocellular carcinoma (HCC). In (a) the activity is concentrated in the two liver tumors, indicating a high tumor to normal ratio and no shunting to the lungs. In (b) there is massive lung shunting. If this latter patient were given yttrium treatment, radiation pneumonitis would be very likely to occur. (Courtesy of Dr Stephen Ho.)

regimen known as PIAF (platinum, interferon, Adriamycin, and 5-FU) has been shown to convert about 10–20% of unresectable tumors to resectable ones, but this regimen should remain experimental until controlled trials have been completed. It is noteworthy that the most common primary liver tumor in childhood, hepatoblastoma, is significantly more chemosensitive, and it is now common practice to administer chemotherapy before surgical resection. The most usual regimen is PLADO (cisplatin 80 mg/m<sup>2</sup> over 24 h by continuous infusion) and doxorubicin (60 mg/m<sup>2</sup> as an intravenous infusion over 48 h). Using this regimen, more than 80% of cases of hepatoblastoma will achieve remission, and initially unresectable tumors can often be resected after four courses.

The last decade has seen interest in altering the hormonal environment of the tumors and small studies suggested survival benefit from antiandrogenic, antiestrogenic agents and octreotide. However, recent large-scale prospective randomized controlled studies have generally failed to find any support for these contentions. It should be recognized that these systemic treatments tend to be studied in patients with advanced disease and the possibility that they would be more effective if instituted earlier can not be excluded.

### Screening programs for hepatocellular carcinoma

Whole population screening is not an option, but there are well-defined high risk groups – patients who have chronic liver disease and those who carry the HBV and/or HCV – who may benefit from HCC surveillance programs (although in a proportion of patients who develop HCC, the underlying liver disease is ‘silent’ and would thus never present for surveillance). The lifetime risk of HCC development for a male who has cirrhosis in any of these groups is in the range of 10–40%. In such an individual, who has access to the necessary resources, it seems likely that a 3–6 monthly AFP estimation and ultrasound examination will lead to the detection of tumor development before it becomes clinically apparent. If liver transplantation is available, a case can be made for screening all those at risk. If not then screening is only appropriate in those with good liver function, who would be candidates for resection with curative intent if a tumor is detected.

Surveillance programs are commonly promoted in the West, but it should be stressed that, at the time of writing, this practice is not supported by evidence showing that it is either

cost effective or leads to improved survival and the practical problems associated with surveillance should not be underestimated. Other, non-HCC lesions may be picked up, including macroregenerative nodules, dysplastic nodules and hemangiomas, and these are not easy to confirm histologically particularly when small. Furthermore the patient undergoing resection or transplantation faces a 5–10% chance of perioperative mortality, whereas without operation the survival with an asymptotically detected tumor may be up to 3 years.

### OTHER PRIMARY LIVER TUMORS

The ratio of HCC:cholangiocarcinoma:other primary malignant liver tumors is in the order of 100:10:1. It follows that in most units physicians and pathologists will have only a very limited experience of ‘other’ primary liver tumors. It is, however, important that such diagnoses are established accurately since some of the tumors described below have a significantly better prognosis than HCC and are more often cured by surgery. Histologic review by a pathologist who has a special interest in liver tumors is always recommended if there is any doubt whatsoever about the diagnosis. Fibrolamellar carcinoma is considered a ‘variant’ of HCC and has been described above.

### Sarcomas

Angiosarcomas present in a manner similar to those of other malignant tumors of the liver, usually with hepatomegaly, pain and weight loss, or hemoperitoneum. In about 25% of cases a history of occupational exposure to vinyl chloride (VC), thorotrast or arsenic can be obtained. Precursor features are well recognized and include periportal and subcapsular fibrosis and evidence of activation of hepatocytes in the sinusoid lining cells. Exposure to VC is now strictly controlled during the production of polyvinyl chloride so that the number of cases is likely to decrease significantly. A similar situation exists with thorotrast, a radiologic contrast medium containing a colloidal suspension of thorium dioxide (a powerful emitter of  $\alpha$  particles), which was first used in the 1920s but has been withdrawn since 1950. The minimum period between exposure and tumor development is 15 years, but cases are still being encountered more than 40 years after the agent was withdrawn from use. The radioactive emission of the thorotrast can be readily detected by autoradiography and the particles of thorotrast can be seen by



electron microscopy. The tumors are usually multifocal and, apart from the occasional patient with localized disease where the tumor is amenable to resection, the prognosis is less than 1 year with no therapy having been shown to alter the natural history.

### **Epithelioid hemangioendotheliomas**

Epithelioid hemangioendotheliomas represent the malignant end of a spectrum starting with the typical hemangioma. There are no specific presenting features and they are most often seen in young and middle-aged females. The tumors are usually multiple at presentation and metastases are detectable in about half of all cases. Although the tumor may appear epithelioid on routine histologic examination, its endothelial origins can be confirmed by staining for factor-VIII-related antigen, and the malignant cells have characteristic ultrastructural features. The prognosis is very variable. Most patients will die from the tumor, but prolonged survival periods of up to 10 years, even without treatment, have been reported. The standard surgical approaches of resection where this is possible, or liver transplantation, are still recommended.

### **Primary hepatic lymphomas**

Such tumors are even rarer than those mentioned above. Their diagnosis remains controversial since it is often difficult to establish that they are truly primary. Occasionally cases can be cured by resection or palliated with chemotherapy, but the prognosis is very poor in the majority of cases.

### **Malignant liver tumors in childhood – hepatoblastoma**

Malignant liver tumors are rare in children, but when present are usually hepatoblastomas. Of hepatoblastomas, 90% occur within the first 5 years of life and they outnumber HCC in this age group by a ratio of 2:1. The usual presentation is with an enlarging upper abdominal mass together with pain, weight loss and anorexia. Association with congenital abnormalities is well described as are cases of precocious puberty related to  $\beta$ -human chorionic gonadotropin (HCG) production by the tumor cells. Serum AFP is consistently elevated, often to very high levels. It accurately reflects tumor mass and is useful in monitoring treatment. On imaging the tumor is hypervascular, usually solitary and often exhibits calcification.

Primary liver tumors in childhood are uniformly fatal without resection. As with adult HCC, the main hope of cure is complete surgical resection. About 50% will be suitable for resection and half of these will survive long term. However, the tumor is highly sensitive to chemotherapy (usually Adriamycin and cisplatin) and postoperative adjuvant therapy, even in the presence of incomplete resection, has been found to improve survival rates. Currently, in several centers, preoperative chemotherapy is given to all patients irrespective of the perceived degree of operability. Long-term survival rates in the order of 80% are being achieved. All children who have hepatoblastoma should be managed in centers conversant with a multimodality approach to treatment.

## **SECONDARY LIVER TUMORS**

Until recently the approach to the patient who has hepatic metastases has been largely nihilistic and the detection of metastases immediately heralded withdrawal of all active

therapy. However, it is now recognized that, with careful selection, a small percentage of patients may be cured by surgery, a proportion may have their survival prolonged, and others can be offered a useful improvement in quality of life by cytotoxic chemotherapy. Equally, however, administration of cytotoxic therapy to patients who have poor performance status and widely disseminated disease may significantly decrease their quality of life during their few remaining months. A carefully balanced approach is therefore needed. Hepatic metastases are often only one site of metastatic disease and the overall management of such cases is the purview of the oncologist. The following section briefly summarizes the situation for those in which the liver is the sole or predominant site of disease.

Liver metastases are found at 1% of all autopsies. After lymph nodes, the liver is the most common site of metastatic disease, being involved in 40% of adult patients who have primary extrahepatic malignancy and up to 75% of those with primary tumors drained by the portal venous system (pancreas, large bowel and stomach). About 20% present with synchronous metastases (the figure is higher if metastases are sought by intraoperative US) and a further 20% of hepatic metastases develop during the course of the disease. The tumors may be solitary or multiple, but unlike HCC they tend to be umbilicated and infiltrate the normal liver tissue (Fig. 29.14). Around 25% of cases will have solitary liver metastases and thus be candidates for some form of surgical resection; this figure is much lower for other primary tumors such as those of breast and lung.

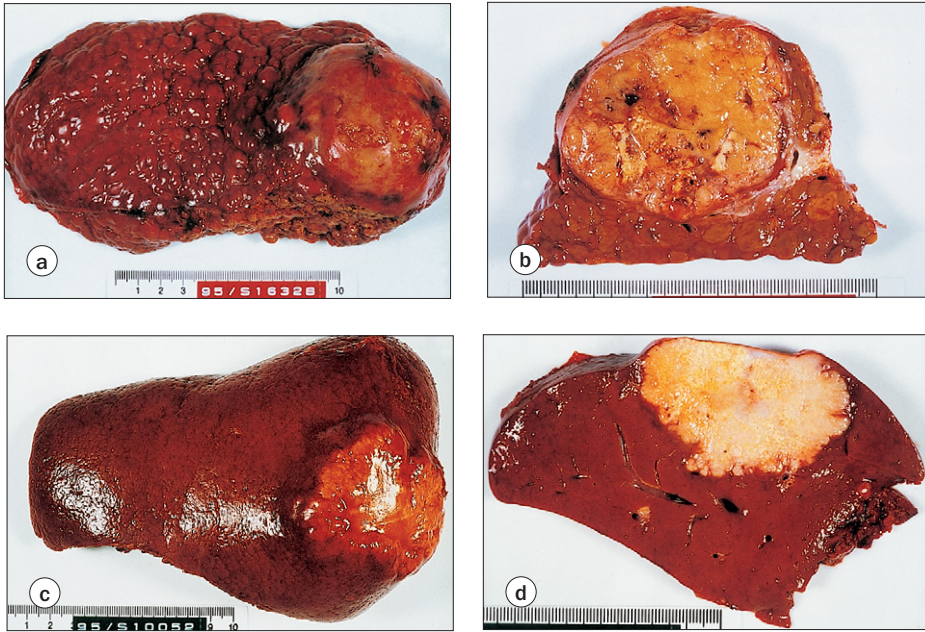
### **Clinical features and disease associations**

Patients who have hepatic metastases present with pain, weight loss, anorexia and hard hepatomegaly in which discrete masses can often be palpated. The abdomen may be distended with ascites and there may be other, extrahepatic, signs of malignancy. These are all signs of advanced disease. Patients who have hepatic metastases who may be candidates for surgical resection often have their tumors detected before symptoms develop. Certain metastatic tumors, particularly carcinoid and other 'neuroendocrine' tumors including islet cell carcinomas, appear to involve the liver with relatively little disruption of hepatic function (see below).

### **Diagnosis**

Routine liver tests are not very helpful in diagnosing liver metastases, although metastases are unlikely if all routine liver tests are entirely within their reference ranges. Any abnormality of standard liver function, particularly a raised activity of hepatic alkaline phosphatase, is an indication for further imaging of the liver.

Contrast-enhanced CT of the abdomen is now widely considered as a routine investigation in patients presenting with colorectal cancer and has sensitivity in the order of 85%. With intraoperative US scanning the sensitivity reaches 100% (by definition, as this is the gold standard by which other techniques are judged). If isolated hepatic metastases are detected, CT of the chest and pelvis should be undertaken, if not part of the initial investigation. It should be stressed that the figures quoted above refer to the detection of metastases when the reporting radiologist has been specifically requested to examine the liver with a view to detecting tumors. Metastases show lower



**Figure 29.14 Comparison of the macroscopic appearances of primary and secondary liver tumors.** Note that the primary tumor (hepatocellular carcinoma (HCC)) arises as a well-circumscribed, solitary nodule in an extensively macrocirrhotic liver and bulges from the surface of the liver (a & b). In marked contrast the secondary tumor arises in a non-cirrhotic liver, is infiltrating in nature, and umbilicated (c & d). (Courtesy of Dr CT Liew.)

attenuation than the surrounding liver, both before and after contrast, and often show peripheral ring enhancement on ultrasound examination (Fig. 29.15). In contrast to primary liver tumors, metastases tend to be umbilicated and do not expand and distort the liver surface to the same extent (Fig. 29.15). About 20% of patients who have hepatic metastases from colorectal cancer will show calcification on CT or US and as such seem to have a significantly better prognosis. Estimation of the serum concentration of CEA is used mainly to detect recurrence after resection of the primary colorectal tumor and, as such should be a part of routine initial investigation. A rising CEA level in this situation usually heralds metastases, most often in the liver.

All too frequently patients present with malignant liver disease (usually reported as adenocarcinoma on biopsy), but no obvious primary extrahepatic site can be detected after full

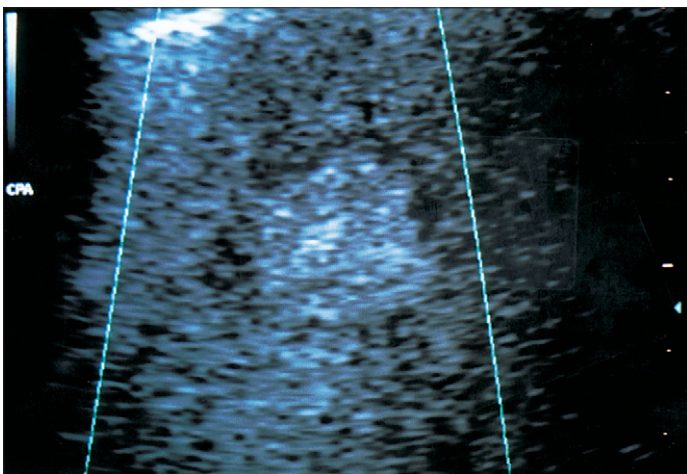
physical examination. Extensive radiologic investigations such as barium meal, barium enema and intravenous pyelography are not indicated as the occult tumor is seldom situated in the gut or kidney and both false-positive and false-negative results are frequent. CT scanning is the most rewarding investigation, but in a proportion of these patients the primary is never identified.

### Histology

Histologic confirmation of suspected hepatic metastases is not always indicated, particularly when active treatment is not contemplated. However, if the source of the primary tumor is not known, biopsies are usually done to exclude some of the more treatable forms of metastatic disease such as lymphoma or breast cancer. In addition, when the lesion is solitary, biopsy is indicated unless resection is to be undertaken. The presence of metastases obviously has major implications for the patient, but this diagnosis should not be presumed if a solitary lesion is detected as this may be an unrelated benign lesion. When the primary tumor from which a hepatic metastasis has originated is not apparent, histologic examination may provide a clue to the site of the primary (Fig. 29.16). Adenocarcinomas are usually of colorectal or pancreatic origin, but intrahepatic cholangiocarcinomas also have a similar appearance. Anaplastic tumors are usually of bronchial origin. Recently developed histological methods involving special stains, immunocytochemistry, and electron microscopy often permit determination of the origin of these highly anaplastic tumors. Thus, the CK7/CK20 profile often gives clues as to the primary site of the lesion, and positive nuclear staining for TTF1 is suggestive of a primary lung tumor.

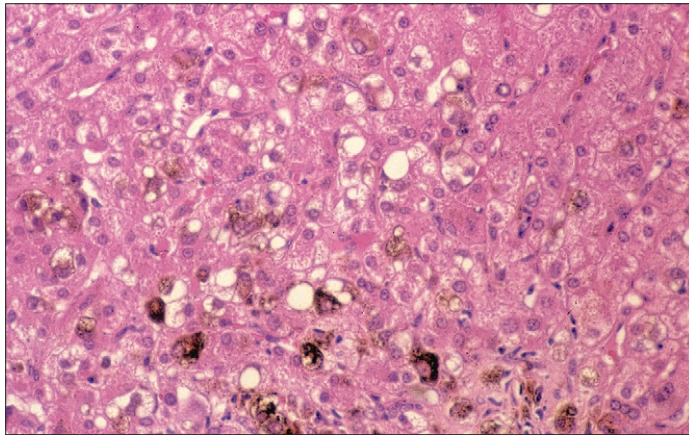
### Natural history and prognosis

In the absence of treatment there is an inverse linear relation between percentage of liver involved and log survival time (from diagnosis) with secondary liver tumors. About 50% of patients are alive at 3 months and less than 10% at 1 year, although individual cases may show wide variations. Patients who have multiple metastases have a significantly worse prognosis than



**Figure 29.15 Characteristic liver secondary seen on ultrasound examination as a 'target' lesion with a hypoechoic rim and a hyperechoic center.** (Courtesy of Dr Wei Tse Yang.)





**Figure 29.16 Hepatic metastasis for a primary melanoma.** The site of a secondary tumor can seldom be confidently made on routine histologic examination. Here the presence of melanin confirms metastasis from a primary melanoma. (Courtesy of Dr Bernard Portmann.)

those who have solitary metastases, as do those who have any evidence of other, extrahepatic, metastases. Among those who have solitary lesions receiving no active treatment, a 20% 3-year survival rate has been quoted. This observation should not be forgotten when assessing the efficacy of surgical resection of solitary metastases in uncontrolled studies. Metastases from primary carcinoid tumors are the only secondary tumors to exhibit a significantly better prognosis, survival periods of up to 10 years not being uncommon. With the wide use of more sophisticated radiologic techniques the diagnosis is being established earlier, often while the patient is asymptomatic, and this makes for an apparent increase in survival.

### Management

As with primary tumors, the first aim of investigation is to identify that small subgroup that might benefit from surgical resection, the only hope of cure. In about 25% of cases of metastatic colorectal cancer disease is confined to the liver and overall, about 20% of all patients who have hepatic metastases will be candidates for surgical resection with curative intent. A review of the literature shows that survival at 1, 3, 5 and 10 years is of the order of 85, 50, 35 and 20% respectively. Although the complication rate can be high, the perioperative mortality should now be well below 5%. The precise indications vary from unit to unit. Some consider that any cases in which all macroscopic disease can be removed should be candidates. Others confine surgical resection to those who have solitary lesions, or at most, three small lesions confined to one lobe. Such decisions remain a matter of philosophy and resource. Resection appears less likely to be curative as the tumor size increases, the number of nodules decreases, and as the time between resection of the primary tumor and the detection of liver metastases decreases. An involved margin after resection is usually a harbinger of subsequent recurrences. The optimal follow-up strategy remains unclear and although there is now evidence from randomized controlled trials that post operative adjuvant therapy with intravenous and/or intra-arterial administration of 5-FU based therapy decreases the recurrence rate and improves survival, this approach has not yet entered routine practice.

Surgical resection of hepatic metastases is an area of intense research at present and much of the current dogma is being actively challenged. The wider application of 2-[fluorine-18] fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) and intraoperative ultrasound scanning prior to resection is frequently demonstrating lesions not detected on the original imaging. Several groups are examining the possibility, in carefully selected cases, of resecting both the liver and lung lesions, and combining surgical resection with RFA when the complete surgical resection cannot be attained. Preoperative cytotoxic chemotherapy, to render an initially unresectable lesion resectable, is also an area of intense investigation.

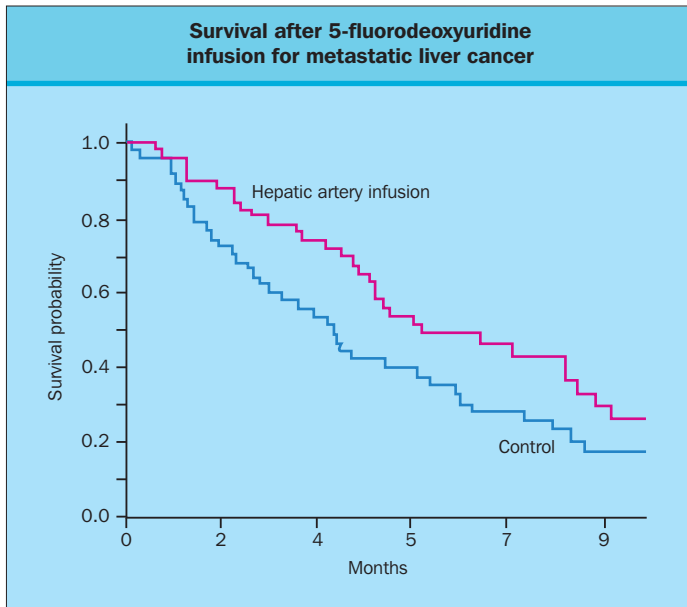
### Management of inoperable hepatic metastases

Steady incremental advances are improving the outlook for patients with unresectable metastatic colorectal cancer. 5-FU in combination with folinic acid (FA) remains the foundation of treatment and the standard infusional regimens lead to response rates of around 20% and an improvement in median survival from 6 months to 12 months. Two recently introduced drugs (oxaliplatin and irinotecan), in combination with 5-FU-FA, can each increase the response rate to 30–50% with a median overall survival of 15–20 months. With the addition of bevacizumab [an anti-vascular endothelial growth factor (anti-VEGF) monoclonal antibody] the figure for median survival appears now to exceed 20 months. Similar results can be obtained with hepatic arterial infusion of 5-FU-FA although the toxicity is quite distinct from that seen with systemic administration of 5-FU. Nausea, vomiting, diarrhea and myelosuppression are all uncommon. The toxicity relates to ulcer disease and hepatic toxicity. The most common lesion is a sclerosing cholangitis, probably due to primary damage of the blood vessels feeding the bile ducts, which also derive their blood supply from the hepatic artery. The overall cost, inconvenience, and side-effects of chronic intra-arterial chemotherapy still limit this approach. However, in highly motivated patients, with good performance status and modest disease burden, controlled studies have shown that significant improvement in quality of life can be obtained (Fig. 29.17).

### Hepatic metastases from the neuroendocrine tumors

Hepatic metastases often dominate the clinical picture in patients with neuroendocrine tumors particularly carcinoid tumors, even in the absence of the 'carcinoid syndrome'. The syndrome, which affects about 20% of patients with carcinoid tumors, comprises facial flushing, abdominal cramps and diarrhea and, less often, wheezing, and right-sided cardiac complications attributable to the episodic release of vasoactive peptides such as serotonin, kallikrein, 5-hydroxytryptophan and neurotensin. Since the liver can readily metabolize serotonin, the primary peptide produced, the carcinoid syndrome only develops when the liver is bypassed or there are metastases within the liver; the development of the carcinoid syndrome thus invariably implies metastatic spread from a primary lesion in the small bowel to the liver. Abdominal pain or discomfort is often due to hepatic involvement although the pain may be due to obstruction by the primary small bowel tumor. Diagnosis is based on an elevated level of urinary 5-hydroxyindoleacetic acid (5-HIAA), the characteristic histological pattern of a neuroendocrine tumor involving the liver (staining for synaptophysin





**Figure 29.17 Improvement in survival, with a normal quality of life after chronic infusion with 5-fluorodeoxyuridine (FUDR).** Reproduced from Allen-Marsh TG et al (94) by permission of Elsevier.

and CD 56 are very characteristic as is chromogranin, although the latter marker can be negative in poorly differentiated tumors), and disease distribution can be assessed by an octreotide scan. Because the synthetic analogs of somatostatin bind with high affinity to somatostatin receptors present on the great majority of carcinoid tumors, radiolabeled octreotide can be used for localization tumors, being more sensitive than conventional imaging procedures such as CT scanning, and being positive in over 80% of cases. It is highly characteristic that the patient's general clinical state and standard liver function tests can be remarkably good, even in the presence of massive liver involvement.

In the absence of symptoms, there is probably no indication for active treatment of hepatic carcinoid metastases unless complete surgical resection is feasible. Symptoms of the carcinoid syndrome may be controlled by reduction of tumor mass (by surgery, arterial embolization, or cytotoxic chemotherapy) or pharmacologic interference with production or action of the tumor products. Some of these approaches may have unpleasant side-effects and should be employed only when symptoms become severe.

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By the time the carcinoid syndrome has developed, the tumor is usually too widespread for curative resection to be attempted, but it may be feasible in 5–10% of cases. 'Debulking' surgery or 'shelling out' metastases may relieve symptoms, but as better approaches to pharmacologic control of symptoms become available and with the advent of hepatic artery embolization, it is being used much less widely. Embolization, with or without cytotoxic chemotherapy (doxorubicin, streptozocin and 5-FU) in various combinations control symptoms in most patients when disease is predominant in, or confined to, the liver. About 80% of patients achieve complete resolution of symptoms, which may last for 1 month to several years (median, 1 year). The procedure can be repeated and some patients have had multiple embolizations over several years. Liver transplantation has been employed in carefully selected cases.

### Pharmacological control

$\alpha$ -Methyl-dopa and parachlorophenylalanine, which inhibit key enzymes in the biosynthesis of serotonin from tryptophan, have now been largely superseded by the long-acting somatostatin analog octreotide. Octreotide is usually dramatically effective against diarrhea and frequently against flushing too, with associated decrease in the 5-HIAA levels. The disadvantage has been that it has needed to be administered subcutaneously two or three times per day (50–100  $\mu$ g). Most patients, however, learn to administer the drug themselves, and effective slow release formulations that only require monthly injections are now available. The aim of therapy is symptom control; significant tumor shrinkage occurs in less than 10% of cases. Some authorities believe that somatostatin analogs have a role in carcinoid tumors even in the absence of the syndrome, where they may 'stabilize the disease'. When octreotide becomes ineffective, the addition of interferon- $\alpha$  may further control symptoms.

Control of diarrhea with codeine phosphate or loperamide and careful avoidance of precipitating factors such as alcohol, stress and certain foods may also be effective. Streptozocin is the most active cytotoxic agent to have been used but it leads to a reduction in tumor mass in only 30% of cases. The addition of other agents, such as adriamycin and 5-FU, may give rather higher response rates but at the cost of considerably greater toxicity. Cytotoxic chemotherapy should be the treatment of last resort and only used after embolization and pharmacologic approaches have failed. All these approaches to decreasing tumor bulk may be associated with the massive release of vasoactive peptides from the tumor tissue. To prevent the resultant complications, patients are treated with octreotide.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 30

## Vascular Diseases of the Liver

Dominique-Charles Valla

### INTRODUCTION

The vasculature of the liver is unusual in that it has both a venous and an arterial blood supply. Poorly oxygenated blood from the low pressure portal venous system supplies the hepatic parenchyma. Richly oxygenated blood from the high pressure arterial side is mainly directed to the bile ducts. Arterial blood is eventually collected into small veins that drain into the intrahepatic portal venous system. Thus, the hepatic sinusoids are exclusively supplied with venous blood, 65% of which comes directly from the portal vein, and 35% indirectly from the hepatic arteries. There is a poorly understood mechanism whereby the hepatic artery and portal vein work together to maintain a constant blood flow to the liver. Sinusoids drain into central veins which in turn drain into hepatic veins entering the inferior vena cava close to the right atrium.

In this chapter, diseases that affect the vascular structures of the liver will be considered with the exception of parenchymal or biliary diseases that secondarily affect the hepatic vasculature. Likewise, vascular disorders that occur secondary to systemic circulatory disturbances such as cardiac failure or cardiogenic shock will not be discussed further.

### HEPATIC ARTERIAL DISEASES

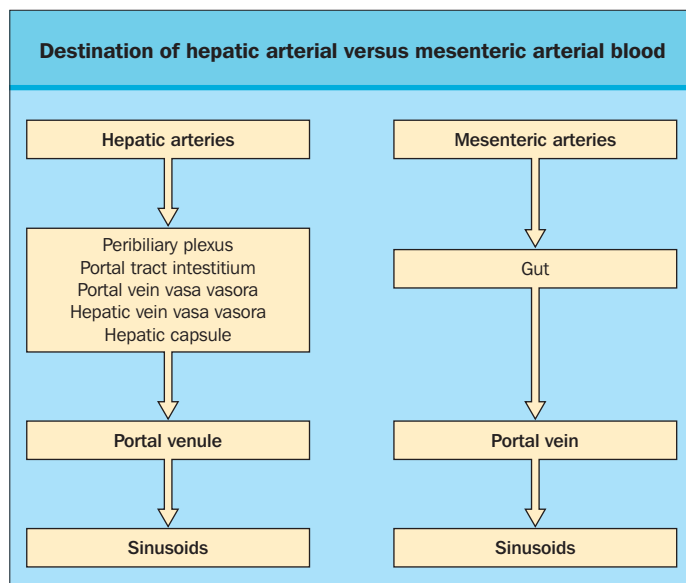
#### Hepatic artery occlusion

Over 50% of hepatic arterial blood flow is distributed to the intrahepatic bile ducts (through the peribiliary plexus), with the rest going to the large blood vessels (through the vasa vasora), to the portal tracts, and to the liver capsule (Fig. 30.1). In addition to the main hepatic arteries that enter the liver at the hepatic hilum, there are numerous accessory arteries that directly penetrate the liver through the capsule. The branches of the hepatic arteries are interconnected. The peribiliary plexus drains into small veins that ultimately reach the intrahepatic portal venous system.

Hepatic arterial occlusion is relatively uncommon. The above characteristics explain why hepatic arterial obstruction gives rise to clinical sequelae only in particular circumstances. In essence, ischemic bile duct damage occurs when the peribiliary plexus is involved or when all main and accessory arteries are simultaneously blocked. Concurrent impairment of portal venous and hepatic arterial inflow results in liver infarction.

#### Etiology and pathogenesis

Occlusion of the main arteries may result from arterial constriction within a malignant tumor (most frequently a carcinoma of the bile duct or pancreas). Iatrogenic occlusion may be intentional or unintentional, and occur in the context of surgery (liga-



**Figure 30.1 Destination of hepatic arterial and mesenteric arterial blood.** There are striking similarities. Because over 50% of hepatic arterial blood is destined for the bile ducts, occlusion of the hepatic arteries can impact not only on the hepatic parenchyma but also on the bile ducts.

tion, suture, anastomosis, injury), or percutaneous catheterization (therapeutic embolization, inadvertent dissection). Stenosis or occlusion can be related to blunt or penetrating trauma; to systemic arteritis (e.g. polyarteritis nodosa, giant cell arteritis, Wegener granulomatosis, Behçet disease and systemic lupus erythematosus); or to systemic infection (e.g. bacterial endocarditis, tuberculosis, syphilis). Atheroma of the hepatic artery is rare compared with other arteries.

Outside the liver transplant setting, occlusion limited to the main hepatic arteries or their large branches is without consequences. The opening of pre-existing intrahepatic or extrahepatic anastomoses is seen at imaging within a few hours of the occlusion. In the context of decreased portal blood inflow, such as circulatory failure or portal vein occlusion, sudden obstruction of a main artery results in parenchymal infarction. When hepatic arterial occlusion gives rise to infarction, this is usually segmental with the central pale area surrounded by a hemorrhagic region.

Selective injury to the peribiliary plexus can be observed after transcatheter embolization of small-sized particles (less than 120  $\mu\text{m}$  in diameter); after intrahepatic arterial infusion of toxic substances (fluoruridine or alcohol); and in patients with systemic disorders characterized by microcirculatory impairment (e.g. sickle-cell disease, systemic lupus erythematosus,



antiphospholipid syndrome, paroxysmal nocturnal hemoglobinuria, polyarteritis nodosa). Injury to the peribiliary plexus induces ischemic bile duct injury, with destruction of the biliary epithelium. The cellular debris can mix with bile components to form biliary casts. There may be complete necrosis of the bile duct wall with extravasated bile forming around bile infarcts within the liver. Bile infarcts can become cystic (bilomas), or superinfected. Healing of the areas of necrosis can give rise to fibrous stenoses or, conversely, to cystic dilatation of the bile ducts. When areas of stenosis or dilatation are multiple, this aspect of the biliary tree mimics the findings that are seen in primary sclerosing cholangitis.

In the setting of liver transplantation, the bile ducts and the liver are particularly vulnerable to arterial occlusion. This can be explained by the devascularization of the liver capsule (preventing collaterals from developing from accessory arteries) and to preservation injury to the endothelium of the peribiliary plexus. Arterial thrombosis may contribute to the formation of a stricture at the site of bile duct reconstruction.

### **Clinical features**

The classic features of hepatic infarction are severe right upper quadrant abdominal pain accompanied by tachycardia, hypotension, fever and leukocytosis. Serum aminotransferase levels are markedly increased.

Ischemic bile duct injury can develop in the absence of clinical features and be revealed by increased serum alkaline phosphatase levels, several weeks or months after arterial occlusion has occurred. High fever, jaundice and right upper quadrant pain may be present when there is acute severe necrosis of the bile ducts, particularly with biliary cast formation. Bilomas may be silent, and only detected at imaging; or they may become infected giving rise to the features of single or multiple liver abscesses. Unpredictable episodes of recurrent cholangitis occur when there are stenoses or dilatation, particularly when there are secondary stones. In some patients, chronic cholestasis leads to secondary biliary fibrosis and portal hypertension.

### **Diagnosis**

The diagnosis of hepatic infarction is established by computed tomography (CT) or magnetic resonance (MR) imaging showing a triangular zone with a peripheral base without any enhancement at the arterial and portal phase of contrast injection. An associated portal vein thrombosis or an episode of shock may be present.

Bile duct injury is suggested by ultrasonography (US) or CT, and confirmed by MR cholangiography. In selected cases, endoscopic retrograde cholangiography is needed to show diffuse beading, or single or multiple narrowing that usually predominates at the confluence of the left and right hepatic ducts and in the upper part of the main bile duct. The main differential diagnosis is primary sclerosing cholangitis. Bilomas are demonstrated using US, CT or MR imaging. A superinfected biloma is difficult to differentiate from an abscess of other origin.

The diagnosis of arterial thrombosis should be suspected whenever the above features are found in a patient with a history of hepatic surgery, radiologic intervention involving the hepatic artery, abdominal trauma, systemic vasculitis or infection. Diagnosis will usually be suggested by Doppler ultrasonography and

confirmed using CT or MR angiography. Arteriography is considered in selected cases or as a first step when being performed as part of a therapeutic procedure.

### **Natural history and prognosis**

The outcome for patients who have hepatic artery occlusion depends to a great extent on the anatomic position of the occlusion, the rate of the occlusion, and the status of the portal circulation. The underlying condition is a major determinant of prognosis.

Focal infarction at the periphery of the liver has little clinical sequelae. The infarcted area undergoes atrophy while there is hypertrophy of the rest of the parenchyma. However, when several segments of the liver are involved, either contiguously or as multiple lesions, liver failure may develop.

Similarly, focal bile duct lesions have limited clinical consequences if they are located at the periphery of the biliary tree. The parenchymal area corresponding to the bile duct lesion may undergo atrophy or abscess formation. When the bile duct lesions are centrally located, they will impact on the whole liver and transplantation may be required.

If hepatic artery occlusion follows soon after liver transplantation, the outcome is poor without urgent recanalization of the hepatic artery or retransplantation.

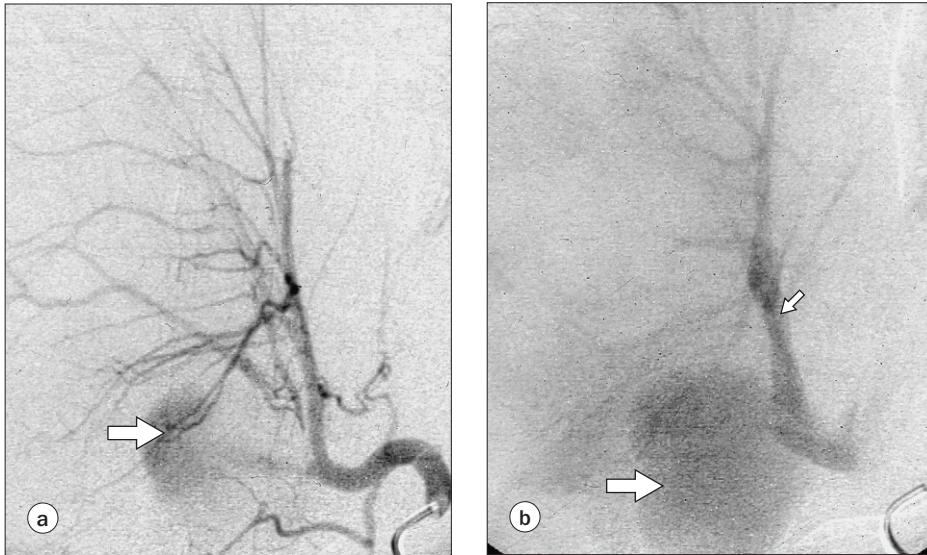
### **Management**

The management of patients who have hepatic artery occlusion depends upon the clinical sequelae. If acute liver failure develops, then management should be instituted as described in Chapter 33. Bile duct damage may require percutaneous drainage of bilomas or abscesses, biliary drainage and insertion of stents for stenoses as described in Chapters 20, 28 and 33. In patients who have less severe complications, management may be expectant. Anticoagulation should be started in patients with portal venous thrombosis. Antibiotics should be used to prevent secondary infection in infarcted liver tissue and whenever there is acute cholangitis.

In the transplant patient with hepatic artery occlusion, reconstruction or recanalization of the hepatic artery must be considered urgently, using interventional radiology or surgery, as discussed later in this chapter. Outside the transplant setting, a decision to attempt recanalization must take into consideration the severity of the clinical sequelae, the location and the mechanism of the occlusion, and the association with portal vein thrombosis.

### **Hepatic artery aneurysms**

Aneurysm of the hepatic artery is a rare condition, the cause of which may be: atheroma, fibromuscular dysplasia, polyarteritis nodosa, systemic infections (e.g. endocarditis, tuberculosis, and syphilis), or intra-abdominal sepsis; due to trauma [for example following liver biopsy or transjugular intrahepatic portosystemic shunt (TIPS) insertion] or congenital. The aneurysms can be either intra- or extrahepatic. They may show considerable variation in size. A distinction is made between a true aneurysm where the dilatation involves all three layers of the arterial wall, and a false aneurysm where the dilatation results from a rupture of the intima and of the media with preservation of the adventitia. Most aneurysms due to trauma or infection are of the false type. Dissections and aneurysms may thrombose or may



**Figure 30.2 Hepatic arterial aneurysm.**

(a) Traumatic hepatic arterial aneurysm filling with radiographic contrast (arrow). (b) The same aneurysm now filled (arrow). Early filling of a portal vein branch has occurred via an arteriovenous fistula (small arrow).

rupture into the peritoneal cavity, the liver, the bile ducts (causing hemobilia), or the intra or extrahepatic portal vein (causing arterioportal fistula).

Hepatic artery aneurysms are most common in middle-aged men and are often asymptomatic for long periods. The most common symptom is abdominal pain which can be severe particularly if there is arterial dissection. The risk of rupture increases with the size and the number of aneurysms. A diameter below 2 cm indicates a low risk of rupture. When compared with atheroma, fibromuscular dysplasia and polyarteritis nodosa have a greater risk of rupture. Pregnancy can precipitate the rupture of pre-existing aneurysms. Large extrahepatic aneurysms may cause obstructive jaundice or portal vein thrombosis.

Doppler-US, and CT or MR angiography are the key diagnostic investigations as described in Chapter 8. The use of arteriography should be limited to therapeutic evaluation.

The prognosis for a ruptured hepatic artery aneurysm is poor; mortality as high as 40% has been reported. Expectant management with imaging follow-up at regular intervals is appropriate in patients with silent intrahepatic aneurysms less than 2 cm in diameter. Percutaneous transarterial embolization or stenting has become the preferred therapy for large or symptomatic lesions. A ruptured aneurysm is best managed using emergency embolization therapy after appropriate resuscitation. Surgery can be used where interventional radiology fails or is unsuitable. (Fig. 30.2).

### **Solitary arterioportal fistula**

In most cases, an arterioportal fistula is produced by the rupture of a false aneurysm of the hepatic artery into the neighboring portal vein branch (see above for causes of false aneurysm). Previous needle liver biopsy is probably the most common cause of the small arterioportal fistulas detected with current imaging techniques. Abdominal trauma is the most common cause of large arterioportal fistulas. Small peripheral arterioportal fistulae are common findings at CT or MR imaging in patients with cirrhosis or hepatocellular carcinoma.

Even with large arterioportal fistulae, the increase in portal pressure is usually moderate and does not reach a clinically significant level. In patients where an arterioportal fistula is associated with clinically significant portal hypertension, underlying chronic liver disease should be considered. Such an association may not be totally fortuitous since traumatic injury is more common in alcoholics or drug addicts. Large fistulae can induce mesenteric to hepatic arterial stealing resulting in intestinal ischemia. A hepatic bruit is usually present. The diagnosis is usually made by either Doppler-US or by CT or MR imaging.

Treatment of arterioportal fistulae depends on their size and position. Small intrahepatic fistulae may require no treatment. Extrahepatic fistulae can be managed with embolization using a transarterial or a transhepatic portal approach. However, spreading or dislodgment of the embolized material can lead to portal or mesenteric vein thrombosis.

### **Hereditary hemorrhagic telangiectasia (HHT)**

Hereditary hemorrhagic telangiectasia is characterized by malformations of the small vessels resulting in arteriovenous fistulae. The condition is inherited as an autosomal dominant trait with one of two possible genes involved: endoglin on chromosome 9, and an activin receptor-like kinase on chromosome 12. Both genes code for a receptor of the transforming growth factor (TGF)- $\beta$  superfamily and are mainly expressed on endothelial cells. Involvement of the skin and of nasal and gastrointestinal mucosae is common. Hepatic involvement is rare. Fistulae may be predominantly arteriovenous or arterioportal. Under the microscope, randomly distributed telangiectasias are surrounded by various degrees of fibrous tissue. Nodular hyperplasia and sinusoidal fibrosis are common. Among HHT patients liver involvement without obvious extrahepatic involvement is rare.

Massive arteriovenous shunting within the liver may cause high output cardiac failure in the absence of liver dysfunction or portal hypertension. Ischemic bile duct injury may occur due to blood-stealing away from the peribiliary plexus. When present, portal hypertension is related to either the association of nodular

regenerative hyperplasia and perisinusoidal fibrosis, or to arterio-portal shunting, or to post-transfusion chronic viral hepatitis. Ascites is rare.

The diagnosis of HHT is based on the cardinal features of the disease (recurrent epistaxis, cutaneous telangiectasias, familial history). Hepatic involvement is demonstrated by Doppler-US on the basis of a marked enlargement of the hepatic arteries with increased blood flow velocity. CT or MR imaging usually confirm the enlargement of the hepatic arteries and allow better characterization of gross architectural changes including large regenerative nodules.

Embolization of the hepatic artery has been associated with both encouraging short-term results, and severe – sometimes lethal – ischemic damage to the liver or bile ducts. Therefore, embolization should be considered only in patients with severe biliary involvement or progressive cardiac dysfunction when liver transplantation is contraindicated. Although liver transplantation will not cure the other complications of HHT, its results are good provided it is performed before severe cardiac dysfunction has occurred.

PORTAL VEIN DISEASE

Disorders of the portal vein include aneurysm, fistulae and obstruction. The so-called portal vein cavernoma corresponds to the network of hepatopetal collaterals that develop following permanent obstruction of the portal vein, of its main radicles, or of its main branches.

Portal aneurysm

Aneurysm is usually defined as an increase in portal vein diameter taking a fusiform or a saccular aspect. This is an extremely uncommon condition the cause of which is unknown; it may be congenital. Aneurysms may rupture or thrombose. Most cases of portal aneurysm are recognized fortuitously at abdominal imaging. Expectant management is reasonable. Acute thrombosis of a previously normal portal vein can be associated with a marked but transient enlargement of the thrombosed portion which may lead to an erroneous diagnosis of pre-existing portal aneurysm.

Spontaneous portacaval fistula

These fistulae are characterized by a large-sized communication between the portal vein and the inferior vena cava in the absence of chronic liver disease. Two entities have been described. In the first entity, ‘congenital absence of the portal vein’, the extrahepatic portal vein is not seen at mesenteric or celiac angiography because of a total fistula between a mesenteric vein and the inferior vena cava. In the second entity, one or several intrahepatic fistulae are demonstrated. For both entities, evidence for a congenital anomaly stems from their recognition in neonates screened for congenital galactosemia (portosystemic shunting induces hypergalactosemia), and from their association with other developmental anomalies. However, similar intrahepatic shunts can develop in patients with acquired chronic liver disease suggesting that a block in the intrahepatic circulation may actually cause intrauterine development of a portacaval fistula. In most cases, there is no evidence for familial transmission.

Enlargement of the hepatic arteries suggests compensatory arterIALIZATION. Morphometry may show a paucity of intra-hepatic portal veins. Macroregenerative nodules (resembling focal nodular hyperplasia) or nodular regenerative hyperplasia may develop which may be explained by an imbalance between increased arterial perfusion and abolished portal inflow. There are anecdotal reports of adenoma, hepatoblastoma or hepatocarcinoma.

Although liver function is well maintained, patients may develop portal-systemic encephalopathy, usually after the age of 50.

Diagnosis is established by Doppler-US and CT or MR angiography. Most patients will not require any treatment. In patients with debilitating portal-systemic encephalopathy, percutaneous transhepatic embolization of the shunt should probably be attempted first, keeping liver transplantation as an ultimate option. Proper characterization of the macronodules may require limited surgical resection. Large hepatic resections should be avoided because there is concern that regeneration can be impaired by the lack of portal inflow.

Extrahepatic portal vein obstruction

Etiology and pathogenesis

Primary adenocarcinomas of the liver, pancreas or bile ducts may invade or constrict the portal vein. When not related to a tumor, portal venous obstruction is due to thrombosis. Causes of portal vein thrombosis (PVT) can be systemic or local. Causes are usually not apparent at the time of presentation. Therefore, investigations have to be systematic. As showed in Table 30.1, all the known hereditary or acquired thrombogenic factors have been incriminated, with primary myeloproliferative disorders and protein S deficiency as the leading causes. Chronic portal

Thrombogenic conditions that cause portal vein thrombosis or primary Budd-Chiari syndrome		
	Approximate prevalence in patients with	
	Portal vein thrombosis (%)	Budd-Chiari syndrome (%)
Myeloproliferative disorder	25	50
Antiphospholipid syndrome	13	20
Paroxysmal nocturnal hemoglobinuria	1	5
Behçet disease	0	5
Factor V Leiden	5	25
Factor II gene mutation	13	5
Primary protein C deficiency	3	25
Primary protein S deficiency	25	3
Primary antithrombin deficiency	1	1
Homozygous C677T MTHFR mutation	13	13
Combination of two or more of the above factors	15	25

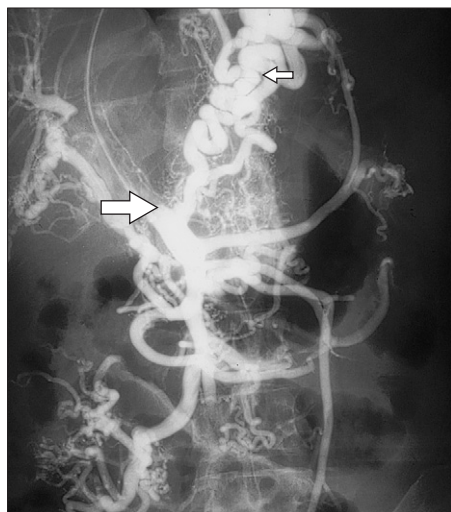
Table 30.1 Thrombogenic conditions that cause portal vein thrombosis or primary Budd-Chiari syndrome.



hypertension per se induces a decrease in the levels of protein C, protein S or antithrombin, which makes recognition of a primary defect difficult. Likewise, iron deficiency, hypersplenism and hemodilution may mask the peripheral blood features of myeloproliferative disorders. It is unclear whether pregnancy or administration of estrogen/progesterone precipitates PVT. Local factors are identified in 30% of patients at the time when they present with acute thrombosis, and in a lesser proportion when they present at the late stage of portal cavernoma. Common local factors include portal venous injury (e.g. cannulation, section, ligation, anastomosis, splenectomy); portal venous stasis (due to cirrhosis, obliterative portal venopathy or hepatic venous outflow block); and inflammatory conditions in the splanchnic area (e.g. appendicitis, diverticulitis, inflammatory bowel disease, pancreatitis, cholangitis, liver abscesses). Surgery for portal hypertension has been a major provider of PVT in the past. Several systemic causes, or systemic and local causes are frequently combined. Currently, about 25% of PVT cases remain unexplained despite extensive investigation.

In patients with cirrhosis but no hepatocellular carcinoma, the risk of extrahepatic PVT is related to the severity of liver disease, being less than 1% in Child-Pugh class A patients, and up to 15% in candidates for liver transplantation or portosystemic shunting. In the native livers from patients coming to transplant, about 50% of intrahepatic portal veins are found to be thrombosed. Decreased portal blood velocity, which occurs with progressive worsening of cirrhosis, probably explains PVT in patients with advanced liver disease. However, there is evidence that extrahepatic PVT in a Child-Pugh class A patient is precipitated by an underlying or incidental thrombogenic state.

Upstream from the portal vein thrombus, preserved mesenteric venous arches prevent ischemic damage to the gut, whereas involvement of these arches produces intestinal ischemia. Downstream, the liver is protected from clinically significant ischemia by an increased arterial blood flow (the buffer response), and by a rapid development of hepatopetal collaterals (the cavernoma) (Fig. 30.3). These collaterals, however, do not relieve the extrahepatic block, so portal hypertension ensues.



**Figure 30.3**  
**Transhepatic venogram**  
**showing thrombosis in**  
**the portal vein (arrow).**  
There is widespread  
collateral circulation  
(small arrow).

There are particular consequences to the obstruction of one major branch of the portal vein, with the other branches remaining patent. In the obstructed territory, the hepatic parenchyma undergoes atrophy due to apoptosis and necrosis. Sinusoids are dilated. In the unobstructed territory, however, the hepatic parenchyma undergoes hypertrophy related to hepatocellular proliferation. The mechanisms signaling this adaptation are not yet understood.

### **Clinical features**

#### **Acute thrombosis**

Many cases of extrahepatic PVT are now recognized at the acute stage of recent thrombosis (acute pylephlebitis) in patients with abdominal pain, often associated with fever. When thrombosis is precipitated by an abscess in the splanchnic area, spiking fever with chills and positive blood cultures indicate septic pylephlebitis. Patients in whom portal vein thrombosis complicates intra-abdominal inflammation or malignancy may present with obvious features of the underlying disorder such as a perforated viscus or necrotizing pancreatitis.

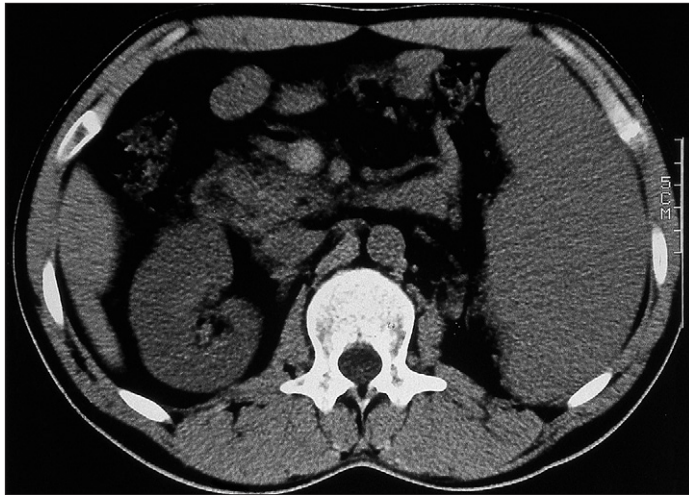
#### **Chronic portal vein thrombosis**

When the initial episode goes unnoticed, the condition is recognized at the stage of portal cavernoma, usually as a fortuitous discovery of portal hypertension in the investigation of thrombocytopenia, splenic enlargement, or esophageal varices. Gastrointestinal bleeding has become an uncommon presentation of PVT. Presentation with ascites or encephalopathy is rare, although these features may transiently accompany gastrointestinal bleeding. Biliary complications may be the presenting features of a portal cavernoma.

### **Investigations and diagnosis**

Liver specific tests are generally normal in patients who have portal vein thrombosis not secondary to parenchymal liver disease, although mild abnormalities may be present in adults who have portal vein thrombosis. In such patients liver biopsy typically shows normal hepatic parenchyma. However, some patients with superimposed or pre-existing intrahepatic portal vein thrombosis may show some histological alterations (see below). Complete blood counts may show thrombocytopenia and leukopenia with or without anemia related to hypersplenism. However, blood cell counts may be increased or high normal which, in a patient with massive splenic enlargement, suggests that a primary myeloproliferative disorder is present. Wedged hepatic venous pressure in portal hypertension due to portal vein thrombosis is normal, but where the thrombosis is secondary to hepatic disease, wedged hepatic venous pressure may be elevated.

CT scan and Doppler-US are the key diagnostic procedures, as described in Chapter 8. At the acute stage, diagnosis is based on the absence of flow in the portal vein lumen which is occupied by solid material. Differential diagnosis includes a tumor invading the vein. At CT scanning, spontaneous hyperdensity of the portal vein prior to contrast injection indicates recent thrombosis (Fig. 30.4). Arterial phase enhancement of the endoluminal material indicates invasion by a malignant tumor. At the late stage, diagnosis of portal cavernoma is made by showing that the normal portal vein is replaced by winding venous structures. There may be calcifications of the portal vein wall indicating previous mural thrombi.



**Figure 30.4 Uninjected computed tomography (CT) scan showing spontaneous hyperdensity in the mesenteric vein.** This is diagnostic of recent venous thrombosis. Note that the spleen is enlarged due to a myeloproliferative disorder.

Identification of a possible cause is difficult. Local factors are investigated with abdominal imaging. Due to acute portal hypertension, intestinal and gallbladder walls are thickened and the head of the pancreas is enlarged in the absence of ischemic damage or primary inflammatory lesions.

Underlying prothrombotic disorders require systematic hematological investigations. Even without direct proof from laboratory investigation, a family or personal history of deep vein thrombosis represents circumstantial but significant evidence for an underlying prothrombotic condition. A myeloproliferative disorder may be readily identified by traditional hematologic investigations, but in some patients the culture of bone marrow cells is necessary to identify increased spontaneous formation of erythroid colonies. Primary deficiency in antithrombin, protein C or protein S is firmly established when screening discloses an affected first degree relative.

#### **Natural history and prognosis**

When there is extension, or recurrence, of thrombosis into the small mesenteric veins, there is a risk of intestinal necrosis, peritonitis and multiorgan failure. At this stage, even after resection of the infarcted gut, mortality rates may reach 50%.

Recurrent gastrointestinal bleeding from portal hypertension is the major complication of long-standing PVT. The prognostic factors for bleeding include the size of the esophageal varices and a past history of bleeding. The second major complication is recurrent thrombosis, usually in the portal venous territory and less commonly in other venous beds. The main prognostic factor for recurrent thrombosis is the presence of an underlying prothrombotic condition. Compression of the bile ducts by portal collaterals is emerging as an important source of morbidity including bacterial cholangitis, cholecystitis, and chronic cholestasis. The likelihood of these biliary complications increases with time elapsed from initial obstruction.

The prognosis of patients who have chronic portal vein thrombosis is to a large degree dependent upon any associated underlying disorder, such as cirrhosis. In those patients in whom no associated condition is identified, the outlook is good.

Tolerance, for example, to gastrointestinal bleeding is better than in patients who have cirrhosis because of the preserved liver function. In children who present with variceal bleeding secondary to portal vein thrombosis, the risk of hemorrhage decreases after adolescence and hepatic dysfunction may occur years later associated with atrophy and portal tract fibrosis.

#### **Management**

PVT treatment is discussed differently at the stage of recent thrombosis versus at the stage of cavernoma. Recanalization of a recently thrombosed portal vein can be expected in over 75% of the cases promptly treated with anticoagulation alone. Spontaneous recanalization appears to be rare. Anticoagulation should be maintained for at least 6 months before concluding lack of success. When reperfusion has been achieved, current data support continued anticoagulation in patients with permanent underlying prothrombotic disorders and no contraindication. Successful thrombolysis for acute portal vein thrombosis has been reported. However, the high rate of reperfusion on anticoagulation alone and the risk of dreadful complications from thrombolysis question the value of the latter treatment.

In patients with portal cavernoma, pharmacological or endoscopic treatments for prevention of first or recurrent gastrointestinal bleeding appear useful. There is some evidence that anticoagulation can be given for decreasing the risk of recurrent thrombosis without increasing the risk or the severity of gastrointestinal bleeding. Therefore, anticoagulation can be proposed for patients with portal cavernoma, particularly when there is an underlying thrombogenic condition and when no treatable local factor has been documented. The outcome is excellent in patients treated according to the above considerations. In elderly patients mortality is largely related to unlinked diseases.

In occasional cases, portal venous angioplasty may be successfully undertaken. A TIPS is not normally indicated in this situation and would usually be impossible to establish.

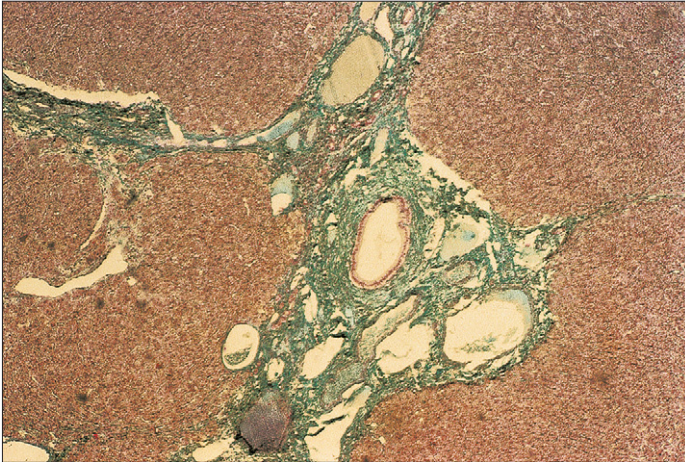
#### **Obstruction of intrahepatic portal veins *Schistosomiasis***

Worldwide, the major cause for the obstruction of intrahepatic portal veins is schistosomiasis, which is beyond the scope of this chapter. In brief, adult worms residing in the small mesenteric veins lay their eggs in the intestinal wall for fecal elimination. When infestation is massive, eggs are transported downstream along the portal venous system to the liver where they block the small intrahepatic portal veins. The granulomatous reaction leads to fibrosis of the portal tracts and neighboring sinusoids. Portal hypertension ensues. In many patients, concurrent hepatitis C or hepatitis B add their own lesion to the formation of the intrahepatic block.

#### **Obstructive portal venopathy**

Obstructive portal venopathy is similarly characterized by the obstruction of small intrahepatic portal veins. It has been reported under numerous other names, including non-cirrhotic intrahepatic portal hypertension in clinically-based reports, and hepatoportal sclerosis or non-cirrhotic portal fibrosis in pathologic studies. In Western countries, exposure to arsenicals, vinyl chloride monomer, and thorium sulfate has been recognized to cause obliterative portal venopathy in the past. Recent





**Figure 30.5 Histologic features of obliterative portal venopathy.** The normal portal vein is no longer visible. It should be much larger than the interlobular bile duct that is visible at the center of the portal tract. The normal portal vein is replaced by several thin walled channels that constitute a microscopic cavernoma. There are slender fibrous expansions of the portal tract.

studies indicate that an underlying thrombogenic condition similar to those discussed above for extrahepatic portal vein thrombosis is found in over half the patients. Still, a proportion of cases remain cryptogenic. It is generally admitted that the obstruction is related to phlebitis or thrombosis.

The portal tracts are devoid of normal veins. The small intrahepatic portal veins are absent, thrombosed or replaced by a fibrous scar. Characteristically, multiple small vascular channels (a microscopic equivalent to the cavernoma) are found within the portal tracts, at their periphery, or at random within the lobule (Fig. 30.5). Fibrous enlargement of the portal tracts, nodulation of the parenchyma, patchy sinusoidal dilatation and sinusoidal fibrosis are common. The atrophy of the parenchyma is reflected by the approximation of the portal tracts and of the central veins.

### **Clinical features and prognosis**

The clinical presentation almost always includes portal hypertension. Liver failure is rare or occurs after a course of several decades. The prognosis is better than for cirrhotic portal hypertension, and is mostly related to accompanying diseases. Superimposed extrahepatic PVT is seen in half the patients. There is no specific therapy. Anticoagulation can be proposed when an underlying thrombogenic condition is present. Portal hypertension can be managed as recommended for cirrhotic patients.

### **Diagnosis**

Diagnosis is suspected when portal hypertension is found in the absence of extrahepatic portal vein thrombosis and in the absence of the common causes of cirrhosis. Liver biopsy is the clue to the diagnosis by showing the abnormal portal veins or, in the case of schistosomiasis, the ova. The hepatic venous pressure gradient can be normal or raised. A gradient below 12 mmHg in a patient with previous bleeding from portal hypertension and a patent main portal vein suggests that small intrahepatic portal veins are obstructed.

### **Splenic vein thrombosis**

The most common causes of splenic vein thrombosis are pancreatitis with or without pancreatic pseudocysts and pancreatic tumors. Retroperitoneal infections or fibrosis, cirrhosis, and upper abdominal surgery are unusual causes. When no obvious local cause is found, an underlying prothrombotic disorder should be suspected as for PVT.

Thrombosis of the splenic vein gives rise to the development of collateral vessels between the spleen and the portal vein, namely portoportal shunts, or between the spleen and the vena cava or azygos vein, namely portosystemic shunts. In particular, a collateral circulation develops via the short gastric veins into the gastric fundus, and then to the left gastric vein and portal vein. The result is prominent gastric varices, with few or no varices in the lower esophagus. The classic clinical feature is splenomegaly.

In many patients splenic vein thrombosis is identified following gastrointestinal bleeding usually from gastric varices since esophageal varices are less common. Other patients may present with features related to the associated underlying disorder such as pancreatic neoplasia or chronic pancreatitis. In some patients, diagnosis is made fortuitously in the investigation of splenomegaly, gastric varices or collateral veins at abdominal imaging.

The diagnosis would generally be confirmed by Doppler-US, but CT or MR angiography may be required. Hematologic investigations may reveal evidence of hypersplenism.

Prognosis depends to a large extent on the underlying predisposing cause. In those without serious underlying pathology the prognosis is good.

In patients in whom thrombosis is confined to the splenic vein, splenectomy is the definitive treatment and is curative. However, in patients with underlying thrombogenic factors, splenectomy may initiate portal or mesenteric vein thrombosis. Endoscopic treatment using a cyanoacrylate glue injection may be superior to band ligation or sclerosis. Prophylactic splenectomy has been proposed for patients in whom splenic vein thrombosis was identified before variceal bleeding. The benefit:risk ratio of this approach is unclear, and generally, splenectomy is reserved for those patients who have bled.

### **HEPATIC VENOUS OUTLET OBSTRUCTION**

Budd-Chiari syndrome (BCS) is characterized by an obstruction of the hepatic venous outflow tract at the level of small hepatic veins, large hepatic veins, or suprahepatic segment of the inferior vena cava (IVC). Secondary BCS is related to invasion or compression of the veins by an extravenous lesion, whereas primary BCS is related to primary phlebitis or thrombosis. Veno-occlusive disease is now considered not to be a primary disorder of the hepatic veins and will be discussed below as sinusoidal obstruction syndrome.

### **Etiology and pathogenesis**

BCS is a rare condition. Causes of secondary BCS include malignancies prone to invade their venous outflow tract (adenocarcinoma of the liver, kidney or adrenals), or developing in the vicinity of the terminal portion of the IVC (leiomyosarcoma and myxoma). Alveolar hydatid disease behaves like a malignant tumor of the liver. Focal nodular hyperplasia or liver





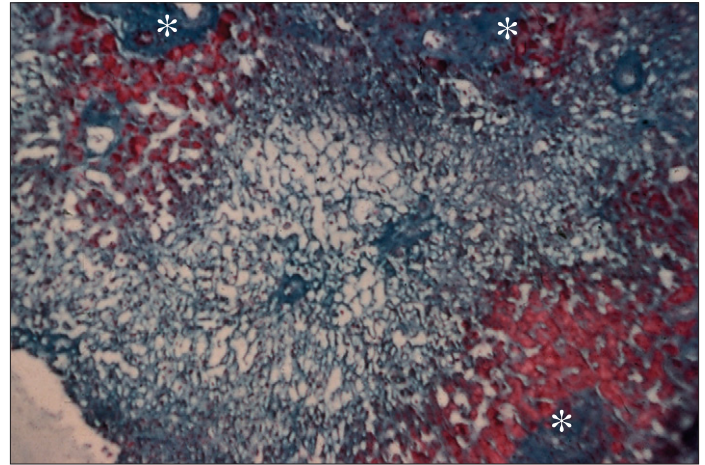
**Figure 30.6 Membranous stenosis of the left hepatic vein ostium.** Low magnification view of the termination of the left hepatic vein (small arrows) into the inferior vena cava (arrowheads) in a patient with a myeloproliferative disorder. There is a 'web' occluding the ostium.

cysts can block the termination of the hepatic veins in the IVC but rarely produce clinically significant obstruction, probably because of their slow development.

As shown in Table 30.1, half the cases of primary BCS are related to a myeloproliferative disease, 25% to factor V Leiden mutation and 25% to protein C deficiency. All other thrombotic disorders have also been implicated. BCS is particularly common in patients with paroxysmal nocturnal hemoglobinuria. There is good evidence for an association with some inflammatory disorders (e.g. granulomatous venulitis, Behçet disease, inflammatory bowel disease, amebic abscess), with oral contraceptive use and with pregnancy. The local factors that cause thrombosis at this uncommon site usually remain unidentified. A combination of causes is common (25% of cases).

Hepatic vein or IVC involvement by thrombosis can take three different aspects depending on the stage at which it is discovered: a fresh thrombus, a localized fibrous or membranous stenosis, or complete obliteration of the vein. In the Far East, a membranous obstruction of the terminal IVC is the predominant form of BCS whereas in the West, a thrombus or a localized stenosis of the hepatic veins appears to be more frequent (Fig. 30.6). In rare cases, thrombosis is limited to the small hepatic veins.

Obstruction of the hepatic venous outflow tract has dual consequences. First, sinusoidal pressure rises, explaining congestion (Fig. 30.7), portal hypertension, increased lymph production (resulting in the formation of protein-rich ascitic fluid), and the development of collaterals that bypass the obstructed portion of the hepatic veins or inferior vena cava. Second, there may be a sudden interruption in hepatic venous blood outflow, explaining ischemic necrosis (Fig. 30.7) and the resulting liver insufficiency. Hepatic perfusion is restored through the following mechanisms: increased arterial blood flow; increased portal pressure; the redistribution of intrahepatic portal flow from



**Figure 30.7 Histologic features of hepatic venous outflow block.** Centrilobular necrosis is visible as a loss of hepatocytes in the center of the photograph while hepatocytes are preserved in the periportal areas (indicated by asterisks). There is also marked dilatation of the centrilobular sinusoidal remnants (Masson trichrome).

obstructed areas toward unobstructed areas and the development of intrahepatic and extrahepatic venous collaterals (which should be distinguished from portosystemic collaterals). Fibrosis develops in the areas of ischemic necrosis.

At the advanced stage of the disease, thrombosed intrahepatic portal veins are found in an irregular distribution. The areas where both portal veins and hepatic veins are thrombosed undergo infarction or parenchymal extinction (i.e. transformation into a fibrous area devoid of parenchymal cells). By contrast, the areas with thrombosed portal veins, but patent hepatic veins, undergo hypertrophy (nodular regenerative hyperplasia), sometimes in an exuberant form (regenerative macronodules). Extrahepatic PVT occurs in about 20% of cases.

### Clinical features

Hepatic vein or IVC thrombosis can run a long asymptomatic course and be discovered incidentally in up to 20% of cases. There is little clinicopathologic correlation, an acute presentation being frequently associated with histopathologic features of chronic damage with superimposed features of recent injury. The acute presentation includes upper abdominal pain, hepatomegaly, ascites of recent onset, increased serum aminotransferases, decreased coagulation factors, and impaired renal function. A chronic presentation as decompensated liver disease is most common (60% of cases).

### Diagnosis

Diagnosis should be considered whenever patients with a known prothrombotic disorder develop liver disease; whenever patients with acute and severe liver disease have an enlarged painful liver and massive ascites; in patients with ascitic fluid protein content more than 3.0 g/dL; and in all patients presenting with chronic liver disease that remains unexplained once all common viral, autoimmune and metabolic causes have been ruled out.

Diagnosis is made on the basis of the findings at Doppler-US and MR imaging. The most specific and sensitive signs are an altered flow pattern within the hepatic veins, a hepatic venous



**Figure 30.8 Color Doppler ultrasound of abnormal collateral.** The abnormal collateral (colored in red, i.e. flowing toward the probe at the top) connects an obstructed right hepatic vein (arrow) to a patent middle hepatic vein (colored in blue, i.e. flowing from the probe).

collateral circulation (Fig. 30.8), and patchy alterations in the parenchymal perfusion pattern. Hepatic venography, which is not required for diagnosis, still remains an irreplaceable tool for delineating the venous lesion and planning therapeutic interventions (Fig. 30.9).

Usually, the underlying thrombogenic factor is not evident at presentation and consequentially systematic investigation is needed. Similar to PVT patients, myeloproliferative disorders in BCS patients frequently lack the classical peripheral blood features. Bone marrow biopsy and, in some patients, the culture of bone marrow cells may be necessary. In many patients, liver failure will hamper the recognition of primary anomalies in the coagulation system.

### **Course and prognosis**

The course is often unpredictable. In rare patients, complete remission occurs spontaneously, with or without subsequent recurrence. In other patients, there is an unremitting progression to liver failure and portal hypertension. Spontaneous mortality, three decades ago, was estimated to be 60% at 1 year. Recent analyses adjusting for treatment variables indicate that age and Child-Pugh score (or its components) are independent predictors for survival. Portal vein thrombosis makes prognosis poor.

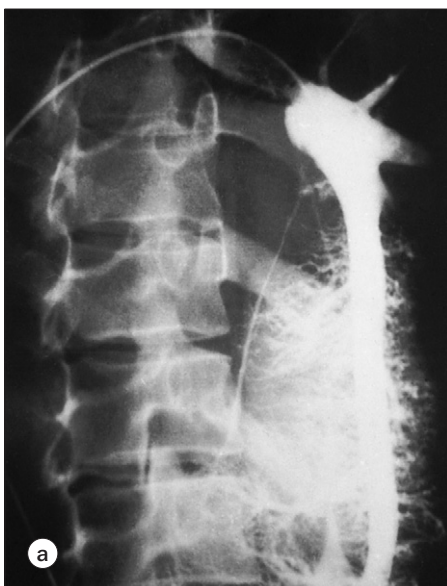
In 60% of patients with well-controlled BCS, regenerative macronodules produce multiple hypervascular tumors that are generally multiple and irregular in size. Exclusion of hepatocellular carcinoma can be difficult. Hepatocellular carcinoma may occur but has been described almost exclusively in patients originating from tropical or subtropical areas, and having long standing IVC obstruction with well-compensated liver disease.

Myeloproliferative disorders may undergo late transformation into acute leukemia or myelofibrosis with myeloid metaplasia. This is a matter of concern for long-term outcome. Similarly, paroxysmal nocturnal hemoglobinuria or the antiphospholipid syndrome, two other causes of BCS may lead to lethal extra-hepatic complications.

### **Management**

Due to the rarity of the disease, therapy for hepatic vein thrombosis has not been properly evaluated. Treatment of the underlying prothrombotic state should be instituted without delay. Anticoagulant therapy is logical and has probably improved the outcome of the disease over the last two decades. Ascites and portal hypertensive bleeding can be treated as recommended for patients with cirrhosis.

Several means are available to re-establish a low pressure outflow of hepatic blood. Angioplasty of the hepatic veins or IVC can be performed percutaneously (Fig. 30.9). Restenosis, which is infrequent for IVC, but common for the hepatic veins,



**Figure 30.9 Retrograde hepatic venography.** (a) In a patient with myeloproliferative disease and a short length of stenosis on the trunk of the middle and left hepatic veins. (b) The stenosis is better outlined during an unsuccessful attempt at percutaneous angioplasty.



can be prevented or cured by stent insertion. Limited data indicate that pharmacologic thrombolysis is more efficient when delivered in situ, early after formation of the thrombus, and combined with the angioplasty of an associated stenosis. Side to side portosystemic shunts aim to transform the portal vein from an inflow tract into an outflow tract. Surgical shunts have impacted little on survival, probably because of a high operative mortality (about 25%) and a high incidence of secondary shunt dysfunction (about 30%). An additional shortcoming of surgical shunts is the need to use long prosthetic grafts to reach the superior vena cava or the right atrium when the IVC is occluded or severely compressed by the hypertrophied caudate lobe. TIPS have achieved excellent results with low mortality but their long-term efficacy is still unclear. Covered stents are less likely to undergo stenosis or obstruction than conventional stents. TIPS can be inserted transcavally or through a hepatic vein stump. The problems of IVC compression by the hypertrophied caudate lobe are circumvented by the insertion of the TIPS above a caval stenosis. In patients with severe liver disease, transplantation appears to give better results than surgical portosystemic shunts, but there is limited data for a valuable comparison.

In symptomatic patients with a short-length stenosis on the hepatic veins or IVC, percutaneous angioplasty (with or without stenting, with or without thrombolysis) can be attempted first. Next, when angioplasty fails or is not feasible, TIPS should be attempted. When a TIPS attempt has failed, surgical shunting is proposed for patients with good liver function but disabling symptoms. Liver transplantation is recommended for patients with advanced liver disease in whom TIPS has failed.

Recent series included patients seen during a 10–25 year period who received various forms of therapy. They showed a varied outcome: about half the patients had a 10-year survival rate of >90%, while the other half had a 50% 10-year mortality rate. Most fatalities occurred in the first 12 or 18 months following presentation. Current data suggest that the overall 10-year mortality rate could be <20%. The long-term outcome may be strongly dependent on the underlying disorder that caused the hepatic vein or the IVC thrombosis.

## DISORDERS OF THE SINUSOIDS

Disorders that exclusively or predominantly affect the sinusoids are rare. They include the sinusoidal obstruction syndrome (SOS, formerly called veno-occlusive disease), sinusoidal dilatation, peliosis hepatis, perisinusoidal fibrosis and sinusoidal accumulation of various materials.

### Sinusoidal obstruction syndrome

#### *Etiology and pathogenesis*

The causes of SOS include exposure to certain toxic substances (e.g. pyrrolizidine alkaloids, azathioprine and various chemotherapeutic agents), hepatic irradiation, or the combination of irradiation and chemotherapy (as used in myeloablative therapy). Pyrrolizidine alkaloids are found in many plant species. When poisoning is caused by ingestion of contaminated flour, the disease may be epidemic. Ingestion of alkaloid-containing plant infusions or decoction (mainly as a herbal tea) is responsible for most endemic and sporadic cases. Hepatotoxicity is dose dependent. SOS caused by irradiation has its clinical onset 2–5 weeks

after irradiation. Although sensitivity to liver irradiation varies from one patient to another, a direct relationship is suggested by the limitation of the lesion to the irradiated part of the liver and by the dose-dependent prevalence of the lesion. SOS rarely occurs below 30 Gy, if irradiation is not associated with administration of antineoplastic drugs. Azathioprine and the closely related compounds 6-mercaptopurine and 6-thioguanine have been implicated in several cases of SOS. The clinical onset takes place after 6–108 months of continuous administration. Most patients are renal transplant recipients who also take corticosteroids. Several other drugs have been implicated, such as mitomycin C, BCNU (carmustine), indicine-*N*-oxide, mustine hydrochloride, vincristine, and doxorubicin; however, their role has not always been clearly established.

Myeloablative therapy for stem cell transplantation has become the major cause of sinusoidal obstruction syndrome. Depending on the myeloablative conditioning regimen, the incidence varies from 0 to 50%, the prevalence being highest with regimens of cyclophosphamide and total body irradiation of greater than 13.2 Gy. The following factors have been shown to increase the risk of SOS: chronic infection with hepatitis C virus, treatment with norethisterone, previous chemotherapy and irradiation to the abdomen. There is also inter-individual variation in the risk in relation to drug metabolism.

SOS is characterized by an initial, acute damage to endothelial cells which is followed by their detachment and embolization in the central area of the lobule where they cause a postsinusoidal outflow block. Histologically, the subendothelial space of the central and subendothelial veins is widened and occupied by diverse cellular debris. There is congestion and necrosis within the centrilobular area, followed by subendothelial deposition of fibrous tissue in central and sublobular veins, and in sinusoids.

### *Clinical and laboratory features*

The features mimic BCS. In the context of myeloablative therapy, the onset of the syndrome is heralded by fluid retention and tender hepatomegaly, beginning 10–20 days after the initiation of cyclophosphamide regimens and later after other myeloablative regimens. Increased serum bilirubin follows, usually before the 30th day. Serum aminotransferases are increased.

### *Diagnosis*

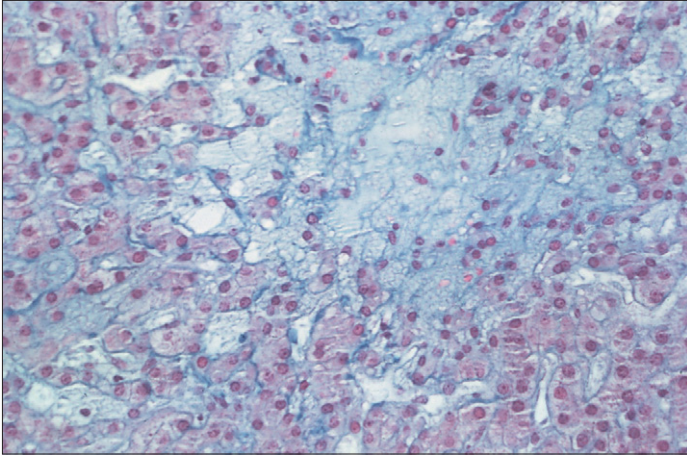
The clinical diagnosis of SOS requires that other possible causes of liver disease are ruled out (particularly severe sepsis and hyperacute graft versus host disease). When the association with other liver diseases is possible, transvenous liver biopsy coupled with hepatic venous pressure gradient measurement are useful.

### *Course and prognosis*

Severity varies from patient to patient, ranging from silent forms to fulminant hepatic failure. Some patients completely recover while others develop lethal forms, usually in association with multiorgan failure.

In the context of myeloablative therapy, reported mortality rates have varied between 0 and 67%. However, the highest figures may be a reflection of death with SOS in fragile patients rather than death from SOS. Actually, recovery from SOS may occur in up to 70% of cases. A poor prognosis is correlated with





**Figure 30.10** Histologic features of peliosis hepatis. (Masson trichrome).

high serum aminotransferase levels, high hepatic venous pressure gradient and development of portal vein thrombosis, renal insufficiency and decreased oxygen saturation.

### Management

Besides alterations of the conditioning regimen in high risk patients, there is no established way to prevent SOS related to myeloablative therapy. Likewise, no therapy has been established to be of clinically significant benefit once SOS has developed. Uncontrolled data have suggested the potential benefit of defibrotide, *N*-acetyl-cysteine and prostaglandin E1.

### Peliosis hepatis

Peliosis hepatis is characterized by blood-filled cavities of various sizes, bordered by hepatocellular plates, randomly distributed throughout the hepatic lobule. The hepatocytes that border the peliotic cavities are often atrophic or compressed. Peliosis hepatis, sinusoidal dilatation, perisinusoidal fibrosis and nodular regenerative hyperplasia may coexist. The peliotic cavities consist of dilated sinusoids and the space of Disse (**Fig. 30.10**).

The pathogenesis is unknown. Indirect evidence suggests two mechanisms:

- obstruction at the junctions of sinusoids and central veins, and
- a primary lesion of the sinusoidal (mainly endothelial) cells.

Peliosis hepatis is frequently associated with an extrahepatic disease. Most of these associated diseases share the following features: a chronic debilitating condition frequently associated with severe infections, leading commonly to the administration of androgenic-anabolic steroids. Several drugs and chemicals have been incriminated; however, rather than a direct toxic effect, these substances may only potentiate the action of yet unidentified endogenous factors. In patients with acquired immunodeficiency syndrome, an infectious form of peliosis hepatis has been characterized under the name of bacillary peliosis hepatis. It is related to infection by *Bartonella henselae* and is cured by erythromycin.

Peliosis hepatis is associated with portal hypertension attributed to an intrahepatic block as demonstrated by an increased hepatic venous pressure gradient. The mechanism for increased intrahepatic resistance is unknown. Perisinusoidal fibrosis is a

late sequela after resolution of the peliotic cavities but can be demonstrated at electron microscopy in the early stages. Perisinusoidal fibrosis could thus explain the intrahepatic block observed in peliosis hepatis.

Mild lesions induce no signs or symptoms, whereas in severe lesions, hepatomegaly, ascites, esophageal varices or liver failure are present. Liver tests are usually mildly impaired. A marked increase in serum alkaline phosphatase and  $\gamma$ -glutamyltranspeptidase activities can occur.

At laparoscopy, peliosis hepatis can be recognized as dark blebs on the surface of the liver. Macroscopic blood-filled cavities can be demonstrated by US, CT or MR imaging. Liver biopsy is required to demonstrate the blood lakes.

Death results from intraperitoneal bleeding, liver failure or a complication of the associated conditions but not from portal hypertension. Symptomatic peliosis hepatis should be regarded as a feature of a bad prognosis, even though it is rarely the direct cause of death.

There is no specific treatment.

### Sinusoidal dilatation

The lesion of sinusoidal dilatation differs from peliosis hepatis in the following characteristics:

- the ectatic sinusoids do not take the cystic appearance that is typical of peliosis hepatis; and
- the dilatation is not randomly distributed but affects a distinct part (pericentral and midzonal or periportal) of the lobule.

However, the separation between the two entities may be difficult to distinguish morphologically and etiologically.

As in peliosis hepatis, sinusoidal dilatation has been reported alone or in association with nodular regenerative hyperplasia and perisinusoidal fibrosis. The causes are almost similar to those associated with peliosis hepatis and nodular regenerative hyperplasia. In recent series, the antiphospholipid syndrome was the most common cause of sinusoidal dilatation unrelated to drugs or Crohn disease. In the past, oral contraceptives have been considered a cause of sinusoidal dilatation. However, an underlying inflammatory condition was almost always associated. The pathogenesis remains unelucidated.

Clinical features of sinusoidal dilatation may be absent or might include right upper quadrant pain, fever and hepatomegaly. Ascites and esophageal varices have been reported. The erythrocyte sedimentation rate and serum  $\gamma$ -globulin are almost constantly raised. Liver specific test results are moderately abnormal. Perisinusoidal fibrosis may persist as a late sequela. In a few cases, the disease progresses to fibrosis and cirrhosis with esophageal varices, splenomegaly and hypersplenism.

The diagnosis raises the same difficulties as that of peliosis hepatis. Diffuse, mottled alterations in hepatic perfusion can be seen at CT or MR imaging persisting through all phases of contrast injection. In the absence of hepatic venous outflow block or portal vein obstruction, this pattern is suggestive of sinusoidal dilatation. Liver biopsy cannot be avoided to exclude malignant infiltration of the liver or obstruction limited to the small hepatic veins. The differential diagnosis of right upper quadrant pain with fever and mild or absent abnormalities of liver tests includes acute cholecystitis, perihepatitis, acute hepatic venous outflow block and right heart failure. US plays a major role in this differential diagnosis.

There is no specific treatment for this benign condition. The manifestations disappear with the resolution of the associated acute or chronic disease.

### Sinusoidal fibrosis

Pure sinusoidal fibrosis predominating in the perivenous area is usually associated with venous scarring. The main causes are resolved steatohepatitis (alcoholic or non-alcoholic), and right sided heart failure or constrictive pericarditis. Chronic intoxication with vitamin A is a prototype for pure sinusoidal fibrosis without zonal predominance. A distinctive feature is the hypertrophy of the lipid-laden stellate cells. Chronic therapy with methotrexate has been related to sinusoidal fibrosis although the distinction from coexistent alcoholic or metabolic liver disease is difficult. Mastocytosis may produce an almost pure aspect of perisinusoidal fibrosis. All causes of obliterative portal venopathy have been associated with pure sinusoidal fibrosis, which raises the issue of portal vein lesions having been missed in small-sized biopsy specimens where pure sinusoidal fibrosis was found.

### Infiltration of the sinusoids

The perisinusoidal space can be infiltrated by deposition of

various substances including immune globulin light chain (myeloma), various amyloid substances (AL type in myeloma, AA type in chronic inflammatory disorders), or immune globulin A (alcoholics). Liver enlargement and cholestatic liver tests are the main manifestations of these lesions.

The sinusoidal lumen can be the site of fibrin deposition in conditions where intravascular activation of coagulation occurs, such as pre-eclampsia (see Ch. 31), the hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura. Liver hematoma and intraperitoneal rupture may occur although rarely. Upper abdominal pain occurring in the setting of the HELLP syndrome (hemolysis, elevated liver function and low platelets) heralds the development of intra- or extrahepatic bleeding.

Diffuse infiltration of the sinusoids with abnormal cells may occur in patients with breast carcinoma, undifferentiated carcinoma, lymphomas and leukemias, mastocytosis, and the hemophagocytic syndrome. In rare instances, the disorders present with apparently pure liver involvement. Liver enlargement is constant. Ascites and acute liver failure are frequent and the prognosis is poor.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 31

## Liver Diseases in Pregnancy

Rebecca W. Van Dyke

Liver diseases that occur during pregnancy can be divided into three categories: liver diseases that are unique to pregnancy; liver diseases that occur more commonly with or because of pregnancy; and acute or chronic liver diseases that exist coincidentally with pregnancy (Table 31.1). The first category contains diseases such as intrahepatic cholestasis of pregnancy (ICP), pre-eclampsia/eclampsia/HELLP syndrome (named for the laboratory features of hemolysis, elevated liver enzymes and low platelet count), and acute fatty liver of pregnancy (AFLP). The latter two categories include many types of liver diseases; however, this chapter will focus on liver diseases that are affected by the pregnant state or liver diseases that have special therapeutic implications in the setting of pregnancy. For example, the pregnant state is a risk factor for common problems such as the development of gallstones and biliary colic as well as rare diseases, such as Herpes simplex hepatitis and Budd-Chiari syndrome. Other liver diseases can be more devastating for the pregnant woman (e.g. hepatitis E) or may have, like hepatitis B, implications for the newborn infant. Finally, in any patient with chronic liver disease, the consequences of pregnancy must be considered carefully, both for the mother and the child.

### EPIDEMIOLOGY

Liver disease is uncommon during pregnancy; however, jaundice, which is often a sign of significant liver disease, is reported in

approximately 1 in 1500 to 1 in 5000 pregnancies. The most common reason for jaundice during pregnancy in the USA is viral hepatitis, a reflection of the prevalence of viral hepatitis in the general population. Other than hepatitis E, which is rarely encountered in the USA, the incidence and course of hepatitis A, B and C are not different in the pregnant patient as opposed to the non-pregnant patient. The second most common cause of jaundice is a disease unique to pregnancy, intrahepatic cholestasis of pregnancy, which accounts for up to 20% of jaundice in pregnancy. The most common cause of new abnormal liver tests in pregnancy is pre-eclampsia-related liver disease.

### NORMAL CHANGES IN LIVER TESTS AND PHYSICAL EXAMINATION DURING PREGNANCY

Although most liver functions are not affected by normal pregnancy, some laboratory and physical findings occur during normal pregnancy that may be mistaken for evidence of liver disease. On physical examination, normal pregnant women may have peripheral edema as a result of an expanded intravascular volume, decreased serum albumin and/or pressure exerted by a gravid uterus. Spider nevi and palmar erythema are common findings in normal pregnancy, occurring in up to 67% of women, particularly in the second and third trimester. These vascular phenomena are related to increases in serum estrogen levels and generally disappear after delivery. Transient small esophageal varices have been observed in some pregnant women without liver disease due to compression of the inferior vena cava and increased flow in the azygous system.

During pregnancy a number of biochemical tests may also be affected (Table 31.2). Plasma albumin levels are decreased as a result of increasing plasma volume. Serum alkaline phosphatase and leucine aminopeptidase values increase throughout pregnancy to as much as two times the upper limits of normal as a result of placental production. By contrast, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values do not normally exceed the upper limits of normal, thus consistent increases are a reliable clue to liver disease. Other liver tests such as the prothrombin time, 5' nucleotidase,  $\gamma$ -glutamyl-transpeptidase and bilirubin either remain unchanged or may actually decrease with normal pregnancy. A variety of other serum proteins and lipids either increase or decrease during normal pregnancy (Table 31.2). Finally, pregnancy is associated with a mild subclinical cholestasis as indicated by decreases in bile formation and in hepatic transport of bile salts; however these changes are not detected with routine laboratory studies.

Liver biopsies from normal pregnant women may show modest changes, including reactive Kupffer cells, variation in nuclear size

Liver disease in pregnancy		
<i>Liver diseases unique to pregnancy</i>	<i>Liver diseases occurring more commonly in pregnancy</i>	<i>Pre-existing liver diseases coincident with pregnancy</i>
Hyperemesis gravidarum	Gallstones (symptomatic)	Hepatitis B and C
Intrahepatic cholestasis of pregnancy	Fulminant hepatitis E	Autoimmune hepatitis
Pre-eclampsia/HELLP	Budd-Chiari syndrome	Wilson disease
Acute fatty liver of pregnancy	Herpes simplex hepatitis	Cirrhosis Liver transplantation Liver adenoma

Table 31.1 Liver disease in pregnancy.

Expected changes in laboratory tests in normal pregnancy		
Values increase	Values not changed	Values decrease
Alkaline phosphatase (to 2 ×)	Alanine and aspartate aminotransferases	Albumin
Leucine aminopeptidase (to 2 ×)	Prothrombin time	Antithrombin III
Bile acids (to 2 ×)		Protein S
Globulins		γ-glutamyltranspeptidase
Fibrinogen		
Triglycerides		

Table 31.2 Expected changes in laboratory tests in normal pregnancy.

and shape, proliferation of smooth endoplasmic reticulum and giant mitochondria, which are of no known clinical significance.

## PATHOPHYSIOLOGY AND CLINICAL FEATURES

### Liver diseases unique to pregnancy

#### Hyperemesis gravidarum

Hyperemesis gravidarum is an idiopathic syndrome of severe nausea and vomiting that generally occurs in the first trimester of pregnancy, typically before 10 weeks of gestation. Risk factors for hyperemesis gravidarum include young age, obesity, nulliparity, tobacco use and twin gestation. In many patients with severe vomiting and dehydration, abnormal biochemical liver tests may be observed, usually elevations of serum bilirubin and/or aminotransferases. The elevations are usually mild with bilirubin levels up to 4 mg/dL and aminotransferases levels up to 200 U/L. Liver biopsy shows mild fatty change, mild cholestasis or no abnormality. Liver test abnormalities resolve with resolution of vomiting and dehydration.

Cessation of oral feeding may be necessary to reduce nausea and vomiting and total parenteral nutrition may be necessary in severe cases.

#### Intrahepatic cholestasis of pregnancy

ICP, also called benign cholestasis of pregnancy, cholestasis of pregnancy or obstetric cholestasis, occurs in 0.2–1.5% of pregnant women and usually presents in the third trimester at a mean gestation time of 29 weeks (Table 31.3). Patients may have a family or personal history of pruritus during pregnancy or prior oral contraceptive use and the disease is more common in Scandinavia and Chile. Patients typically present with generalized pruritus. As with other pruritic syndromes, the itching is usually worse at night and can become very intense, leading to skin excoriations, infections, insomnia, fatigue and depression.

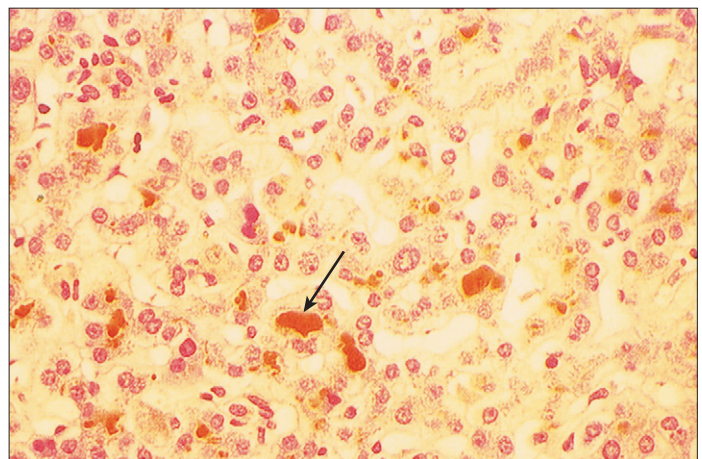
Frank jaundice occurs in about 10–25% of patients, usually after the onset of pruritus. The jaundice is generally not severe, and bilirubin levels rarely rise above 6 mg/dL. Alkaline phosphatase levels usually rise above what is expected from normal

Clinical findings in intrahepatic cholestasis of pregnancy		
Incidence	0.2–1.5% of pregnant women	
Onset	3rd trimester (median onset, 29 weeks)	
Symptoms and signs	Pruritus	100%
	Jaundice	10–25%
Laboratory findings		
Alkaline phosphatase	3–5-fold increase	
Bilirubin	1–4 mg/dL	
Aminotransferases	2–4-fold increase	
Serum bile salts	3–10-fold increase	
Prothrombin time	Modest increase in 20% of patients	

Table 31.3 Clinical findings in intrahepatic cholestasis of pregnancy.

pregnancy to three to five times the upper limits of normal and this alkaline phosphatase is of hepatic, not placental, origin. Serum aminotransferases can also increase from 2- to 10-fold. Serum bile acids are increased to 10- to 100-fold. In severe cases, with clinically obvious jaundice, steatorrhea and vitamin K deficiency may occur owing to intestinal bile salt deficiency. Liver biopsy, which is rarely necessary, shows a 'bland' cholestasis with minimal or no inflammation (Fig. 31.1). Imaging studies such as ultrasound or computed tomography (CT) scans are normal but usually are not necessary.

In both humans and animals, exogenous estrogen impairs hepatic uptake and biliary secretion of bilirubin and bile salts. ICP is thought to be due to an increased sensitivity to these cholestatic effects of estrogen, likely in conjunction with minor mutations in genes coding for hepatic canalicular transporters or other proteins involved in bile formation. For example, in one family, ICP occurred in six women, all of whom had a mutation in the gene for the MDR3 canalicular phospholipid pump. ICP appears to be decreasing in prevalence, however, suggesting environmental factors may play a role as well.



**Figure 31.1** Liver biopsy from a patient with intrahepatic cholestasis. Cholestatic changes are shown including bile accumulation in canalicular spaces (arrow).

Medical treatments for pruritus include divided doses of ursodeoxycholic acid (10–15 mg/kg/day) or cholestyramine (8–16 g/day). These drugs should be started early in symptomatic cases as they may take time to be fully effective. No maternal or fetal toxicities related to these agents have been observed, other than an increase in maternal steatorrhea with cholestyramine. Indeed, data suggests that fetal outcomes also may be improved by treatment of mothers with ursodeoxycholic acid. Other suggested treatments for which efficacy is less well established include S-adenosyl-L-methionine (800 mg/day i.v.), dexamethasone (12 mg/day for 7 days and then tapered over 3 days) and phenobarbital (90 mg at bedtime). The treatment of choice for severe ICP is delivery of the infant, which is followed by resolution of pruritus. However the risks to an immature fetus of early delivery should be balanced against maternal symptoms. Finally, vitamin K should be administered to jaundiced women near the time of delivery to decrease postpartum bleeding, especially for women who received cholestyramine.

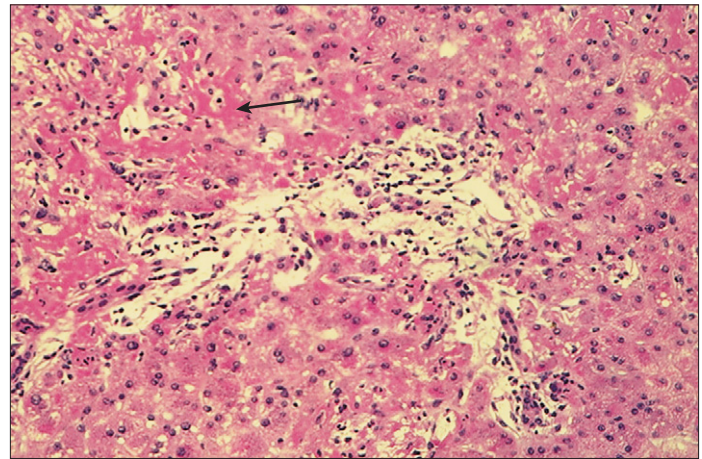
Infants of mothers with ICP appear to be at increased risk of fetal distress, low birth weight, sudden intrauterine death and perinatal mortality. Thus, pregnancies should be monitored closely from at least 34 weeks on, although fetal stress tests may not prevent all sudden fetal deaths. Prompt delivery should take place as soon as fetal distress is identified. In the absence of evidence of fetal distress, delivery is recommended by 36 weeks for severe cases (such as women with jaundice) and by 37 weeks for women with mild disease.

While ICP resolves quickly after delivery, it will likely recur in future pregnancies or if women use oral contraceptives, although its severity may vary. Women who have experienced ICP have a greater lifetime risk of developing gallstones.

### Pre-eclampsia

Pre-eclampsia, a common disease in pregnancy, is characterized by the triad of hypertension, proteinuria and edema. It begins in the late second trimester, third trimester or in the first few days postpartum, occurs in 7–10% of all pregnancies and is the major cause of maternal morbidity and mortality. Eclampsia is a more severe stage of the disease with extreme hypertension ( $\geq 160/100$ ), proteinuria ( $\geq 5$  g/24 h) and seizures. Pre-eclampsia has been associated with a spectrum of liver injury, including the HELLP syndrome and hepatic infarcts, hematomas or rupture. The liver complications of pre-eclampsia account for up to 70% of new abnormal liver tests during pregnancy and up to 20% of maternal mortality is due to pre-eclampsia. Risk factors for the development of pre-eclampsia/eclampsia include procoagulant disorders (such as antiphospholipid antibody syndrome), pre-existing hypertension, extremes of childbearing age, first pregnancy and the presence of multiple gestations.

Although the pathogenesis is unclear, pre-eclampsia/eclampsia is a systemic disease of abnormal endothelial reactivity that can affect multiple organs. The initial event may be abnormal trophoblastic implantation leading to reduced placental perfusion. Mothers exhibit endothelial dysfunction, decreased plasma volume, increased systemic vascular resistance, decreased perfusion of various organs, abnormal endothelial reactivity with vasospasm, deposition of fibrin in vascular spaces and activation of the coagulation cascade. These lead to ischemic damage of multiple organs, including the liver in about 10% of cases. On liver biopsy, fibrin thrombi are present in the periportal sinusoids



**Figure 31.2 Liver biopsy from a patient with pre-eclampsia.** Characteristic fibrin thrombi (arrow) are seen in the periportal sinusoidal space.

with ischemic hepatocyte necrosis and periportal hemorrhage with little inflammation (**Fig. 31.2**). Occasional patients also exhibit microvesicular fatty change.

The frequency and severity of liver function test abnormalities, such as aminotransferase elevations, reflects the degree of hepatocellular necrosis that correlates somewhat with the severity of the underlying pre-eclampsia/eclampsia. HELLP syndrome, the most common clinical manifestation of pre-eclampsia-related liver disease, denotes a subset of women who exhibit evidence of microangiopathic hemolytic anemia, thrombocytopenia (platelet count less than 100 000), and elevated liver tests. HELLP syndrome occurs in about 10% of women with pre-eclampsia with an overall incidence of 0.5–1.0% of pregnancies (**Table 31.4**). Patients with HELLP syndrome may present with asymptomatic laboratory abnormalities or with liver-related symptoms including nausea and vomiting or right upper quadrant pain and tenderness. Characteristic laboratory abnormalities include increases in serum aminotransferases from 5 to 100 times normal. Because of the associated hemolysis, serum lactic dehydrogenase (LDH) and bilirubin are also elevated and red blood cell morphology is often abnormal. The prothrombin time may be normal despite these abnormal liver enzymes or it may be elevated. Antithrombin III levels are characteristically low and evidence of intravascular coagulopathy may develop, especially in severe disease. Renal involvement is indicated by elevated blood urea nitrogen (BUN) and creatinine levels. Liver biopsy is diagnostic but usually not indicated as the diagnosis is often made clinically.

Pre-eclampsia also affects the fetus, which is at high risk of growth retardation and sudden fetal death due to poor placental perfusion or infarction. Since maternal pre-eclampsia and liver involvement resolve quickly after delivery, the principal treatment for both mother and child involves rapid assessment of fetal lung maturity, administration of corticosteroids to improve fetal lung function and to speed improvement of HELLP (if time permits) and prompt delivery. Mothers should be observed closely after delivery as the disease may transiently worsen for 1–2 days. Although maternal morbidity can be considerable, maternal mortality is usually 2.5% or less. Fetal outcome is less



Clinical findings in HELLP* syndrome	
Incidence	~ 1% of all pregnancies, especially first pregnancies ~ 10% of women with pre-eclampsia
Onset	3rd trimester (median onset, 33 weeks)
Symptoms and signs	
Hypertension, proteinuria and edema	~100%
Headache	up to 60%
Nausea and vomiting	35%
Abdominal pain	80%
Laboratory findings	
Thrombocytopenia	100%, early onset
Abnormal red blood cells	Frequent (fragments, schistocytes)
Prothrombin time	Modest increase (late)
Fibrin degradation products	Increased (early)
Aminotransferases	5- to 100-fold increase
Alkaline phosphatase	Up to ~2-fold increase
Bilirubin	Low, unless extensive hemolysis and/or hepatic necrosis occur
Serum creatinine	Mild increase
*HELLP, laboratory features of hemolysis, elevated liver enzymes and low platelet count.	

Table 31.4 Clinical findings in HELLP syndrome.

good with fetal losses of 6–25% depending on disease severity. HELLP syndrome recurs in about 3–5% of subsequent pregnancies. It is recommended that women who had HELLP syndrome be screened for procoagulant disorders.

Hepatic infarction, subcapsular hematoma, and hepatic rupture are estimated to occur in 1:15 000–45 000 pregnancies and in 1–2% of women with pre-eclampsia. Patients usually present in the third trimester or the immediate postpartum period with abdominal pain, hypotension, fever, leukocytosis, nausea, vomiting, shock and abnormal liver tests similar to those in severe HELLP syndrome. These likely represent a progression from the milder HELLP syndrome to confluent hepatic infarction, to subcapsular hematoma, and subsequently, to hepatic rupture. Because it may be difficult to differentiate a contained subcapsular hematoma from free hepatic rupture, on clinical grounds, CT is very helpful in the evaluation of these patients (Fig. 31.3). Patients with hepatic infarction or contained liver hematoma should undergo volume resuscitation, if necessary, and urgent delivery. Contained hematomas do not need specific treatment and resolve slowly after delivery. For hepatic rupture, a cesarean section should be performed emergently with volume resuscitation and surgical drainage and packing of the liver. Selected cases may be treated by hepatic artery embolization. Emergent liver transplantation has been performed in a few cases. Due to the severity of maternal disease, fetal loss is as high as 60–70% and maternal mortality from hepatic rupture is up to 50%.

Acute fatty liver of pregnancy

AFLP occurs in approximately 1 in 900–6000 pregnancies primarily in the third trimester or the first few days postpartum. AFLP is one of a group of diseases known as the hepatic

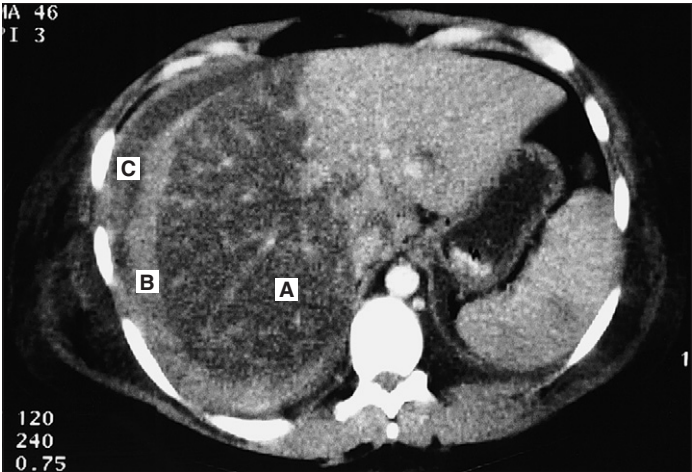


Figure 31.3 Contrast enhanced computed tomography scan of a patient with pre-eclampsia. The patient developed hepatic infarction with segmental hypoperfusion of the right lobe (A) and subcapsular hematoma (B) that ruptured, creating extrahepatic hemorrhage (C).

microvesicular steatoses that includes Reye syndrome, Jamaican vomiting sickness and toxicity of several drugs (tetracycline, sodium valproate and fialuridine). They are characterized by infiltration of hepatocytes with microvesicular droplets of fat and profound liver dysfunction (manifested by changes in PT, glucose, bilirubin) with modest increases in AST/ALT levels.

The pathogenesis of AFLP is thought to involve abnormalities in  $\beta$ -oxidation of fatty acids, in mitochondrial function and in ATP syntheses. Hepatic metabolism of triglycerides and fatty acids increases greatly during pregnancy. AFLP may occur when this increased load of fatty acids is delivered to a liver that also exhibits impaired mitochondrial  $\beta$ -oxidation of fatty acids due to elevated estrogens and one or more other factors including genetic abnormalities in acyl-CoA dehydrogenases, inflammatory cytokines and/or drugs such as salicylates or non-steroidal anti-inflammatory drugs. Under these conditions, fatty acids and reactive oxygen species may cause damage to mitochondria and reduce ATP synthesis leading to energy deprivation in hepatocytes, reduction in many hepatocyte functions, lactic acidosis and liver cell dysfunction. Most cases of AFLP are sporadic and non-recurring. However genetic abnormalities in fatty acid oxidation, especially long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, account for up to 20% of cases. For example, when a mother heterozygous for LCHAD deficiency is pregnant with a homozygously affected fetus, AFLP occurs in most pregnancies. Therefore it is recommended that all infants and mothers who experience AFLP be tested for LCHAD deficiency. A list of clinical laboratories that perform this test can be found at [www.genetests.org](http://www.genetests.org).

The early clinical signs of AFLP are non-specific but include fatigue, malaise, nausea and vomiting accompanied by abdominal pain, particularly in the right upper quadrant or epigastrium (Table 31.5). Signs of liver failure such as hepatic encephalopathy may ensue within days. Physical findings include right upper quadrant tenderness, jaundice and a normal or small liver. Signs and symptoms of pre-eclampsia are seen in 30–40% of cases. The development of coma portends a poor outcome.

Laboratory studies show relatively modest increases in AST and ALT, rarely greater than 10 times normal, increased

Clinical findings in acute fatty liver of pregnancy	
Incidence	1 in 900–6000 pregnancies
Onset	3rd trimester (median onset, 35 weeks)
Symptoms and signs	
Nausea and vomiting	Up to 80%
Abdominal pain	60%
Hypertension, proteinuria, edema	20–30%
Jaundice	90% (if disease severe)
Encephalopathy	70% (if disease severe)
Hypoglycemia	25% (if disease severe)
Laboratory findings	
Alkaline phosphatase	2- to 5-fold increase
Bilirubin	2–30 mg/dL
Aminotransferases	5- to 10-fold increase
Prothrombin time	Marked increase in severe disease to >20 s
Platelet count	Decrease late in severe disease with evidence of DIC*
Serum uric acid	Modest elevation
*DIC, Disseminated intravascular coagulation.	

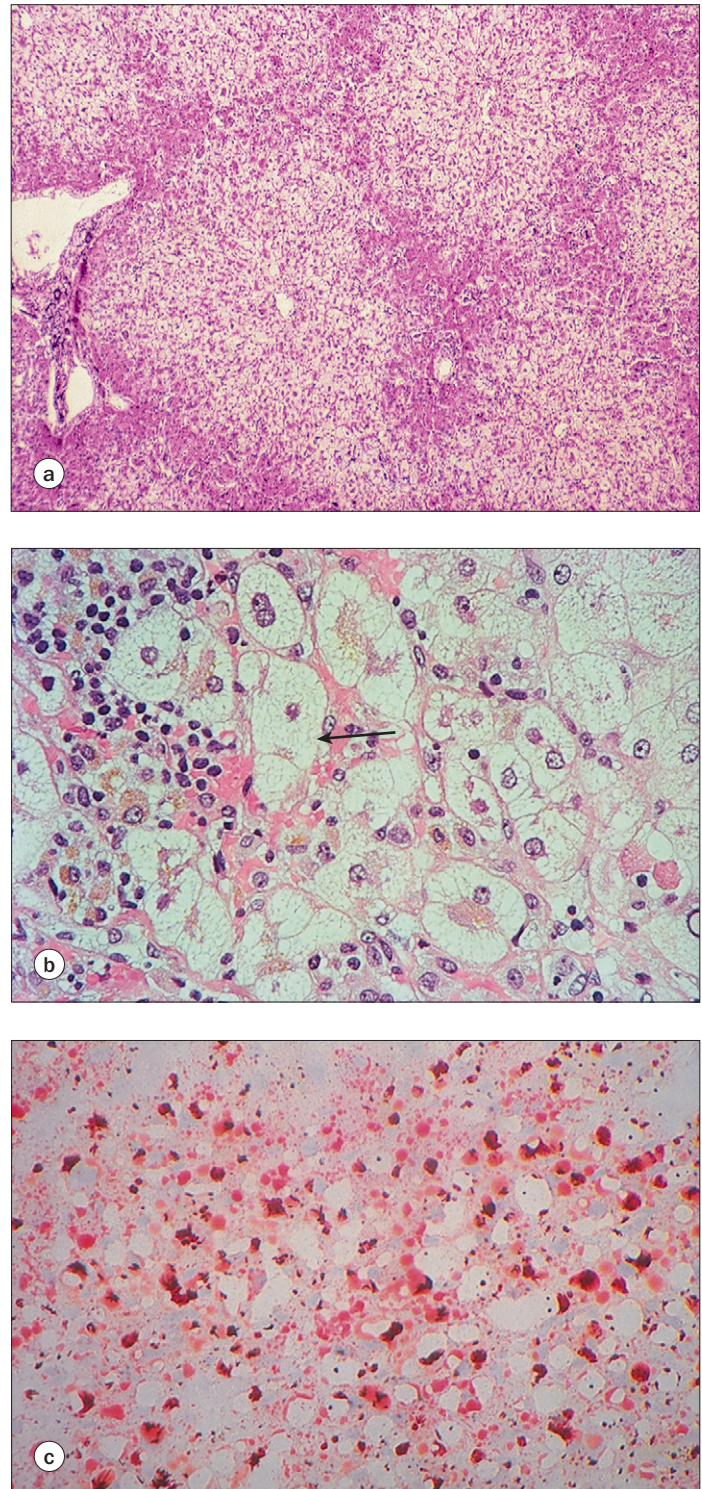
**Table 31.5 Clinical findings in acute fatty liver of pregnancy.**

PT and international normalized ratio (INR), bilirubin levels and hypoglycemia. Elevated uric acid levels and renal insufficiency are common, possibly owing to microvesicular steatosis in renal tubular cells. Disseminated intravascular coagulation (DIC) may occur in severe cases.

On liver biopsy, the pathognomonic finding is that of centrilobular microvesicular fatty infiltration with little or no inflammation (Fig. 31.4a, b). The periportal hepatocytes are relatively spared. A frozen section of the liver biopsy stained for fat with an oil red O stain can be very helpful, especially if fatty infiltration is not obvious on a hematoxylin and eosin stain (Fig. 31.4c).

Ultrasound examination may show a hyperechoic liver and CT may identify a fatty liver with a liver density less than that of the spleen, but these imaging studies have poor sensitivity for AFLP and are often normal or non-specific.

Since AFLP resolves after delivery, the principal management involves fetal assessment and prompt delivery. In mild cases involving an immature fetus, gestation could be allowed to continue for some days but only with close monitoring by an experienced team of obstetricians and physicians expert in the care of liver failure. Like pre-eclampsia, AFLP can worsen transiently in the immediate postpartum period but then resolves rapidly. Maternal morbidity may be considerable and a few patients have required liver transplants. However, most women recover and maternal and fetal mortality are in the 5–20% range. Postpartum hemorrhage is common in women with coagulopathy. AFLP can recur in subsequent pregnancies, although this is very rare unless genetic abnormalities in fatty acid oxidation are present.



**Figure 31.4 Liver biopsy from a patient with acute fatty liver of pregnancy.** Microvesicular fatty change in pericentral hepatocytes (arrow); (a) low power, (b) high power, (c) Oil red O stain.

### Liver diseases occurring more commonly with pregnancy

#### Cholesterol gallstones

Pregnancy is a risk factor for the development of biliary sludge and cholesterol gallstones. This may be related to pregnancy-induced decreases in gallbladder motility due to elevated progesterone levels as well as increases in biliary cholesterol



secretion and possibly impaired bile salt excretion related to high estrogen levels. Indeed, ultrasound studies have documented transient formation of sludge and stones in 33% and 10% of normal pregnancies, respectively. Women with a history of ICP are at even higher risk of gallstone formation. Moreover, gallstones are more likely to cause symptoms during pregnancy. Clinically, pregnant women with gallstone disease present in a manner similar to that of the non-pregnant patient, and the management is the same, although elective cholecystectomy is usually deferred until after delivery. Endoscopic retrograde cholangiopancreatography (ERCP) and laparoscopic cholecystectomy can be performed during pregnancy with excellent maternal outcome and low fetal loss rates (<5–10%).

### **Budd-Chiari syndrome**

Budd-Chiari syndrome, or hepatic vein thrombosis, may occur during pregnancy. Pregnancy, like the use of oral contraceptives, is thought to contribute to a hypercoagulable state that increases the risk of thrombotic diseases. In addition to pregnancy, patients with Budd-Chiari syndrome may also have other underlying hypercoagulable states (e.g. mutations in coagulation factors, myeloproliferative disorders) that are uncovered during pregnancy. The syndrome typically occurs postpartum with the sudden onset of abdominal pain and ascites. The diagnosis is confirmed by venography. Liver biopsy shows centrilobular congestion. Treatment options are discussed elsewhere in this book (Ch. 37).

### **Herpes simplex virus**

Herpes simplex virus (HSV) hepatitis is a rare acute hepatitis that occurs predominantly in pregnant women and immunocompromised patients. It represents a form of disseminated primary herpes infection, and most cases are caused by HSV type 2. All of the reported cases have occurred in the second or third trimester. Systemic symptoms are common, including fever, chills, malaise and nausea. Mucocutaneous lesions characteristic of HSV, if present, are helpful in making the diagnosis. Anicteric liver failure is the hallmark of this disease, often associated with considerable right upper quadrant pain. The serum aminotransferases are usually extremely elevated, rising to levels of 25- to 40-fold normal. In contrast, serum bilirubin usually is only mildly elevated to a level about 2- to 3-fold that of normal. Marked hepatocellular dysfunction is as indicated by an increased INR. Although CT may show multiple non-enhancing low-density lesions, these are not specific findings. A liver biopsy is usually diagnostic, and both immunoperoxidase staining for HSV and viral culture should be performed. Histologically, the liver shows patchy and confluent hepatic necrosis with hemorrhage but little inflammation. At the periphery of the necrosis, there may be intranuclear eosinophilic Cowdry type A inclusions (Fig. 31.5). Early recognition is essential as treatment with aciclovir is effective if begun early. After delivery, the infant should also receive of treatment. Maternal and fetal mortality can be as high as 50%.

### **Other liver diseases that occur coincidentally with pregnancy**

#### **Acute viral hepatitis**

Any liver disease can exist concurrently with pregnancy. Acute viral hepatitis in well-nourished patients is not different in pregnant patients except that the rate of fetal loss is higher in severely affected women. The exception is hepatitis E in which

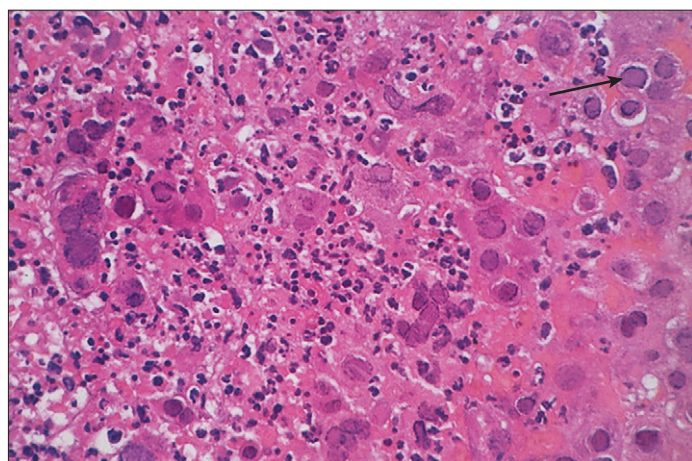
pregnant women experience a very high rate (20% or greater) of fulminant liver failure.

### **Pre-existing liver disease**

Patients with pre-existing liver disease, such as Wilson disease, autoimmune hepatitis, hepatitis C, chronic hepatitis B and alcoholic liver disease, have lower rates of fertility; however, pregnancies do occur. In general, pregnancy does not change the course of the underlying liver disease. However during pregnancy women with hepatitis C usually exhibit normalization of elevated AST and ALT values with return to prepregnant values after delivery.

The outcome of pregnancy may depend on the clinical condition of the patient. In Wilson disease and autoimmune hepatitis, it is important to not stop therapy as these diseases can flare during pregnancy with potentially serious adverse effects on both mother and fetus. Drugs such as azathioprine or penicillamine may have rare teratogenic effects on the fetus, but the risk of this possibility is far outweighed by the benefit of continued therapy. In general, a higher incidence of fetal wastage and premature births occurs in these pregnancies, probably related to the poorer clinical condition of the mothers. Further, up to 43% of women with autoimmune hepatitis experience a flare of their disease within 6 months of delivery.

Pregnant women with cirrhosis have approximately a 25% chance of experiencing variceal bleeding during the pregnancy, especially in the latter half of gestation. This increased risk probably reflects the increased circulatory volume of late pregnancy. In addition, as the gravid uterus enlarges, there may be an obstruction of the inferior vena cava with peripheral venous return forced through collateral systems. Patients should be aware of the risk; screening for varices could be performed prior to pregnancy and the option of a therapeutic abortion may be considered. In patients with documented varices, multiple different therapies have been used with variable results.  $\beta$ -blocker therapy, shunt surgery (for women with non-cirrhotic portal hypertension), and elective sclerotherapy have all been used successfully to treat variceal bleeding in pregnancy, but no prospective trials are available to assess the true efficacy of these



**Figure 31.5** Liver biopsy from a patient with Herpes simplex hepatitis. Widespread hepatocyte necrosis is shown with many characteristic eosinophilic intranuclear viral inclusions (arrow).



treatments. There have been no reports, as yet, of using trans-jugular intrahepatic portosystemic shunts (TIPS) or variceal band ligation in pregnancy, but these therapeutic modalities may also be considered. In patients who are actively bleeding from esophageal varices, standard therapy should be undertaken including volume resuscitation, intravenous octreotide, variceal banding or sclerotherapy and, if necessary, a Sengstaken-Blakemore tube and placement of a TIPS. The use of vasopressin should be avoided, given the risk of inducing labor with this medication. Maternal mortality can be 10% or higher and these women experience higher rates of fetal loss and premature birth.

### Vertical transmission of virus

In cases of acute or chronic viral hepatitis, vertical transmission of virus from mother to child is an important issue. Transplacental transfer of hepatitis A is extremely rare. The infant is at risk of infection during delivery if the acute infection occurs within 2 weeks before or after delivery. If that is the case, a single intramuscular injection of immune serum globulin shortly after birth is recommended.

Transplacental transmission of hepatitis B has not been reported, but vertical transmission at the time of delivery is common with the risk estimated to be approximately 25% if the mother is hepatitis B surface antigen (HBsAg)-positive and 80–90% if she is also hepatitis B e antigen (HBeAg)-positive. Many infants infected at birth develop chronic infections and perpetuate infection to the next generation. All pregnant women should be screened for HBsAg and infants born to HBsAg-positive mothers, regardless of HBeAg status, should be given both active and passive immunization with hepatitis B vaccine (0.5 ml i.m. at the time of birth and at 1 and 6 months of age) and hepatitis B immune globulin (0.5 ml i.m. at the time of birth). Although this regimen is highly effective, babies born to highly viremic mothers, who exhibit HBV DNA of  $1.2 \times 10^9$  viral particles (or gene equivalents)/mL, may experience better protection if mothers also are treated with lamivudine during the last 1–2 months of pregnancy. Other family members should be tested and vaccinated if negative for HBsAg and HBsAB.

Since hepatitis B is a common infection worldwide, and because of lifelong risks of infections, universal vaccination has been recommended in the USA for all infants. All other infants born in the USA (to mothers without hepatitis B infection) should receive three doses of hepatitis B vaccine, one dose at about 0–2 months of age, one at 1–4 months of age (at least 1 month after the first dose) and the last dose at 6–18 months of age.

Vertical transmission of hepatitis C is uncommon, occurring in about 8% of births, but is greatly increased in infants born to women with HIV infection. No treatment is available to prevent this vertical transmission, however some infants appear able to control and eliminate this virus even if they are infected at birth. Babies born to women with hepatitis C receive maternal antibody against hepatitis C and may only express true infection at 6 months of age or greater. Therefore it is recommended that infants born to infected mothers be checked for hepatitis C, using a polymerase chain reaction (PCR)-based test for hepatitis C RNA, at 3, 6 and 18 months of age and only those positive at 6 or 18 months be considered chronically infected. Treatment for those infants that do become chronically infected is being evaluated.

### Liver transplantation

Liver transplantation often allows return of normal menstrual function in women of child-bearing age and pregnancies have been reported as early as several months after successful transplant. Pregnancies after liver transplantation are considered high risk with rates of maternal pregnancy-related hypertension and pre-eclampsia of at least 25%. Although immunosuppressive drugs are potentially teratogenic, few congenital anomalies have been described. Pregnancy after transplant does result in increased fetal growth retardation, fetal loss and premature birth. However if the liver graft is functioning well and mother and fetus are followed closely by an experienced team, most mothers and babies have done well. Reports from small series indicate a risk of rejection of about 20% during or immediately after pregnancy, although most episodes were mild and treatable.

### Liver adenomas

Liver adenomas may enlarge under the influence of estrogen during pregnancy and potentially might become symptomatic and/or rupture. The actual risk of rupture during pregnancy is unknown but is likely related to size and/or rapid growth. Large adenomas identified prior to pregnancy could be considered for surgical resection. Treatment of a ruptured adenoma during pregnancy includes volume resuscitation, rapid delivery and use of angiographic and/or surgical means to stop bleeding and remove the adenoma.

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of liver disease in pregnancy includes all types of liver diseases, but in this section we focus on diseases that are either unique to pregnancy or more common in pregnancy. Diagnosis can be divided based on the stage of pregnancy (Table 31.6). In the first trimester of pregnancy, the only disease

Differential diagnosis of liver disease according to stage of pregnancy		
First trimester	Second trimester	Third trimester
Acute viral hepatitis	Acute viral hepatitis	Acute viral hepatitis
Hyperemesis gravidarum	Gallstone disease	Intrahepatic cholestasis of pregnancy*
Gallstone disease	Herpes simplex hepatitis	Acute fatty liver of pregnancy*
		Pre-eclampsia related liver disease (HELLP, hepatic infarction, hematoma, rupture)*
		Gallstone disease
		Herpes simplex hepatitis
*These can rarely occur in the second trimester or immediately postpartum. HELLP, laboratory features of hemolysis, elevated liver enzymes and low platelet count.		

**Table 31.6** Differential diagnosis of liver disease according to stage of pregnancy.

Diagnostic features distinguishing causes of liver disease in pregnancy					
Tools	<i>Intrahepatic cholestasis of pregnancy</i>	<i>Pre-eclampsia/eclampsia related liver disease</i>	<i>Acute fatty liver of pregnancy</i>	<i>Viral hepatitis</i>	<i>Biliary tract disease</i>
Onset (trimester)	3	3 or postpartum	3 or postpartum	Any	Any
Family history	Often	No	Rare	No	No
Recurrence in subsequent pregnancy	Likely	Possible	Rare	No	Possible if stones not removed
Symptoms	Pruritus	Malaise, nausea, vomiting, abdominal discomfort, fever, headache	Malaise, anorexia, nausea, vomiting, abdominal discomfort, somnolence	Malaise, nausea, vomiting	Nausea, abdominal pain, fever
Signs	Skin excoriations Jaundice	Pre-eclampsia (hypertension, proteinuria, edema) Evidence of shock may indicate hepatic rupture	Up to 40% with concurrent pre-eclampsia Hepatic encephalopathy Bleeding due to coagulopathy Signs of liver failure	Liver tenderness Jaundice Signs of liver failure (uncommon)	RUQ pain/tenderness Jaundice
Serum liver tests	Bilirubin rarely >6 mg/dL Alkaline phosphatase 3–5 normal Serum transaminase level variable, 2–10 × normal Increased PT (rare)	Variable elevations of AST/ALT (5–100 × normal) Extreme elevations of transaminase suggests hepatic infarction Bilirubin variable	Variable bilirubin of up to 10 × normal Variable serum aminotransferase levels, rarely greater than 10 × normal Increased PT	Significant elevations of aminotransferases from 5 to 25 times normal Variable degree of liver dysfunction	Elevated bilirubin Elevated alkaline phosphatase
Serum tests: Other	Serum bile acid 10–100 × normal	Hemolysis, thrombocytopenia Renal insufficiency Increased uric acid DIC	Renal insufficiency Hypoglycemia Increased uric acid DIC Leukocytosis	Hepatitis serology: anti-HAV IgM, anti-HBc IgM, HBsAg, HBeAg, anti-HCV, and HCV RNA	None
Hepatic imaging	Normal	Useful for diagnosing hepatic infarcts, hematomas, and rupture	Can show fatty liver but not sensitive	Not useful	Useful to show gallstones, sludge and/or dilated bile ducts if present
Liver biopsy	Cholestasis with little or no inflammation	Periportal hemorrhage with sinusoidal fibrin deposition	Microvesicular fatty infiltration of centrilobular hepatocytes	Intense portal and periportal inflammation with lobular necrosis	Normal or periportal edema, PMN infiltration, cholestasis
ALT, alanine aminotransferase; AST, aspartate aminotransferase; DIC, disseminated intravascular coagulation; HAV, hepatitis A virus; HBc, hepatitis B core; HBeAg, hepatitis B e antigen; HBs Ag, hepatitis B surface antigen; HCV, hepatitis C virus; IgM, immunoglobulin M; PMN, polymorphonuclear neutrophil; PT, prothrombin time; RUQ, right upper quadrant.					

Table 31.7 Diagnostic features distinguishing causes of liver disease in pregnancy.

unique to pregnancy is hyperemesis gravidarum, which should be easily differentiated from acute viral hepatitis by more severe nausea and vomiting in the former and much more elevated AST/ALT values in the latter and by serologic testing for hepatitis viruses. Gallstone disease, like hyperemesis gravidarum, may present with nausea and vomiting and mild liver test abnormalities, but significant abdominal pain should only be present in the former.

In the second trimester of pregnancy, the possibility of herpes simplex hepatitis should be considered in patients with fever,

abdominal pain and marked elevations in serum aminotransferases and mild elevations of bilirubin. This disease may present similar to other causes of viral hepatitis (e.g. hepatitis A and B) but can be differentiated by serology, liver biopsy and, if present, skin lesions.

In the third trimester of pregnancy, various diseases can occur, which may be differentiated based on clinical presentation (Tables 31.6 and 31.7). A cholestatic pattern of liver tests in the setting of pruritus will differentiate ICP from other liver diseases (e.g. HELLP, acute fatty liver of pregnancy and viral hepatitis).

Treatment of diseases unique to pregnancy	
Disease	Treatment
Hyperemesis gravidarum	Supportive care with rehydration, correction of underlying metabolic and electrolyte imbalance Nutritional support with total parenteral nutrition in severe cases
Intrahepatic cholestasis of pregnancy	Ursodeoxycholic acid (10–15 mg/kg/day or 1 g/day) Cholestyramine (8–16 g/day) Parenteral vitamin K, especially near the time of delivery Monitor beginning at 34 weeks with prompt delivery for fetal distress or by 36 weeks (severe disease) or 37 weeks (mild disease)
Pre-eclampsia related liver disease/HELLP*	Treatment of pre-eclampsia Corticosteroids (in selected cases) Prompt delivery
Hepatic hematoma/rupture	Close observation and prompt delivery for contained hematoma Prompt delivery with cesarean section for patients with suspected rupture Laparotomy with drainage and packing of rupture Hepatic artery embolization for rupture in selected cases
Acute fatty liver of pregnancy	Prompt delivery Supportive care of liver failure
*HELLP, laboratory features of hemolysis, elevated liver enzymes and low platelet count.	

**Table 31.8 Treatment of diseases unique to pregnancy.**

Fulminant hepatic failure can result from acute fatty liver of pregnancy, viral hepatitis or severe HELLP syndrome, and these can be differentiated with serology and/or liver biopsy. Differentiation of acute viral hepatitis from ICP, HELLP syndrome, and acute fatty liver of pregnancy is particularly important, because the treatment for the latter three is early delivery. A summary of the treatments for liver disease unique to pregnancy is shown in **Table 31.8**.

## PROGNOSIS

For patients with chronic liver disease who become pregnant, the maternal outcome and prognosis depend greatly on the underlying liver disease. Pregnancy does not alter the outcome of most liver diseases, but the presence of cirrhosis and portal hypertension greatly increases the risk of gastrointestinal bleeding, which then portends a poor prognosis. For the fetus, the perinatal mortality is increased, with a higher incidence of prematurity, stillbirth and intrauterine growth retardation, but there is no increased risk of birth defects.

For patients with hyperemesis gravidarum, maternal survival is excellent if dehydration, metabolic disorders and malnutrition are treated (**Table 31.9**). Mean infant birth weight is decreased if the hyperemesis is severe, for example, with a greater than 3% decrease in maternal body weight. In ICP, maternal survival is not affected, but there is up to a 70% chance of a recurrence with subsequent pregnancies. Furthermore, there is some perinatal morbidity and mortality, owing to prematurity, stillbirth and fetal death. The prognosis for HELLP and AFLP is somewhat worse, with maternal mortality rates of 2–20%. This is an improvement on historical reports, likely caused by advances in intensive care, early diagnosis, early delivery and recognition of milder cases. Recurrence has been variable with rates of 3–27% for HELLP and is rare, but not absent, for AFLP.

Prognosis of liver diseases unique to pregnancy		
Disease	Maternal outcome	Fetal outcome
Hyperemesis gravidarum	Excellent maternal outcome with good supportive care	Decreased mean birth weight in severe cases
Intrahepatic cholestasis of pregnancy	Resolves with delivery Increased risk of postpartum hemorrhage Recurrence is common with subsequent pregnancies and/or use of oral contraceptives Increased lifetime risk of gallstone formation	Increased risk of intrauterine growth retardation (10%), meconium staining, spontaneous preterm labor, and intrapartum fetal distress resulting in increased perinatal mortality (2% or more)
Pre-eclampsia related liver disease/HELLP*	Adverse effect on maternal outcome with mortality of 2–3% No long-term sequelae	Intrauterine growth retardation. Increased perinatal mortality of 6–25%, related to severity of maternal disease
Acute fatty liver of pregnancy	Maternal mortality of 2–20%. No long-term sequelae Recurrence is rare in absence of genetic mutation in fatty acid metabolism	5–20% or higher fetal mortality, related to severity of maternal disease
*HELLP, laboratory features of hemolysis, elevated liver enzymes and low platelet count.		

**Table 31.9 Prognosis of liver diseases unique to pregnancy.**



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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 32

## Drug- and Toxin-Induced Liver Disease

Suzanne Norris

### INTRODUCTION

Drug-induced liver diseases are clinicopathologic syndromes in which hepatotoxicity is caused by drugs and other foreign chemicals. Such syndromes are a potential complication of nearly every medication that is prescribed, as the liver is central to the metabolic disposal of virtually all drugs and toxins. The severity varies from minor non-specific injury to hepatic necrosis resulting in acute liver failure, chronic hepatitis, cirrhosis and liver tumors. Removal of the offending drug usually ameliorates the injury, but ongoing exposure increases the likelihood of progression to liver failure or chronic liver disease. Early recognition of hepatic drug reactions is therefore of great importance for clinicians. Some types of drug-induced liver disease result from dose-dependent toxic mechanisms. Most, however, are rare and are idiosyncratic drug reactions occurring with conventional therapeutic doses. The resultant clinical syndromes and histopathology mimic all known hepatobiliary diseases and one drug may act through a variety of mechanisms, leading to different histologic appearances in different patients. It is therefore likely that multiple pathogenic mechanisms operate or that the response of the liver to injury may vary between individuals, thereby resulting in diverse clinicopathologic entities. Drug-induced liver injury must therefore always be included in the differential diagnosis of liver disease of unknown etiology.

### EPIDEMIOLOGY

Reliable data on the risk and frequency of drug-induced liver injury are rare. Large population studies are required to reliably determine the frequency of a rare event. Thus, detecting a relative risk of 2 for a given event in a cohort study, assuming a level of significance of 0.05, a power of 0.9, and a background incidence in the control group of 0.001, requires a total of 63 000 patients in both the exposed and unexposed groups. Record linkage studies represent a possible solution to this problem. These involve linkage of prescriptions for a particular drug with hospital discharge diagnoses or disease registries, while unexposed patients serve as a control group. Using this technique, the relative risk for acute liver failure associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs) has been estimated to be 2.3 with an absolute risk of 9 cases/100 000 person years.

Comprehensive hospital drug monitoring is a way to determine frequencies of adverse drug reactions in hospitalized patients. Using this type of epidemiologic study, 20 patients with drug-induced hepatitis were diagnosed among 66 995 patients monitored, indicating a frequency of 1:3350 in hospitalized patients. This figure is clearly higher than that

Frequencies of drug-induced liver diseases	
Drug	Frequency (cases per 100 000 exposed)
Isoniazid	1000–2500
Chlorpromazine	500–1000
Dantrolene	1000
Valproic acid	3–200
Ketoconazole	7–9
Phenytoin	<10
Diclofenac	1–5
Amoxicillin and clavulanic acid	0.1–0.5

**Table 32.1** Frequencies of drug-induced liver diseases. Modified from Farrell (94).

observed for hepatotoxicity in general practice where a prevalence of 0.3/100 000 patients has been reported. In several studies, the frequency of drug-induced liver disease was determined prospectively in patients admitted to the hospital. These studies suggest that drug-induced liver injury accounts for between 2 and 5% of hospital admissions for jaundice in the USA, and between 2 and 3% of all hospital admissions due to adverse drug reactions. Drugs are disproportionately represented amongst the more serious and fatal forms of liver disease. In the USA, drugs account for up to 30% of cases of acute liver failure. Suspected drug-induced liver injury accounts for 4–7% of all reports of adverse drug effects voluntarily reported to central registries. While between 600 and 1000 drugs have been implicated in the etiology of a wide variety of liver diseases, only a few agents produce clinically significant liver injury in more than 0.1% of individuals exposed. For most drugs, the risk of liver injury is in the range of 1–10/100 000 individuals exposed, although there are some notable exceptions (Table 32.1).

### BASIC PRINCIPLES OF DRUG METABOLISM

The vulnerability of the liver to damage by drugs and toxins is a consequence of its central role in metabolism and its constant exposure to ingested foreign substances, the metabolism of which may result in noxious intermediates that can cause or augment liver injury. Many drugs and toxins are lipophilic, entering the body through the gastrointestinal tract, and require conversion from water-insoluble (non-polar) to water-soluble (polar) metabolites for their elimination in urine and bile. The

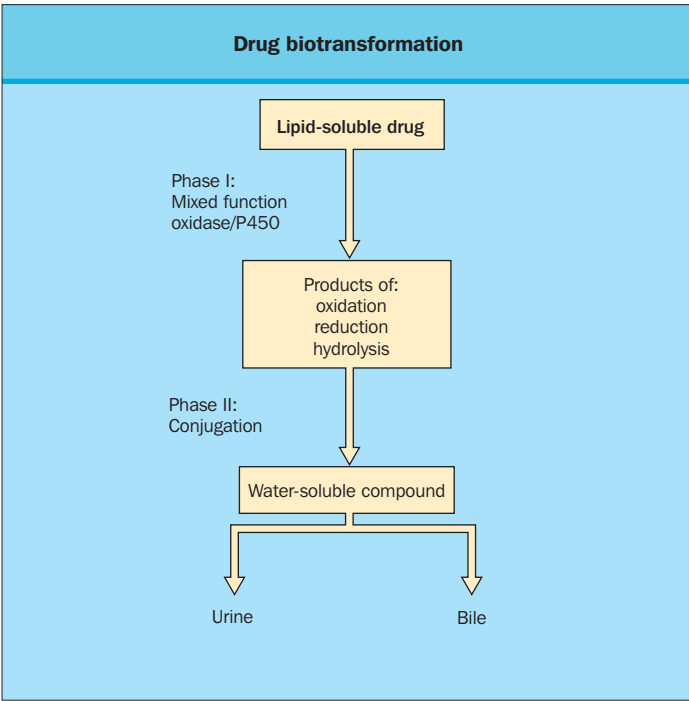


Figure 32.1 Schematic representation of drug biotransformation.

liver is uniquely suited to metabolize lipophilic drugs due to the fenestrations in the endothelium lining the sinusoidal spaces (see Ch. 2). These fenestrations allow the passive diffusion of most blood-borne proteins from the sinusoid to the space of Disse where protein-bound drugs come into contact with the hepatocyte plasma membrane. From here, the proteins can be actively transported or diffuse passively into the hepatocyte where the drugs are metabolized and then excreted into the space of Disse, sinusoids, and ultimately the systemic circulation to be excreted by the kidney. Alternatively, the metabolites may be excreted in bile.

Biotransformation is the process by which therapeutic agents are rendered more hydrophilic. The enzyme systems responsible for biotransformation are located in the smooth endoplasmic reticulum of the hepatocyte and include mixed function oxidase or mono-oxygenase (MFO), cytochrome c-reductase and cytochrome P450. Biotransformation consists of two phases (Fig. 32.1). Phase I prepares the compound for conjugation by providing polar groups through hydroxylation or oxidation, thereby modifying the primary structure of the drug. In phase II, the resulting metabolite is conjugated with glutathione (GSH), glucuronate, sulfate, glycine or water, which enhances its water solubility. The conjugate can then be excreted. Differences between phase I and II pathways of metabolism are demonstrated in Table 32.2.

The key enzyme in phase I reactions is cytochrome P450, a large multigene family of enzymes with nearly 300 members. P450 enzymes are composed of an apoprotein and a heme group which binds oxygen after electron-transfer reactions from nicotinamide adenine dinucleotide phosphate (NADP), reduced form (NADPH), resulting in hydroxylation, dealkylation or dehalogenation. Each group of genes forms a family whose isoenzymes function in a similar fashion, and three distinct P450 gene families (termed CYP1, CYP2 and CYP3) account for the

Differences between phase I and phase II pathways of drug metabolism		
	Phase I Oxidation	Phase II Conjugation
Enzyme system	Cytochrome P450	Acetyltransferases Glucuronyltransferases Sulfotransferases
Location in liver	Centrilobular	Periportal
Location within cell	Microsomal	Can be non-microsomal
Location within microsomal membrane	Surface	Interior
Endoplasmic reticulum	Smooth	Rough

Table 32.2 Differences between phase I and phase II pathways of drug metabolism.

majority of phase I metabolism. Each family contains members that are more than 40% homologous in terms of amino acid sequence. These families are subdivided into subfamilies which share greater than 55% amino acid sequence homology. Each isoenzyme has specific substrates on which it can act; for example, CYP2E1 is the major isoenzyme responsible for the production of metabolites of acetaminophen (paracetamol), while CYP3A is concerned with the metabolism of cyclosporine. There are marked differences between patients in their ability to perform phase I liver metabolism of some drugs, and genetic differences in the catalytic activity of P450 isoenzymes may determine idiosyncratic reactions to drugs. One example is the poor metabolism of the antihypertensive agent, debrisoquin (debrisoquine), which occurs in approximately 5% of the Caucasian population. The debrisoquin (debrisoquine) 'poor metabolizer' phenotype is inherited as an autosomal recessive trait and results from genetic defects in several alleles for a single P450 enzyme, CYP2D6, leading to its deficiency. This enzyme system also metabolizes most neuroleptics and  $\beta$  blockers. More recently, it has been reported that approximately 2% of Caucasians are 'rapid extensive metabolizers' of debrisoquin (debrisoquine) and these patients demonstrate rapid metabolism of other drugs that are CYP2D6 substrates. The rate of hepatic drug metabolism may also be affected by compounds that accelerate the metabolism of certain drugs through induction of the cytochrome P450 system (Table 32.3).

Thus, rifampin (rifampicin) and antiepileptic medication cause induction of CYP3A4 and accelerate metabolism of sex hormones and as a result, reduce the efficacy of the oral contraceptive pill. P450 induction also leads to accelerated production of toxic metabolites, as demonstrated by ethanol which induces CYP2E1 and enhances the toxicity of acetaminophen. Cigarette smoke is also a potent inducer of certain P450 enzyme species.

RISK FACTORS FOR DRUG-INDUCED HEPATOTOXICITY

Individual susceptibility to drug- and toxin-induced liver injury may be affected by a variety of host factors such as age, sex,



Characteristics of human liver P450		
P450	Drug substrates	Probable inducers
CYP1A2	Caffeine Acetaminophen Theophylline Cimetidine	Cigarette smoke Omeprazole
CYP2C	Omeprazole Diazepam Tolbutamide Phenylbutazone Tienilic acid	
CYP2D6	Debrisoquine Codeine Fluoxetine Quinidine Most $\beta$ blockers Many neuroleptics	
CYP2E1	Acetaminophen Ethanol	Ethanol Isoniazid
CYP3A4	Cyclosporin A Erythromycin Ketoconazole Estrogens Nifedipine Acetaminophen Midazolam/triazolam Tamoxifen Lovastatin	Rifampin Glucocorticoids Antiepileptic medication

**Table 32.3 Characteristics of human liver P450.**

genetic make up, pregnancy, nutritional status and co-existing disease (Table 32.4).

The increased susceptibility of the elderly to hepatotoxicity from drugs may result from age-related changes including reduced hepatic blood flow, decreased activity of the hepatic cytochrome P450 enzyme system, and reduced renal clearance. A linear increase in hepatic drug toxicity from less than 10 cases/million persons/year in the first two decades to approximately 40 cases in the eighth decade of life has been documented. Typical examples for drugs exhibiting increased toxicity in older patients include NSAIDs, isoniazid, chlorpromazine, penicillins, methotrexate and halothane. The frequency of hepatic injury is higher in children or young adults with valproate, erythromycin and aspirin.

Women are particularly predisposed to drug-induced hepatitis, for example chronic hepatitis due to nitrofurantoin or diclofenac, while cholestatic drug reactions are equally found in both sexes. However, male renal transplant recipients are more predisposed to azathioprine-induced liver disease than females. Obesity may increase the risk of hepatotoxicity through prolonged hepatic exposure to fat-soluble drugs released from stores in adipose tissues. Impairment of GSH synthesis and its depletion in the hepatocyte in patients with malnutrition and chronic alcoholism may be responsible for their increased susceptibility to acetaminophen toxicity. GSH depletion in patients with acquired immune deficiency syndrome may also be associated with the increased susceptibility of this patient group

Host variables for individual susceptibility to drug-induced liver injury		
Host factor	Increased susceptibility	
Age	Infancy Old age	Valproic acid, salicylates NSAIDs, isoniazid, nitrofurantoin
Sex	Male  Female	Amoxicillin–clavulanic acid, azathioprine Isoniazid, zidovudine, halothane, methylidopa
Racial background	Asian/African	Isoniazid
Pre-existing disease	Liver Renal AIDS	Acetaminophen, isoniazid NSAIDs Oxacillin, trimethoprim– sulfamethoxazole
Nutrition	Obesity Fasting	Halothane, methotrexate Acetaminophen
Pregnancy		Acetaminophen
Other drugs	Alcohol Isoniazid Antiepileptics	Acetaminophen Acetaminophen Acetaminophen
Genetic polymorphism/defect		
	Slow acetylators	Isoniazid
	Urea cycle	Valproic acid
	Mitochondrial $\beta$ -oxidation	Valproic acid
	UDP glucuronosyl transferase	Acetaminophen
	HLA phenotypes	See text
HLA, human lymphocyte antigen; NSAIDs, non-steroidal anti-inflammatory drugs; UDP, uridine diphosphate.		

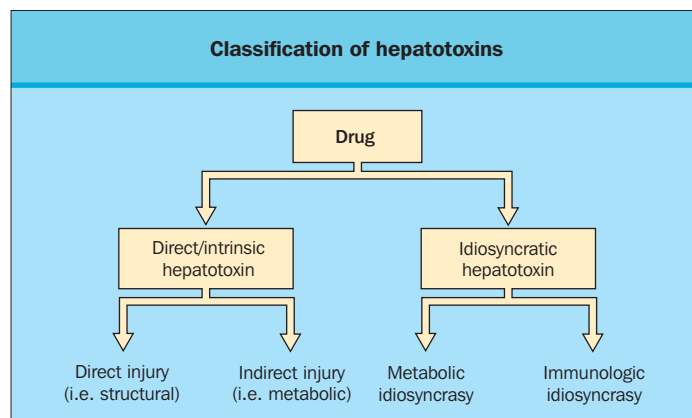
**Table 32.4 Host variables for individual susceptibility to drug-induced liver injury.**

to drug toxicity from oxacillin and sulfa drugs. The association between hypoalbuminemia and hepatotoxicity from aspirin may reflect the reduced binding of aspirin to plasma proteins and subsequent increased tissue levels. Inborn errors of metabolism, such as defects in mitochondrial  $\beta$ -oxidation, have been associated with microvesicular fat infiltration in children exposed to aspirin or valproic acid. Deficient uridine diphosphate (UDP) glucuronosyl transferase activity in patients with Gilbert syndrome may be associated with increased sensitivity to acetaminophen.

## **PATHOGENESIS OF DRUG-INDUCED HEPATOTOXICITY**

Drugs having the potential to cause hepatotoxicity can be divided into intrinsic or predictable hepatotoxins (type A reaction) and unpredictable or idiosyncratic hepatotoxins (type B reactions) (Fig. 32.2).

Type A reactions produce liver injury in a predictable, dose-dependent manner that can be reproduced in experimental animals. Characteristically, these toxins cause liver necrosis that



**Figure 32.2 Classification of hepatotoxins.**

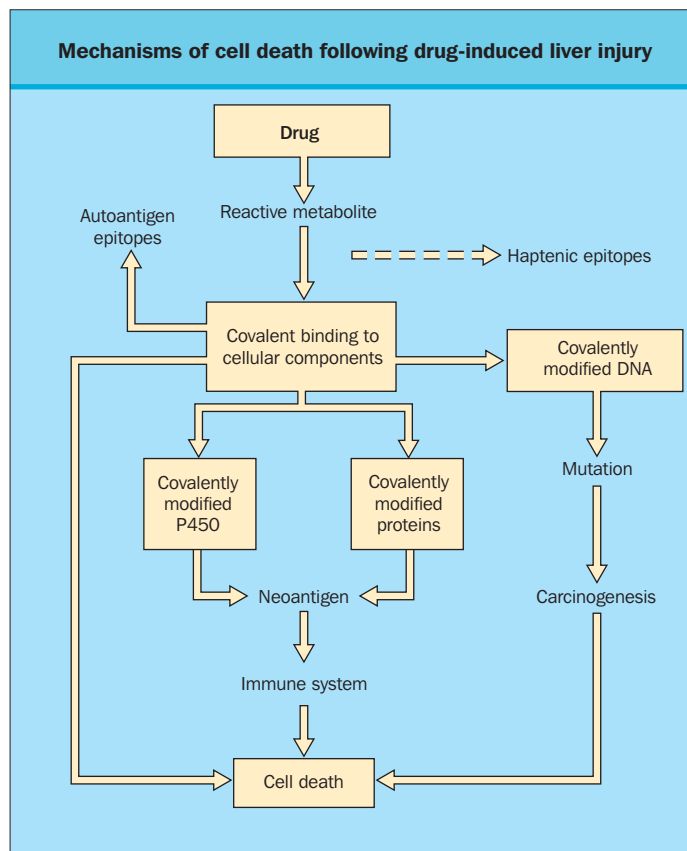
affects predominantly one area of the liver lobule, for example acetaminophen causes centrilobular or zone 3 necrosis. In contrast, type B reactions produce liver injury in an unpredictable manner (while administered within an acceptable therapeutic range e.g. with isoniazid), are not dose-dependent, are not reproducible in animal models, and produce a more diffuse form of liver injury.

Most intrinsic or type A hepatotoxins produce injury through the toxic effects of their reactive metabolites after phase I biotransformation. These reactive metabolites, which include electrophilic radicals, free radicals or reactive oxygen, can form covalent bonds with cellular molecules such as proteins, lipids and nucleic acids, with subsequent disruption of their function. This cellular distortion produces liver injury by inactivating key enzymes or by forming protein–drug complexes that are potential targets for immune-mediated liver injury (Fig. 32.3).

In the past, covalent binding has been considered to be a key mechanism of drug-induced liver injury; it appears to be the main mechanism by which acetaminophen causes necrosis and by which electrophilic metabolites of carcinogens distort DNA. However, the extent of covalent binding of drug intermediates to cellular proteins does not correlate with the level of hepatic necrosis in experimental models, questioning the importance of this mechanism. Nevertheless, the specificity of the proteins covalently bound by metabolites may be more important than the extent of covalent modification.

Oxidative processes are also important in the development of cell damage through the generation of oxidative stress and an imbalance between pro-oxidants and antioxidants. Electrophilic metabolites of acetaminophen metabolism can act as cellular oxidants resulting in oxidation of thiol groups in proteins. Oxidative stress is also an important mechanism for activating apoptosis. GSH, a major intracellular antioxidant that protects against the toxic effects of covalent binding, is important for the conjugation of several metabolites (such as acetaminophen) and facilitates their excretion in the bile. Depletion of GSH can therefore potentiate or augment hepatic injury. Lipid peroxidation is another major mechanism by which free radicals and active oxygen cause cellular injury. Peroxidative injury may also result from the generation of superoxide anions.

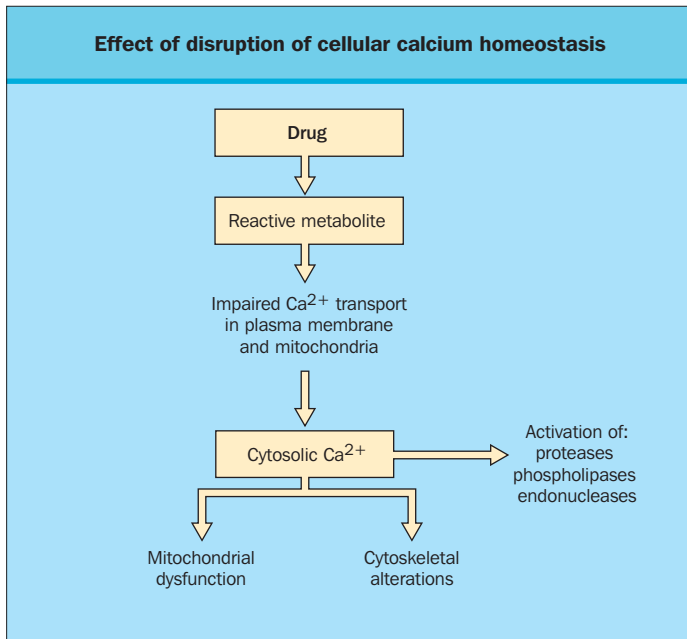
Most of the intrinsic hepatotoxins (such as acetaminophen) produce cellular injury as an indirect consequence of depletion of essential molecules or interference with biochemical path-



**Figure 32.3 Mechanisms of cell death following drug-induced liver injury.**

ways essential for cell integrity, which subsequently results in secondary structural damage. However, some intrinsic hepatotoxins produce a direct destructive effect on hepatocyte structure through the effects of peroxidation [such as carbon tetrachloride ( $\text{CCl}_4$ )] (Fig. 32.2). This structural alteration leads to secondary metabolic defects. Irrespective of whether the initial insult is metabolic or structural, ultimately it results in cell death. It is uncertain whether a final common pathway exists for drug-induced liver injury. Disruption of cellular calcium homeostasis, impairment of energy production and depletion of ATP have all been postulated to be key determinants of cell death (Fig. 32.4).

Activated hepatic macrophages release proteases, nitrogen radicals, superoxide and cytokines, which also recruit lymphocytes and neutrophils from the systemic circulation to the hepatic inflammatory response. Cytokines produced by activated macrophages can be hepatotoxins in their own right, for example tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) which is thought to exert its hepatotoxic effect by producing intramitochondrial oxidative stress. Studies have reported that anti-TNF- $\alpha$  antibodies protect against  $\text{CCl}_4$ -induced hepatotoxicity in rats. Release of cytokines such as interleukin-1 (IL-1), TNF and IL-8 can also lead to injury to endothelial and stellate cells. IL-8 and endothelial damage promote neutrophil adherence and induce further damage by the release of proteases and reactive oxygen intermediates by the neutrophils. Thus, not only the initial formation of toxic metabolites determines the ultimate extent of liver disease, but also the interplay among hepatocytes, non-parenchymal cells and neutrophils.



**Figure 32.4** Effect of disruption of cellular calcium homeostasis.

Immunologic mechanisms may also be involved in idiosyncratic drug-induced hepatotoxicity (type B reactions). The term immunologic idiosyncrasy assumes an allergic basis for drug-induced liver injury. However, the mechanism of the immune response is still not fully understood. The basis of the injury is thought to be hypersensitivity which develops after a period of 1–5 weeks, recurs quickly on readministration of the drug, and is accompanied by fever, rash, eosinophilia, lymphocytosis and an inflammatory infiltration of the liver. In some cases, the liver is involved as part of a systemic hypersensitivity reaction. For drugs to induce an immune response, it is generally assumed that they covalently alter an endogenous macromolecule forming a carrier hapten-conjugate, which then acts as an immunogen and elicits a humoral and/or a cellular immune response directed against the liver. The immune response may be directed against three types of antigenic determinants (epitopes) of the altered macromolecule: epitopes that include the bound drug metabolite (haptenic epitopes); novel epitopes of the carrier molecule, namely neoantigens that result from the covalent modification; and native or autoantigen epitopes normally seen as self molecules but rendered immunogenic by covalent modifications (Fig. 32.3).

Autoantibodies have been documented in the sera of patients taking the antihypertensive diuretic, tienilic acid (Table 32.5). These patients developed a high incidence of anti-liver-kidney microsomal type 2 (anti-LKM<sub>2</sub>) autoantibodies that recognize epitopes specific to CYP2C9, the major cytochrome P450 involved in the biotransformation of tienilic acid. Anti-LKM<sub>2</sub> autoantibodies are highly specific for drug-induced autoimmune-like chronic hepatitis and have a different pattern of immunofluorescence staining from the anti-LKM<sub>1</sub> autoantibodies found in type 2 autoimmune hepatitis. Another example of autoantibodies associated with drug-induced liver injury is the recognition of the E2 subunit of pyruvate dehydrogenase complex (PDC) by sera of patients with a history of halothane hepatitis. E2, the major autoantigen recognized by antimitochondrial

Drug-induced liver disease and autoantibody expression	
Drug	Autoantibody
Nitrofurantoin	ANA, SMA
Methyldopa	ANA, SMA
Chlorpromazine	ANA (AMA negative)
Tienilic acid	LKM <sub>2</sub>
Diclofenac	ANA
Sulfonamides	ANA
Isoniazid	AMA (E6 moiety)
Halothane	PDH (E2 moiety)
Nimesulide	ANA
Alverine	ANA
ANA, antinuclear antibody; SMA, smooth muscle antibody; AMA, antimitochondrial antibody; LKM <sub>2</sub> , liver kidney microsomal type 2; PDG, pyruvate dehydrogenase.	

**Table 32.5** Drug-induced liver disease and autoantibody expression.

antibodies in primary biliary cirrhosis, mimics the epitope common to several trifluoroacetylated proteins produced as a result of halothane exposure, representing an example of molecular mimicry. Non-tissue-specific autoantibodies, such as antinuclear and antismooth muscle antibodies, may occur in drug hepatitis caused by nitrofurantoin, methyldopa, and minocycline. Major histocompatibility antigens (MHC) class I and class II molecules present antigenic peptides to T lymphocytes, and as human leukocyte antigen (HLA) molecule expression is genetically polymorphic it is possible that certain haplotypes may modulate the immune response. Weak associations have been reported with the MHC complex HLA-A11 for halothane-induced and diclofenac-induced hepatotoxicity, and HLA-DR6 for nitrofurantoin-induced liver injury. Other drugs that are thought to mediate immunologic-mediated liver damage include phenytoin, dapsone and sulfonamides. Idiosyncratic hepatotoxicity not associated with hypersensitivity is thought to depend on metabolic idiosyncrasy and examples include isoniazid and valproic acid. Table 32.6 compares the features that suggest immunoallergy or metabolic idiosyncrasy.

Hepatotoxicity is normally prevented or limited by a number of protective mechanisms. Cytochrome P450 can be inactivated by the reactive metabolites themselves when they covalently bind to the apoprotein of cytochrome P450. Reactive epoxides undergo transformation by epoxide hydrolases to stable compounds. Other enzymes such as glutathione peroxidase and catalase protect against lipid peroxidation. Failure of these protective mechanisms therefore contributes to drug-induced liver injury.

## PATTERNS OF DRUG-INDUCED HEPATOTOXICITY

Two broad categories of liver injury are commonly recognized i.e. cholestasis and hepatocellular (or cytolytic) damage. The former is characterized by a predominantly elevated alkaline phosphatase and  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), while in the latter the



Comparison of drug-induced immunologic and metabolic idiosyncrasy		
Feature	Immunologic type	Metabolic type
Frequency	0.01% exposed	0.1–2% exposed
Gender	More common in women	Slightly more common in women
Latent period	Relatively constant	Highly variable
Response to rechallenge	Invariably fever in 12–72 h	Abnormal liver function tests after 3–30 days
Fever	Common	Less striking
Rash, arthralgia	Common	Rare
Eosinophilia	20–70% cases	Less than 10% cases
Granulomas	Common	Rare
Autoantigens	Common	Rare
Examples	Diclofenac Methyldopa	Isoniazid Ketoconazole

**Table 32.6 Comparison of drug-induced immunologic and metabolic idiosyncrasy.** Modified from Farrell (94).

Morphologic classification of drug-induced acute liver injury	
Type of injury	Examples
<b>Hepatocellular (cytolytic)</b>	
Zonal necrosis	Acetaminophen, CCl <sub>4</sub> , halothane
Steatosis: macrovesicular microvesicular	Ethanol, methotrexate Tetracycline, valproic acid, methotrexate
Hepatitis	Methyldopa, isoniazid
<b>Cholestatic</b>	
Hepatocanalicular (pericholangitis)	Amoxicillin–clavulanic acid, chlorpromazine
Canalicular (non-inflammatory)	Estrogens, 17 $\alpha$ -substituted steroids (anabolic)
<b>Vascular</b>	
Hepatic vein occlusion	Estrogens
Veno-occlusive disease	Antineoplastic agents, pyrrolizidine alkaloids
Peliosis hepatitis	Anabolic steroids

**Table 32.7 Morphologic classification of drug-induced acute liver injury.**

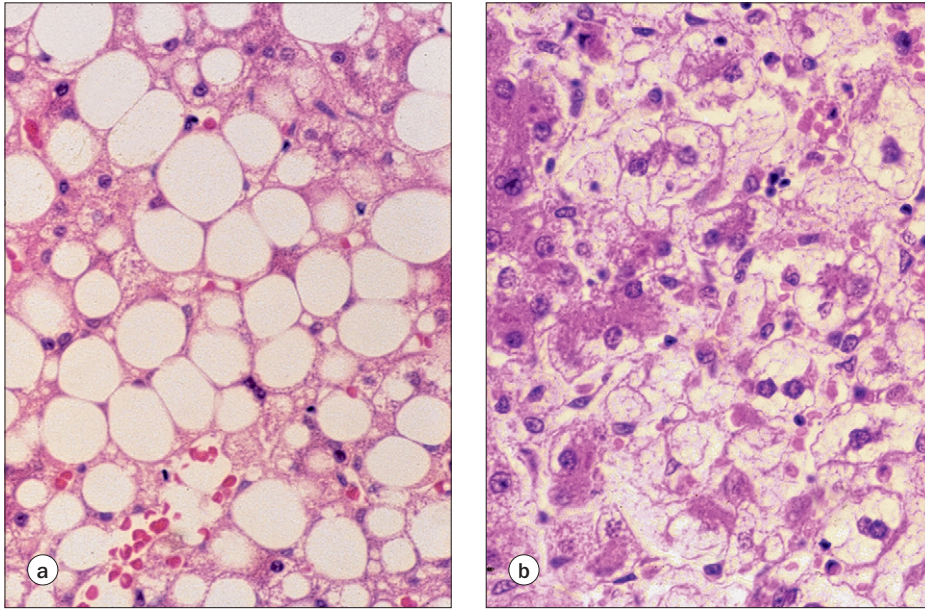
aminotransferases, alanine (ALT) and aspartate (AST), are significantly elevated, although mixed patterns of abnormalities are common. Hepatocellular injury can be acute or chronic and includes necrosis, steatosis and hepatitis in various combinations. A variety of chronic lesions related to vascular damage, neoplastic change or fibrosis has also been described. These patterns of hepatotoxicity are summarized in Tables 32.7 and 32.8.

Morphologic classification of drug-related chronic liver injury	
Type of injury	Examples
<b>Chronic hepatitis</b>	
Autoimmune-like	Methyldopa, dantrolene, sulfonamides, diclofenac
Viral hepatitis-like	Amiodarone, isoniazid, halothane, aspirin
<b>Chronic cholestasis</b>	
Ductal (sclerosing cholangitis)	Floxuridine (FUDR)
Ductopenia	Flucloxacillin, tricyclic antidepressants
Hepatocanalicular	Chlorpromazine, barbituates, cimetidine, phenytoin, tolbutamide, amitriptyline
<b>Granulomas</b> (more than 50 drugs implicated)	Sulfa drugs, allopurinol, carbamazepine, chlorpromazine, diazepam, diltiazem, gold, phenytoin, penicillin, nitrofurantoin, tolbutamide, quinidine, isoniazid
<b>Chronic steatosis</b>	Ethanol, methotrexate, antineoplastic agents
<b>Phospholipidosis</b>	Amiodarone, perhexiline maleate, thioridazine
<b>Vascular</b>	
Nodular regenerative hyperplasia	Azathioprine, 6-thioguanine
Non-cirrhotic portal hypertension	Vinyl chloride, azathioprine, 6-thioguanine
Hepatic vein occlusion	Estrogens
Veno-occlusive disease	Antineoplastic agents, pyrrolizidine alkaloids
Peliosis hepatitis	Anabolic steroids
<b>Neoplasms</b>	
Adenoma	Estrogens
Hepatocellular carcinoma	Estrogens, anabolic steroids, vinyl chloride
Angiosarcoma	Vinyl chloride, thorotrast
<b>Fibrosis</b>	Methotrexate, vitamin A, vinyl chloride

**Table 32.8 Morphologic classification of drug-induced chronic liver injury.**

### Necrosis

Necrosis induced by intrinsic hepatotoxins is often zonal (centrilobular or zone 3, midzonal or zone 2, and periportal or zone 1), whereas that produced by idiosyncratic reactions is more diffuse and similar to that observed with viral hepatitis. Some drugs such as halothane are associated with diffuse as well as zonal necrosis. The predominance of necrosis in selected areas is not fully understood, but zone 3 necrosis is thought to relate to the abundance of the cytochrome P450 metabolizing systems in centrilobular hepatocytes and a vulnerability of this area to hypoxia. This distribution is likely to be influenced by other factors such as rate of drug uptake, drug concentration and



**Figure 32.5 Steatosis.** (a) Macrovesicular steatosis; (b) microvesicular steatosis.

concentrations of cellular protective components such as GSH. Examples of drugs or toxins causing zone 3 necrosis include acetaminophen,  $\text{CCl}_4$ , chloroform, *Amanita phalloides* toxin, and pyrrolizidine alkaloids. Periportal or zone 1 necrosis is characteristic of ferrous sulfate overdoses, yellow phosphorus and toxins such as allyl alcohol. Agents causing midzonal necrosis include beryllium and furosemide (frusemide).

### Steatosis

Steatotic liver injury frequently precedes liver necrosis and two types are described according to their pathologic patterns. Steatosis may predominate in either the centrilobular or periportal region. Occasionally, the fat cells may coalesce to form fatty cysts or become surrounded by histiocytes to form lipogranulomas. Triglyceride is the predominant lipid that accumulates and this occurs when the rate of formation exceeds that of disposal into lipoproteins or hydrolysis to fatty acids. In macrovesicular steatosis, the hepatocyte contains a single large droplet of fat, which displaces the nucleus to the periphery of the cell giving it the appearance of an adipocyte (Fig. 32.5a). This pattern of fat is also seen with obesity, diabetes, ethanol, malnutrition and jejunoileal bypass.

In microvesicular steatosis, the hepatocyte contains numerous small droplets of fat that leave the nucleus centrally placed so that the cell retains its hepatocyte-like appearance (Fig. 32.5b). The pathogenesis appears related to inhibition of the mitochondrial oxidation of fatty acids. Candidate drugs include valproic acid and tetracycline. Another form of lipid accumulation is phospholipidosis, which occurs when amphiphilic drugs accumulate within lysosomes forming a stable complex with phospholipids. Phospholipidosis can histologically resemble alcoholic hepatitis. The liver injury induced is usually mild but can be associated with Mallory hyaline, and can progress to overt disease. Cirrhosis has been observed in patients taking amiodarone and perhexiline maleate. Alcohol-like liver injury without phospholipidosis has also been reported in patients taking nifedipine and diltiazem.

### Hepatitis

Non-specific hepatitis is typical of many types of drug-induced hepatotoxicity. It lacks the typical appearance of autoimmune hepatitis and is associated with scattered foci of necrosis and a variable inflammatory infiltrate. It is usually not associated with progressive liver disease. Chronic hepatitis that resembles autoimmune hepatitis in its histologic and clinical features has been described for a number of drugs (see Table 32.8). Pathologic changes include portal and periportal inflammatory infiltration by lymphocytes and plasma cells, often associated with degeneration of surrounding cells (piecemeal necrosis). Auto-antibodies are frequently present in the sera. Granulomatous hepatitis is characterized by aggregates of epithelioid histiocytes and accompanying inflammatory cells. Drug-induced granulomas are usually non-caseating and their presence suggests an immunologic idiosyncrasy (see Table 32.6).

### CHOLESTASIS

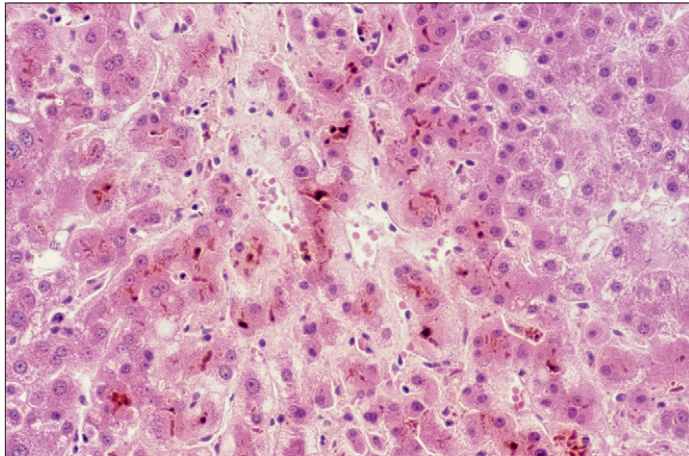
Intrahepatic cholestasis is a common manifestation of hepatotoxicity. It is predominantly noted as centrilobular bile staining of hepatocytes and as bile casts in the canaliculi. If the cholestasis is severe, the periportal area is also involved and bile plugs occur. Several forms of cholestatic liver disease have been described (see Table 32.7 and Table 32.8).

#### Bland, pure or canalicular cholestasis

This is characterized by a bland accumulation of bile in canaliculi and cells with little or no necrosis or inflammatory features (Fig. 32.6). Anabolic and contraceptive steroids are candidate drugs.

#### Hepatocanalicular cholestasis

This form of cholestasis is accompanied by a portal and lobular inflammatory infiltrate. Necrosis may also occur. This type of liver injury is observed with chlorpromazine and erythromycin.



**Figure 32.6** Bland or pure cholestasis following anabolic steroid use.

### Ductopenic cholestasis

An inflammatory cholestasis may also be accompanied by destruction of the small bile ducts resulting in ductopenic cholestasis or 'vanishing bile duct syndrome'. Drugs implicated in this form of liver injury include: chlorpromazine, tricyclic antidepressants, flucloxacillin, haloperidol, thiabendazole, tolbutamide and carbamazepine. In some cases, the bile duct destruction occurs without an inflammatory infiltration, but frank cholangitis with neutrophilic infiltration of bile ducts is more common. This form of liver injury can persist for months and even years before resolving, but may progress to secondary biliary cirrhosis.

### Ductular or cholangiolar cholestasis

Another form of cholestasis associated with bile casts in the cholangiocytes is known as ductular or cholangiolar cholestasis and is exemplified by benoxaprofen.

### Ductal cholestasis

Ductal cholestasis associated with intra- and extrahepatic sclerosing cholangitis has been described following intra-arterial administration of floxuridine and fluorouracil for treatment of metastatic liver carcinoma.

## OTHER CHRONIC LESIONS

Chronic forms of liver disease almost always depend on continued exposure to the drug rather than a self-perpetuating process instigated by the initial insult. However, in a few cases cirrhosis may develop despite discontinuation of the drug; this may occur with amiodarone and perhexiline maleate treatment as they can persist for many months in the liver. Portal hypertension has also been described following drug-induced liver injury due to the development of portal fibrosis. This non-cirrhotic portal hypertension, or hepatoportal sclerosis, has been described with vitamin A intoxication, chronic exposure to arsenicals, vinyl chloride and copper sulfate. Periportal fibrosis has also been reported following methotrexate administration. Portal hypertension due to vascular lesions may also result from drug administration such as oral contraceptive-induced hepatic vein occlusion and antimetabolite-induced veno-occlusive disease (see Table 32.8).

Drug administration has been linked to tumor development, both benign and malignant disease. Oral contraceptives and anabolic steroids have been implicated in the pathogenesis of hepatic adenomas and hepatocellular carcinoma. Angiosarcomas have been reported in those exposed to vinyl chloride and arsenic. Cholangiocarcinoma has been reported in those using anabolic and contraceptive steroids but a causal relationship has not been definitively established.

## DRUG-INDUCED LIVER INJURY ACCORDING TO DRUG CLASS

### Steroids: anabolic and contraceptive drugs

Natural and synthetic steroids have a number of effects on the liver:

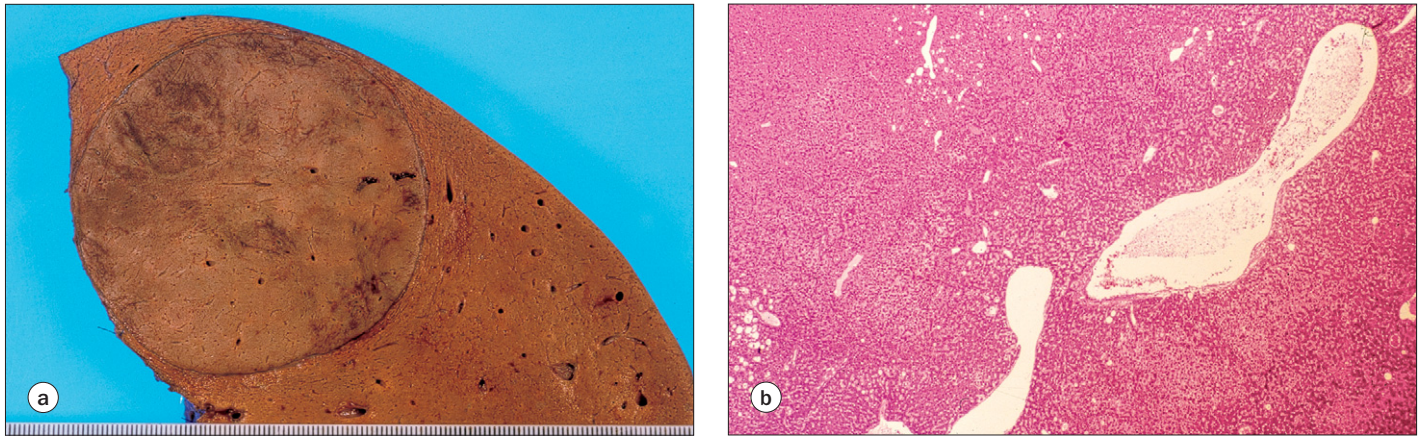
- pure cholestasis;
- development of tumors, hepatic adenoma, and hepatocellular carcinoma;
- architectural disturbance with the development of focal nodular hyperplasia (FNH);
- increased incidence of gallstones;
- hepatic vein occlusion; and
- peliosis hepatis.

Many of these effects have been reported for both oral contraceptive steroids (OCS) and anabolic steroids. Both groups of drugs are intrinsic hepatotoxins and the presence of an alkyl or ethinyl group on carbon 17 appears to be essential for the development of cholestatic liver disease. However, there are some differences in the type of resultant liver injury. Anabolic and androgenic steroids have been more frequently associated with malignant tumors, while contraceptive steroids are more commonly associated with benign liver tumors. Hepatic vein occlusion may relate to thrombogenic effects of the estrogen component of contraceptive steroids. Anabolic steroids more commonly produce peliosis hepatis.

Oral contraceptive steroids are associated with pure cholestasis in approximately 10/100 000 women exposed in Western Europe and 25/100 000 in Chile and Scandinavia, and it occurs within 2–3 months of commencing therapy. The estrogen component is probably responsible for the cholestasis. The injury is dose-dependent and is less common with low-dose preparations. Oral contraceptive steroid-induced cholestasis occurs in women with a history of cholestasis of pregnancy, and has also been observed in sisters, suggesting a genetic component. The mechanism by which estrogens and related steroids produce cholestasis is uncertain, but it appears to result from interference with bile excretion. Decreased bile flow, biliary secretion of bile acids, plasma membrane fluidity, and  $\text{Na}^+$ - and  $\text{K}^+$ -ATPase activity have all been reported in experimental animals. Biliary excretion of sulfobromophthalein (BSP) is also impaired. Estrogens may also alter the membrane lipid composition. Following cessation of OCS, the cholestasis resolves and the prognosis is excellent. Pure cholestasis may also complicate the use of stanozolol, a C-17 substituted testosterone, and danazol, a C-17 substituted androgen used in endometriosis.

The risk of developing hepatic adenomas and hepatocellular carcinoma appears to be increased by OCS and anabolic/androgenic use (Fig. 32.7), and most cases are associated with 17- $\alpha$ -alkylated steroids.





**Figure 32.7** Hepatic adenoma secondary to long-term oral contraceptive steroid (OCS) usage. (a) Macropathology; (b) micropathology.

Adenomas have also been reported with norethisterone, a progestin, and clomifene. The true incidence of OCS-related adenomas is unknown but the risk seems to be time-dependent and the relative risk is increased 116-fold at 5 years. Adenomas may regress after cessation of the drug, but can recur with readministration of OCS or with pregnancy. Estrogen-associated adenomas are highly vascular and have a tendency to hemorrhage into the tumor or into the peritoneal cavity. Whether adenomas progress to hepatocellular carcinoma (HCC) is controversial. Foci of dysplasia or malignancy have been described in resected adenomas, and the incidence of HCC is increased when OCS have been used for periods in excess of 5 years. However, other cofactors may be important in the pathogenesis of HCC in OCS users and anabolic steroid users, such as hepatitis B and alcohol excess. Like adenomas, steroid-induced HCC may regress after cessation of the drugs. Oral contraceptive steroid use has also been linked to the development of epithelioid hemangioendothelioma, and the enlargement of existing hemangiomas. Angiosarcomas have been reported with anabolic steroid use.

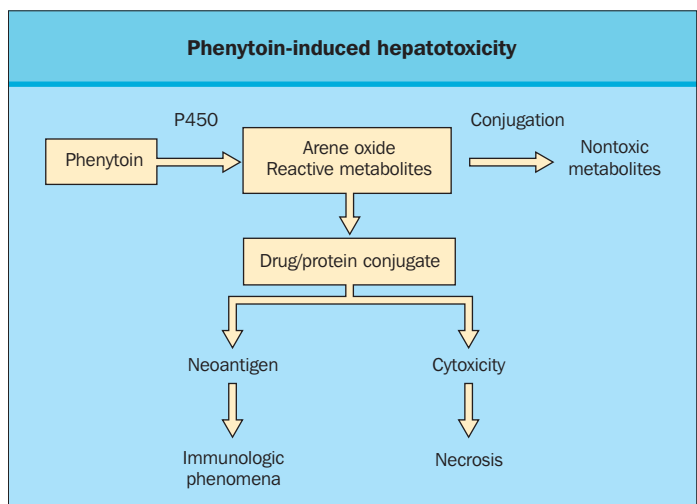
The risk of hepatic vein occlusion, or Budd-Chiari syndrome, is increased 2.5-fold in OCS users, consistent with their perceived thromboembolic potential. This thrombogenic trait is ascribed to the estrogenic component of the drug which may exacerbate an underlying thrombogenic disorder. Peliosis hepatis is characterized by blood-filled cavities within the hepatic parenchyma which may be lined by sinusoidal endothelium, and numerous cases have been reported in association with anabolic/androgenic steroids.

### Anticonvulsant and psychoactive drugs

#### Anticonvulsant drugs

##### Phenytoin

Phenytoin causes both acute and chronic hepatotoxicity. While most patients are adults, hepatotoxic effects have been reported in patients as young as 8 months. Clinical symptoms and signs occur within 6 weeks of administration and include rash, fever, lymphadenopathy, and eosinophilia, suggesting a hypersensitivity or immunologic basis to the injury. This is supported by the occasional occurrence of a pseudomononucleosis syndrome of lymphadenopathy, lymphocytosis, and serum sickness-like features. Some patients exhibit manifestations of Stevens-Johnson syndrome. Histologically, the lesion resembles



**Figure 32.8** Phenytoin-induced hepatotoxicity.

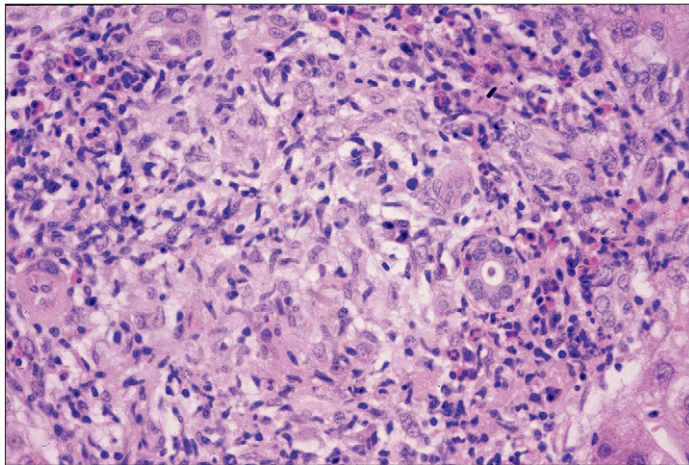
acute viral hepatitis with diffuse hepatocellular degeneration, foci of necrosis, and an inflammatory infiltrate. Granulomas have also been described. However, phenytoin-induced liver injury may also result from the adverse effects of toxic intermediates, as it is metabolized by the cytochrome P450 system with the production of reactive arene oxide metabolites, which may subsequently bind covalently to tissue macromolecules, producing liver injury (Fig. 32.8).

Differences in individual susceptibilities to phenytoin-induced liver damage may be due to genetic differences in rates of biotransformation, as deficiencies in epoxide hydrolase activity (required for detoxification) have been reported in those affected and their family members.

##### Carbamazepine

Carbamazepine is similar to phenytoin in its structure and it also utilizes epoxide hydrolase in its biotransformation. Minor liver dysfunction is seen in approximately 5–10% of asymptomatic subjects using the drug. Liver damage begins within 1 month of commencing therapy and hepatocellular, cholestatic and granulomatous reactions (Fig. 32.9) have been described.

Carbamazepine may also cause cholangiolitis and has been reported as a cause of ductopenia.



**Figure 32.9 Carbamazepine-induced hepatic granulomatous liver disease.**

#### Valproic acid

Valproic acid-induced liver injury has a particular predilection for children, especially for those less than 2 years of age. Symptoms occur between 10 and 12 weeks of therapy and seem to be dose-related in some cases. Between 1978 and 1984, the fatality rate from acute liver failure was approximately 1:500 in children up to 2 years of age and 1:12 000 in older children and adults receiving antiepileptic polytherapy. In patients treated with valproic acid as a monotherapy, the fatality rate was lower, reaching 1:7000 in children up to 2 years of age and 1:45 000 in older children and adults. Since 1984, the fatality rate has dropped, probably as a consequence of improved knowledge about the adverse effects of valproic acid. Risk factors for acute liver failure include concomitant treatment with enzyme inducers [e.g. carbamazepine, phenytoin, phenobarbital and rifampin (rifampicin)], concomitant treatment with drugs that inhibit hepatic  $\beta$ -oxidation (e.g. aspirin), and pre-existing metabolic diseases associated with impaired hepatic fatty acid oxidation. Thus, polypharmacy may also be a factor in valproate-induced liver injury. The pathologic lesions include submassive necrosis with microvesicular steatosis and bile duct injury. The mechanism of injury is uncertain. A branched medium-chain fatty acid itself, valproic acid inhibits mitochondrial oxidation of long-chain fatty acids, with reduction in hepatocellular acetyl coenzyme A and impairment of urea cycle enzymes. Patients with inborn errors in the urea cycle are susceptible to valproate hepatotoxicity. There is no specific antidote for valproate-associated hepatotoxicity. Because most patients with valproate-induced acute liver failure have secondary carnitine deficiency, treatment with carnitine has been studied but its effectiveness remains unproven.

#### Psychoactive drugs

##### Chlorpromazine

Chlorpromazine causes cholestatic jaundice in 1% of those taking the drug, but subclinical liver dysfunction may occur in as many as 40–50% of chronic users. It occurs within 5 weeks of therapy and is heralded by a prodrome of flu-like symptoms, followed by a rash and fever in up to 60% of cases. The histologic pattern includes cholestasis in zone 3 and portal inflammation with eosinophils in 20–50% of cases. Granulomas have been described. The prognosis is generally good, but a small

number of patients develop a prolonged cholestatic syndrome with hypercholesterolemia and xanthelasmata. In these patients, the histology can resemble primary biliary cirrhosis, but anti-mitochondrial antibodies are negative, although some patients have antinuclear antibodies in their sera. The clinical features suggest a hypersensitivity reaction, but chlorpromazine and its metabolites do directly interfere with bile secretion. Altered membrane fluidity, impaired membrane  $\text{Na}^+$ - and  $\text{K}^+$ -ATPase activity and impaired solute transport are changes that contribute to the development of chlorpromazine-induced cholestasis. Furthermore, chlorpromazine affects the cytoskeleton, causing dilatation and diverticuli in the canaliculi.

#### Antidepressants: tricyclic antidepressants

Many members of this class of psychotropic drugs (imipramine, amineptine, amitriptyline, and desipramine) are hepatotoxic and asymptomatic liver dysfunction may occur in approximately 10% of users. The injury can be either cholestatic or hepatocellular, although amitriptyline has caused a prolonged cholestatic lesion with portal fibrosis and inflammation. The mechanism of hepatotoxicity is thought to be due to reactive arylating intermediates.

#### Antidepressants: monoamine oxidase inhibitors

Hepatotoxicity is commonly seen in this group with jaundice reported in 1–2% of patients. Iproniazid, an amine oxidase inhibitor, was originally used to treat tuberculosis, but occasionally resulted in fatal liver necrosis with a mortality of 20% in those who developed jaundice. Clinical features occur within 4–5 weeks of treatment. Histology reveals diffuse hepatocellular necrosis that can progress to massive necrosis in some patients, resembling that associated with acute viral hepatitis. Iproniazid hepatitis is usually associated with anti-M6 antimitochondrial antibody. Angiosarcomas have been reported in experimental animals following administration of phenylhydrazine.

#### Cocaine

Cocaine use may result in severe liver damage with associated systemic failure, rhabdomyolysis and renal failure. Pathologic examination reveals zone 3 centrilobular necrosis but also microvesicular steatosis, suggesting impaired fatty acid oxidation. It is metabolized by the cytochrome P450 enzyme system to the active hepatotoxic metabolite norcocaine nitroxide which causes oxidative stress. Hepatotoxicity may also be due to hepatic ischemia as cocaine increases the systemic levels of norepinephrine and epinephrine (noradrenaline and adrenaline), which reduce hepatic artery blood flow.

#### Ecstasy (3,4-methylenedioxymethamphetamine)

Several case reports have described an acute hepatotoxic syndrome similar to that seen with cocaine, that is, hyperthermia, rhabdomyolysis, and hepatic necrosis (see Ch. 33). Chronic ecstasy ingestion has been associated with recurrent episodes of acute hepatitis.

#### Antibiotics

##### Antibacterial agents

##### Tetracyclines

Tetracyclines can cause severe acute liver injury. The majority of cases have been in pregnant women where tetracycline was



used to treat urinary tract infections. However, males and non-pregnant women are also susceptible to tetracycline-induced liver damage, especially at higher doses of more than 1.5 g/day. Within 2–3 days of intravenous administration of tetracycline, jaundice occurs with non-specific symptoms such as malaise, anorexia and vomiting.

Histologically, the main lesion is microvesicular fat (see Fig. 32.5b) resembling that seen in fatty liver of pregnancy, Reye syndrome and valproic acid hepatotoxicity. Also, tetracycline in high doses impairs hepatic mitochondrial oxidation. The mechanism of injury appears to be a toxic effect of the drug or its metabolites rather than an immunologic effect, and suggested methods of injury include failure of ATP synthesis and inhibition of protein synthesis. Similar toxic effects can be seen with oxytetracycline and chlortetracycline. Minocycline has been reported to cause liver injury with predominantly allergic features.

### **Erythromycin**

Administration of erythromycin estolate causes hepatocellular liver injury in up to 15% of patients and results in cholestatic jaundice in approximately 1–3% of recipients. The risk of cholestatic jaundice is lower with erythromycin stearate and propionate, and is calculated at 3.6/100 000 users for all erythromycin preparations. Symptoms occur between 1 and 3 weeks after commencing treatment and rash, fever, and eosinophilia in tissue and blood have suggested an allergic mechanism of injury. However, erythromycin estolate can impair bile flow and interfere with canalicular membrane Na<sup>+</sup>- and K<sup>+</sup>-ATPases, which indicates a direct hepatotoxic effect. Erythromycin toxicity may also present as an acute cholangitis or cholecystitis. Liver biopsy reveals centrilobular cholestasis and a portal infiltration rich with eosinophils.

### **Flucloxacillin**

Hepatotoxicity due to penicillins is rare, and very few cases of penicillin-G-induced liver injury have been reported. However, several semisynthetic penicillin derivatives have been associated with cholestatic or hepatocellular injury which rapidly improves on withdrawal of the drug. Flucloxacillin produces cholestatic liver disease. The risk of liver injury is estimated at 7.6/100 000 users. Symptoms usually occur within 3 weeks of treatment, and there is often a lag period between the cessation of drug administration and onset of symptoms. While most patients fully recover, some develop a syndrome of prolonged cholestasis with ductopenia, and portal and bridging fibrosis that can progress to cirrhosis.

### **Ampicillin and amoxicillin**

These semisynthetic penicillins have little hepatotoxicity when used alone. However, when amoxicillin is used in combination with clavulanic acid, the risk of cholestasis is 1/100 000 users. Symptoms occur within 1–2 weeks of treatment but can be delayed for up to 6 weeks following withdrawal of the drug. Histology reveals cholestasis with minimal inflammation or necrosis. Granulomas may be present. The mechanism of injury probably relates to immunologic idiosyncrasy.

### **Sulfonamides**

Sulfa drugs are associated with a wide spectrum of pathologic changes in the liver. The precise risk of injury following

administration of these drugs is unknown, but the incidence of hepatic injury appears to be less than 5%. Patients infected with human immunodeficiency virus (HIV) carry a higher risk for immunologic toxic reactions, including liver injury. The risk for sulfonamide-induced liver injury appears to be increased in slow acetylators. The clinical pattern of sulfonamide hepatotoxicity is usually cholestatic or mixed hepatocellular-cholestatic and the clinical course can resemble viral hepatitis. Hypersensitivity phenomenon such as a rash, fever, arthralgia, and eosinophilia are not uncommon. Sulfonamides in combination with other drugs also cause liver injury. Sulfasalazine induces allergic-type hepatitis with low levels of serum complement and circulating immune complexes, mimicking serum sickness. Although the sulphyridine is believed to be responsible for the liver injury, the 5-aminosalicylate moiety may also play a part (see below). Pyrimethamine-sulfadoxine, used as an antimalaria prophylaxis, causes hepatic necrosis and fatalities has been described from massive necrosis. Granulomatous hepatitis has also been described. Trimethoprim-sulfamethoxazole can lead to cholestatic hepatotoxicity, and fatalities due to acute liver failure have been recorded. However, the majority of patients fully recover when the drug is withdrawn.

### **Fluoroquinolones**

Asymptomatic elevations of aminotransferases have been observed quite frequently with fluoroquinolones. Hepatocellular liver injury with spontaneous resolution has been reported for norfloxacin. Acute liver failure has been described in patients taking ciprofloxacin and enoxacin. Cholestatic liver injury was occurs with ofloxacin, enoxacin and ciprofloxacin, but recovery within 1–2 months is the norm. Patients with liver cirrhosis appear to have an increased risk for developing seizures during treatment with pefloxacin.

### **Nitrofurantoin**

This antibiotic is associated with a wide variety of adverse effects such as peripheral neuropathy, pulmonary infiltration, and skin reactions and it can also lead to cholestatic hepatitis with hypersensitivity features suggesting an underlying immunoallergic mechanism as the basis for the injury. It has also been associated with chronic hepatitis and cirrhosis, particularly in women over the age of 40 years who have taken the drug for longer than 4–6 months. The chronic hepatitis is associated with a number of autoimmune features such as hyperglobulinemia and positive antinuclear and antismooth muscle autoantibodies. Associations with HLA-DR6, HLA-DR2 and HLA-B8 have been reported.

### **Antifungal agents**

#### **Ketoconazole**

Ketoconazole has been well-documented as a hepatotoxic drug, with an estimated risk of injury of 1/15 000 cases. Asymptomatic liver dysfunction occurs in 5–10% of users. Symptoms occur within 1–6 months of treatment and are commoner in women. Liver biopsy reveals a diffuse hepatocellular necrosis, occasionally with bridging necrosis, but fatalities are rare.

#### **Griseofulvin**

This antifungal agent produces hepatocellular carcinoma and a porphyria-like syndrome in experimental animal models.



Humans taking this drug may develop porphyrinuria and those with acute intermittent porphyria may relapse. Cholestatic injury has also been documented in humans, and alcohol-like lesions in mice.

### Antituberculous agents

Most of the antituberculous drugs can cause hepatic damage in susceptible individuals, but as these drugs are administered in combination to prevent resistant bacterial strains emerging, it may be difficult to attribute liver dysfunction to a particular agent. Furthermore, drug interactions can potentiate hepatotoxicity.

#### Isoniazid

Isoniazid is one of the most important causes of drug-induced liver injury, and two types of injury are observed. Mild hepatotoxicity is seen in approximately 10–20% of users and occurs in the first few months of treatment. It produces a mild non-specific focal hepatitis (Fig. 32.10) that is clinically unapparent and that resolves despite continuation of the drug.

Isoniazid-induced hepatitis occurs in approximately 1% of all users, but in 2% of those over 50 years of age. It appears to be more common in women and alcoholics. For two-thirds of patients, symptoms begin within the first 3 months of treatment, but this can be delayed for up to 12 months. Clinically, the symptoms and signs are those of an acute viral illness. There is a rising serum transaminase level and histologic findings include acute hepatitis, bridging necrosis, submassive necrosis and chronic hepatitis. The drug must be stopped once hepatitis is diagnosed. The mortality for clinically jaundiced patients is 10%. The mechanism of injury involves the production of toxic metabolites. Isoniazid first undergoes biotransformation in the liver by acetylation, producing the harmless acetylisoniazid which then undergoes hydrolysis to monoacetylhydrazine. This product is oxidized by the cytochrome P450 system to form reactive metabolites that covalently bind to tissue macromolecules. It has been suggested that the rate of acetylation of isoniazid to subsequent toxic metabolites may be important for individual susceptibility to hepatotoxicity and rapid acetylators appear to be at greatest risk of injury. However, it has also been suggested that slow acetylation status may be as important a risk factor for hepatic injury.

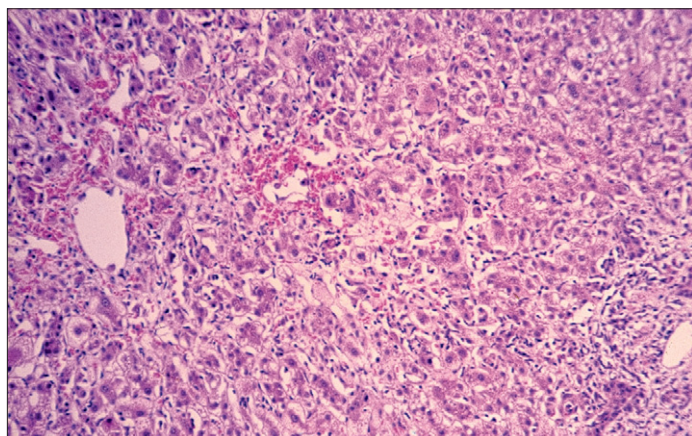


Figure 32.10 Mild hepatitis secondary to isoniazid administration.

#### Rifampin (rifampicin)

Rifampin (rifampicin)-induced liver injury occurs in approximately 1–4% of users and occurs within 3 weeks of therapy. It is usually given in combination with isoniazid. Clinically, it produces an acute hepatitis similar to that seen with isoniazid. The histology reveals hepatocellular injury and necrosis is common. Rifampin (rifampicin) impairs the hepatic uptake of bile acids and bilirubin in a dose-dependent manner, and impaired BSP excretion is observed in experimental animals. It induces drug-metabolizing systems in the endoplasmic reticulum and this may explain the higher incidence of hepatotoxicity in recipients of combination therapy compared with those using monotherapy.

### Analgesic and anti-inflammatory drugs

#### Acetaminophen

Acetaminophen (paracetamol) is a widely used analgesic and antipyretic drug that has a good safety profile when used at the recommended dose (0.5–4 g daily). Higher doses, however, can lead to liver damage in one of two ways. The commonest is intentional overdosing and this is commonly encountered in the USA and UK. Hepatotoxicity is also seen with therapeutic use involving doses above the recommended range (4–8 g daily), when other factors are present that influence the susceptible individual. These include chronic alcohol ingestion, co-ingestion of drugs that induce the cytochrome P450 metabolizing system, pre-existing liver disease, and conditions that are associated with low GSH stores such as malnutrition. Individuals with Gilbert syndrome may also have an increased susceptibility to acetaminophen-related liver injury due to their deficiency of hepatic UDP-glucuronyltransferase. Acetaminophen is an intrinsic hepatotoxin; the effect on the liver is predictable and dose-dependent.

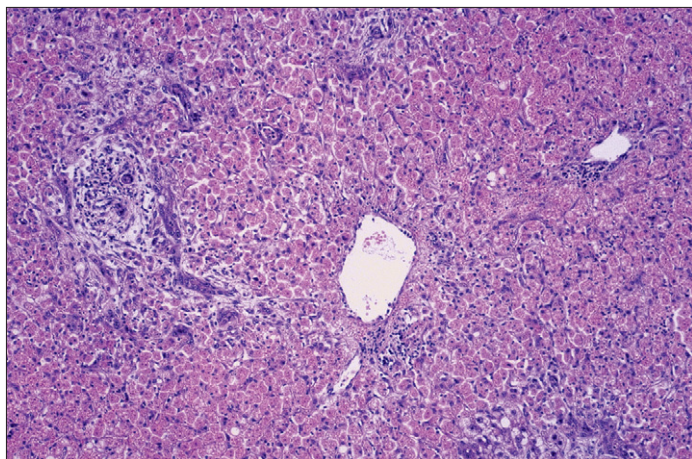
Clinically, there are three phases:

- phase 1 (1–24 h) when the patient complains of nausea vomiting and diaphoresis;
- phase 2 (24–48 h) during which time there may be no symptoms or clinical evidence of liver disease, and
- phase 3 (3–10 days) with the onset of overt acute liver dysfunction or failure (see Ch. 33).

The characteristic histologic lesion is centrilobular or zone 3 necrosis that may develop into submassive or massive hepatic necrosis (Fig. 32.11).

The zone 3 location of the initial histologic change is a consequence of the location in this area of the cytochrome P450 enzyme that is involved in metabolizing the drug (CYP2E1). At low doses, acetaminophen is largely conjugated with sulfate or glucuronic acid (Fig. 32.12) and these conjugates are then excreted in the urine.

A small proportion of the drug (approximately 5%) is metabolized by the CYP2E1 isoenzyme resulting in the formation of the highly toxic and reactive electrophile *N*-acetyl-*p*-benzoquinone imine (NAPQI). This is rendered harmless by conjugation with GSH and, after further conversion to acetylcysteine derivatives, is excreted in the urine as a mercapturic acid (Fig. 32.12). GSH, therefore, normally provides protection against the toxic intermediate at low levels of acetaminophen ingestion. However, with larger doses of acetaminophen, the sulfate and glucuronide pathways are saturated and therefore a larger amount of drug is available for



**Figure 32.11** Acetaminophen-induced submassive hepatic necrosis.

biotransformation via the P450 enzyme system. Subsequent detoxification depends on conjugation with GSH and therefore tissue levels of GSH are critical to the development of hepatic necrosis. When doses of acetaminophen are large enough to deplete GSH stores by more than 70%, detoxification of NAPQI cannot be sustained; it can then covalently bind to tissue macromolecules which are essential for cellular homeostasis, resulting in structural and functional disruption of key cellular components by oxidation of protein and non-protein thiols. Lipid peroxidation is a late event and may be the result rather than the cause of tissue necrosis. Through the covalent modification of plasma membrane and mitochondrial proteins, intracellular calcium homeostasis is destroyed, resulting in a cascade of endonuclease-, phospholipase-, and protease-induced cellular damage that ends in cell death. *N*-acetylcysteine and methionine increase hepatic GSH stores by promoting GSH synthesis. Other agents that inhibit the CYP2E1 isoenzyme, such as cimetidine, offer protection against liver injury in experimental animals, but do not appear to be of benefit in humans.

### Salicylates

Aspirin is another intrinsic hepatotoxin with a dose-dependent and reversible form of liver injury. Hepatic injury may occur with high therapeutic doses in children and adults with chronic

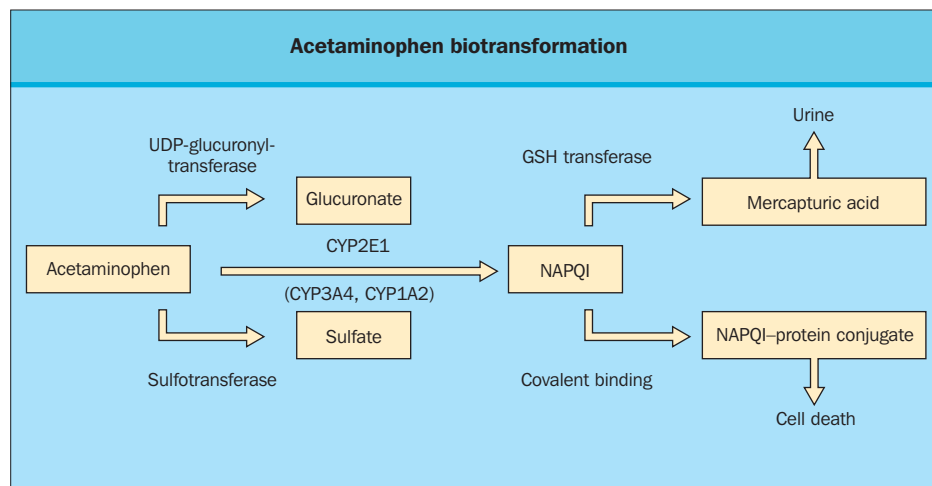
rheumatologic or collagen vascular diseases, or following a deliberate overdose. The injury is usually associated with serum salicylate levels of greater than 2.5 mmol/L (25 mg/dL) but can occur at lower serum drug levels. Hypoalbuminemia may predispose to aspirin-induced hepatotoxicity by increasing the unbound fraction of the drug. The hepatotoxic effects of aspirin may be involved in the development of Reye syndrome. In most cases, it presents as an asymptomatic dysfunction in liver function and jaundice occurs in less than 5% of cases. Liver biopsy is compatible with a non-specific focal hepatitis with minimal inflammatory changes in the portal areas. Recovery is complete on cessation of drug ingestion.

### Non-steroidal anti-inflammatory drugs

The hepatotoxicity induced by this group of drugs is not uniform. Most cause hepatocellular damage but several lead to cholestasis. Likewise, the frequency of liver damage that they produce is variable; for example, mefenamic acid rarely causes liver injury unlike ibufenac, which caused fatal liver damage and was ultimately withdrawn from the market. Although the mechanism of injury appears to be idiosyncrasy, for some drugs it is immunologically mediated with accompanying hypersensitivity phenomena. Others appear to have a metabolic basis for their injury. The risk of hepatotoxicity for the group as a whole is estimated to be approximately 3.7–9.0/100 000 users. The incidence was highest for sulindac (148/100 000 users), lowest for ibuprofen (1.6/100 000 users), and intermediate for mefenamic acid (2.5/100 000 users), diclofenac (3.6/100 000 users), and naproxen (3.8/100 000 users). Associated risk factors for liver injury include old age, renal disease and alcohol excess.

### Diclofenac

Women and patients with osteoarthritis appear to be more susceptible than others to diclofenac-induced hepatotoxicity which causes asymptomatic liver dysfunction in 15% of users. Duration of administration before the onset of liver dysfunction is widely variable, ranging from 1 to 14 months. Allergic features are rare. Liver biopsy demonstrates a non-specific acute hepatitis with zonal necrosis. Recovery is usual but fatalities have been recorded. Diclofenac has also been associated with an autoimmune-like chronic hepatitis. Monitoring of serum



**Figure 32.12** Schematic representation of acetaminophen biotransformation.

aminotransferases every 3 months is recommended in patients with prolonged ingestion of diclofenac.

#### **Phenylbutazone**

This drug has been associated with clinically apparent liver injury in 0.25% of users. Symptoms usually begin within 6 weeks of treatment and are similar to a viral hepatitis. Rash, fever and arthralgia are common. The histology may vary from hepatocellular necrosis to bridging necrosis to granulomatous hepatitis. Less commonly, cholestasis with minimal hepatocellular features can be seen.

#### **Sulindac**

As with diclofenac, the mechanism of injury with sulindac appears to be hypersensitivity-based. The illness begins within 6 weeks of drug administration. The liver biopsy reveals a cholestatic injury in most patients, although hepatocellular features have been recorded in some patients. Approximately 5% of jaundiced cases are fatal. Recovery may take months.

#### **Ibuprofen**

This drug appears to be one of the least commonly implicated NSAIDs in the generation of liver injury despite its frequent use. It is associated with hepatocellular injury that has many of the clinical hallmarks of the hypersensitivity syndrome.

#### **Nimesulide**

This agent is a new NSAID of the sulfonanilide class and is a more selective cyclo-oxygenase type 2 (cox-2) inhibitor than cox-1 inhibitor. Nimesulide-induced hepatotoxicity may occur as hepatocellular necrosis or pure cholestasis. Autoantibodies may be detected in serum and some patients demonstrate hypersensitivity features.

### **Cardiovascular drugs**

#### **Antihypertensive agents**

##### **Methyldopa**

Both acute and chronic liver disease has been described in patients taking methyldopa, although the incidence has fallen as it has been replaced by newer antihypertensive agents. Asymptomatic liver dysfunction occurs in 5–30% of users within 1–6 weeks, and may resolve despite continued use of the drug. Overt hepatic disease occurs in 1% of users. The commonest histologic lesion resembles acute viral hepatitis with hepatocellular necrosis. Portal and periportal inflammation is prominent. More importantly, methyldopa can cause an autoimmune-like chronic hepatitis that has been reported to progress to fibrosis and macronodular cirrhosis in some patients.

The mechanism of injury appears to be both an immunologic and metabolic idiosyncrasy. Methyldopa undergoes biotransformation via the cytochrome P450 system by oxidation to a reactive metabolite thought to be a quinone or semiquinone. These intermediates may covalently bind to cellular macromolecules, resulting in a conjugate that can act as a neoantigen becoming a target for immune recognition. Evidence for immunologic disturbance is suggested by a positive Coomb test, positive autoantibodies and inhibition of cytotoxic T-cell function. Despite these findings, clinical evidence of hypersensitivity is rare. The mortality is similar to that for other causes of drug-

induced hepatocellular injury, with a rate of 10% in those who develop clinically apparent jaundice.

#### **Angiotensin-converting enzyme inhibitors**

This class of drugs occasionally produces liver damage. Acute hepatocellular injury occurs over a widely variable period of time. The mechanism of injury is unknown although hypersensitivity phenomena have been described with captopril. Cross-reactivity of hepatotoxicity can occur between captopril and enalapril.

#### **Thiazide diuretics**

Despite widespread use, thiazide diuretics rarely cause liver injury, but cholestatic hepatitis has been reported with chlor-thiazide. In mice, furosemide causes zone 3 necrosis.

#### **Hydralazine**

This vasodilator can cause a variety of liver injuries including hepatocellular hepatitis and granulomatous hepatitis, particularly on a background of a lupus-like syndrome. The drug can also produce an effect mimicking acute cholangitis. Non-specific organ antibodies are found in the serum and anti-liver microsomal antibodies directed against CYP1A2 have been reported.

### **Antiarrhythmic agents**

#### **Amiodarone**

Amiodarone is an amphiphilic drug with a lipophilic ring complex and a hydrophilic side chain, a structure that results in its accumulation in lysosomes. Within the lysosomes, it binds to phospholipids and prevents their degradation by phospholipases, thereby producing secondary phospholipid storage. This results in increased density on computed tomography (CT) scanning due to the iodine content of the stored drug. Toxicity is not confined to the liver and lysosomal inclusions are also found in other tissues. The range of liver injury sustained with amiodarone is diverse, and both acute and chronic liver disease has been reported. Approximately 15–50% of users develop an asymptomatic rise in liver tests, while clinically apparent disease occurs in less than 3% of patients. Jaundice is rare. Acute hepatitis in those taking the drug for a few weeks usually resolves upon cessation of therapy. Chronic injury can be insidious and patients may present with complications of cirrhosis. Histologic appearances include granulomas, cholangitis and micronodular cirrhosis, but the typical appearance is that of alcoholic hepatitis with steatosis, focal necrosis, centrilobular fibrosis, Mallory hyaline and polymorphonuclear infiltration (**Figs 32.13 and 32.14**).

Interference with mitochondrial fatty acid oxidation by amiodarone in animal models may be a factor in the pathogenesis of this pseudoalcoholic liver injury.

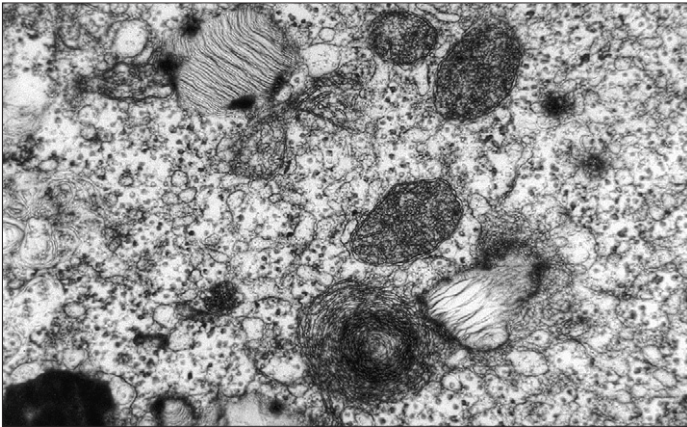
#### **Quinidine**

Quinidine administration may result in liver injury within 6–12 days of treatment. The clinical symptoms are similar to those of acute viral hepatitis and liver biopsy confirms hepatocellular necrosis. Granulomas have also been described.

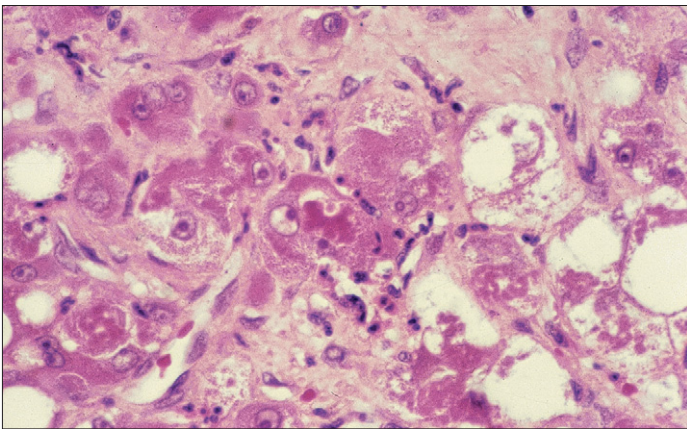
#### **Calcium channel blockers**

Verapamil has been reported to have hepatotoxic effects within 2–3 weeks of commencing therapy, producing a mixed





**Figure 32.13** Amiodarone-induced phospholipidosis. Electron microscopy reveals phospholipidic inclusions.



**Figure 32.14** Amiodarone-induced liver disease demonstrating Mallory bodies and hepatic fibrosis.

cholestatic and hepatocellular pattern. Nifedipine can cause steatosis and zone 3 necrosis. It has also been implicated in pseudoalcoholic liver disease. Diltiazem may rarely cause fatal hepatotoxicity.

### **Anti-anginal agents**

#### **$\beta$ -Adrenergic blocking drugs**

Most of the drugs in this group rarely induce liver disease. However, labetalol can cause hepatocellular necrosis within 2 months of treatment, and fatalities have been recorded. Metoprolol is metabolized by the CYP2D6 isoenzyme, which is also responsible for debrisoquin (debrisoquine) metabolism. It induces an acute hepatitis.

#### **Perhexiline maleate**

A lipophilic drug that accumulates in lysosomes, this agent produces pseudoalcoholic liver disease similar to that described for amiodarone, which can also progress to cirrhosis despite cessation of drug ingestion. Deficiency of the CYP2D6 metabolizing isoenzyme, which is responsible for perhexiline hydroxylation, may be a predisposing factor for perhexiline-induced liver damage. Susceptibility to perhexiline hepatotoxicity has been associated with HLA-B8 phenotype.

### **Antihyperlipidemic agents**

#### **3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins)**

Asymptomatic elevation in aminotransferases occurs in 1–5% of users in the first few weeks of treatment and this abnormality is usually dose-related. The mechanism of liver function abnormality may be the accumulation of metabolites whose conversion to cholesterol has been blocked by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A reductase enzyme.

#### **Clofibrate**

Clofibrate may also produce asymptomatic elevation in aminotransferases. Cholestasis, granulomatous inflammation and an increased incidence of gallstones have been reported following clofibrate administration. In animal models they cause an increase in hepatocyte peroxisomes that may be related to the development of hepatic neoplasms in these animals.

### **Immunomodulatory drugs**

Immunomodulatory drugs, a group of drugs that includes anti-neoplastic drugs, antimetabolites, immunosuppressant agents and antiviral drugs, produce a wide spectrum of hepatotoxicity ranging from mild hepatocellular hepatitis to cirrhosis. However, it can be difficult to identify the cause of the injury due to the multiple potential causes of liver dysfunction in patients receiving these drugs. The differential diagnosis may include the underlying disease itself, metastatic disease, opportunistic infection, sepsis and polypharmacy with multiple drug interactions.

### **Antimetabolites**

#### **Methotrexate**

The potential for hepatotoxicity with long-term use of methotrexate includes fatty liver, liver fibrosis and cirrhosis. The risk of liver injury seems to be related to the duration of therapy but other contributing factors may include obesity, diabetes, alcohol excess, underlying liver disease and renal impairment. Several studies show that the risk for development of fibrosis or cirrhosis is dose-dependent. For every gram of methotrexate ingested, the risk for progression of one histologic grade is approximately 6%. The progression of methotrexate-induced liver injury is usually subclinical, and it has been argued that a liver biopsy should be performed after cumulative doses in excess of 1.5 g. Patients with rheumatoid arthritis appear to have lower susceptibility to methotrexate-induced hepatotoxicity. Although fibrosis can progress despite the liver tests being normal, monitoring of liver function is advisable as methotrexate has been associated with hepatocellular toxicity and acute liver failure. Histologic abnormalities include steatosis, necrosis, a mixed portal inflammatory infiltration and ultimately cirrhosis. The mechanism of liver injury is unclear.

#### **Antipurines**

Azathioprine, frequently used to treat chronic liver disease, is metabolized in the liver to produce 6-mercaptopurine. Liver injury induced by this drug ranges from portal inflammation with cholestasis to peliosis hepatis, nodular regenerative hyperplasia, veno-occlusive disease and hepatoportal sclerosis (or idiopathic portal hypertension), suggesting a susceptibility of the vascular endothelium to injury by azathioprine. There is a

strong association between male renal transplant recipients and azathioprine-induced veno-occlusive disease. After kidney transplantation, liver injury appeared 4–143 months after surgery at an average dose of 2.5 mg/kg/day in approximately 2% of the patients. 6-Mercaptopurine, used to treat leukemia, is also hepatotoxic. It can produce jaundice in 5–40% of users, although hepatocellular injury is more common than cholestatic injury. 6-Thioguanine produces histologic hepatotoxicity similar to that described for azathioprine, in particular veno-occlusive disease, nodular regenerative hyperplasia and hepatoportal sclerosis. Cytosine arabinoside produces a mild cholestatic injury.

#### Antipyrimidines

One of the most important drugs that causes hepatotoxicity in this group is 5-fluorouracil, which produces little liver damage when administered orally as a single agent. However, the co-administration of a pyrimidine synthesis inhibitor (phosphonoacetyl-L-aspartate), used to enhance the efficacy of 5-fluorouracil, results in cholestatic injury. A derivative of 5-fluorouracil, floxuridine, has been reported to cause irreversible sclerosing cholangitis and cholecystitis when infused into the hepatic artery as treatment for metastatic liver disease.

#### Alkylating agents

Many drugs in this group are capable of hepatic injury. Cyclophosphamide, used in high dosage or in combination with other agents for bone marrow pre-conditioning, can lead to veno-occlusive disease (Fig. 32.15).

Busulfan, at high doses used in bone marrow preconditioning, produces a similar lesion but may also induce a cholestatic hepatitis. The nitrogen mustards are rarely hepatotoxic. Chlorambucil can produce hepatocellular injury that has been reported to progress to cirrhosis.

#### Antiviral agents

##### Nucleoside analogs

Zidovudine (AZT) hepatotoxicity has been documented in patients with HIV. The typical histologic abnormality is a severe macrovesicular steatosis with minimal necrosis or inflammation. However, acute liver failure has been reported following its use. The mechanism of injury is uncertain, but seems to relate to a toxic effect on mitochondrial function with subsequent fat accumulation, lactic acidosis and multiorgan failure.

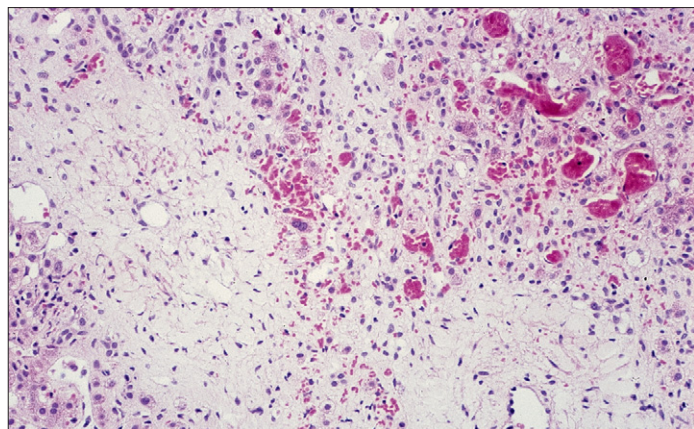


Figure 32.15 Cyclophosphamide-induced veno-occlusive disease.

#### Naturally occurring immunomodulatory agents

##### L-asparaginase

This agent usually induces a mild steatosis that resolves with cessation of the drug. Large doses can cause hepatic necrosis. Toxicity may result from impaired hepatic protein synthesis as deamination of asparagine is facilitated by asparaginase, resulting in depletion of asparagine stores.

##### Alkaloids

These agents rarely cause liver injury, but when used in combination with irradiation, vincristine and vinblastine can lead to a severe hepatitis. Pyrrolizidine alkaloids induce veno-occlusive disease. Etoposide and other podophylline alkaloids in high doses can cause hepatic necrosis.

##### Adriamycin (doxorubicin hydrochloride)

This agent and the related minomycin have been implicated in the development of veno-occlusive disease. Bleomycin can induce steatosis.

##### Cyclosporin

Hepatotoxicity is a fairly trivial component of the side-effect profile of cyclosporin but both hepatocellular and cholestatic liver disease have been described in patients after organ transplantation or with autoimmune disease. Most patients have increased serum bile acids and some also have increased conjugated serum bilirubin concentrations, which may be caused by inhibition of canalicular excretion of anions. Cyclosporin-associated liver injury is dose-dependent and usually disappears upon reduction of the dose; only a few patients have developed chronic liver disease. Both cholestatic and hepatocellular liver injury was more pronounced in patients with parenteral nutrition, which may be a risk factor for cyclosporin-associated liver injury.

##### Tacrolimus

The context of tacrolimus hepatotoxicity is very similar to that for cyclosporin. Liver toxicity is predominantly cholestatic and considered to be dose-dependent. Decreased hepatic activity of CYP3A has been identified as a risk factor for renal toxicity with tacrolimus. The dose-dependent nature of the hepatotoxicity raises the possibility that decreased activity of CYP3A could also be a risk factor for liver injury.

#### Anesthetic agents

##### Halothane

Halothane is the prototype for anesthetic-induced hepatotoxicity. The risk of liver injury ranges from 1/35 000 to 1/10 000 anesthetics administered. Risk factors for developing halothane-induced hepatotoxicity include obesity, female sex, advancing age and repeated exposure to the agent (implying a sensitization process). The risk of injury has been reported to be 7/10 000 in those who had previously experienced this anesthetic agent. Asymptomatic liver dysfunction occurs in approximately 20–25% of those exposed. Non-specific symptoms appear within 2 weeks of exposure (but may occur as quickly as 2–3 days in those previously exposed) and resemble those of acute viral hepatitis. Histologic features include zone 3 necrosis that can progress to massive necrosis. Other less severe lesions include spotty necrosis, diffuse hepatitis and bridging necrosis. Increased



incidence after repeated exposure, shortened period between exposure and the onset of symptoms, and clinical and histologic findings suggest an immunologic mechanism. However, the centrilobular location of hepatocyte necrosis suggests a toxic mechanism produced by biotransformation. In humans, 20% of halothane is metabolized, producing bromide ion and trifluoroacetic acid (TFA), which are not hepatotoxic. In animal studies, reactive metabolites of halothane covalently bound to tissue macromolecules and induced liver injury only in the presence of tissue hypoxemia. TFA-protein conjugates have been demonstrated in the sera of patients who have been exposed to halothane anesthesia and seem to act as neoantigens, producing antibodies in patients with severe liver injury. These antibodies may cross-react with endogenous proteins; antibodies to the E2 component of PDH have been reported. This may explain the hypersensitivity phenomena such as fever, rash, eosinophilia and increased frequency of toxicity with repeated exposure.

#### **Other anesthetic agents**

Enflurane-induced liver injury is less common than that due to halothane but produces a similar clinical and histologic picture. Isoflurane is metabolized less extensively than halothane and therefore is less commonly implicated as a cause of anesthesia-related liver dysfunction.

#### **Industrial and naturally occurring toxins**

##### **Carbon tetrachloride**

Occupational exposure to  $\text{CCl}_4$  may occur in those in contact with dry-cleaning, grain fumigants or working with fire-fighting equipment. Inhalation and ingestion are the commonest modes of exposure. Starvation and alcohol excess appear to enhance the toxicity of  $\text{CCl}_4$ , possibly by inducing the microsomal metabolizing enzyme system, in particular the CYP2E1 isoenzyme. Hypoxia enhances the conversion of  $\text{CCl}_4$  to the reactive metabolite  $\text{CCl}_3$ . Symptoms occur within 24 h of exposure, and jaundice appears after 2–4 days. Renal impairment often coexists. Histology shows zone 3 necrosis and fatty change is not uncommon.

##### **Yellow phosphorus**

Yellow phosphorus-induced hepatic injury occurs by inhalation or ingestion of rat poison, and produces a clinical syndrome similar to that described for  $\text{CCl}_4$ . It is characterized by rapidly deteriorating liver and renal function, and can lead to acute liver failure within 3–4 days of exposure. The breath, vomitus and feces typically have a garlic odor and are phosphorescent. The pathologic findings are zone 1 necrosis with macrosteatosis.

##### **Selenium**

At low concentrations this essential trace element protects against lipid peroxidation. However, in higher concentrations selenium is hepatotoxic and inhibits protein synthesis with subsequent deficiency of methionine and cysteine. Acute toxicity results in microvascular steatosis. Like phosphorus toxicity, the breath and excreta have a garlic odor.

##### **Vinyl chloride**

Vinyl chloride has been associated with a range of liver pathologies, including angiosarcoma, fibrosis and hepatportal sclerosis. Liver injury appears to be related to duration of exposure, but other factors may include smoking and alcohol.

The most characteristic histologic lesion is capsular and sub-capsular fibrosis. Angiosarcomas are often peripherally placed and associated with terminal retroperitoneal hemorrhage. Vinyl chloride at a low concentration can be metabolized by alcohol dehydrogenase, but at higher levels, metabolism is performed by the microsomal enzyme systems. Extrahepatic manifestations of vinyl chloride toxicity include Raynaud phenomenon and scleroderma-like skin changes.

#### **Pesticides (insecticides, herbicides, fungicides)**

Pesticides have rarely produced significant liver injury in humans despite experimental evidence of perivenular necrosis in rodents. However, their potential for hepatotoxicity and hepatocarcinogenesis is a concern as they are stored for a long time in body fat and in the liver. The pesticide dichlorodiphenyl-trichloroethane (DDT) is listed by the World Health Organization as a carcinogen. Cholestasis with bile duct degeneration and necrosis has been reported following paraquat ingestion, but the clinical relevance of this is superseded by the pulmonary and gastrointestinal manifestations.

#### **Arsenic**

Acute ingestion of this poison produces acute and chronic liver injury. Features of hypersensitivity are prominent and the histologic abnormalities include hepatocellular necrosis and severe steatosis. Alternatively, patients may present with a cholestatic syndrome, reflected histologically by cholestasis and portal infiltration, which may progress to a primary biliary cirrhosis-like entity. Chronic arsenic exposure is associated with hepatosclerosis, cirrhosis, hepatocellular carcinoma and angiosarcoma.

#### **Mushroom poisoning**

Ingestion of *Amanita phalloides* (death cap) and *Amanita verna* (destroying angel) is associated with severe liver injury, especially in children. *Amanita phalloides* is recognized by its green-brown cap, white gills and stem. Toxicity is due to the amatoxins, which bind to RNA polymerase and inhibit the formation of messenger RNA, and phallotoxins, which impair cell membrane function by interfering with the polymerization-depolymerization cycle. As little as three mushrooms can be fatal as they contain approximately 7 mg of amatoxins. Symptoms occur within 8–12 h of ingestion followed by a period of apparent improvement before clinically overt liver dysfunction occurs (see Ch. 33). Histology reveals zone 3 hemorrhagic necrosis with severe steatosis. In those that survive, a minority can progress to have a histologic picture of chronic autoimmune-like hepatitis with autoantibodies, hyperglobulinemia and circulating immune complexes in the serum.

#### **Aflatoxins**

Aflatoxins are derived from the *Aspergillus* species and have been identified as a contaminant of nuts, corn, wheat, barley, soy beans and rice. There are 12 naturally occurring parent forms, and they have been classified into two major groups: aflatoxin B and aflatoxin G. The former toxin is the most common aflatoxin contaminant found in food. Hepatotoxicity is due to impairment of RNA synthesis, possibly due to inhibition of RNA polymerase or interaction with nuclear chromatin. Ingestion of aflatoxin-contaminated food results in acute hepatotoxicity heralded by nausea, vomiting and jaundice with subsequent development of portal hypertension. Liver biopsy demonstrates cholestasis with



periductal fibrosis. Chronic ingestion in animals results in veno-occlusive disease and hepatocellular carcinoma; aflatoxins are also carcinogenic in humans. They have been postulated as cocarcinogens with hepatitis B virus.

### Herbal medicines

The increasing use of alternative medicines has led to an increased awareness of their potential toxicity. The association with hepatotoxicity may be difficult to establish as many patients fail to disclose the use of herbal remedies on routine enquiry. Germander, which is used to treat obesity, has been associated with acute hepatitis and acute liver failure. Symptoms of abdominal pain and jaundice develop within 3–18 weeks of germander administration in capsular form or as tea. Histology reveals a non-specific hepatocellular necrosis. Symptoms resolve quickly on withdrawal of the agent, but rapidly reappear upon re-exposure. Animal studies have reported depletion of GSH and protein thiols due to germander-derived reactive metabolites, activated by the cytochrome P450 enzyme system. Chinese herbal remedies have also been associated with acute hepatitis, in particular xiao-chai-hu-tang, used as an antidote to the common cold, but also used to treat liver diseases in Japan. Acute hepatitis may also follow administration of mistletoe (*Viscus album*) used to treat asthma, skullcap (*Scutellaria*) and valerian (*Valeriana officinalis*) used to treat stress, and chaparral leaf. Pyrrolizidine, ingested as Bush Tea in the Caribbean, causes veno-occlusive disease.

### Miscellaneous drugs

#### Retinoids

Both etretinate and its pro-drug acitretin can cause asymptomatic hepatocellular liver injury in up to 20% of patients at the beginning of therapy. In most patients, elevated aminotransferases normalize despite continued administration of the drug; liver histology is not available in such patients. However, some patients develop chronic hepatitis that can progress to cirrhosis and chronic liver failure, necessitating transplantation.

#### Tetrahydroaminoacridine (tacrine)

This drug is a centrally active non-competitive inhibitor of acetyl cholinesterase, which is used for the treatment of patients with Alzheimer disease. It is associated with hepatocellular liver injury in approximately 50% of patients but does not normally cause cholestatic liver injury. Liver injury manifests typically 6–8 weeks after exposure, and most patients stay free of symptoms. In patients with aminotransferases more than three times the upper limit of normal or jaundice, treatment should be withdrawn. The mechanism of toxicity is uncoupling of oxidative phosphorylation of hepatic mitochondria.

## CLINICAL FEATURES OF DRUG-INDUCED LIVER INJURY

There are few, if any, clinical features associated with drug-induced hepatotoxicity that are pathognomonic for drug-induced injury to the liver. Many drugs cause a non-specific viral hepatitis-type picture with an initial prodrome of nausea, anorexia and vomiting followed by abdominal pain and jaundice (Fig. 32.16).

Fever, rash, and arthralgia are hallmarks of hypersensitivity which may indicate an immunologic idiosyncrasy to drugs, but

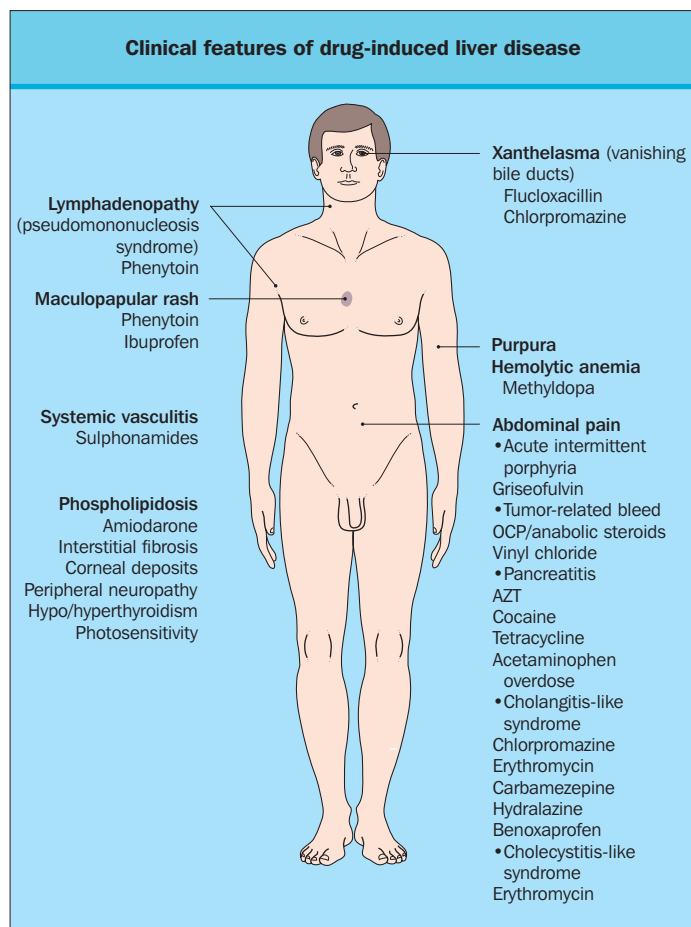


Figure 32.16 Clinical associations of drug-induced liver disease.

these signs also occur with viral hepatitis. Classical indicators of toxin-induced liver injury, such as the garlic odor of breath, vomitus and feces of yellow phosphorus hepatotoxicity, are rare. Syndromes of acute liver disease occur in three phases as characterized by injury due to acetaminophen, hepatotoxic mushrooms and  $\text{CCl}_4$ : early severe gastrointestinal and neurologic symptoms; a period of apparent improvement; and finally overt liver injury with subsequent multiorgan involvement. Some drugs mimic clinical entities such as cholecystitis (erythromycin) and cholangitis (chlorpromazine), pseudomononucleosis, lymphadenopathy and serum sickness-like syndromes (phenytoin, sulfonamides, sulindac, dapsone), bone marrow suppression (immunomodulatory drugs), and systemic vasculitis (allopurinol, sulfonamides). Hepatic adenomas may present as a painless abdominal mass or hemoperitoneum following rupture of the tumor.

## DIAGNOSIS OF DRUG-INDUCED LIVER INJURY

Because the majority of clinical manifestations of drug-induced liver injury are non-specific, making the correct diagnosis at an early stage is extremely difficult. Furthermore, liver deterioration may represent progression of the underlying disease, a complication of the underlying disease, or an unrelated clinical episode such as sepsis. In addition, the administration of multiple drugs in seriously ill patients further complicates the process of drug-induced liver damage. A low threshold of suspicion is there-

fore necessary to make the correct diagnosis at an early stage in the illness when withdrawal of the offending agent should result in complete recovery. Central to the diagnosis is a thorough history including recent and past drug exposure (including herbal remedies, vitamins and homeopathic medicines), and occupational hazards with exposure to potential toxins. Ideally, the diagnosis should be based on a history of illness subsequent to drug ingestion and improvement on cessation of therapy. Confirmation of the diagnosis by a drug rechallenge is rarely justifiable. In recent years, a clinical scale for the diagnosis of drug-induced hepatitis has been developed and validated (Table 32.9).

## Investigations

### Liver tests

It has been suggested that drug-induced liver injury is diagnosed as hepatocellular when the ALT and AST are greater than twice the upper limit of normal or when the ALT/alkaline phosphatase ratio is equal to or greater than 5. The injury is cholestatic where the alkaline phosphatase is twice the upper limit of normal or where the ALT/alkaline phosphatase ratio is less than or equal to 2. However, many drug reactions frequently cause a mixed pattern of liver function abnormality. Hypoalbuminemia is particularly associated with salicylate toxicity, but this may just reflect the severity of the underlying liver disease, if present.

### Full blood count

Lymphocytosis and eosinophilia are associated with immunologic phenomena. Leukopenia and thrombocytopenia may reflect bone marrow suppression as a result of drug toxicity or as a secondary toxic event following liver failure. A low platelet count may also indicate disseminated intravascular coagulation (DIC) which may follow acute toxic injury to the liver.

### Coagulation screen

A prolonged international normalized ratio (INR) or activated partial thromboplastin time (APTT) reflects deteriorating liver function. These indices are the most useful prognostic markers of acute liver failure following acetaminophen overdose. A DIC screen (fibrinogen, fibrin degradation products or D-dimers) is also useful.

### C-reactive protein

This is an acute phase protein which can be elevated as a non-specific response to a hypersensitivity reaction.

### Autoimmune screen

Autoantibodies found in association with drug-related liver impairment are listed in Table 32.5. Elevated immunoglobulin-G (IgG) levels are observed with autoimmune-like syndromes. Increased IgE levels have been described following gold and carbamazepine administration.

### Electrolytes and renal function

A markedly elevated  $K^+$  may indicate rhabdomyolysis associated with acute liver failure, in particular, cocaine-induced liver injury.

### Toxicology screen

This can be useful to aid the diagnosis of a suspected drug overdose.

Drug-induced liver injury diagnostic scale	
	Score
<b>Temporal relationship between drug intake and the onset of clinical picture</b>	
Time from drug intake until the onset of first clinical or laboratory manifestations:	
4 days to 8 weeks (or less than 4 days in cases of re-exposure)	3
Less than 4 days or more than 8 weeks	1
Time from withdrawal of the drug until the onset of manifestations:	
0–7 days	3
8–15 days	0
More than 15 days*	–3
Time from withdrawal of the drug until normalization of laboratory values**:	
Less than 6 months (cholestatic or mixed patterns) or 2 months (hepatocellular)	3
More than 6 months (cholestatic or mixed) or 2 months hepatocellular	0
<b>Exclusion of alternative causes***</b>	
Viral hepatitis	
Alcoholic liver disease	
Biliary tree obstruction	
Other (pregnancy, acute hypotension):	
Complete exclusion	3
Partial exclusion	0
Possible alternative cause detected	–1
Probable alternative detected	–3
<b>Extrahepatic manifestations</b>	
Rash, fever, arthralgia, eosinophilia (>6%), cytopenia:	
4 or more	3
2 or 3	2
1	1
None	0
<b>Intentional or accidental re-exposure to the drug</b>	
Positive rechallenge test	3
Negative or absent rechallenge test	0
<b>Previous report in the literature of cases of DILI associated with the drug:</b>	
Yes	2
No (drugs marketed for up to 5 years)	0
No (drugs marketed for more than 5 years)	–3
<b>Total score</b>	
*Except cases of prolonged persistence of the drug in the body after drug withdrawal (e.g. amiodarone). **Normalization: decrease to values below 2× the upper limit of normal values. ***Use the exclusion criteria considered appropriate in each case. Definite drug associated injury >17, probable 14–17, possible 10–13, unlikely 6–9, excluded <6.	

**Table 32.9 Drug-induced liver injury (DILI) diagnostic scale.** Description of the component elements and scores attributed. Modified from Maria (97).

Others

Amylase, creatine phosphokinase (CPK) and lactate may indicate muscle necrosis secondary to rhabdomyolysis. A rapidly rising lactate accompanies impaired mitochondrial fatty acid oxidation.

Radiology

Ultrasound and CT examination can be useful to rule out non-drug-related causes of liver dysfunction. Hepatic adenomas and carcinomas can be diagnosed and subsequently confirmed by angiography. Fatty infiltration of the liver may be first suggested by ultrasound screening. Amiodarone produces a classical CT appearance with increased liver density due to the iodine content of the retained drug stored within the lysosomes. Examination of hepatic vein patency by CT and Doppler studies may aid the diagnosis of drug-related Budd-Chiari and veno-occlusive disease.

Liver biopsy

Histologic features suggestive of drug-related liver disease are listed in Table 32.10.

MANAGEMENT

The main treatment for drug-induced hepatotoxicity is withdrawal of the offending agent. While the majority of patients will recover completely, for some the adverse effects continue to deteriorate, despite cessation of therapy. The basic principles of management involve supportive care and alleviation of symptoms such as pruritus. Colestyramine, antihistamines, opiate antagonists, and odansatron may alleviate pruritus, but

Histologic features that implicate a drug-induced etiology
Zonal necrosis
Microvesicular steatosis
Granulomas
Eosinophilic inflammatory infiltration
Pure cholestasis
Peliosis hepatis
Veno-occlusive disease

Table 32.10 Histologic features that implicate a drug-induced etiology.

some patients may require plasmapheresis. Rifampin (rifampicin) has also been used to treat pruritus. Corticosteroids have no established role in the treatment of drug-related liver injury, but may be used in the setting of immunologic idiosyncrasy to suppress features of hypersensitivity or in autoimmune-like syndromes. Specific antidotes exist for overdose of some drugs such as acetaminophen, where N-acetylcysteine can replenish hepatic GSH stores. Carnitine has been suggested in cases where defects in mitochondrial fatty acid defects are prominent, but there is little evidence to indicate that this is of major benefit. Hyperbaric oxygen has been suggested as an adjunct to therapy in cases of CCl<sub>4</sub> toxicity as hypoxemia potentiates the hepatotoxic effects. Liver transplantation may ultimately be necessary if clinical and biochemical parameters continue to deteriorate (see Ch. 37).

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 33

## Acute Liver Failure

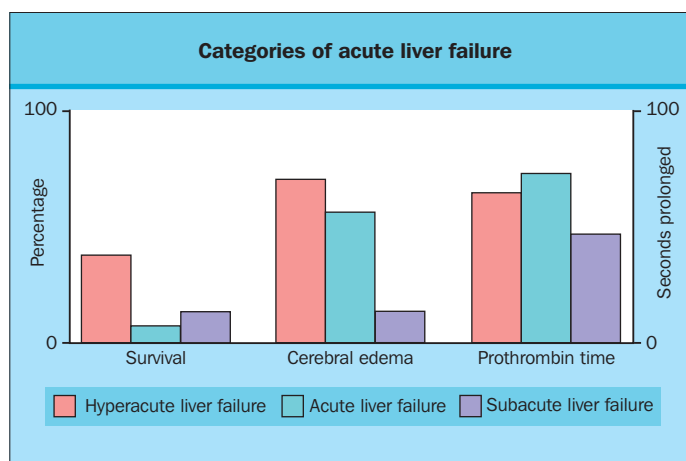
John G. O'Grady

Acute liver failure is a complex medical emergency that evolves after a catastrophic insult to the liver. The liver damage is sufficiently severe to trigger the development of encephalopathy within a matter of days or weeks of the liver injury. Acute liver failure is a heterogeneous condition incorporating a range of clinical syndromes. The dominant factors that give rise to this heterogeneity include the underlying etiology, the age of the patient and the duration of time over which the disease evolves. The natural history of the condition is very variable within this spectrum and survival rates without recourse to transplantation range from 10 to 90% for different cohorts. The treatment involves an integrated, multidisciplinary strategy with the hepatologist, intensivist and transplant surgeon playing pivotal roles. The key components of the management are the assessment of the severity of the disease and the associated prognosis, prevention or treatment of the complications that may arise and the use of transplantation when spontaneous survival is considered unlikely. Consequently, this condition should ideally be managed in specialist centers where these integrated care protocols are achieving survival rates in the range of 40 to in excess of 90% depending on the underlying etiology.

### DEFINITIONS

The terms fulminant hepatic failure and acute liver failure are used interchangeably. Fulminant hepatic failure was first used in the late 1960s and was defined as the development of encephalopathy within 8 weeks of the onset of symptoms in patients with no previous history of liver disease. Late-onset hepatic failure was later described for a complementary group of patients in whom the first symptom to encephalopathy interval ranged between 9 and 26 weeks. A French nomenclature from the 1980s defined fulminant hepatic failure as the onset of encephalopathy within 2 weeks of the onset of jaundice (rather than the less precise first symptom), and subfulminant hepatic failure as the development of encephalopathy 3–12 weeks after becoming jaundiced.

In the 1990s another classification was proposed in an attempt to achieve a closer alignment between the patient subgroups and the associated natural history and pattern of clinical features observed (Fig. 33.1). This used the core term acute liver failure to describe the development of encephalopathy within 12 weeks of the onset of jaundice, and prefixed the terms hyper- and sub- to describe subgroups with distinct clinical characteristics in which this interval is up to 7 days and 5–12 weeks, respectively. This definition excluded patients with previous symptomatic liver disease but allowed the inclusion of subclinical chronic liver disease characteristic of Wilson disease and some patients with hepatitis B related liver failure.



**Figure 33.1 Categories of acute liver failure.** Hyperacute liver failure has the best prognosis despite the high incidence of cerebral edema and marked prolongation of prothrombin time. Acute liver failure shares the same characteristics but has a much poorer prognosis. The outcome in subacute liver failure is poor despite the low incidence of cerebral edema and less marked prolongation of prothrombin time. The survival figures relate to the period 1973–1985 before liver transplantation confused natural history studies, and the survival rate for the hyperacute group is now considerably higher.

Hyperacute liver failure is defined as the development of encephalopathy within 7 days of the onset of jaundice. This group has the highest likelihood of recovery with medical management despite the characteristic rapid deterioration, high incidence of cerebral edema and severe prolongation of prothrombin time that are observed. In acute liver failure the encephalopathy develops between 8 and 28 days after the onset of jaundice. This group has a high mortality, a high incidence of cerebral edema and prolongation of prothrombin time that is as severe as in hyperacute liver failure. The interval between the onset of jaundice and the development of encephalopathy in subacute liver failure ranges from 4 to 12 weeks. This group also has a high mortality rate despite a very low incidence of cerebral edema and much less severe prolongation of prothrombin time.

Young children with acute liver failure may not develop classical encephalopathy until late in the disease process. Increasingly in this group the diagnosis of acute liver failure is made on the basis of a coagulopathy. It has also been argued that in adults with severe liver damage, drugs like benzodiazepines may mimic encephalopathy and that coagulopathy is a more reliable parameter to differentiate severe hepatitis or liver injury from acute liver failure.

ETIOLOGY AND PATHOGENESIS

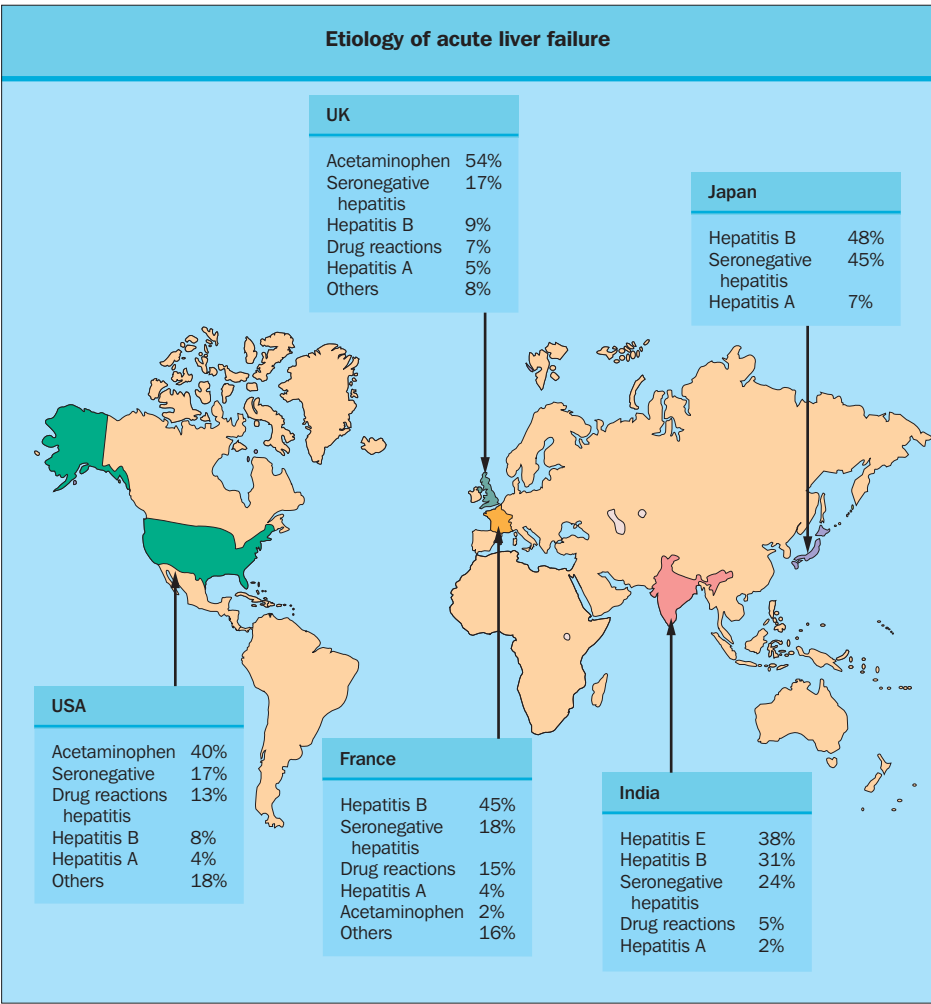
There is considerable geographic variation in the etiology of acute liver failure (Fig. 33.2). Viruses and drugs account for the majority of cases. However, a significant number of patients have no definable viral cause and have no history of exposure to drugs or toxins. This condition is referred to as seronegative hepatitis in this chapter but is also known as non-A-E hepatitis and acute liver failure of indeterminate etiology. The overall incidence of acute liver failure complicating acute hepatitis in the USA is 0.9% and this equates to about 2000 deaths annually. Most of the drug-induced cases are rare idiosyncratic reactions, but some like acetaminophen are in part dose related toxic events.

Viral

Acute liver failure is a very uncommon complication of hepatitis A infection, occurring in 0.14–0.35% of hospitalized cases, and in 0.4% of all cases seen in the USA. As hospitalized and notified cases represent only a proportion of all cases of acute hepatitis A, the real prevalence is probably considerably lower. Exposure to hepatitis A in childhood is associated with a very low mortality and induces immunity. However, in Western countries the overwhelming majority of the population now enters adulthood with no immunity against hepatitis A. The risk of acute liver failure is significantly increased in adults and there is a positive

correlation with age. This trend may be reversed by vaccination of populations at risk. The diagnosis of acute hepatitis A is made by the detection of the immunoglobulin M (IgM) antibody in serum and this is present in 95% of cases at the time of presentation. The remaining 5% become positive on repeat testing.

The incidence of acute liver failure following acute hepatitis B is 1–4% of hospitalized patients. Women appear to have a higher risk and a number of interesting cases have been described where sequential female sexual partners of hepatitis B carriers have developed acute liver failure. In early studies, hepatitis D co-infection or superinfection was thought to increase the risk of acute liver failure as hepatitis D virus (HDV) was found in 34–43% of patients with acute liver failure due to hepatitis B, as compared to 4–19% of less severe cases. However, the prevalence of hepatitis D appears to have decreased dramatically in recent years. The presence of the IgM antibody to hepatitis B virus (HBV) core-antigen in serum is the most accurate way of diagnosing acute liver failure due to de novo hepatitis B infection. Hepatitis B surface antigen (HBsAg) may be undetectable in serum at the time of presentation in a minority of patients, and HBV DNA levels tend to be low in most instances. These observations reflect the role of an aggressive immune response to the HBV in the pathogenesis of the acute liver failure. It is now recognized that acute liver failure-like syndromes occur in chronic hepatitis B in association with spontaneous surges in



**Figure 33.2 Etiology of acute liver failure.** There are considerable geographic variations in the etiology of acute liver failure. Acetaminophen is especially common in the UK, while hepatitis B and hepatitis E are particularly prominent in France and India, respectively.

viral replication (HBV DNA levels typically markedly elevated), seroconversion from hepatitis B 'e' antigen (HBeAg) to hepatitis B 'e' antibody (HBeAb) and with exposure to immunosuppressive or cytotoxic drugs. Studies of the role of mutants, especially the pre-core mutants that are unable to secrete HBeAg, have failed to conclusively link them to the pathogenesis of hepatitis B related acute liver failure. Vaccination and antiviral therapy with drugs such as lamivudine and adefovir should alter the observed pattern of hepatitis B related acute liver failure.

The risk of developing acute liver failure after exposure to hepatitis C appears to be very low and in a number of studies of patients with acute liver failure the overall hepatitis C positivity rate was only 2%. Contrary reports have emerged from series of Japanese and Hispanic Californian patients, but these populations have relatively high carrier rates for hepatitis C and the association is probably not causal. Post-transfusion hepatitis, which is usually due to hepatitis C, rarely leads to acute liver failure. It has been suggested that chronic hepatitis C infection may be a cofactor with acute hepatitis B infection increasing the risk of developing acute liver failure.

Seronegative hepatitis is a common cause of acute liver failure in some parts of the Western world. The diagnosis remains one of exclusion. There is now considerable doubt whether this is a viral infection as most cases are sporadic and unidentified toxins or autoimmune processes may be the underlying mechanisms. Middle-aged females are most frequently affected and the risk of developing acute liver failure has been calculated at 2.3–4.7% of hospitalized cases.

Hepatitis E is common in parts of Asia and Africa and the risk of developing acute liver failure ranges from 0.6–2.8 to over 20% in pregnant women, being particularly high during the third trimester. Hepatitis E is also encountered in Europe and the USA and may account for up to 8% of cases that would previously have been described as seronegative hepatitis. A history of travel to a high endemic area was not always present in these cases. Unusual causes of viral acute liver failure include herpes simplex 1 and 2, herpesvirus-6, varicella zoster, Epstein-Barr virus and cytomegalovirus. The cases due to herpes simplex may have external manifestations of herpetic infection and there is often a positive therapeutic response to aciclovir. Herpes simplex was implicated in 3% of cases of adult cases of acute liver failure in France.

## Drugs

Acetaminophen (paracetamol) overdose is the commonest cause of acute liver failure in the UK and the USA. It appears to be increasing in other English speaking countries but remains rare in continental Europe and the rest of the world. It is usually taken with suicidal or parasuicidal intent, but up to 8% cases in the UK resulted from the therapeutic use of acetaminophen. This is either because of unintentional overdosing or accelerated metabolism in people with liver enzyme induction as a consequence of anti-epileptic therapy or regular alcohol usage. The pattern in the USA may be somewhat different as one small urban study suggested that 70% of cases were suicidal and 30% were unintentional in undernourished or alcoholic subjects. The patients developing acute liver failure represent only about 2–5% of those presenting at hospital with acetaminophen overdoses. The median dose of acetaminophen causing acute liver failure in the UK was 40 g (range 5–210 g), and the mortality

Drug causes of acute liver failure	
Category 1	Category 2
Acetaminophen	Benoxypfen
Halothane	Phenytoin
Isoniazid/rifampin (rifampicin)	Isoflurane
Non-steroidal anti-inflammatory drugs (NSAIDs)	Enflurane
	Tetracycline
Sulfonamides	Allopurinol
Flutamide	Ketoconazole
Sodium valproate	Monoamine oxidase inhibitors (MAOIs)
Carbamazepine	Disulfiram
Ecstasy	Methyldopa
	Amiodarone
	Tricyclic antidepressants
	Propylthiouracil
	Gold
	2, 3-Dideoxyinosine (ddI)

**Table 33.1 Drug causes of acute liver failure.** The drugs that cause acute liver failure are divided into two categories; category 1 includes frequent causes and category 2 includes occasional causes.

was highest at doses exceeding 48 g. Unintentional overdosing in the USA averaged 12 g of acetaminophen, as compared with 20 g in the suicidal group, but resulted in higher rates of coma (33 versus 6%) and death (19 versus 2%).

Estimates of the risk of developing acute liver failure as a result of an idiosyncratic reaction range from 0.001% for non-steroidal anti-inflammatory drugs to 1% for the isoniazid/rifampin (rifampicin) combination. The diagnosis is made on the basis of a temporal relationship between exposure to the drug and the development of acute liver failure. The more common offending drugs are listed in **Table 33.1**. Most cases develop during the first exposure to the drug but some, like halothane, occur in sensitized individuals on the second or subsequent exposure. Non-therapeutic drugs also cause acute liver failure. Ecstasy (methylenedioxymethamphetamine) has been associated with a number of clinical syndromes ranging from rapidly progressive acute liver failure associated with malignant hyperthermia to subacute liver failure.

## Other etiologies

Acute liver failure associated with pregnancy tends to occur during the third trimester. Although three discrete entities have been described, in reality considerable overlap is frequently observed. Acute fatty liver of pregnancy usually occurs in primigravids carrying a male fetus, and is characterized by severe microvesicular steatosis, which may be detectable by ultrasound. Serum uric acid levels are markedly elevated in these patients. The HELLP syndrome is defined as the combination of hemolysis, elevated liver enzymes and low platelets. Acute liver failure complicating pre-eclampsia or eclampsia typically exhibits





**Figure 33.3** A classical view of the poisonous *Amanita phalloides*.

very high serum transaminase levels and abnormal tissue perfusion patterns on computed tomography (CT) scanning that reflect the microvascular infarction characteristic of this condition.

Wilson disease may present as acute liver failure, usually during the second decade of life. It is characterized clinically by a Coomb negative hemolytic anemia and demonstrable Kayser-Fleischer rings in the majority of cases. The serum ceruloplasmin levels are usually, but not invariably, low and the serum and urinary copper levels are increased. Although the latter is the most specific feature of Wilson disease, increases of lesser magnitude can occur in other causes of acute liver failure. A serum alkaline phosphatase/total bilirubin ratio of  $<2.0$  had been suggested as an accurate discriminator of Wilson disease from other causes of acute liver failure.

Poisoning with *Amanita phalloides* (mushrooms) is most commonly seen in central Europe, South Africa and the west coast of the USA (**Figure 33.3**). Severe diarrhea, often with vomiting, is a typical feature and commences 5 h or more after ingestion of the mushrooms. Liver failure develops 4–5 days later. Auto-immune chronic hepatitis may present as acute liver failure but it is usually beyond rescue with corticosteroid or other immunosuppressive therapy. Anti-liver-kidney-microsomal antibodies may be the only detectable autoantibody in these cases. The diagnosis may be difficult to establish as autoantibodies in low titers are found in a proportion of patients with seronegative hepatitis. The Budd-Chiari syndrome may present with acute liver failure and the diagnosis is suggested by hepatomegaly and confirmed by the demonstration of hepatic vein thrombosis. Malignancy infiltration, especially with lymphoma, is another rare cause of acute liver failure that is typically associated with hepatomegaly. Ischemic hepatitis is being increasingly recognized as a cause of acute liver failure, especially in older patients. Other unusual causes of acute liver failure include heatstroke and sepsis.

### Pediatric causes

Children are at risk of developing most of the causes of acute liver failure discussed above but also have some unique underlying causes especially in the category of metabolic disease. Neonatal hemochromatosis presents within the first few weeks of life and has been treated by liver transplantation as early as

5 days of age. Acute liver failure due to mitochondrial disorders may be triggered by bacterial infections and are characterized by high lactate levels in blood. Other metabolic causes include tyrosinemia, galactosemia and fructose intolerance. Young infants can develop acute liver failure with viral infections such as adenovirus, coxsackie and cytomegalovirus.

## CLINICAL SYNDROME

Acute liver failure causes a syndrome of multisystem failure potentially involving all of the major body systems. Jaundice is present in most patients but some cases of hyperacute liver failure develop encephalopathy before jaundice becomes clinically apparent. Most of the other classical signs of liver failure are notable by their absence. Feter and asterix are not prominent features of the encephalopathy of acute liver failure, although are more likely to be seen with subacute liver failure. Portal hypertension can occur but clinical manifestations are unusual. Ascites is characteristic of Wilson disease and can occur in subacute liver failure, whilst bleeding from esophageal varices has been documented occasionally.

### Encephalopathy

All patients with acute liver failure have some degree of encephalopathy. Patients with grades 1 and 2 encephalopathy exhibit degrees of drowsiness or disorientation, but they can be roused and they respond appropriately to verbal stimuli. Progression to grade 3 encephalopathy is often heralded by a short period of extreme agitation before the patient becomes very confused and at best obeys simple commands. Grade 4 encephalopathy signifies deep coma with the patients being responsive at best to painful stimuli. In acetaminophen-induced acute liver failure, the encephalopathy develops on the third or fourth day after drug ingestion, but in other etiologies the onset and rate of progression of the encephalopathy is variable.

The pathogenesis of the encephalopathy is incompletely understood but is likely to be multifactorial (Ch. 11). Ammonia levels are classically elevated in serum but in amounts inadequate to independently cause encephalopathy. Furthermore, there is an absence of a close correlation between the ammonia levels and grade of encephalopathy. Phenols, fatty acids, middle-molecular weight substances and mercaptans have also been proposed as possible causative agents. These toxins interfere with neuronal energy metabolism, and may also contribute to alterations in blood–brain barrier permeability by direct toxicity. The inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (GABA) is increased in acute liver failure and this may act independently or in synergy with the benzodiazepine receptor, which forms a supramolecular complex with the GABA receptor on neuronal plasma membranes. Other possible mechanisms include the development of false neurotransmitters e.g. octopamine, and imbalances in the ratios of plasma and intracerebral amino acids.

### Intracranial hypertension

Early clinical studies suggested intracranial hypertension occurred in 70% of patients with hyperacute liver failure, 55% of patients with acute liver failure and 15% of patients with subacute liver failure who developed grade 3–4 encephalopathy.

Main changes seen with intracranial hypertension			
<i>Baseline intracranial pressure</i>	<i>Early</i> Normal	<i>Intermediate</i> Increased	<i>Late</i> Increased
Surges in intracranial pressure	Yes	Possible	May occur
Mean arterial pressure	Normal or increased	Normal or low	Low
Cerebral perfusion pressure	Normal	Normal or low	Low
Cerebral oxygen metabolism	Normal or increased	Normal or reduced	Reduced
Cerebral autoregulation	Maintained	Lost	Lost
Potential cause of death	Brainstem herniation	Brainstem herniation	Hypoxic brain

**Table 33.2 Intracranial hypertension.** This is a breakdown of the main changes in the key parameters as intracranial hypertension progresses through a spectrum of increasing severity from early to late stages.

However, there is some evidence that the incidence of cerebral edema is decreasing with modern management protocols. Nevertheless, it remains a major cause of death and frequently disqualifies patients from transplantation.

The term intracranial hypertension covers a range of events including increased blood flow, astrocyte swelling, brainstem herniation and hypoxic injury through sustained impairment of cerebral perfusion (Table 33.2). In the early phase, the baseline intracranial pressure is normal but sudden surges in pressure occur either spontaneously or in response to tactile or auditory stimuli. There is an associated increase in mean arterial pressure so that cerebral perfusion pressure is maintained and neuronal oxygenation is satisfactory. The risk to life at this stage is by means of classical brainstem herniation. With time, the baseline intracranial pressure gradually increases but sudden surges may continue to increase the pressure to dangerous levels. Autoregulation is lost and the systemic arterial pressure response becomes less predictable so that cerebral perfusion is variably reduced with an associated reduction in oxygen delivery to the brain. In the later stages, either the intracranial pressure is so high or the mean arterial pressure is so low that adequate cerebral perfusion no longer occurs and death results from hypoxic brain damage.

The clinical features of cerebral edema include systemic hypertension, ‘decerebrate’ posturing, hyperventilation, abnormal pupillary reflexes and ultimately impairment of brainstem reflexes and functions. Papilledema however is rarely seen. The outcome with medical management is either full recovery or death, although a few survivors with residual neurologic deficits have been described. Failure of neurological recovery has been observed following otherwise successful liver transplantation.

### Renal failure

This occurs in 75% of patients developing grade 4 encephalopathy following an acetaminophen overdose and 30% of other etiologies of acute liver failure. Renal failure after an acetaminophen overdose is a consequence of direct renal toxicity and develops early in the course of the illness. Early renal dysfunction is also seen in Wilson disease and pregnancy related syndromes. In the other etiologies, renal impairment develops relatively late and progresses from a stage of ‘functional’ or prerenal failure (urinary sodium <10 mmol/L, urine/plasma osmolality ratio >1.1) to

acute tubular necrosis. Urea synthesis is impaired in acute liver failure and serum creatinine levels are preferred for the purposes of monitoring renal function.

### Metabolic disorders

Hypoglycemia is common and can induce reversible impairment of consciousness prior to the onset of classical encephalopathy. The signs and symptoms of hypoglycemia are often masked and regular blood glucose monitoring is required. Metabolic acidosis is present in 30% of patients developing acute liver failure after an acetaminophen overdose and is associated with a particularly high mortality – >90% if the pH of arterial blood is <7.30 on the second or subsequent days after the overdose. This acidosis precedes the onset of encephalopathy and is independent of renal function. In contrast, a metabolic acidosis is found in 5% of patients with other etiologies of acute liver failure, occurring later in the disease process and also associated with a poor outcome. Increased serum lactate levels have been documented in patients with metabolic acidosis, and these correlate inversely with mean arterial pressure, systemic vascular resistance and oxygen extraction ratios. The hyperlactatemia possibly reflects tissue hypoxia resulting from impaired oxygen extraction as a result of microvascular shunting of blood away from actively respiring tissues. In most etiologies of acute liver failure, alkalosis is the dominant acid–base abnormality and it may be associated with hypokalemia. Hyponatremia may reflect sodium depletion in patients with vomiting or it may be dilutional due to excessive antidiuretic hormone secretion or intracellular sodium shifts. Hypophosphatemia is most frequently encountered in acetaminophen-induced acute liver failure when renal function is preserved.

### Hemodynamics

The hemodynamic changes in acute liver failure are very similar to those observed in the systemic inflammatory response syndrome (SIRS). The early hemodynamic profile reflects a hyperdynamic circulation with increased cardiac output and reduced systemic peripheral vascular resistance. Profound vasodilatation may cause relative hypovolemia and invasive monitoring is used to determine appropriate fluid regimens and adequate intravascular volumes. Progressive disease leads to circulatory failure either as a result of a falling cardiac output or an inability to

maintain an adequate mean arterial pressure. This is a common cause of death in acute liver failure. Cardiac dysrhythmias occur and may be due to a definable precipitating event e.g. hypo- or hyperkalemia, acidosis, hypoxia, or cardiac irritation by a catheter, especially Swan-Ganz catheters.

### Pulmonary complications

Patients with grade 3–4 encephalopathy are usually intubated and mechanically ventilated to provide airway protection and to facilitate monitoring and treatment. Prior to intubation, hyper-ventilation may be observed due to early intracranial hypertension or as a response to a coexisting metabolic acidosis. Impaired oxygen exchange is common, usually as a result of acute lung injury with or without infection. Sputum plugs and intrapulmonary hemorrhage can also contribute to the challenge of maintaining adequate oxygenation.

### Hematology

The liver is responsible for the synthesis of most of the coagulation factors (except factor VIII which is produced by endothelial cells) and some of the inhibitors of coagulation and fibrinolysis. In acute liver failure circulating levels of fibrinogen, prothrombin and factors V, VII, IX and X are reduced, and the prothrombin time is widely used as an indicator of the severity of liver damage. In addition to decreased synthesis of coagulation factors by the liver, there is evidence of increased peripheral consumption. Overt disseminated intravascular coagulation (DIC) is occasionally observed, especially in the pregnancy related syndromes, but sensitive investigative techniques point to the presence of a low-grade process in most patients. Both quantitative and qualitative defects in platelet function are well described in acute liver failure and platelet counts of  $<100 \times 10^9/L$  are seen in up to 70% of patients. Platelet aggregation is impaired, but there is an increase in platelet adhesiveness, a pattern that may be due to increased levels of circulating von Willebrand factor.

There is a fairly poor correlation between the laboratory and clinical manifestations of the coagulopathy. The highest risk of bleeding is seen in those with an associated thrombocytopenia or a frank DIC syndrome. Gastrointestinal hemorrhage was common and was attributed to gastric erosions, but the incidence and severity have been decreased by gastric mucosal protection regimens. The other main sites include nasopharynx, lungs, kidneys, retroperitoneum and skin puncture sites.

Anemia not related to bleeding may be due to hemolysis or bone marrow disease. A Coomb negative hemolytic anemia is a characteristic of Wilson disease and a Coomb positive hemolytic anemia may be seen in acute liver failure secondary to autoimmune hepatitis. Aplastic anemia is associated with seronegative hepatitis especially in younger patients, and has been seen in up to one-third of cases in pediatric series. This may be related to parvovirus B19 infection, although this is not necessarily the cause of the associated acute liver failure. Erythrophagocytosis is an increasingly recognized occurrence in acute liver failure.

### Infection

Patients with acute liver failure are at increased risk of infections and this has been calculated as twice the expected rate for similarly ill patients without liver disease. It is another of the

common causes of death playing an integral role in the evolving cycle of hemodynamic instability and multisystem failure. It also frequently disqualifies potential candidates from emergency liver transplantation. Infection may be difficult to detect with confidence as there is a poor correlation between the presence of infection and body temperature or white cell counts. Clinical studies found that patients with at least grade 2 encephalopathy had proven bacterial infection in up to 80% and fungal infection in 32% of cases. The source of positive cultures included blood, urine, sputum and vascular cannulae. The predominant bacteria were *Staphylococcus aureus*, streptococci and coliform bacteria, while *Candida* species accounted for most of the fungal infections. The fungal infections were particularly difficult to diagnose and were detected antemortem in only 50% of cases. The risk factors for both bacterial and fungal sepsis include coexisting renal failure, cholestasis, treatment with thiopentone and liver transplantation. Surveillance cultures are required on a daily basis.

### Diagnosis

The diagnosis of acute liver failure is a clinical one based on the detection of evidence of encephalopathy in patients with acute liver disease. Formal psychometric testing may be useful in patients with subacute liver failure to detect subtle changes to mental state, but in most patients the diagnosis of encephalopathy is made on overt clinical criteria. Commonly hypoglycemia and unusually uremia may mimic hepatic encephalopathy in patients with acute liver failure, and these need to be excluded before the diagnosis is confirmed.

The etiology of acute liver failure must be accurately identified and the appropriate investigations are outlined in **Table 33.4**. The laboratory investigation of acute liver failure is dealt with in **Table 33.5**. Histologic assessment of liver tissue may aid the diagnosis of the cause of acute liver failure but this is often only available after death or transplantation. Confluent necrosis is the commonest histological finding and this may be zonal or involve all of the parenchyma. Necrosis that is zonal within the acinus and coagulative or eosinophilic is more likely to be secondary to a toxic insult or ischemia (**Fig. 33.3**). The features of necrosis and parenchymal collapse may be interspersed with evidence of regeneration (**Fig. 33.4**). The regeneration may either occur in a diffuse pattern of small areas throughout the liver or in randomly occurring larger nodules that give the 'map-like pattern' that has been described in this condition (**Figs 33.5 and 33.6**). The latter pattern is most commonly seen in patients with subacute liver failure. The defined viral causes of acute liver failure have similar histologic appearances and in true acute hepatitis B infection there are usually no tissue markers detectable to implicate hepatitis B as the cause. The detection of hepatitis B markers suggests seroconversion to HBsAb positivity, a surge in hepatitis B replication or superinfection with  $\delta$  viruses or viruses unrelated to hepatitis B as the cause of the acute liver failure.

Histologic features may suggest specific diagnoses including sodium valproate toxicity, malignant infiltration, Wilson disease, pregnancy related syndromes and the Budd-Chiari syndrome. Sodium valproate toxicity is characterized by microvesicular steatosis. Screening for malignant infiltration as the cause of acute liver failure is one of the stronger indications for performing



Underlying etiology of acute liver failure		
<i>Etiology</i>	<i>Investigation</i>	<i>Comment</i>
Hepatitis A virus (HAV)	IgM anti-HAV	95% positive initially – 100% on repeat testing
Hepatitis B virus (HBV) acute infection	IgM anti-core	Always positive but HBsAg often undetectable
seroconversion	Full HBV profile	HbsAg positive, HBeAg negative, HBeAb initially negative, HBV DNA negative
increased replication	Full HBV screen	HBsAg positive, HBeAg positive, HBV DNA markedly elevated
Hepatitis D virus (HDV)	IgM anti-HDV	IgM anti-core may be positive (co-infection) or negative (superinfection)
Hepatitis E virus (HEV)	Anti-HEV	IgM antibody test not routinely available
Seronegative hepatitis	All tests	Diagnosis of exclusion
Acetaminophen	Drug levels in blood	May be negative on third or subsequent days after overdose
Halothane	Antibody test	Diagnosis usually made on clinical grounds
Idiosyncratic drug reactions	Eosinophil count	Most diagnoses based on temporal relationship
Ecstasy	Blood, urine, hair analysis	Medium-term exposure can be mapped from analysis of hair
Autoimmune	Autoantibodies, IgGs	High titers or anti-KLM suggest diagnosis
Pregnancy-related syndromes: fatty liver	Ultrasound, uric acid, histology	First pregnancy
HELLP syndrome toxemia	Platelet count	Disseminated intravascular coagulation a prominent feature
	Serum aminotransferases	Very high transaminase, appropriate obstetric history
Wilson disease	Urinary copper, ceruloplasmin	Deeply jaundiced, anemic, second interruption in drug compliance
<i>Amanita phalloides</i>		History of ingestion of mushrooms, diarrhea
Budd-Chiari syndrome	Ultrasound or venography	Ascites, prominent caudate lobe on imaging
Malignancy	Imaging and histology	Imaging may be interpreted as normal
Ischemic hepatitis	Aminotransferases	Aminotransferases very high
Heatstroke	Myoglobinuria	Rhabdomyolysis a prominent feature

**Table 33.3 Underlying etiology of acute liver failure.** The investigations required to establish the underlying cause of acute liver failure.

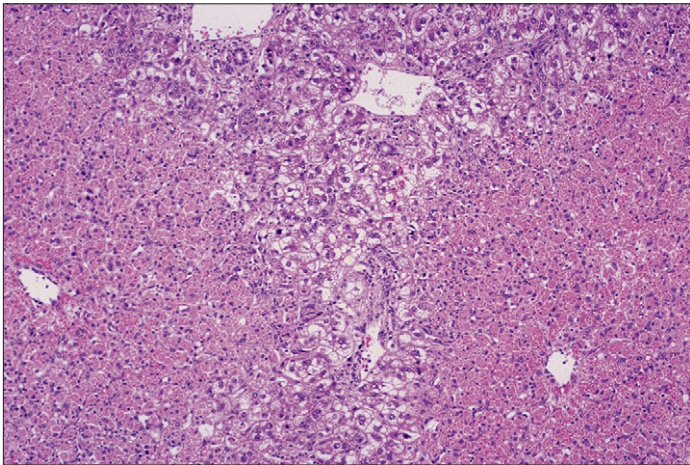
a liver biopsy in this condition. The benefit from a histologic diagnosis is strongest when lymphoma is the underlying diagnosis, as this may be responsive to chemotherapy. Patients with Wilson disease presenting as acute liver failure usually have established cirrhosis, commonly associated with interface hepatitis resembling autoimmune disease, hepatocyte ballooning and steatosis. Liver histology may be very useful in making a precise diagnosis within the spectrum of pregnancy related liver diseases, ranging from the fatty infiltration characteristic of acute fatty liver of pregnancy (**Fig. 33.7**), to the fibrin microthrombi and associated necrosis that is a feature of liver dysfunction secondary to pre-eclampsia or eclampsia. The histologic features of the Budd-Chiari syndrome are extreme sinusoidal dilatation, congestion and coagulative necrosis.

## PROGNOSIS

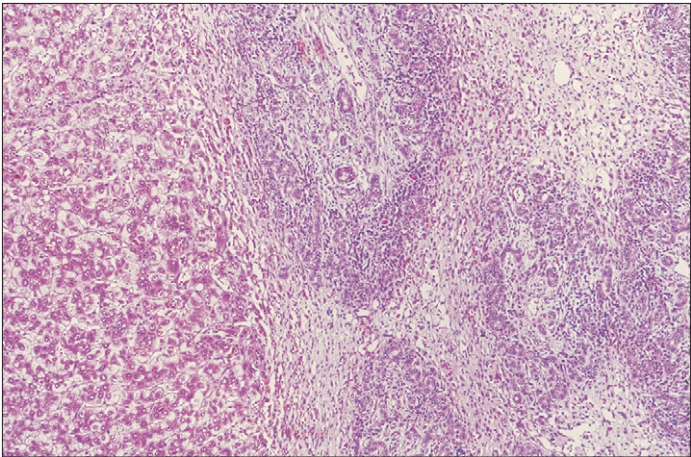
The range of parameters that correlates with prognosis in acute liver failure is extensive. The grade of encephalopathy, not unsurprisingly, correlates strongly with outcome and this is true for both the grade of encephalopathy at the time of presentation to a specialist unit and the maximum grade attained. The prognosis deteriorates further when grade 4 encephalopathy is complicated by cerebral edema and even further when the latter coexists with renal failure (**Fig. 33.8**). Reliance on the development of these clinical complications to determine prognosis is not helpful when defining the scope and application of liver transplantation in this condition. Furthermore, subsets of patients have very poor prognoses without the development of cerebral or renal

Interpretation of laboratory parameters in acute liver failure	
Investigation	Interpretation
Hemoglobin	Hemorrhage, hemolytic or non-hemolytic anemia, aids fluid replacement
White cell count	Partial indicator of presence of sepsis
Platelet count	Important risk factor for bleeding, DIC syndrome, erythrohemophagocytosis, may be very low in acetaminophen cases
Serum bilirubin	Very variable, levels > 700 µmol/L (41 g/dL) indicate hemolysis
Aminotransferases	Very variable and of no prognostic value
Serum albumin	Normal early but falls with disease progression, very low in hyperthermia
Prothrombin time	Very variable but of strong prognostic value
Serum sodium	Often low and may reflect sodium deficiency or dilution
Serum potassium	Low initially but rising with onset of renal failure
Serum phosphate	Low in acetaminophen cases with preserved renal function
Blood glucose	Regular estimations the only reliable screen for hypoglycemia
Acid–base status	Alkalosis common but acidosis associated with a poor prognosis
Arterial oxygen	Best screen for pulmonary disease even when chest X-ray is normal
Serum creatinine	Best indicator of renal dysfunction
Serum amylase	Essential to screen for co-existing pancreatitis

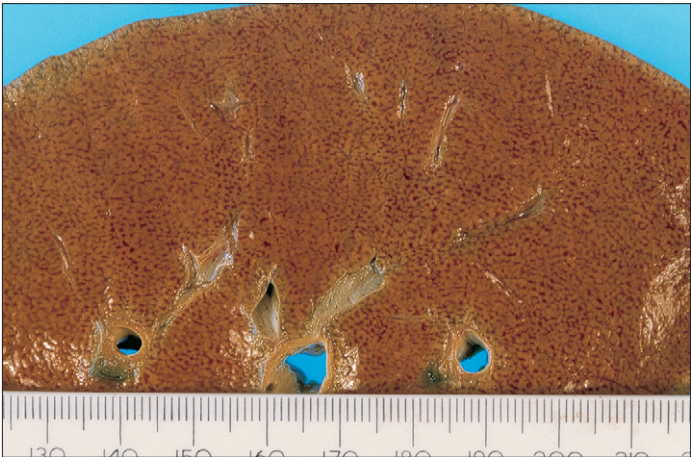
**Table 33.4 Interpretation of laboratory parameters.** Abnormalities in investigations are interpreted in the context of acute liver failure. DIC, disseminated intravascular coagulation.



**Figure 33.4 Liver histology.** Submassive eosinophilic necrosis following an acetaminophen overdose showing few viable hepatocytes. This appearance can underestimate the capacity for regeneration to occur and the likelihood of spontaneous recovery. (Courtesy of Professor B. Portmann.)



**Figure 33.5 Liver histology.** Adjacent regeneration (right) and severe collapse (left) occurring in a patient who has subacute liver failure. This appearance can overestimate the likelihood of spontaneous recovery. (Courtesy of Professor B. Portmann.)

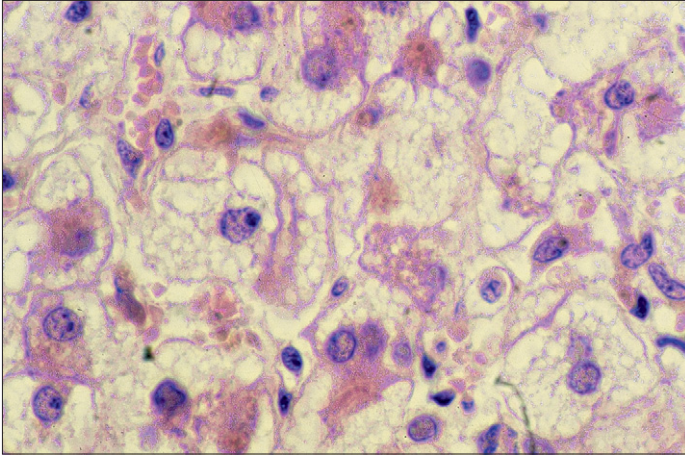


**Figure 33.6 Macroscopic appearance of liver.** The uniformly shrunken liver is characteristic of hyperacute liver failure. (Courtesy of Professor B. Portmann.)

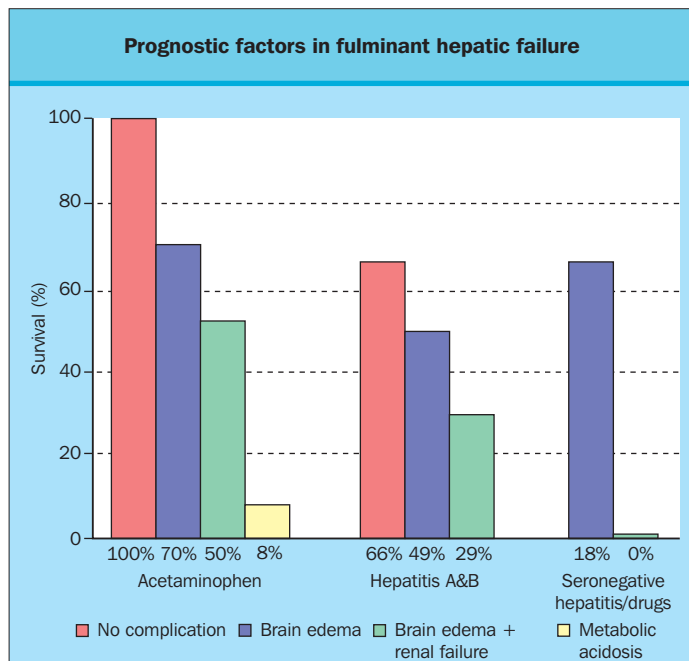


**Figure 33.7 Macroscopic appearance of liver.** The areas of regeneration randomly interspersed with collapse gives the 'map-like' pattern. The areas of regeneration may achieve significant volumes without improving the clinical condition. (Courtesy of Professor B. Portmann.)





**Figure 33.8 Diagnostic biopsy.** This biopsy from a pregnant woman who has acute liver failure shows extensive fatty infiltration and is an example of the minority of biopsies that establish a specific diagnosis. (Courtesy of Professor B. Portmann.)

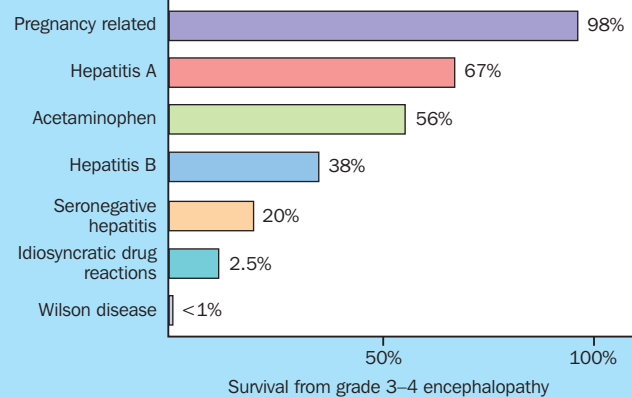


**Figure 33.9 Prognostic factors in fulminant hepatic failure (FHF).** Survival is related to etiology of the syndrome as well as to the development of complications. Data from O'Grady et al (89).

failure. The use of transplantation intensified the need for early indicators of prognosis so that those in need of this intervention could be identified as quickly as possible.

The prognosis of acute liver failure varies greatly with the underlying etiology (Fig. 33.9), as well as a number of other factors. Determination of prognosis drives two key management issues when assessing patients with acute liver failure i.e. the need for referral to specialist centers and the indications for transplantation. Indications for referral to specialist units have been suggested for acetaminophen and other etiologies of acute liver failure (Tables 33.6 and 33.7). Separate criteria have been identified for use within specialist centers to identify the cohort

#### Survival for different etiologies of acute liver failure



**Figure 33.10 Impact of etiology on prognosis.** The survival rates attained at King's College Hospital with medical management of patients progressing to acute liver failure is given for the underlying etiology. Similar results have been reported from Japan.

#### Referral to specialist units following acetaminophen ingestion

Any of these criteria should prompt referral

Day 2	Day 3	Day 4
Arterial pH <7.30	Arterial pH <7.30	INR >6 or PT >100 s
INR >3.0 or PT >50 s	INR >4.5 or PT > 75 s	Progressive rise in PT to any level
Oliguria	Oliguria	Oliguria
Creatinine >200 μmol/L (1.5 mg/dL)	Creatinine >200 μmol/L (1.5 mg/dL)	Creatinine >300 μmol/L (2.3 mg/dL)
Hypoglycemia	Encephalopathy	Encephalopathy
	Severe thrombocytopenia	Severe thrombocytopenia

**Table 33.5 Referral to specialist units.** Criteria are suggested to select patients with acetaminophen-induced liver damage for referral to a specialist center. INR, international normalized ratio; PT, prothrombin time.

most in need of liver transplantation; these King's College criteria, which are widely used, are given in Tables 33.8 and 33.9. One of the problems with the criteria is the difficulty applying the coagulation parameters outside the UK. The scale on which prothrombin times are reported in the USA is dramatically shorter than in the UK reflecting differences in technique and the reagents used. The use of the INR (international normalized ratio) did not entirely resolve this problem because of loss of accuracy at higher readings. In France, the factor V levels are used in preference to either the prothrombin time or INR. Factor V levels <20% in patients under the age of 30 years and <30% in older patients are indicative of a poor prognosis once



Referral to specialist units in non-acetaminophen etiologies		
The presence of any of the following criteria should prompt referral		
<i>Hyperacute</i>	<i>Acute</i>	<i>Subacute</i>
Encephalopathy	Encephalopathy	Encephalopathy
Hypoglycemia	Hypoglycemia	Hypoglycemia (less common)
PT >30 s	PT >30 s	PT >20 s
INR >2.0	INR >2.0	INR >1.5
Renal failure	Renal failure	Renal failure
Hyperpyrexia		Serum sodium <130 mmol/L (130 mEq/L)
		Shrinking liver volume

**Table 33.6 Referral to specialist units.** Criteria are suggested to select patients with non-acetaminophen-induced liver damage for referral to a specialist center. INR, international normalized ratio; PT, prothrombin time.

Indicators of a poor prognosis in acetaminophen-induced acute liver failure			
<i>Parameter</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>Positive predictive</i>
Arterial pH <7.30	49%	99%	81%
All 3 of the following concomitantly:			
Prothrombin time >100 s or INR >6.5, Creatinine >300 μmol/L (2.3 mg/dL) and Grade 3–4 encephalopathy	45%	94%	67%

**Table 33.7 Indicators of a poor prognosis.** The King's College criteria indicating a poor prognosis in acetaminophen-induced acute liver failure. INR, international normalized ratio.

encephalopathy develops. Factor V levels have not been used extensively in clinical practice outside France. A study from the UK assessed the prognostic value of factor V levels and validated their use in non-acetaminophen causes of acute liver failure, but found that they were not discriminatory in cases secondary to acetaminophen.

The King's College criteria, which are early indicators of prognosis, pre-empt advanced encephalopathy and have a major advantage in that largely they can be applied quickly and before the patient progresses to the advanced stages of encephalopathy. In non-acetaminophen cases, etiology, the age of the patient and the interval between the onset of jaundice and the development of encephalopathy are the static variables used to assess prognosis (Table 33.9). These are combined with two commonly used dynamic parameters, serum bilirubin and prothrombin time, to complete the model. In acetaminophen cases, the pH of arterial blood has the strongest predictive value, and a pH <7.30 suggests a very poor prognosis. In patients who did not

Indicators of a poor prognosis in nonacetaminophen etiologies of acute liver failure			
<i>Parameter</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>Positive predictive</i>
Prothrombin time >100 s or INR >6.7	34%	100%	46%
Any 3 of the following:			
Unfavorable etiology (seronegative hepatitis or drug reaction), age <10 or >40 years, acute or subacute categories, serum bilirubin > 300 μmol/L (2.3 mg/dL), PT >50 s or INR >3.5	93%	90%	92%

**Table 33.8 Indicators of a poor prognosis.** The King's College criteria indicating a poor prognosis in causes of acute liver failure other than acetaminophen. INR, international normalized ratio; PT, prothrombin time.

develop acidosis, the coexistence of a prothrombin time >100 s (INR 6.7), serum creatinine >300 μmol/L and grade 3 encephalopathy was necessary to be reasonably certain of a poor prognosis. However, despite the prompt identification of patients with a poor prognosis after an acetaminophen overdose, liver transplantation could only be achieved in a minority of cases because of very rapid progression of the disease. The criteria are still used as originally described with a few exceptions. In the original analysis, the discriminatory power of a metabolic acidosis with an arterial pH <7.30 on the second or subsequent day after an acetaminophen overdose was very strong (95% mortality), but the more liberal use of *N*-acetylcysteine and aggressive early rehydration appear to have improved the outcome in these patients. As a result, the interpretation of a transient acidosis in isolation from other prognostic indicators is more cautious. Serum lactate levels above 3.5 before resuscitation and 3.0 after resuscitation also indicate a poor prognosis in acetaminophen-induced acute liver failure. The King's College criteria were not validated in a number of rarer etiologies particularly pregnancy-related syndromes, Wilson disease and *Amanita phalloides* poisoning.

Assessment of the volume of viable hepatocytes by histologic examination is considered by some to be of prognostic value. The critical mass that suggests a good prognosis has been calculated at between 25 and 40%. This parameter has been used in isolation and in combination with other criteria to select patients for liver transplantation, but the potential for sampling error is considerable. A biopsy taken from an area of total collapse will show few viable hepatocytes even though the adjacent tissue may be regenerating. In addition, the poor prognosis in patients with subacute liver failure may not be apparent from the relative healthy appearance of a biopsy taken from a regenerative nodule (Fig. 33.4).

A small liver on clinical or radiologic assessment, or more particularly a liver that is found to be shrinking rapidly, is a poor prognostic indicator. This feature is especially useful in subacute

liver failure when the degree of encephalopathy and the severity of the derangement of coagulation may not be particularly marked. In Japan, CT scanning has been used to assess both the size of the liver and the functional reserve and this was useful in determining prognosis. Serial ultrasonic assessments are commonly used to detect changes in liver size, but the ease with which this can be done has to be weighted against the relatively subjective assessment of liver volume with this technique.

A number of parameters have been shown to have prognostic value in certain circumstances. Patients with acute hepatitis B who have cleared HBsAg from serum are more likely to survive than those with persistent HBsAg in blood. Serum  $\alpha$ -fetoprotein levels are higher in patients surviving acute liver failure due to hepatitis B. Serial arterial ketone body ratios have also been shown to identify likely survivors in a number of studies. Although these parameters are of academic interest they are not routinely used to guide decision making in clinical practice.

## MANAGEMENT

### Overall strategy

The management starts with identification of etiology and an initial assessment of prognosis. Appropriate patients should then be referred to specialist centers where appropriate monitoring is instituted and a decision on the need for immediate liver transplantation is made. Patients are then monitored for the complications that may develop and these are treated as they emerge to the point of recovery, death or transplantation. Patients not initially considered for transplantation may change status on the basis of prognostic indicators or the pattern of clinical complications that emerges. Likewise, patients listed for transplantation may develop complications that preclude this intervention or occasionally may show unexpected signs of recovery before a donor organ becomes available. The final decision on transplantation is made when an organ is available. The potential to develop 'bridges to transplantation' using hepatectomy and/or extracorporeal liver support devices is likely to be increasingly tested in the forthcoming decade.

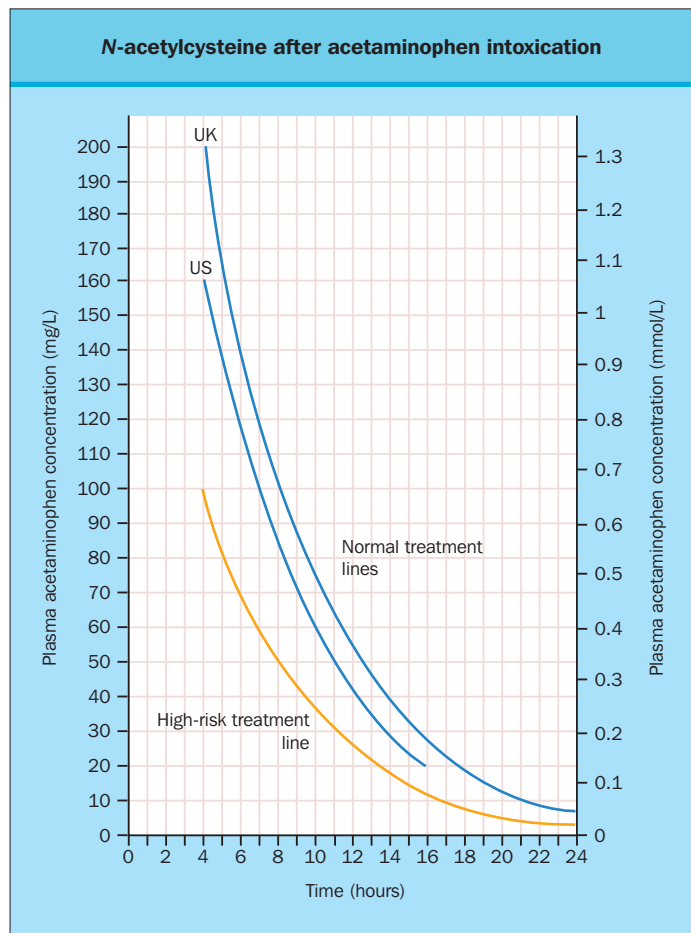
### General measures

There has been scant reward from decades of research activity seeking the panacea for acute liver failure. Efforts have focused on reducing tissue injury, removing accumulated toxins and promoting hepatocyte regeneration (**Table 33.10**). Initial promising reports of efficacy followed by disappointing negative trials have been a recurring theme. There is no role for corticosteroid therapy and controlled studies suggest that in general they are contraindicated. Occasional patients with late-onset hepatic failure are steroid responsive but they are not yet readily identifiable on the basis of serologic markers. Insulin and glucagon infusions have been used to promote hepatic regeneration without convincing evidence of efficacy. Circulating interferon levels were markedly reduced in one study of patients with acute liver failure of viral etiology, and improved survival rates were reported for patients treated with interferon for 3 or more days. However, this observation has also been refuted in a larger study. Similarly an initial enthusiastic report of the benefits of prostaglandins could not be reproduced within the construct of a controlled trial.

There are a few drugs with well-defined roles in specific etiologies of acute liver failure. *N*-acetylcysteine is established in the management of acetaminophen-induced acute liver failure. It was initially used to prevent liver damage when administered orally within 8 h and intravenously within 15 h of acetaminophen ingestion. The drug is given on the basis of the level of acetaminophen in blood by time after the ingestion of the drug. Standard curves have been developed in the UK and the USA to indicate when *N*-acetylcysteine is indicated, and a third line has recently been proposed for high-risk patients (**Fig. 33.10**). The latter include patients taking enzyme inducing drugs e.g. antiepileptic therapy, regular alcohol consumers and malnourished patients. An extended role for this drug in acetaminophen-induced acute liver failure was first suggested by a retrospective study of 100 patients showing that *N*-acetylcysteine given 10–36 h after drug ingestion was associated with a significant decrease in mortality (37 versus 58%), despite the development of comparable prolongations in prothrombin time. These observations were confirmed in a prospective

General management measures in acute liver failure		
Measure	Controlled trials	Comment
Corticosteroids	Yes	Old study but no benefit seen
Interferon	No	Initial positive report but no benefit seen in larger study
Insulin and glucagon	Yes	Early anecdotal suggestion of benefit but not confirmed in controlled study
Prostaglandin E <sub>1</sub>	Yes	No survival benefit when controlled trial was performed
<i>N</i> -acetylcysteine	Yes	Beneficial in acetaminophen cases, broader use not yet confirm in clinical studies
Bowel decontamination	Yes	Inconclusive results
Charcoal hemoperfusion	Yes	No survival benefit, re-emerging as component of some extracorporeal circuits incorporating hepatocytes
Resin hemoperfusion	No	Preliminary studies only, inconclusive
Extracorporeal circuits	Yes	One large negative trial

**Table 33.9 General management measures.** Almost all specific treatments applied to acute liver failure have been found to be ineffective.



**Figure 33.11 N-acetylcysteine after acetaminophen intoxication.** The nomogram shows the blood levels that indicate a high risk of hepatotoxicity after acetaminophen ingestion in the UK and the USA. The lowest line is used in high-risk patients including the malnourished, chronic alcohol consumers and patients taking enzyme inducing drugs (e.g. antiepileptics).

controlled trial of 50 patients, in which patients in the active treatment limb were commenced on *N*-acetylcysteine 36–80 h after drug ingestion. Although adequate studies of the overall effect of *N*-acetylcysteine on survival have not been reported for other etiologies of acute liver failure, it is increasingly used empirically in ischemic and toxic insults, as well as a modulator of cellular oxygen metabolism. Penicillin, and possibly silymarin, should be added at the earliest opportunity to the standard supportive measures in patients with *Amanita phalloides* toxicity.

Charcoal hemoperfusion was extensively assessed as a system to reduce circulating toxins. A study of 76 patients suggested a significant increase in survival with hemoperfusion when it was commenced while the patient was in grade 3 rather than grade 4 encephalopathy. Subsequently, controlled trials to assess the efficacy and optimal duration of hemoperfusion were carried out in 137 patients. Seventy-five patients with grade 3 encephalopathy were randomized to receive 5 or 10 h of hemoperfusion daily, and there was no difference in survival between the two groups (51.3 versus 50.0%). Sixty-two patients with grade 4 encephalopathy were randomized to receive either no hemoperfusion or 10 h of hemoperfusion daily, and survival rates

were also similar in the two groups (39.3 versus 34.5%). Even though these studies included 137 patients, it has been argued that the numbers may have been inadequate to detect a significant clinical effect, and this illustrates the difficulty in designing controlled studies in acute liver failure. Other non-biological systems that have been inconclusively evaluated include plasmapheresis and albumin dialysis (MARS).

A number of biological extracorporeal bioartificial liver support systems have been developed for clinical assessment in recent years. The hybrid bioartificial liver (BAL) intermittently exposed separated plasma to a cartridge containing porcine liver cells attached to collagen-coated microcarriers after the plasma has been passed through a charcoal column designed to remove substances toxic to the hepatocytes. A controlled trial failed to establish survival benefit in the overall population, although there were indications that subgroups of patients including acetaminophen-induced hepatotoxicity and hyperacute/acute liver failure benefited from therapy with BAL. Another extracorporeal liver assist device (ELAD) continuously exposed whole blood to cartridges containing approximately 200 g of well-differentiated human hepatoblastoma cells. The pilot studies performed suggested a benefit in successfully bridging patients to transplantation but definitive trials have not been performed. Ex-vivo perfusion using pig or human livers that are unsuitable for transplantation has also been described as a bridge to transplantation in a small number of patients. Hepatocyte transfusions are also attracting some interest as a method of augmenting liver function, but as yet no significant clinical outcomes have been reported with this approach.

### Liver transplantation

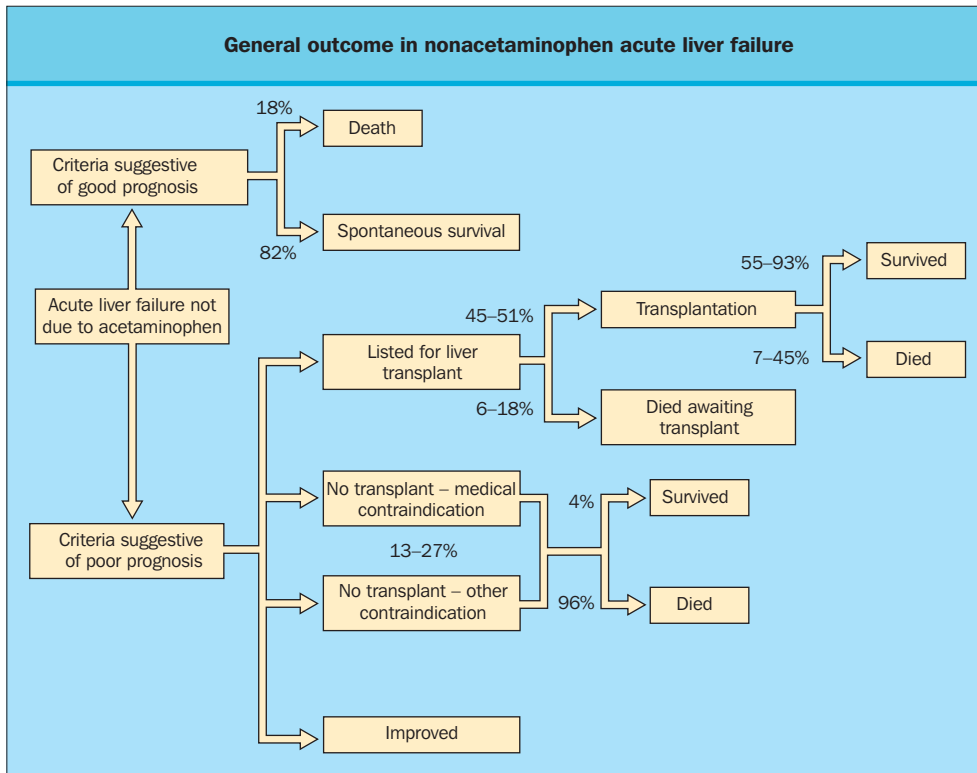
Liver transplantation has revolutionized the management of acute liver failure by offering a lifeline to those cases that have a poor prognosis despite all the advances that have occurred in supportive care. It is now an integral part of the management of acute liver failure.

### Grafts and graft allocation

Acute liver failure now accounts for 5% of all liver transplant activity in the USA and 11% in Europe. Most donor organ allocation systems prioritize acute liver failure; in the United Network for Organ Sharing (UNOS) system it is classified as status 1 with regional sharing of organs for patients in this status and in the UK it is the only primary diagnosis on the 'super-urgent' registration list. Living-related donation is well established in Asia, where cadaveric donation is limited, and is being increasingly used in Western countries. Series from specialized centers in the US, continental Europe and the UK are remarkably consistent in showing that 45–51% of patients admitted with acute liver failure underwent liver transplantation (Fig. 33.11). In 13–27% of cases, liver transplantation was considered to be contraindicated at the time of admission, and between 6 and 18% of patients were removed from the waiting list or died before an organ became available. The UK figures exclude cases due to acetaminophen and only 7–9% of these underwent transplantation.

The waiting times for donor organs are pivotal in determining the policy on liver transplantation in the management of acute liver failure. The USA and UK allocation systems now result in the majority of patients being transplanted within 48 h of





**Figure 33.12** The general outcome for patients who have acute liver failure not due to acetaminophen.

registration. The waiting times also influence the attitude to the use of ABO mismatched grafts, fatty livers and other suboptimal potential grafts. ABO incompatible grafts were used in 11% of adult transplants and 18% of pediatric transplants in the USA. A French group achieved a 93% transplantation rate by adopting a policy of using the first available organ irrespective of quality or blood group and achieved 1- and 5-year survival rates of 68 and 61%, respectively. However, the European data show that the graft survival rate for ABO incompatible liver grafts used for emergency transplantation was only 32% at 1 year, compared with 54 and 49% for matched and unmatched but compatible grafts, respectively.

#### Patient selection

Optimal results require the identification of suitable candidates as soon as possible in the disease process. The development of encephalopathy in association with a progressive coagulopathy is the most commonly used selection criterion. However, survival rates of 39–67% are being achieved with medical management for some etiologies. In these categories, there is considerable risk of performing unnecessary transplants based on this approach. The King's College Hospital criteria indicating a poor prognosis have been used as a method of selecting patients for liver transplantation. This model is sensitive to the urgency with which patients with a poor prognosis need to be identified, is considered user-friendly, does not rely on the development of advanced disease before transplantation is indicated, and maximizes the time available to obtain a suitable donor organ. In addition to recognizing the heterogeneity within the syndromes of acute liver failure, this prognostic model is also sensitive to the paradox that young people with rapidly progressive disease

(hyperacute liver failure) have the best chance of survival without transplantation. Criticisms of this model include its apparent rigidity, non-universal applicability and disservice to marginal cases in the light of the dramatic improvements seen in survival rates after liver transplantation for acute liver failure.

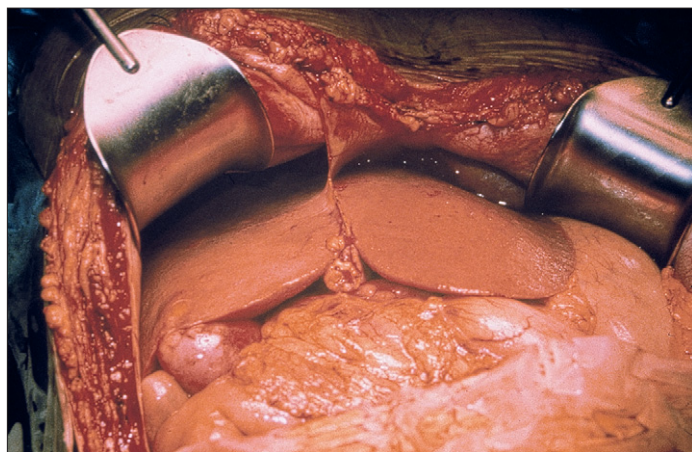
Alternatively, it has been suggested that all patients with acute liver failure should be listed for transplantation and the decision to proceed made in the light of the clinical situation when an organ becomes available. This approach has the attraction of maximizing the delivery of transplantation to this patient population, but it increases the risk of unnecessary transplantation and may result in too much of a valuable resource being unnecessarily diverted to patients with this condition who would otherwise survive without transplantation. Other approaches use factor V levels, liver histology, computed liver volume and unbound plasma Gc levels. A review of the sensitivity and specificity indices for the systems currently in use indicates that the perfect model remains elusive.

The deselection of patients whose clinical condition has deteriorated to the extent that proceeding with transplantation is futile is difficult but important so as not to waste a valuable resource. No definite role has yet been confirmed for intracranial pressure monitoring or cerebral oxygen utilization in deciding the vexed question of when potentially irreversible brain damage should preclude the use of liver transplantation in these patients. Deselecting patients with cerebral perfusion pressures < 40 mmHg for 2 h or more has been advocated and has resulted in a reduced incidence of failure of neurologic recovery after transplantation. However, two studies have documented survival both with and without transplantation in such patients. Accelerating inotropic requirements, uncontrolled

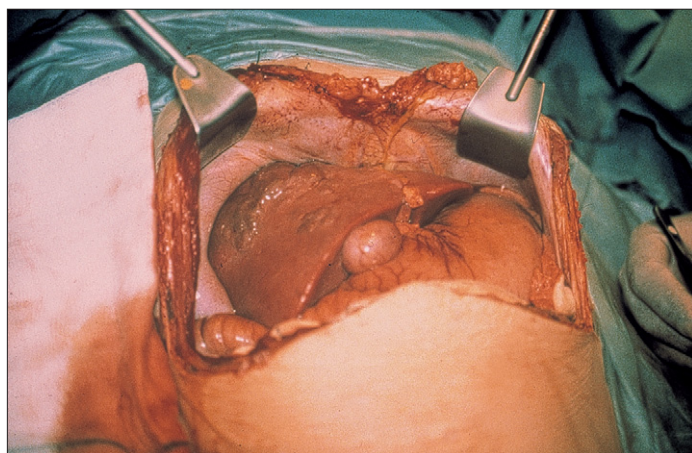
sepsis and severe respiratory failure are other imprecise contraindications to transplantation. These contraindications to transplantation are age sensitive as younger patients are more resilient and more likely to reverse these complications after liver transplantation.

### Transplant operation

The surgical aspects of standard liver transplantation are not as challenging as theoretical considerations might suggest. The repletion of coagulation factors and platelets prior to surgery adequately reverses the clinical coagulopathy in most cases, and intraoperative blood losses are remarkably low. This is in part a reflection of the poor correlation between studies of coagulation factors and the risk of surgical bleeding, but also the absence of portal hypertension. The liver is usually pale and shrunken but the presence of regenerative nodules protruding from the surface of the liver should not persuade the surgeon to abandon transplantation (Figs 33.12 and 33.13). Cerebral edema persists during, and for up to 12 h after, a technically successful transplant operation, and presents one of the major management issues. The dissection phase of the operation and the period immediately



**Figure 33.13 Transplant operation.** The appearance of a liver in a patient undergoing liver transplantation for acute liver failure 5 days after an acetaminophen overdose. The liver is very pale.

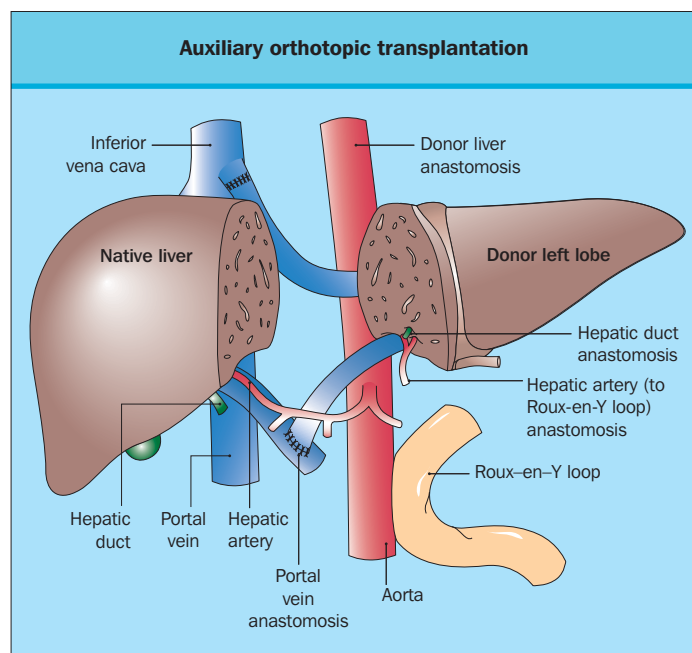


**Figure 33.14 Transplant operation.** The appearance of a liver with regeneration nodules in a patient undergoing liver transplantation for subacute liver failure following seronegative hepatitis.

after reperfusion are the times of greatest risk for increases in intracranial pressure and decreases in cerebral perfusion pressure. In contrast, these parameters often improve dramatically during the anhepatic phase of the transplant operation. Cerebral autoregulation is restored within 48 h of successful transplantation. Monitoring of intracerebral pressure and cerebral perfusion pressure should continue during this period in all patients susceptible to cerebral edema.

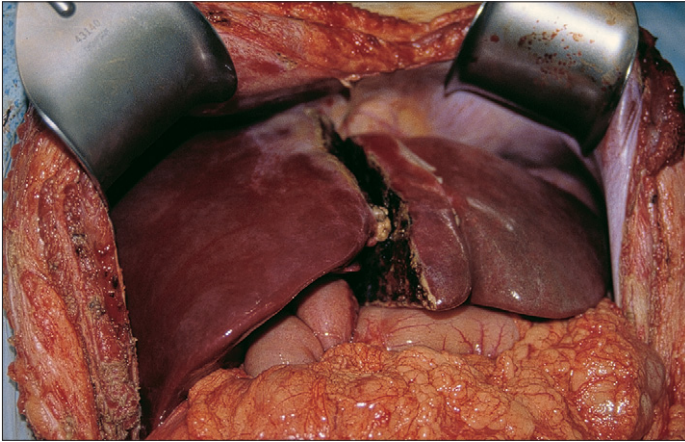
The relative ease of transplant surgery has permitted the use of innovative surgical techniques in these patients. Auxiliary liver transplantation has been pioneered for patients with the potential to recover normal liver function and morphology. In theory, it combines the advantages of transplantation with the ability to withdraw immunosuppression when regeneration has been demonstrated in the native liver. In practice, the prediction of regenerative capacity to normal morphology has not been easy and the perioperative biopsy of the native liver has not been particularly helpful in this regard. Auxiliary liver transplantation may be heterotopic or orthotopic replacing either the right lobe, left lobe or left lateral segment (Fig. 33.14). Current opinion favors the orthotopic approach as it preserves anatomic relationships, maintains autoregulation of portal venous blood flow and is associated with better venous outflow from the graft (Fig. 33.15). Functional studies using radionuclide scans and liver histology are used to assess the degree of regeneration in the native liver (Figs 33.16–33.19). When the native liver returns to normal morphology and function, the auxiliary transplant is sacrificed either passively by withdrawing immunosuppression or actively with surgical resection.

The European data indicate that the outcome of auxiliary liver transplantation for acute liver failure is similar to the overall results achieved with total hepatectomy followed by

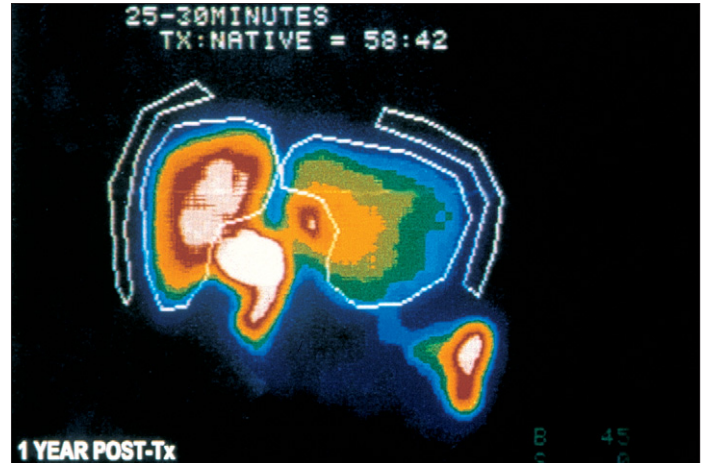


**Figure 33.15 Auxiliary orthotopic transplantation.** The liver can be subdivided to allow transplantation of the right lobe, left lobe or left lateral segment. Most auxiliary transplants use either the right or left lobe.

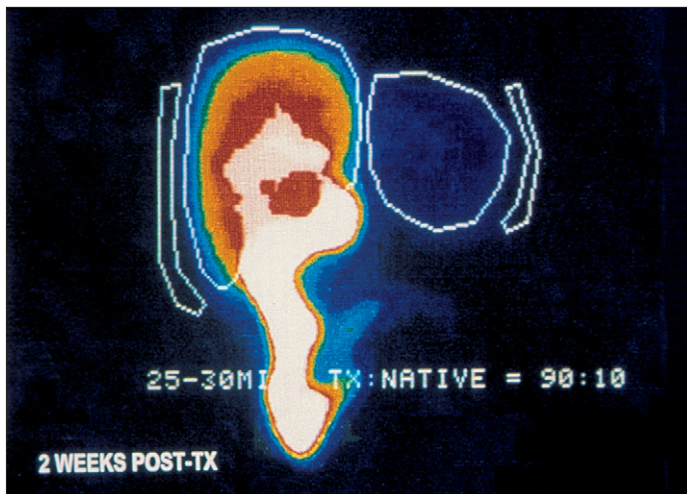




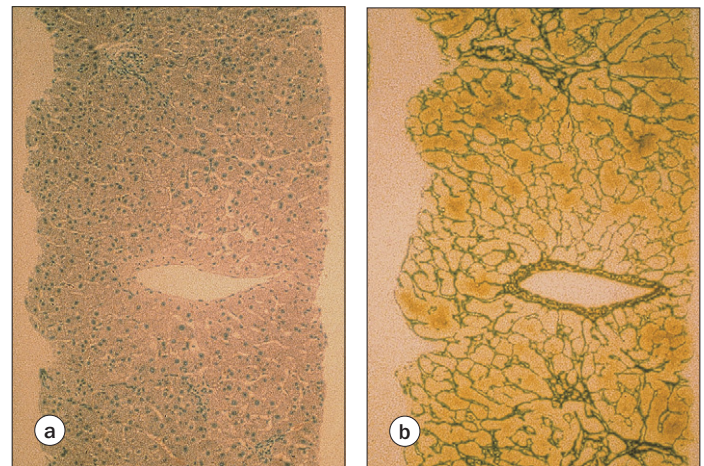
**Figure 33.16 Auxiliary transplant.** The appearance at the end of the operation with a perfused right auxiliary graft in a patient who has acetaminophen-induced acute liver failure.



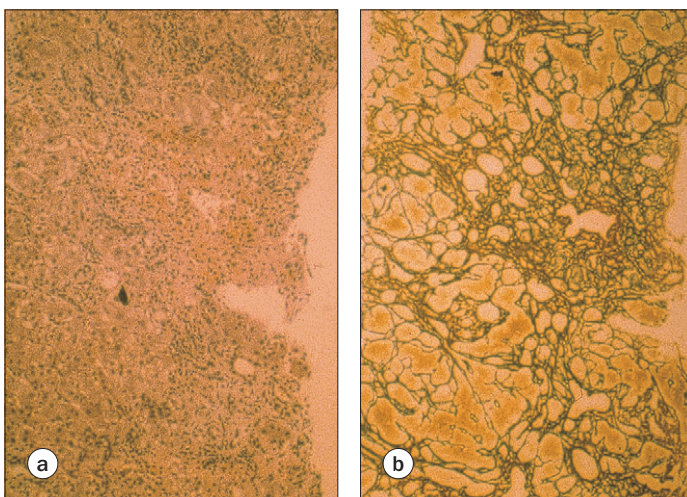
**Figure 33.19 Auxiliary transplant.** Radionuclide scan showing 42% of function in the native left lobe 1 year after auxiliary transplantation.



**Figure 33.17 Auxiliary transplant.** Radionuclide scan showing 90% of function in the right lobe auxiliary transplant 2 weeks after transplantation.



**Figure 33.20 Auxiliary transplant.** Histologic appearance of the native liver 1 year after auxiliary transplantation showing return to normal morphology.

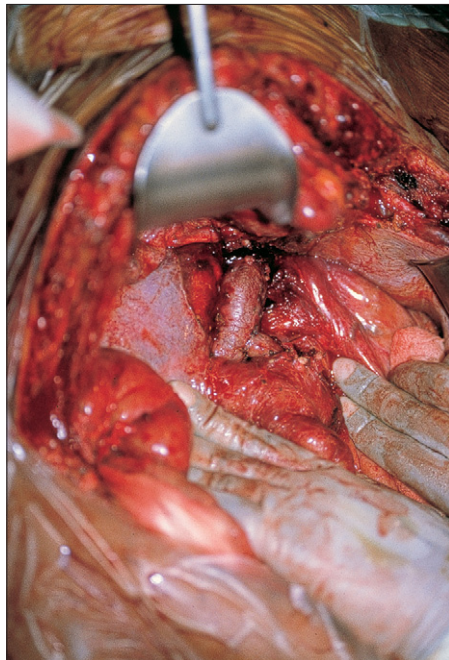


**Figure 33.18 Auxiliary transplant.** Histologic appearances of the native liver at the time of transplantation.

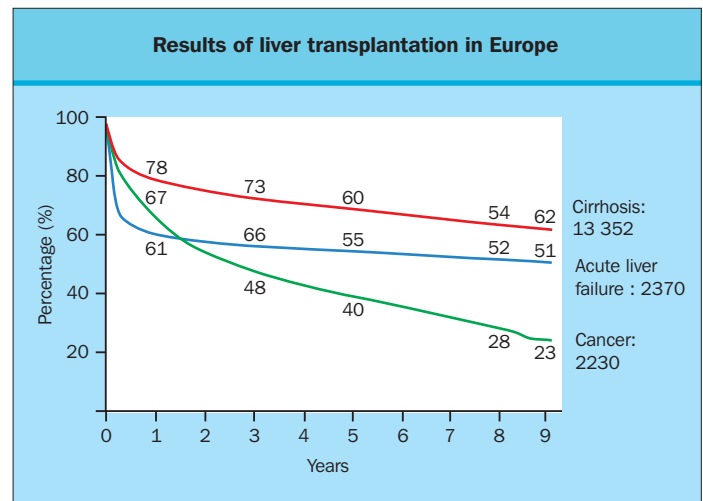
standard transplantation. The expectation is that the results of the auxiliary approach should be superior and, at present, it should be considered that the optimal indications for auxiliary transplantation are unclear. The neurologic and hemodynamic benefits derived from the devascularization or removal of the diseased liver are completely or partially lost with this approach, and in unstable patients, standard orthotopic transplantation may yield higher survival rates. In the short term, patients with subacute liver failure may represent better candidates for auxiliary transplantation, but it has been suggested that this group are more likely to develop fibrosis and cirrhosis in the native liver and this has been observed in 30% of medium-term survivors.

The improvement in hemodynamic and neurologic stability that follows hepatectomy was the justification for the development of the two-staged procedure whereby hepatectomy precedes liver transplantation by a variable period of time. The diseased liver is explanted and a portocaval shunt is fashioned pending the availability of a liver graft (Fig. 33.20). It is often used as an act of desperation for very unstable patients in the





**Figure 33.21 Hepatectomy.** The liver has been removed in an unstable patient who has acute liver failure to reduce the toxic effects of the vascularized necrotic liver. A portocaval shunt has been fashioned. The patient received a liver transplant 17 h later and survived.



**Figure 33.22 Results of transplantation.** The results of transplantation for acute liver failure in Europe are inferior to those of elective transplantation, except for patients who have malignant disease at the time of surgery.

hope that a liver graft will become available. The longest anhepatic time in an ultimate survivor was 21 h and 45 min in a series of cases predominantly carried out for liver graft failure rather than primary acute liver failure. The risk of sepsis, especially fungal infection, increases as the duration between the two procedures is extended, and was the major cause of death in the largest series described. In that experience, transplantation was achieved in only 59% of cases, and some centers using this technique restrict its application to situations where the availability of an organ appears likely within 24 h of the hepatectomy.

The profile of sepsis, including fungal infection, seen in acute liver failure extends into the post-transplant period and is further aggravated by the requirement for immunosuppressive therapy. Patients transplanted for acute liver failure, together with those undergoing retransplantation and those with prolonged stays in intensive care units, routinely receive antimicrobial prophylactic regimens targeted at such high-risk patients. These regimens often include aggressive antifungal prophylaxis with lipid bound amphotericin. Renal function may improve dramatically in the immediate postoperative period but some patients need renal support for many weeks after successful transplantation. The latter scenario is especially common after transplantation for acetaminophen-induced acute liver failure. The potent immunosuppressive agents and some commonly used antimicrobials are potentially nephrotoxic and the use of these agents should be modified to enhance the return of renal function.

### Results

The overall survival rates following transplantation are 63% in the USA and 61% in Europe (Fig. 33.21), and most individual centers that have reported their experience had survival rates falling within the 59–79% range. An exceptional report of a 93% survival rate from one center in the USA suggests that better results are attainable. Patients receiving liver transplants for acute liver failure (median age 28 years) are younger those under-

going elective transplantation (median age 44 years). The age of the patient influences selection for liver transplantation in acute liver failure more than for elective transplantation. The European data indicate that the 1- and 5-year survival rates are 51 and 42%, respectively, for patients aged 60 years or over. The cumulative effects of age and multisystem disease lead to survival rates that are lower and at a level that questions whether it is justifiable to extend the valuable donor organ resource to these patients. However, these figures compare with survival rates of 61 and 55% at the 1 and 5 years for the overall population of patients receiving liver transplants for acute liver failure.

The factors that influence outcome after liver transplantation are multiple. The patient's age has already been addressed. The etiology of the underlying disease correlated with outcome in some centers. The best results were achieved for transplantation for Wilson disease and the worst for idiosyncratic drug reactions. Patients who survived liver transplantation for acetaminophen-induced liver failure received the graft around the fourth day after drug ingestion, as compared with the sixth day in those who succumbed. No patient transplanted on or after the seventh day survived. This reflects the role of sepsis in propagating the continued deterioration in these patients and the inability of liver transplantation to ameliorate this complication. Survivors after transplantation also tend to get the graft sooner after admission to specialist units, and in one study the average waiting time for survivors was 41 h as compared with 67 h for those who died after transplantation. The survival rate decreases with progression through the grades of encephalopathy at the time of transplantation; in one series the figures were 90% for grade 1, 77% for grade 2, 79% for grade 3 and 54% for grade 4. As with liver transplantation in general, renal function correlated with outcome and serum creatinine levels above 200  $\mu\text{mol/L}$  or 1.8 mg/dl were associated with a poorer outcome. Acidosis and Apache III score at the time of transplantation were good indicators of the severity of the patient's illness and correlated with outcome. However, none of these parameters was sufficiently discriminatory to use as criteria to disqualifying individual cases from transplantation.

## Management of specific complications

### Neurologic

The specific management options for encephalopathy are limited. Patients with subacute liver failure may benefit from dietary protein restriction, lactulose or bowel decontamination. However, these approaches are ineffective in the treatment of the more rapidly progressive encephalopathy characteristic of the hyperacute and acute syndromes. No convincing data have emerged to support the use of branched chain amino acids. Clinical and electroencephalogram (EEG) assessed responses to flumazenil have been transient and unpredictable and have not indicated a role for this drug in the management of the encephalopathy of acute liver failure. A transient improvement in encephalopathy has also been noted during many of the studies of extracorporeal liver support devices, but ultimately without survival benefits.

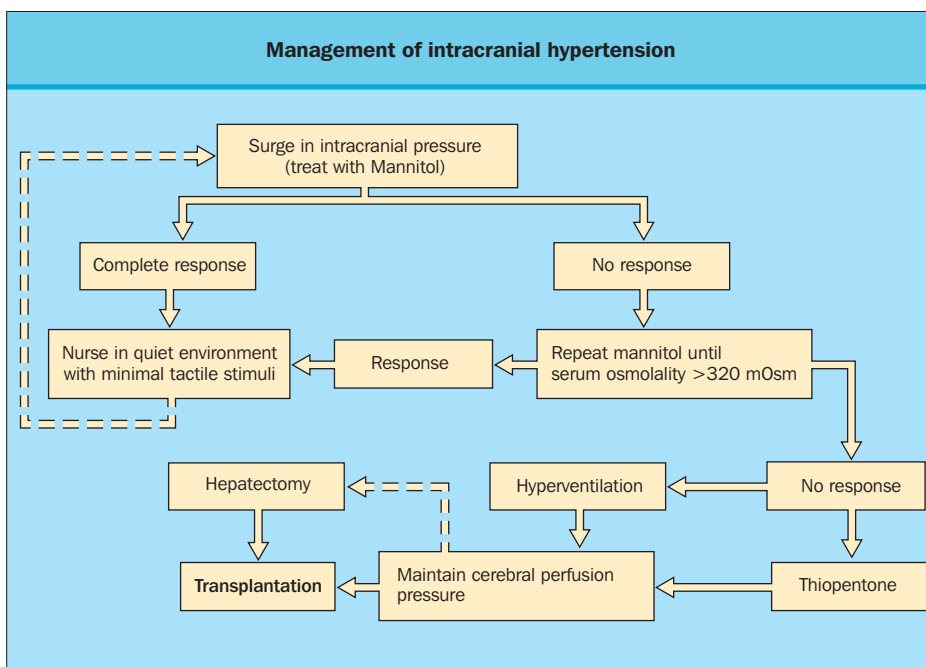
Mannitol is the mainstay of treatment of surges in intracranial pressure that may compromise brainstem function (**Fig. 33.22**). The overall beneficial effect of mannitol was established in a controlled trial and the mechanism of action was considered to be its property as an osmotic diuretic. It has also been suggested that the rapidity of action of mannitol is more consistent with its function of increasing cerebral blood flow being the basis for the therapeutic response. A rapid bolus of 0.3–1.0 g/kg is recommended to achieve the maximal diuretic effect, and in anuric patients a diuresis is simulated by ultrafiltrating three times the administered volume over the subsequent 30 min. This process is repeated as determined by the pattern of clinical relapses until the serum osmolality exceeds 320 mOsm. More recent studies showed that the administration of mannitol was followed by an increase in cerebral blood flow associated with an increase in cerebral metabolic rate for oxygen and reduced brain lactate formation.

Sodium thiopentone (phenobarbital) was shown to control cerebral edema that had become unresponsive to mannitol in an uncontrolled study. A priming dose of between 185 and 250 mg

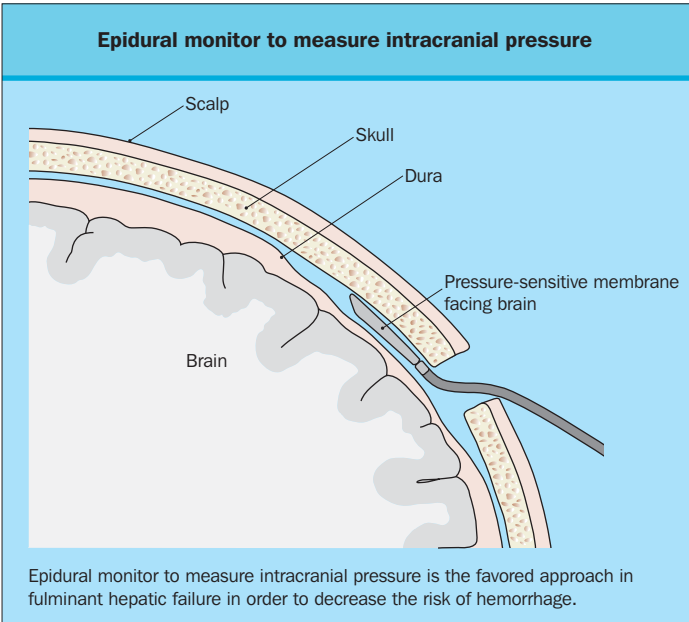
(median 250 mg) given over 15 min was followed by a 4-h infusion of between 50 and 250 mg/h. Another study documented a 73% response rate to barbiturates, but this was followed by an 80% relapse rate after thiopentone was withdrawn. Thiopentone has neither been subjected to a controlled trial nor shown to improve the survival rate. Its role is limited by loss of efficacy and hemodynamic instability following its administration. There is an additional concern that the use of thiopentone results in an increase in infective complications in those surviving this phase of the illness. Acute, but not chronic, hyperventilation was reported to be of benefit. Nevertheless, hyperventilation to partial pressure of CO<sub>2</sub> levels below 25 mmHg is routinely incorporated as a first line treatment in protocols in the USA. Acute hyperventilation using low-tidal volumes (to prevent increases in intrathoracic pressure reducing venous return from the head) combined with 80–100% inspired oxygen may control surges in intracranial pressure that are unresponsive to mannitol and thiopentone. Hypothermia and hypertonic saline infusions have also been shown in small studies to be useful adjuncts in the management of intracranial hypertension.

In the later stages of the neurologic complications, the emphasis of management changes to the preservation of cerebral perfusion pressure, increased oxygen delivery to the brain and manipulation of the neuronal microcirculation to promote cerebral oxygen extraction. The patient is now nursed with the trunk at 0–10 degrees to the horizontal. The options for increasing the mean arterial pressure and consequently improving cerebral perfusion pressure are outlined in the section dealing with hemodynamics. These adjustments are made to maintain a cerebral perfusion pressure greater than 50 mmHg where possible. If it becomes difficult to maintain adequate cerebral perfusion pressure, spontaneous recovery is unlikely without liver transplantation, and hepatectomy is useful to secure transient improvement.

N-acetylcysteine and prostaglandin I<sub>2</sub> infusions have been shown to increase cerebral blood flow and cerebral metabolic



**Figure 33.23 Management of intracranial hypertension.** The therapeutic alternatives in the management of this serious complication of acute liver failure.



**Figure 33.24 Epidural monitor to measure intracranial pressure.** This is the favored approach in acute liver failure in order to decrease the risk of hemorrhage.

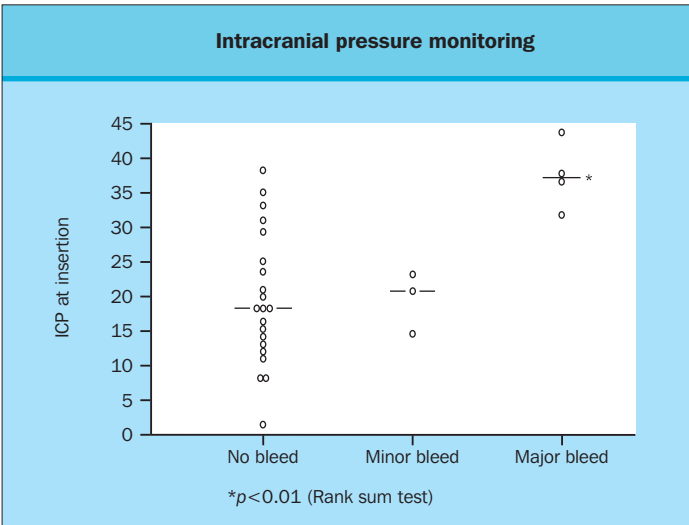
rate for oxygen and are considered to improve microcirculatory stability. Occult seizure activity may contribute to neurologic instability in patients with grade 4 encephalopathy. Phenytoin and diazepam are effective therapies, despite the theoretic consideration that the latter may aggravate the underlying encephalopathy. There is no established role for other diuretics or antihypertensive agents in the management of cerebral edema in acute liver failure.

The use of intracranial pressure monitoring (Fig. 33.23) is controversial and has not been subjected to clinical trials. Early detection of cerebral edema and the facility to constantly monitor this complication help to optimize therapeutic interventions. Intracranial pressure monitoring allows earlier and more accurate detection of pressure changes, especially in the ventilated patient in whom most of the clinical signs are masked. It also facilitates careful monitoring of the intracranial pressure during high-risk therapeutic interventions, such as hemodialysis and tracheal suctioning. It is also considered valuable during orthotopic liver transplantation as increases in intracranial pressure often occur during the dissection and reperfusion phases of the transplant operation. The main points of the arguments for and against intracranial pressure monitoring are outlined in Table 33.11. The most commonly used system places transducers on or through a small nick in the dura. The risk of intracranial hemorrhage is a deterrent, although the studies that have systematically addressed safety have favored the use of intracranial pressure monitoring, especially using fiberoptic extradural or subdural devices. Proponents argue that it has been shown to be effective and relatively safe, despite the attendant coagulopathy. Epidural transducers were associated with a low complication rate at 3.8%, but subdural and parenchymal devices had higher complication rates at 20 and 22% respectively.

One study has shown that high intracranial pressures at the time of insertion of the device were the main risk factor for intracranial hemorrhage, rather than the expected coagulopathy

Arguments for and against intracranial pressure monitoring in acute liver failure	
For	Against
Only way of getting effective insight into condition	Complication rate too high
Allows early diagnosis	Clinical features allow diagnosis
Drives intelligent treatment	Never proven to improve survival
Monitors effect of potentially hazardous interventions	Good management protocols prevent potentially hazardous interventions
Modern devices are easy to place	Requires neurosurgical input
Aggressive correction of coagulopathy not necessary	Aggressive correction of coagulopathy necessary
	False readings may arise and confuse management

**Table 33.10 Intracranial pressure monitoring.** The arguments for and against the use of pressure monitoring in acute liver failure are presented.



**Figure 33.25 Intracranial pressure monitoring (ICP).** The initial intracranial pressures are shown for patients developing major and minor intracranial bleeds after insertion of intracranial pressure monitors. High pressures correlated more strongly with risk than prothrombin times. None of the bleeds was fatal and two of the major bleeds were asymptomatic frontal lobe bleeds detected on scanning.

(Fig. 33.24). This suggests the routine use of intracranial pressure monitoring in all patients with hyperacute and acute (but not subacute because of the low incidence of cerebral edema) liver failure once grade 3–4 encephalopathy develops. Others advocate its use more selectively in patients with evidence of cerebral edema, or those being considered for transplantation.

**Infection**

The high incidence of bacterial and fungal infections is a significant contributor to the mortality rate in acute liver failure and has been implicated in up to 50% of deaths. Clinical trials of prophylactic antibiotics have demonstrated that systemic



antibiotics reduce the incidence of culture-positive bacterial infection by half but this strategy is associated with the emergence of highly resistant organisms in up to 10% of cases. However, this reduction in infection rates was not accompanied by a significant impact on major clinical outcomes (mortality, progression to transplantation) or economic considerations (duration of intensive care unit and hospital stay, overall cost of antimicrobials). Small bowel decontamination was not effective in altering the pattern of infection. Systemic antibiotics are recommended when infection is suspected and the precise regimens used are determined by local antibiotic policy.

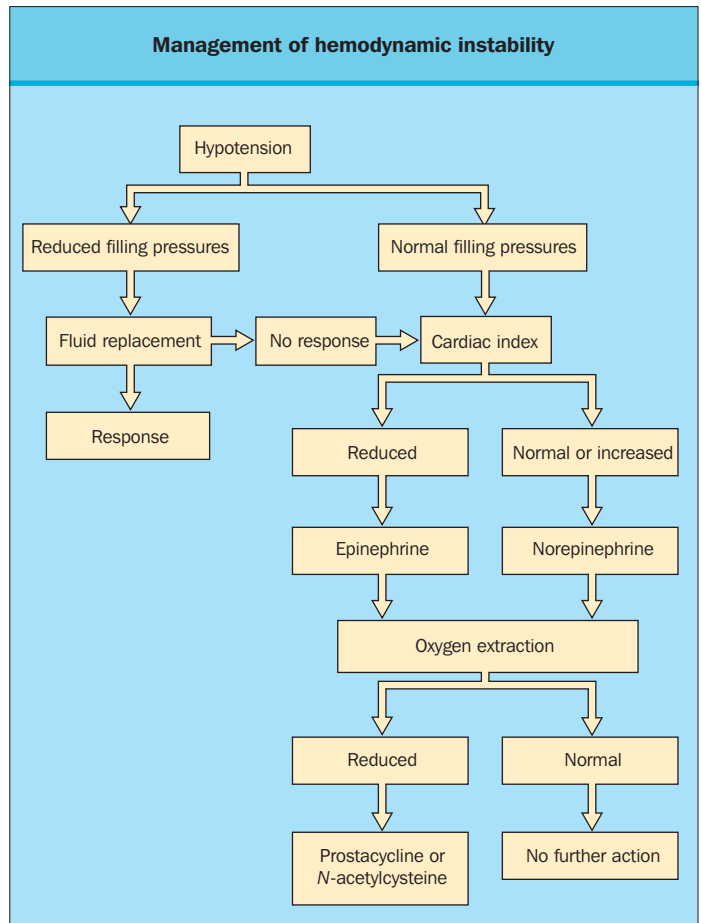
The use of prophylactic systemic antifungal therapy has not been subjected to controlled trials. It may be appropriate in patients with risk factors for systemic fungal infection like renal failure, severe cholestasis, previous or concomitant thiopentone therapy or liver transplantation. Systemic fungal infection is notoriously difficult to diagnose in the setting of acute liver failure, and a high index of suspicion is required especially in the high-risk group outlined above and in those cases where there is an arrest in the recovery of coagulation activity. The latter is a valuable sentinel marker of infection that often predates clinical manifestations by some days. *Candida* spp. account for the vast majority of fungal infections, and there are no data to show superiority of any of the available therapies.

#### Hemodynamic instability and oxygen debt

Circulatory failure is considered to be a significant contraindication to transplantation and a common mode of death in acute liver failure, often occurring against the background of sepsis and multiorgan failure. Invasive hemodynamic monitoring is routinely initiated in patients with grade 3 encephalopathy and filling pressures are normalized with appropriate combinations of colloid, crystalline fluids and blood products. Hypotension occurring despite adequate intravascular volumes (pulmonary capillary wedge pressure 10–14 mmHg) is treated with vasopressor agents, using norepinephrine if the cardiac index exceeds 4.5 L/min/m<sup>2</sup> or epinephrine if the cardiac output needs to be boosted above this threshold. The initial stabilizing dose to achieve a mean arterial pressure above 60 mmHg ranged between 0.2 and 1.8 mg/kg/min of epinephrine and 0.2 and 2.0 mg/kg/min of norepinephrine. Vasopressor agents may cause or aggravate an oxygen debt and prostacyclin infused at a rate of 5 ng/kg/min has been shown to improve parameters of oxygen metabolism (delivery, consumption and extraction ratio) when used in conjunction with both epinephrine and norepinephrine. *N*-acetylcysteine infusion (10 mg/kg/min for 15 min followed by 0.2 mg/kg/min for 4 h) caused less vasodilatation than prostacyclin, independently increased mean arterial pressure and was as effective as prostacyclin in improving oxygen metabolism. The combination of prostacyclin and *N*-acetylcysteine was more beneficial to oxygen metabolism than either drug alone (Fig. 33.25). Patients developing resistance to inotropes have been shown to have a hypoadrenal profile that responds to hydrocortisone.

#### Renal failure

The prophylactic use of dopamine has been common practice but its benefits have been challenged, especially in the setting of profound vasodilatation that is typical of acute liver failure. The appropriate clinical studies have not been performed in this



**Figure 33.26 Management of hemodynamic instability.** A care pathway for the management of hemodynamic instability.

setting. Extracorporeal renal support was required in 75% of cases of acetaminophen-induced acute liver failure and 30% of other etiologies that progressed to grade 3–4 encephalopathy. The metabolic complexity of combined liver and renal failure suggests early intervention with hemodialysis, pre-empting standard indications, is prudent. Continuous filtration or dialysis systems are associated with less hemodynamic instability and run a lower risk of aggravating latent or established cerebral edema than intermittent hemodialysis. The coagulopathy of acute liver failure does not negate the need for anticoagulation and paradoxically heparin requirements have been shown to be increased during hemodialysis. The heparin doses required to prevent platelet depletion showed considerable variation and are best determined by functional assays such as the activated clotting time. Antithrombin III supplementation in a dose of 3000 units prior to hemodialysis reduced heparin requirements. Prostacyclin infusions in doses between 2 and 5 ng/kg/min proved superior to heparin anticoagulation in continuous systems with respect to the functional duration of the filters and the hemorrhagic complications experienced.

#### Coagulopathy

Prophylactic repletion of coagulation factors with fresh frozen plasma has been practiced and requirements of up to a mean of 40 mL/kg/day to maintain the prothrombin time within 5–10 s of normal have been reported. The possible potential advantages

of reduced bleeding and infection (as a consequence of repletion of opsonins) have not been established by clinical studies. A controlled trial of fresh frozen plasma failed to demonstrate an improvement in survival, and was thought to be detrimental in a minority of patients with a consumptive coagulopathy. Fresh frozen plasma administration impedes the use of coagulation studies in the assessment of prognosis and monitoring of disease progression, and other disadvantages associated with aggressive repletion include potential fluid overload and hyperviscosity syndromes. Prophylactic fresh frozen plasma is more commonly used in anticipation of an invasive procedure e.g. insertion of cannulae or intracranial pressure monitors, or liver transplantation. There are limited data available yet on the utility of recombinant factor VIa in this condition. Thrombocytopenia is considered to an important risk factor for hemorrhage and maintenance of platelets counts above  $50\text{--}70 \times 10^9/\text{L}$  has been recommended.

Gastric protection is important and hemorrhage was reduced by prophylaxis with cimetidine in a study performed over 20 years ago. Sucralfate, which has the potential advantage of reducing gastric colonization and pulmonary infection by maintaining gastric acidity, has gained favor even though its efficacy has not been formally assessed. Likewise, the efficacy of ranitidine or proton pump inhibitors which are also widely used to protect the gastric mucosa have not been subjected to formal assessment.

## FURTHER READING

### Etiology

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O'Grady JG, Alexander GJ, Hallyar KM, et al. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology* 1989; 97:439–445. *This seminal article details a scheme to accurately determine prognosis using commonly available variables to rapidly identify which patients will likely need transplantation or not. The scheme has been repeatedly tested by other systems, and has still stood the test of time.*

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### Nutrition

Although the standard patient with acute liver failure is well nourished at the onset of the illness, it is important to institute nutrition as soon as possible. The catabolic rate increases in patients with acute liver failure and this is most apparent in those with complicating sepsis and those undergoing liver transplantation. The theoretical problems that limit nutritional options are legion – gastrointestinal ileus, desire to minimize gastrointestinal protein, difficulty maintaining isoglycemia, fluid restrictions secondary to renal failure, theoretical role of amino-acid ratios in mediating encephalopathy, difficulty handling lipids, aggravation of sepsis by intravenous feeding, etc. Despite all of these considerations, adequate nutrition can be obtained in the majority of patients. An element of enteral nutrition is desirable to help maintain the integrity of the small intestinal mucosa, and this is titrated against the volume of gastric aspirates and the development of diarrhea. Pilot studies showed that parenteral feeding is tolerated considerably better than would be expected from theoretical considerations. Lipid solutions (10%) are cleared from serum and standard amino-acid preparations do not appear to have a clinically relevant impact on the encephalopathy profile. Continuous renal support systems give good flexibility with regard to the management of fluid loads and assiduous attention to the maintenance of feeding lines keeps the septic complications within the expected pattern of frequency.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 34

## The Liver in Systemic Disease

Andrew Ross and Lawrence S. Friedman

The liver is the second largest and most biochemically complex organ in the body. Multiple interactions exist between the liver and other organ systems and, as a result, the liver is often affected by systemic disease processes. This chapter reviews liver involvement in some of these systemic diseases and is limited to those processes not covered in more detail in other chapters.

### ENDOCRINE DISORDERS

#### Diabetes mellitus

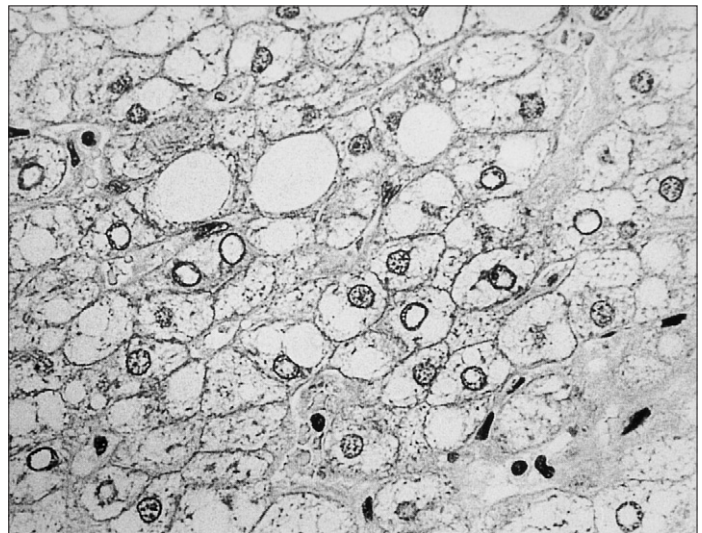
Patients with obesity and diabetes mellitus, especially type II diabetes mellitus, may present with elevated serum aminotransferase levels or massive hepatomegaly (Table 34.1). The most common histologic abnormality observed in affected persons is fatty change, or steatosis, reflected by deposition of large, macronodular fat droplets in liver parenchymal cells. These histologic findings may be found in asymptomatic obese or diabetic patients without liver enzyme elevations and fall within the pathologic spectrum of non-alcoholic fatty liver disease (NAFLD) (Fig. 34.1) (see Ch. 22).

A peculiar type of subcapsular focal fatty change is found in diabetic patients on continuous ambulatory peritoneal dialysis (CAPD). It is thought that glucose and insulin from the dialysis solutions diffuse through the hepatic capsule and accumulate in the thin rim of subcapsular hepatocytes. The high insulin con-

centrations in the subcapsular hepatocytes result in localized steatosis by inhibiting the oxidation of free fatty acids and stimulating the synthesis of triglycerides (Table 34.2).

#### Thyroid disease

The liver plays an integral role in thyroid hormone metabolism through the production of thyroxine-binding globulin (TBG),



**Figure 34.1 Glycogenated nuclei.** Microvesicular and macrovesicular fat are present.

#### Diabetes and associated liver diseases

Hepatic disease caused by diabetes
Glycogen deposition (Mauriac syndrome)
Fatty liver
Cirrhosis
Biliary tract disease
Liver diseases associated with therapy of diabetes
Viral hepatitis (needle stick)
Injury due to oral hypoglycemic drugs
Liver disease associated with diabetes
Hemochromatosis
Chronic active autoimmune hepatitis
Hepatogenic diabetes

**Table 34.1 Diabetes and associated liver diseases**

#### Causes of liver steatosis

Increased fatty acid mobilization
Obesity
Insulin deficiency
Corticosteroid excess
Starvation
Alcohol
Inflammatory bowel disease
Increased fatty acid synthesis
Alcohol
Hyperalimentation
Decreased synthesis of apoproteins
Tetracycline
Yellow phosphorus
Decreased transport and release of lipoproteins
Colchicine
Ethanol
Orotic acid

**Table 34.2 Causes of liver steatosis**



prealbumin and albumin, to which over 88% of circulating thyroid hormone is bound. In addition, the liver is the major site of peripheral thyroid hormone metabolism and is involved in the conjugation, biliary excretion, oxidative deamination and extra-thyroidal deiodination of thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) and the conversion of  $T_4$  to reverse  $T_3$  ( $rT_3$ ). Thyroid hormones are also important for normal hepatic function and bilirubin metabolism. The expression of low-density lipoprotein (LDL) receptors and the activity of hepatic enzymes that are important in lipid metabolism are regulated, in part, by thyroid hormone. In addition, thyroid hormone appears to play an important role in regulating the activity of uridine 5'-diphosphate (UDP) glucuronosyltransferase, the hepatic enzyme that is primarily responsible for the conjugation of bilirubin prior to excretion.

### Hyperthyroidism

Liver injury is common in patients with thyrotoxicosis and can be hepatocellular or cholestatic (Table 34.3). Increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels have been reported in up to 37% of patients with thyrotoxicosis who have no overt signs of hepatic impairment. The liver enzyme elevations are thought to result from an increase in hepatocyte oxygen requirements without a compensatory increase in hepatic blood flow. Liver biopsy specimens in patients with mild hyperthyroidism-related hepatic injury may reveal lobular inflammation with an infiltrate

consisting of polymorphonuclear leukocytes, eosinophils and lymphocytes, hepatocyte nuclear changes, and Kupffer cell hyperplasia. A small percentage of patients may exhibit more progressive injury with centrilobular necrosis and perivenular fibrosis. Clinically, these patients typically present with self-limited hepatitis, but fulminant hepatic failure has been described in patients with severe thyrotoxicosis.

Elevated serum levels of alkaline phosphatase have been described in up to 64% of patients with thyrotoxicosis, but only 17% have an elevated  $\gamma$ -glutamyltranspeptidase level and 5% an elevated total bilirubin level. Liver biopsy specimens from patients with cholestatic thyrotoxicosis-induced liver injury reveal non-specific lobular inflammation with intrahepatocytic cholestasis. Jaundice is rare in patients with thyrotoxicosis, and its presence should prompt a search for other complications of hyperthyroidism, such as cardiac failure, or sepsis.

It is unclear whether hepatic injury in patients with thyrotoxicosis is an effect of thyroid hormone on hepatocytes alone or the result of associated conditions such as congestive heart failure, infection and malnutrition. In all likelihood, the cause is a combination of these factors. In the majority of cases, the hepatic abnormalities reverse following restoration of a euthyroid state.

### Hypothyroidism

The clinical picture of hypothyroidism can be confused with advanced chronic liver disease because of similarities in presenting

Hepatic abnormalities associated with various thyroid conditions and therapies			
Condition	Clinical and laboratory findings	Liver biopsy findings	Outcome
Thyrotoxicosis	Elevated aminotransferase levels (in up to 37% of patients); Elevated serum alkaline phosphatase levels (in up to 64% of patients)	Lobular inflammation; hepatocytes nuclear changes; Kupffer cell hyperplasia  Centrilobular necrosis and perivenular fibrosis in rare cases  Non-specific lobular inflammation; intrahepatocytic cholestasis	Laboratory abnormalities and liver biopsy findings are reversible in the majority of patients with the restoration of the euthyroid state  Fulminant hepatic failure has been reported
Hypothyroidism	Elevated serum AST levels, myalgias, fatigue; elevated serum bilirubin levels; ascites	Centrilobular fibrosis in patients with ascites	Clinical findings and laboratory abnormalities are usually reversible within weeks of the initiation of thyroid replacement therapy
Propylthiouracil therapy	Elevated serum AST and ALT levels (up to 30% of patients)	Non-specific hepatocellular injury  Hepatocellular necrosis in less than 1% of patients	Hepatotoxicity is typically dose-related; full recovery occurs in the majority of patients  Persistent hepatitis occurs in less than 1% of patients  Fulminant hepatic failure has been reported
Methimazole and carbimazole therapy	Rare elevations in serum bilirubin and alkaline phosphatase levels	Intrahepatic cholestasis	Liver test abnormalities and biopsy findings may persist after therapy is discontinued

**Table 34.3 Hepatic abnormalities associated with various thyroid conditions and therapies.** ALT, alanine aminotransferase; AST, aspartate aminotransferase

symptoms and laboratory findings (Table 34.3). Myalgias, fatigue, muscle cramps and an elevated AST level (resulting from myopathy in patients with hypothyroidism) are findings that are common to both liver disease and hypothyroidism. In addition, myxedema coma and ascites with a high serum-ascites albumin gradient (SAAG) can be seen in hypothyroidism. The cause of ascites in patients with hypothyroidism remains in dispute. Although it has long been thought that ascites in patients with hypothyroidism results from right-sided heart failure, right heart pressures are often normal in these patients. However, histologic demonstration of central congestive fibrosis of the liver in patients with myxedema ascites supports a role for right-sided heart failure as an etiology. An alternative explanation for ascites in patients with severe hypothyroidism is enhanced vascular endothelial permeability. Regardless of the underlying pathophysiology, ascites in patients with hypothyroidism typically resolves with thyroid replacement therapy.

Cholestatic jaundice has been described in several case reports of patients with hypothyroidism. Gallstones in patients with hypothyroidism may result from reduced bile flow, hypercholesterolemia, and gallbladder hypotonia. Hepatic dysfunction associated with hypothyroidism typically resolves within weeks of initiation of thyroid replacement therapy.

#### **Liver injury associated with antithyroid therapy**

Propylthiouracil (PTU) can cause hepatocellular injury, which occasionally may be severe, sometimes resulting in hepatic failure or death. Elevated serum AST and ALT levels occur in up to 30% of patients taking PTU, and the effect appears to be dose-related, with peak aminotransferase levels occurring soon after the initiation of therapy (Table 34.3). The elevated aminotransferase levels typically fall following a reduction in the dose of PTU, and the majority of patients experience a full recovery after discontinuation of the drug. In less than 1% of patients, hepatitis persists, with evidence of hepatocellular necrosis on liver biopsy. The hepatitis is an idiosyncratic reaction to PTU that typically occurs in women younger than 30 years of age during the first few months of therapy. Patients usually recover completely after therapy is discontinued, but fulminant hepatic failure requiring liver transplantation has been reported. Patients who experience asymptomatic, mild ( $= 2 \times$  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.

Methimazole and carbimazole cause liver test elevations less frequently than does PTU (Table 34.3). Hepatotoxicity from these agents is typically cholestatic, with elevations in serum bilirubin, alkaline phosphatase, and  $\gamma$ -glutamyltranspeptidase levels.

#### **Thyroid abnormalities resulting from liver disease**

Thyroid test abnormalities are common in patients with liver disease. Patients with cirrhosis often have a reduced level of total and free  $T_3$  with an elevated level of  $rT_3$ , reflecting the 'sick euthyroid' state. The changes result from a decrease in the activity of deiodinase type 1, thereby leading to reduced conversion of  $T_4$  to  $T_3$ . In turn,  $T_4$  is converted to  $rT_3$ . This sequence is regarded as an adaptive hypothyroid state that reduces the basal metabolic rate within hepatocytes and preserves body protein stores.

Patients with acute hepatitis and fulminant hepatic failure initially have increased serum levels of total  $T_4$ ; this is attributed to an increased synthesis of TBG as an acute-phase reactant. Levels of free  $T_4$  remain normal. With increasing severity and duration of liver disease, total  $T_4$  levels may decrease because of reduced hepatic TBG synthesis. Levels of  $T_3$  may vary, but the ratio of free  $T_3$  to free  $T_4$  correlates inversely with the severity of liver disease.

Other forms of chronic liver disease may be associated with thyroid disease. Up to 25% of patients with primary biliary cirrhosis (PBC) have associated autoimmune hypothyroidism. Thyroid dysfunction is also common in patients with autoimmune hepatitis: Grave disease occurs in up to 6% of patients with autoimmune hepatitis, and autoimmune thyroiditis occurs in up to 12%. Patients with primary sclerosing cholangitis also have an increased incidence of Hashimoto thyroiditis, Grave disease and Riedel thyroiditis. Antiviral therapy of chronic hepatitis B and C with  $\alpha$ -interferon and pegylated interferon has been associated with both hypothyroidism and hyperthyroidism. Up to 10% of patients with hepatitis C treated with  $\alpha$ -interferon develop thyroid dysfunction, presumably as a result of the induction of antithyroid and antithyrotropin antibodies by interferon.

## **RHEUMATIC DISEASES**

The incidence 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 abnormalities. In addition, the medications used to treat collagen vascular disorders may cause hepatotoxicity, and distinguishing between drug-induced liver disease and liver disease associated with the underlying systemic rheumatic disease may be difficult.

### **Systemic lupus erythematosus**

Hepatic involvement in systemic lupus erythematosus (SLE) has been difficult to characterize. Initially, hepatic manifestations of SLE were thought to be rare. However, a retrospective observational study performed by Runyon et al (1980) described hepatomegaly in up to 39% of 238 patients with SLE, with associated jaundice in 24%. Overall, 21% of these patients had abnormal liver chemistries 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 in serum of smooth muscle antibodies and anti-mitochondrial antibodies in patients with autoimmune hepatitis; both antibodies are rare in SLE. This distinction is important because patients with autoimmune hepatitis typically have more serious, progressive liver disease as compared to patients with hepatic involvement in SLE. Coexisting liver disease such as viral hepatitis and primary biliary cirrhosis can often be seen in patients with SLE. Findings in two retrospective studies and a prospective study describing the association between SLE and liver disease are summarized in **Table 34.4**.

Hepatomegaly is the most consistent reported clinical finding in patients with hepatic manifestations of SLE. Jaundice and

Liver findings in systemic lupus erythematosus (SLE)	
Number of patients	551
Number with evidence of liver disease	146 (26.5%)
Etiology	
Drug-related	50 (9%)
Liver disease not related to SLE	17 (3%)
Venous congestion	8 (1.5%)
Unknown	72 (13%)
Clinically significant liver disease	22 (4%)
Death due to liver disease	3 (<1%)

Table 34.4 Liver findings in systemic lupus erythematosus (SLE)

splenomegaly have also been described. Non-specific liver chemistry 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 corticosteroid use or other risk factors for steatosis. Additional histologic findings include granulomatous hepatitis, centrilobular necrosis, chronic hepatitis and cirrhosis.

Patients with SLE are especially susceptible to salicylate toxicity from prolonged high-dose salicylate therapy. Salicylate hepatotoxicity in SLE is characterized by diffuse hepatocyte injury, disruption of the limiting plate, and stellate cell activation with a plasma cell-predominant chronic portal infiltrate, occasional lymphoid follicles and fibrosis. A more acute pattern of injury is characterized by centrilobular ballooning of hepatocytes, scattered acidophilic bodies and moderate portal inflammation. Other reported findings include microabscesses from bacterial infection, hemosiderosis from blood transfusion, PBC and drug toxicity. Successful treatment of SLE or discontinuation of offending medications typically leads to improvement in associated hepatic biochemical and histologic abnormalities.

Criteria for treating SLE-related liver disease are not well defined. Liver biochemical abnormalities improve in the majority of patients treated with corticosteroids. In patients who have persistent liver test abnormalities despite treatment, a thorough evaluation, including liver biopsy, should be considered. Attention should be paid to the patient’s medication history, particularly salicylate therapy.

Rheumatoid arthritis

Abnormalities in liver chemistries, primarily elevated serum alkaline phosphatase and  $\gamma$ -glutamyltranspeptidase levels have been reported in up to 6% of patients with rheumatoid arthritis (RA). Elevated alkaline phosphatase levels may also be of bone origin. Liver biopsy findings from patients with abnormal liver chemistries and RA are non-specific and include Kupffer cell hyperplasia, steatosis and a periportal mononuclear cell infiltrate. As in patients with SLE, medications used in the treatment of RA, such as methotrexate, gold salts, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids, may result in hepatotoxicity that is difficult to distinguish from hepatic disease caused by RA.

Sjögren syndrome

Among 300 patients with primary Sjögren syndrome (keratoconjunctivitis sicca) investigated by Skopouli et al (1994) for liver involvement, 7% had abnormal liver chemistries. 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 syndrome is unknown, an overlap between the two conditions has been well described, related perhaps to the similar underlying pathogenic mechanisms of the two diseases. Patients with primary Sjögren syndrome and abnormal liver chemistries should be tested for antimitochondrial antibodies for possible PBC.

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 antibody and the lupus anticoagulant). Diagnosis of APS requires detection of antibodies on two separate occasions 6 months apart. The hepatic manifestations of APS are usually related to vascular abnormalities. Several cases of hepatic vein thrombosis (Budd-Chiari syndrome) have been reported in patients with APS. In addition, a recent report suggests a role for antiphospholipid antibodies in the pathogenesis of nodular regenerative hyperplasia of the liver (see Ch. 35). This rare disorder is characterized by diffuse micronodular transformation of hepatic parenchyma resulting in elevated liver chemistries and portal hypertension.

Scleroderma

While hepatic involvement in scleroderma is rare, limited cutaneous scleroderma, or the CREST (calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome has been described in 3–17% of patients with PBC. The prevalence of PBC in patients with scleroderma is unknown. Scleroderma typically precedes PBC by a mean of 12 years (age 36 years versus 48 years). Raynaud phenomenon is the most common presenting symptom. Anticentromere antibodies are relatively specific for the CREST syndrome and have been found in the serum of 10–29% of patients with PBC but in up to 100% of patients with PBC and CREST syndrome. Up to 90% of patients with scleroderma and PBC also have evidence of Sjögren syndrome; the acronym ‘PACK’ (PBC, anticentromere antibodies, CREST and keratoconjunctivitis sicca) has been used to describe this constellation.

Systemic vasculitis

Many of the systemic vasculitides may involve the liver. Polyarteritis nodosa (PAN) primarily affects small- and medium-sized arteries and is associated with chronic hepatitis B virus infection in over 50% of cases. Although lymphocytic infiltration in the intima and media of the hepatic arteries is rarely detected in percutaneous liver biopsy specimens, this finding can be observed on larger specimens obtained at autopsy in up to 50% of patients with PAN. Hepatic pathology in patients with PAN is generally related to chronic hepatitis B.

Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) typically involve large arteries, and abnormal liver chemistries, usually an elevation of the serum alkaline phosphatase level, are seen in up to one-third of patients. Liver biopsy specimens from



patients with PMR and GCA typically show arterial wall invasion by giant cells.

## SICKLE CELL DISEASE

Homozygous sickle cell anemia, or sickle cell disease (SCD), affects 1 in 600 African-American children. Liver chemistry abnormalities are universal in patients with SCD; most patients with the disease have elevated serum unconjugated bilirubin and AST levels secondary to ongoing hemolysis. Serum alkaline phosphatase elevations are typically of bone origin.

Acute syndromes secondary to SCD are common. Acute sickle hepatic crisis (ASHC) presents with tender hepatomegaly, jaundice, nausea and low-grade fever. Serum AST and ALT levels are usually less than 300 U/L, and serum bilirubin levels are typically less than 15 mg/dL. The syndrome is self-limited and usually resolves following therapy with intravenous fluids and analgesics.

In contrast to ASHC, sickle cell intrahepatic cholestasis (SCIC) is a rare complication of SCD that carries a more ominous prognosis. 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. Differentiating ASHC from SCIC is based on clinical and laboratory findings. Serum AST and ALT levels are usually greater than 1000 U/L in SCIC, and serum bilirubin levels are strikingly high because of a combination of hemolysis, renal failure and intrahepatic cholestasis. The underlying pathophysiology of SCIC is related to sickling of red blood cells within the hepatic sinusoids and resulting anoxic hepatocyte damage. Liver biopsy typically shows ballooning of hepatocytes, sinusoidal 'sickling', and intracanalicular cholestasis. Treatment is largely supportive with the use of exchange transfusion. Successful liver transplantation for SCIC has been reported.

Chronic liver disease associated with SCD is also common. Autopsy series reveal cirrhosis in up to 29% of patients who die of complications of SCD. Most cases of chronic liver disease associated with SCD are thought to be secondary to hepatic iron overload following numerous blood transfusions or from infection by hepatitis B or C virus acquired by transfusion of contaminated blood products before the implementation of universal screening of the blood supply. Following the implementation of universal screening of donated blood in the USA the incidence of viral hepatitis in patients with SCD has decreased substantially.

Patients with SCD are particularly prone to the development of pigmented gallstones. Up to 58% of patients with SCD develop cholelithiasis, and approximately 17% are found to have choledocholithiasis at the time of cholecystectomy.

## AMYLOIDOSIS

The three major categories of amyloidosis are primary amyloidosis, or light-chain amyloidosis (AL); secondary amyloidosis associated with amyloid protein A (AA); and familial amyloidosis. In primary amyloidosis, which includes amyloidosis related to multiple myeloma, amyloid fibrils are

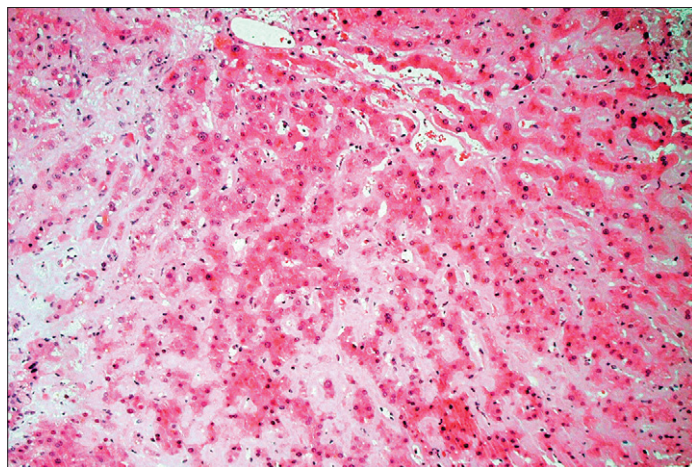
derived from the variable portion of the immunoglobulin light chain (kappa or lambda). In secondary amyloidosis, the amyloid fibrils form as a result of proteolytic cleavage of serum amyloid protein A, an acute-phase reactant. The causes of secondary amyloid include rheumatoid arthritis, osteomyelitis, tuberculosis, Hodgkin disease and other lymphomas, familial Mediterranean fever, long-term heroin abuse, subclinical ankylosing spondylitis and lepromatous leprosy. Familial amyloidosis is rare and includes type I familial amyloid polyneuropathy (FAP), an autosomal dominant disorder caused by a mutation on chromosome 18 that leads to hepatic production of a variant prealbumin, transthyretin (TTR). The disease is manifested by a progressive mixed chronic polyneuropathy (sensory, motor and autonomic) that presents after the age 20 years and is universally fatal. Early recognition is important because liver transplantation has been demonstrated to halt progression and even improve the neurologic symptoms. In all types of amyloidosis, amyloid deposition occurs most commonly in the heart, kidneys and peripheral nervous system; hepatic dysfunction may result from passive congestion caused by cardiac dysfunction.

Regardless of the type of amyloidosis (AL, AA, FAP), the hepatic manifestations of amyloidosis are similar. 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 non-specific and may include weight loss, fatigue, abdominal pain, anorexia, early satiety and nausea. Hepatomegaly is the most common physical finding in hepatic amyloidosis and occurs in up to 81% of patients; purpura and ascites can also be seen. Other stigmata of chronic liver disease, including spider angiomas, palmar erythema and Dupuytren contractures, are rarely observed.

Abnormalities in liver chemistries are common in patients with hepatic amyloidosis. A series from the Mayo Clinic described an elevated serum alkaline phosphatase level in 86% of patients with hepatic amyloidosis, with the level greater than 500 U/L in the majority of cases. Elevated serum AST and bilirubin levels were found in 37 and 21% of cases, respectively. Hepatomegaly on physical examination was associated with a higher likelihood of abnormal liver chemistries. The prothrombin time may be prolonged in patients with primary hepatic amyloidosis; however, the risk of bleeding is not increased. In up to 89% of patients, proteinuria is present on urinalysis. Thrombocytosis and Howell-Jolly bodies can be seen in the peripheral blood smear as a result of amyloid-induced hyposplenism.

The diagnosis of hepatic amyloidosis can be made by transcutaneous liver biopsy, but transcutaneous liver biopsy is associated with an increased bleeding risk in patients with hepatic amyloidosis. Amyloid appears as a pale pink homogenous amorphous protein on liver biopsy when stained with hematoxylin and eosin (Fig. 34.2). Up to 95% of patients suspected of having hepatic amyloidosis have evidence of amyloid deposition on fat pad aspirate or bone marrow biopsy. Because demonstration of hepatic involvement rarely changes 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. A recent trial comparing regimens containing



**Figure 34.2 Deposition of amyloid.** Liver biopsy specimen demonstrating marked deposition of amyloid within the space of Disse with resulting compression of the hepatic trabeculae (hematoxylin and eosin).

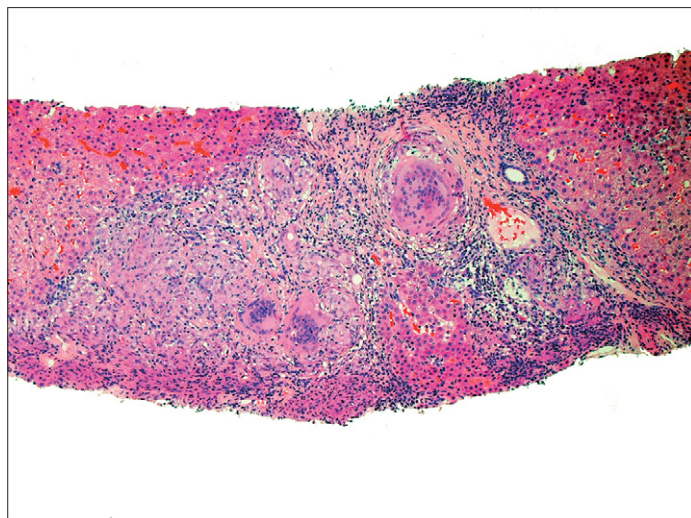
melphalan, prednisone and colchicine in patients with primary amyloidosis found that median survival was significantly longer in patients treated with a combination of melphalan and prednisone than in those treated with colchicine alone or a regimen containing all three drugs. Liver transplantation may be of benefit in rare patients with FAP.

## SARCOIDOSIS

Sarcoidosis is a chronic systemic disorder of unknown etiology that is characterized by an accumulation of T lymphocytes and macrophages leading to granuloma formation in affected organs. Organ dysfunction occurs from distortion of the normal architecture by the inflammatory infiltrate or by development of fibrosis associated with chronic inflammation.

Hepatic involvement is seen in 60–90% of patients with sarcoidosis when liver biopsy is performed. Sarcoidosis accounts for up to 30% of all cases in which hepatic granulomas are present on a liver biopsy specimen. Granulomas typically predominate around portal tracts and show no evidence of caseation necrosis (Fig. 34.3). In the vast majority of patients with hepatic sarcoidosis, liver disease is asymptomatic; however, progressive liver disease characterized by hepatomegaly, cholestasis and the development of portal hypertension has been described. Biochemically, serum immunoglobulin G levels are usually raised, the serum bilirubin is normal, and the alkaline phosphatase is mildly elevated. Serum aminotransferase levels may or may not be elevated. Evidence of mild hepatomegaly can be demonstrated by ultrasound or computed tomography in up to 50% of patients with hepatic sarcoidosis.

In patients with sarcoidosis, the presence of splenomegaly and ascites does not always imply portal hypertension or hepatic involvement. Splenomegaly is most commonly caused by granulomatous infiltration rather than portal hypertension. Ascites formation in sarcoidosis can be related to cor pulmonale resulting from progressive pulmonary disease, hypoalbuminemia resulting from hepatic dysfunction, or, rarely, peritoneal sarcoid granulomas. Hepatic sarcoidosis usually follows a benign clinical course, and there are no data to suggest that corticosteroid administration reduces the risk of progression of the disease.



**Figure 34.3 Portal tract with non-caseating granulomas.** Liver biopsy specimen demonstrating a portal tract with non-caseating granulomas in a patient with sarcoidosis (hematoxylin and eosin).

Therefore, uncomplicated hepatic involvement by sarcoidosis is not an indication for corticosteroid therapy.

In a minority of cases, progressive destruction of the intrahepatic bile ducts may lead to chronic intrahepatic cholestasis. 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.

Biliary cirrhosis may develop in the later stages of hepatic sarcoidosis and patients with end-stage liver disease should be considered for liver transplantation. Portal hypertension is rare in patients with sarcoidosis. In patients with portal hypertension related to hepatic sarcoidosis, complications such as bleeding esophageal varices may be the initial clinical presentation. Affected patients are typically young and black; among Caucasians, women over age 40 are affected most commonly. Liver biopsy specimens in patients presenting with portal hypertension may show fibrosis, cirrhosis or nodular regenerative hyperplasia. Portal hypertension in patients without advanced fibrosis on a liver biopsy specimen has been proposed to result from granulomatous phlebitis or granulomatous infiltration of small portal vein branches with resulting presinusoidal portal hypertension. In some patients, there may be superimposed sinusoidal obstruction from extensive fibrosis. In approximately 25% of patients, portal hypertension is related to secondary biliary cirrhosis. Rarely, associated PBC or primary sclerosing cholangitis account for hepatic fibrosis and portal hypertension in patients with sarcoidosis. The cornerstones of management of the portal hypertension associated with sarcoidosis have been non-selective  $\beta$  blockade and endoscopic management of variceal bleeding. A surgical portosystemic shunt or transjugular intrahepatic portosystemic shunt may be considered in appropriate candidates. Corticosteroids do not appear to affect disease outcome, and in patients with severe hepatic

sarcoidosis and limited extrahepatic disease, liver transplantation should be considered.

## CONGESTIVE HEART FAILURE

The hepatic manifestations of congestive heart failure result from a decrease in hepatic blood flow due to poor left ventricular function; passive hepatic congestion due to impaired right ventricular function; and decreased arterial oxygen saturation. Clinically, congestive heart failure typically manifests as jaundice secondary to hepatic congestion and hepatocellular necrosis secondary to impaired perfusion. 'Cardiac cirrhosis' refers to a characteristic hepatic fibrosis related to prolonged, severe congestive heart failure. A summary of the clinical and biochemical manifestations of hepatic involvement in congestive heart failure is shown in **Table 34.5** and **Table 34.6**.

Whereas decreases in both hepatic blood flow and arterial oxygen saturation can occur in a variety of conditions that result in circulatory collapse, elevated hepatic venous pressure with passive hepatic congestion is usually the result of congestive heart failure. Chronic passive hepatic congestion, or 'congestive hepatopathy', typically presents with hepatomegaly with or without ascites and splenomegaly; other clinical manifestations of chronic liver disease such as spider angiomas and esophageal varices are rare in such patients. Physical examination may reveal a pulsatile liver and hepatjugular reflux. Encephalopathy,

resulting from reduced cerebral perfusion, has been reported to occur in a minority of patients.

Jaundice may be seen and may occasionally be profound (although it is characteristically mild in patients with constrictive pericarditis). The mechanism of hyperbilirubinemia in congestive heart failure is thought to be related to multiple insults including hepatocellular dysfunction, hemolysis, pulmonary infarction and elevated hepatic vein pressures leading to bile canicular obstruction and bile thrombi. In addition, patients with severe congestive heart failure may be treated with medications that can contribute to the development of jaundice.

Liver chemistry abnormalities are typically mild in patients with congestive hepatopathy. An elevated serum bilirubin level, usually no higher than 3 mg/dL, is seen in up to 70% of patients. The alkaline phosphatase level is elevated in only 10% of patients; absence of a significant elevation of the alkaline phosphatase is helpful in excluding obstructive jaundice in patients who present with marked elevations in the serum bilirubin. Elevations in serum aminotransferase levels, typically two to three times the normal value, may be seen in up to one-third of patients. Patients with severe heart failure may present with marked elevations of the aminotransferases and bilirubin that mimic the findings in acute hepatitis. Levels of serum albumin are decreased in up to 50% of patients with congestive heart failure but are rarely less than 2.5 g/dL. Ascites associated with congestive hepatopathy is usually protein-rich, with an ascitic total protein level greater

Clinical features of congestive hepatopathy and hepatic failure		
<i>Clinical features common to both conditions</i>	<i>Features suggestive of congestive hepatopathy</i>	<i>Features suggestive of hepatic failure</i>
Fatigue	Dyspnea	–
Altered mental status	Orthopnea	–
Hepatomegaly	Neck venous distention	Spider angiomas
Ascites	Hepatjugular reflux	Palmar erythema
Edema	Cardiac gallop	Caput medusae
Jaundice	Cardiomegaly on radiograph	–

**Table 34.5 A comparison of the clinical features showing the similarities and differences between congestive hepatopathy and hepatic failure due to chronic liver disease**

Liver test abnormalities in congestive hepatopathy					
<i>Liver test</i>	<i>Abnormal</i>	<i>Right-sided CHF; acute versus chronic</i>	<i>Correlation with clinical or pathologic findings</i>	<i>Improvement with CHF resolution</i>	<i>Normalization with CHF resolution</i>
AST	Common	Acute > chronic	Centrilobular necrosis	1–3 days	3–7 days
ALT	Less common	Acute > chronic	Centrilobular necrosis	1–3 days	3–7 days
Alk phos	Uncommon	Acute = chronic	Hepatomegaly	No	No
Bilirubin	Common	Acute > chronic	Centrilobular necrosis	1–3 days	3–7 days
PT	Common	Acute = chronic	No	Yes	2–3 weeks
Alb	Less common	Acute = chronic	No	1–2 days	Less common

**Table 34.6 Liver test abnormalities in congestive hepatopathy.** CHF, congestive heart failure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; Alb, albumin



than 2.5 g/dL and a serum-ascites albumin gradient greater than 1.1.

Liver biopsy specimens from patients with congestive hepatopathy reveal sinusoidal engorgement, variable degrees of zone 3 hemorrhagic necrosis, steatosis and cholestasis, with occasional bile thrombi in the canaliculi. With chronic or recurrent episodes of congestive heart failure, the reticulin network surrounding the central vein may collapse, and a stromal reaction of fibrotic bands radiating outward from the central vein can be observed. A pattern of 'reverse lobulation' is characteristic of cardiac cirrhosis and occurs when these radiating fibrotic bands reach adjacent central veins (**Fig. 34.4**).

The management of congestive hepatopathy relies on the treatment of the underlying congestive heart failure. Improvements in jaundice, hepatic congestion and ascites are typically seen following institution of diuretic therapy. Optimization of cardiac output may prevent further hepatocellular injury related to poor left ventricular performance. Caution should be utilized in the administration of medications that are metabolized by the liver. Morbidity and mortality related to the hepatic manifestations of congestive heart failure are rare and are more often related to the underlying cardiac diseases. Early treatment of congestive heart failure may result in reversal of the histologic liver abnormalities and avoid progression to cardiac cirrhosis. The presence of cardiac cirrhosis does not portend an unfavorable prognosis; synthetic function appears to be similar to that in patients with passive congestion alone, and portosystemic shunting and variceal bleeding typically do not occur.

Diffuse hepatic injury resulting from acute hypoperfusion of the liver is commonly referred to as ischemic hepatitis. In the majority of cases, ischemic hepatitis occurs in the clinical setting of congestive heart failure, and many of the clinical, laboratory and pathologic features are similar to those seen in patients with congestive hepatopathy. Patients with ischemic hepatitis typically have experienced a period of systemic hypotension prior to the finding of abnormal liver chemistries. Most patients are asymptomatic at presentation, and the diagnosis is only

entertained after routine measurement of liver chemistries. In rare patients, nausea, vomiting, anorexia, malaise, right upper quadrant pain and jaundice may be the presenting symptoms.

Liver biopsy specimens from patients with ischemic hepatitis reveal zone 3 necrosis. Centrilobular architectural collapse varies depending on the duration of ischemia, and necrosis can extend to midzonal hepatocytes. Changes consistent with passive congestion often coexist with those of ischemic hepatitis, especially in patients with severe, decompensated congestive heart failure. A dramatic rise in the levels of serum aminotransferases to 25 to 250 times the upper limit of normal is typical in patients with ischemic hepatitis. Peak serum aminotransferase levels are reached within 1 to 3 days of the hypotensive episode and are usually accompanied by an early rise in serum lactate dehydrogenase (LDH) levels, a finding that may help discriminate ischemic hepatitis from acute viral hepatitis. The serum bilirubin level usually rises to no more than 4 times the upper limit of normal after the serum aminotransferase levels begin to decline. Serum alkaline phosphatase levels usually do not rise above two times the upper limit of normal. Liver biopsy is rarely warranted in these patients, as the diagnosis can usually be made on the basis of clinical and liver chemistry findings.

The course of ischemic hepatitis is usually self-limited, with only mild impairment in hepatic synthetic function, if the patient survives the cardiac insult. Mental status changes in patients with ischemic hepatitis are related to cerebral hypoperfusion rather than hepatic encephalopathy. Hepatic regeneration and recovery of normal function occur in the majority of cases; fulminant hepatic failure leading to death is rare. The prognosis of ischemic hepatitis is related to that of the underlying cardiac disease.

## CONSTRICTIVE PERICARDITIS

Constrictive pericarditis results from diffuse thickening of the pericardium with a consequent restriction in ventricular filling and a rise in right atrial and vena caval pressures. The clinical features and hepatic changes are similar to those in the Budd-Chiari syndrome and cardiac cirrhosis resulting from other causes of congestive heart failure. All three conditions lead to hepatomegaly and frequently splenomegaly, ascites and jaundice in the latter stages. A list of causes of constrictive pericarditis is shown in **Table 34.7**. The diagnosis of hepatopathy caused by constrictive pericarditis is made clinically on the basis of an increased jugular venous pressure with a paradoxical rise in inspiration and characteristic wave form (prominent 'x' and 'y' descents) and a loud third heart sound. A chest radiograph may show pericardial calcification, but computed tomographic scanning or magnetic resonance imaging, together with cardiac catheterization, are usually required to confirm the diagnosis. Cardiac Doppler studies may demonstrate abnormalities in ventricular filling. Treatment is directed toward the pericarditis. If pericardiectomy is possible, the prognosis of the liver disease is good, although recovery is slow. Even the fibrosis of cardiac cirrhosis can partially reverse.

## LYMPHOMA

The liver is part of the reticuloendothelial system and may be involved by lymphomas. Life-threatening complications of



**Figure 34.4 Histologic appearances of cardiac cirrhosis.** Regenerative nodules form within the liver, due to fibrous linking of perivenular areas that come to surround the unaffected portal tract (arrow). This is associated with atrophy of the zone 3 hepatocytes, and sinusoidal dilatation, in contrast to regenerative activity in periportal hepatocytes. (Courtesy of Dr J. Wyatt.)

Causes of constrictive pericarditis	
Infections	Viral Bacterial (including tuberculosis) <i>Toxoplasma</i> Amebic <i>Histoplasma</i> Actinomycosis
Other inflammatory	Post cardiectomy Dressler syndrome
Neoplastic	Primary mesothelioma Secondary – lung, breast, lymphoma
Connective tissue disease	Rheumatoid arthritis, SLE
Chronic renal failure (uremia)	
Physical agents	Trauma, radiotherapy
Idiopathic	

**Table 34.7 Causes of constrictive pericarditis.**

lymphomas involving the liver are rare. Patients with lymphoma are also at risk of hepatotoxicity resulting from systemic chemotherapy and radiation therapy.

### Hodgkin disease

Malignant infiltrates containing Reed-Sternberg cells have been described in up to 14% of patients with Hodgkin disease diagnosed on clinical grounds and up to 50% of patients diagnosed at autopsy. It has been suggested that the frequency of hepatic involvement in Hodgkin disease increases as the disease progresses. Patients with Hodgkin disease may present with hepatomegaly, which occurs in approximately 9% of patients with stage I–II disease and 45% with stage III–IV disease. Splenomegaly and lymphadenopathy may also be detected. Liver biochemical testing may reveal a mild increase in the serum ALT level and a moderate increase in the alkaline phosphatase level. Interestingly, neither physical findings nor abnormal liver chemistry results predict the presence of a Hodgkin infiltrate in the liver. By definition, Hodgkin disease involving the liver signifies stage IIIIE or IV disease and has implications for prognosis and therapy. Therefore, patients in whom hepatic involvement is suspected should undergo liver biopsy. Percutaneous needle biopsy and laparoscopy detect liver involvement with the same frequency as liver biopsy at laparotomy. Hepatic histologic abnormalities in Hodgkin disease occur in up to 60% of patients. Early in the course of the disease, the histologic findings are benign and may be transient.

Jaundice, when present, is secondary to intrahepatic disease in 40–50% and extrahepatic biliary obstruction in 3–10% of cases. Cholestasis has also been described in the absence of extrahepatic obstruction or significant tumor infiltration. The cause in such cases is unclear but may represent a paraneoplastic phenomenon or defect in hepatic microsomal function. In a recent report of patients with Hodgkin disease and ‘idiopathic’ cholestasis, a loss of small intrahepatic bile ducts, not noted on initial assessment, was ultimately discovered. Cholestasis was most pronounced in zone 3 and was not associated with hepatitis. The authors proposed that vanishing bile duct syndrome was the

cause of cholestasis in these cases and in other cases of otherwise idiopathic cholestasis in patients with Hodgkin disease. Release of cytokines from lymphoma cells was proposed as a mechanism. Jaundice has been shown to resolve in some patients with successful treatment of the underlying Hodgkin disease, but persistence despite remission of the primary disease in other patients implies that permanent injury with bile duct loss may have occurred.

### Non-Hodgkin lymphoma

Lymphomatous infiltration of the liver is more common in non-Hodgkin lymphoma (NHL) than in Hodgkin disease. Hepatic involvement has been described in 16–43% of cases, although it is generally not clinically significant. Hepatic involvement is more common with small cell varieties and less frequent with large cell types. Liver chemistry test results may be abnormal, primarily an increase in serum alkaline phosphatase, and evidence of hepatomegaly may be detected. Rarely, NHL can present as a primary hepatic lymphoma. Apart from human immunodeficiency virus-associated lymphomas, primary hepatic lymphomas have a better prognosis than non-hepatic lymphomas because of the possibility of cure with successful resection. In contrast to Hodgkin disease, the yield from laparotomy for detecting hepatic involvement in NHL is higher than that for laparoscopy and percutaneous needle biopsy of the liver.

Jaundice is rare in patients with NHL and in the past was considered a terminal occurrence. Causes of jaundice in patients with NHL are listed in **Table 34.8**. Extrahepatic biliary obstruction is more common in NHL (1.2%) than in Hodgkin disease (0.3%). Biliary obstruction occurs most commonly at the porta hepatitis or in the intrapancreatic bile duct because of the presence of lymph nodes and decreased mobility of the bile duct at these sites. Primary lymphomatous involvement of 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.

Pathogenesis of jaundice in patients with lymphoma	
Cause	Possible mechanism
Extrahepatic biliary obstruction	Hilar nodes Pancreatic obstruction
Intrahepatic causes	Tumor masses/infiltration including acute liver failure Idiopathic cholestasis
Hemolysis	Autoimmune hemolytic anemia
Treatment-related	High-dose chemotherapy High-dose radiotherapy
Infection	Reactivation of viral hepatitis (B, C etc.) Opportunistic infections, especially in immunocompromised individuals

**Table 34.8 Etiology of jaundice in patients with lymphoma.**

### Acute liver failure secondary to lymphoma

Rarely, malignant infiltration of the liver with lymphoma can cause acute liver failure (ALF) (see Ch. 33). Malignant infiltration accounted for 0.44% of all cases of ALF seen at King's College Hospital from 1978 to 1995. Non-Hodgkin lymphoma accounted for 50% and Hodgkin disease for 17% of the malignant causes of ALF in this series. Malignant histiocytosis, acute and chronic leukemias, adenocarcinomas, melanoma and anaplastic tumors from various primary sites have also been reported as causes of ALF.

The pathogenesis of ALF caused by lymphomas and other malignancies includes massive hepatic replacement by non-functioning malignant cells, release of cytokines by tumor cells, Kupffer cell activation, and ischemia from engorgement of hepatic sinusoids with leukocytes and platelets. Prompt diagnosis and treatment of the underlying malignancy during the prodromal period is the key to a successful outcome. The diagnosis can be made from bone marrow or lymph node biopsies, but the absence of peripheral adenopathy in some patients and the predilection of the tumor for the liver emphasizes the importance of a liver biopsy in confirming the diagnosis. It is essential to differentiate these patients from patients with non-malignant causes of ALF, because liver transplantation may be life saving in the latter but is contraindicated in the former.

### Hepatic radiation injury

Radiation therapy is commonly employed in the treatment of lymphomas. Radiation-induced hepatitis occurs 1–3 months after radiation therapy. Patients present with ascites, hepatomegaly and jaundice. Characteristic laboratory findings include elevated

aminotransferase, alkaline phosphatase and bilirubin levels with hypoalbuminemia. Hepatic histology in the first 60 days reveals sinusoidal congestion, hyperemia, hemorrhage and hepatocyte atrophy. After 180 days, biopsy specimens demonstrate resolution except for atrophy of the centrilobular hepatocytes and thickening of the central vein wall. Autopsies of patients who died demonstrated massive hepatic hemorrhage and loss of architecture. Veno-occlusive disease (sinusoidal obstruction syndrome) has also been described as a consequence of radiation exposure (see Ch. 30). The risk of hepatic injury appears to be dose-related. Whole liver radiation tolerance is thought to be 2400 rad (in 300 rad doses) and up to 3000 if smaller daily doses (200 rad) are used. Radiation exposure greater than 3500 rad leads to a significant risk for hepatitis, and at 4000 rad there is a 50% risk of death from hepatitis.

### CELIAC DISEASE

Celiac disease is defined by an intolerance of the small bowel to gluten. Although most symptomatic patients have symptoms related to the gastrointestinal tract, many extraintestinal manifestations have been described including a wide spectrum of hepatobiliary diseases. Hepatic manifestations include asymptomatic elevations of liver chemistries, non-specific hepatitis, NAFLD, and autoimmune and cholestatic liver disease. In addition, celiac disease may be the underlying cause of unexplained elevations of liver enzyme levels. Because most patients do not have overt gastrointestinal symptoms, a high index of suspicion is required. Moreover, in the majority of patients, liver chemistries will normalize on a gluten-free diet.

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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 35

## Non-cirrhotic Portal Hypertension

Shiv Kumar Sarin

### INTRODUCTION

Non-cirrhotic portal hypertension (NCPH) represents a group of disorders in which portal vein pressure is elevated ( $>10$  mmHg) in the absence of cirrhosis of the liver. In these conditions, hepatic parenchymal function is normal but portal hypertension (PHT) exists. Conventionally, the definition not only includes the absence of cirrhosis but also the absence of hepatic venous outflow obstruction, such as exists in veno-occlusive disease and Budd-Chiari syndrome. Hence, NCPH comprises a heterogeneous group of diseases, which are characterized by an increase in portal pressure due to intrahepatic or prehepatic lesions, in the absence of cirrhosis, i.e. regeneration and bridging fibrosis. A second component of the definition is that the hepatic venous pressure gradient (HVPG), i.e. the difference between the wedged hepatic vein pressure (WHVP) and the free hepatic vein pressure (FHVP) is normal ( $<5$  mmHg) or only mildly elevated and significantly lower than the actual portal vein pressure. The pathology in NCPH is generally vascular, present in the portal vein, its branches or in the perisinusoidal area of the liver.

The majority of these diseases, which are grouped under the term NCPH (Table 35.1), have PHT as a late manifestation of the primary disease. These diseases will not be covered individually as several are covered elsewhere in this textbook. However, two diseases, which are particularly common in the developing countries, present only with features of PHT. These two con-

ditions, non-cirrhotic portal fibrosis (NCPF), and extrahepatic portal vein obstruction (EHPVO), are the prototype diseases, which fall under the heading NCPH (Fig. 35.1). For this reason, the pathophysiology, etiology, clinical features, management and natural history of these two diseases are described in detail in this chapter.

### Historical perspective

Banti et al from Northern Italy at the end of the nineteenth century first drew attention to the clinical manifestations of cirrhosis including congestive splenomegaly, anemia, with or without gastrointestinal bleeding and ascites. Awareness of a similar clinical condition, yet histologically distinct from cirrhotic causes of PHT emerged in the Indian subcontinent in the late 1950s with the identification of patients having 'tropical splenomegaly syndrome' or 'Bengal splenomegaly'. In 1962, Indian scientists drew attention to an entity of splenomegaly in the absence of cirrhosis in North Indian patients. Almost at the same time, a similar group of patients were described from Japan. Subsequently, Boyer et al (67), while working at Calcutta, reported a series of similar patients and termed the condition as idiopathic portal hypertension (IPH). Mikkelsen et al (65) described 36 patients in USA with PHT but without cirrhosis who had phlebosclerosis of intra- and extrahepatic portal veins and coined the term hepatportal sclerosis. However, in a majority of these patients, the portal vein was partly or completely occluded. In 1969, a workshop organized by the Indian Council of Medical Research (ICMR) reviewed all

#### Common causes of non-cirrhotic portal hypertension<sup>†</sup>

Extrahepatic portal venous obstruction*
Non-cirrhotic portal fibrosis or idiopathic portal hypertension
Schistosomiasis
Primary or secondary biliary cirrhosis (precirrhotic stage)
Congenital hepatic fibrosis
Veno-occlusive disease*
Nodular regenerative hyperplasia
Partial nodular transformation
Hepatportal sclerosis
Peliosis hepatitis

\*May present acutely.

<sup>†</sup>Most causes are chronic conditions.

Table 35.1 Common causes of non-cirrhotic portal hypertension<sup>†</sup>.

#### Main sites of obstruction in non-cirrhotic portal fibrosis (NCPF) and extrahepatic portal vein obstruction (EHPVO)

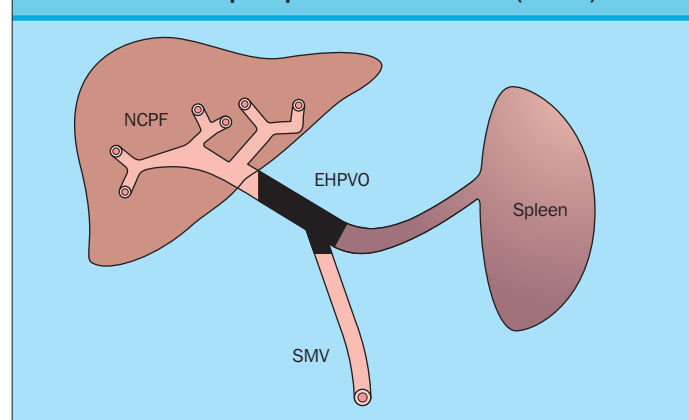


Figure 35.1 Main sites of obstruction in non-cirrhotic portal fibrosis (NCPF) and extrahepatic portal vein obstruction (EHPVO). SMV, superior mesenteric vein.

Comparison between non-cirrhotic portal fibrosis and idiopathic portal hypertension		
	NCPF	IPH
Age (years)	25–35	43–56
Male:female ratio	2:1	1:3
Hematemesis/ melena (%)	94	40
Mass in LUQ (%)	6	40
Ascites (%)	2	10
Autoimmune features	Rare	Common
<i>Pathology</i>		
Irregular parenchymal nodules (%)	53	29
Bile duct proliferation (%)	38	4
Wedge hepatic venous pressure	Normal/mildly raised	Moderately raised
IPH, idiopathic portal hypertension; LUQ, left upper quadrant; NCPF, non-cirrhotic portal fibrosis.		

**Table 35.2 Comparison between non-cirrhotic portal fibrosis and idiopathic portal hypertension.**

available information on this condition, carefully excluding patients with cirrhosis and extrahepatic portal vein thrombosis and termed this distinct clinicopathologic entity 'non-cirrhotic portal fibrosis' (NCPF). While there are some differences, the patients with NCPF and IPH (the term commonly used in Japan) represent the same disease entity (Table 35.2). This condition has since then been reported in varying frequencies from all over the world. However, it clearly is more common in developing countries.

## NON-CIRRHOTIC PORTAL FIBROSIS

### Epidemiology

NCPF accounts for nearly 10–20% of all cases of variceal bleeding in the developing world. The disease is common in those of the lower or lower-middle socioeconomic strata of society. Improved hygiene and standard of living could explain the relative rarity of the disease in the West and its declining incidence in Japan. While in 1985 the reported incidence of IPH in Japan was 0.75/10 population, by 1992 only an average of 11 new patients per year were identified. The incidence of NCPF has not been prospectively studied in the Indian subcontinent but it is believed that the disease is still quite common in this region. In India, almost all series indicate a male predominance. This is in contrast to IPH in Japan, Europe and the USA, where the disease is more common in middle-aged females (Table 35.2). The mean age of NCPF patients is around 30 years, much younger than that of the IPH patients.

### Etiology

The pathogenesis of NCPF is not clear and a number of hypotheses have been proposed.

### Occult portal vein bacteremia

Non-cirrhotic portal fibrosis has been commonly seen in patients from a low socioeconomic background. Thus, intra-abdominal infections at birth and in early childhood are common in these patients and have been alleged to play an important role. Umbilical sepsis, bacterial infections and diarrheal episodes in infancy and in early childhood are proposed to lead to portal pyemia and pylephlebitis, resulting in thrombosis, sclerosis and obstruction of small- and medium-sized portal vein radicals.

The histologic changes seen in the liver in patients with IPH, together with the development of PHT, have been seen after injecting killed non-pathogenic colon bacilli into the portal vein of rabbits and dogs. In a rabbit model of indwelling cannulation of the gastrosplenic vein, repeated injections of *Escherichia coli* lipopolysaccharide also resulted in the development of splenomegaly and a significant increase in the portal pressure. No parenchymal hepatic injury was discernible. The rabbit model also clearly showed a hyporesponsiveness of the vascular bed suggestive of peripheral vasodilatation, and a hyperdynamic circulatory state. The precise mechanism as to how the endotoxemia causes the rise in portal pressure is not clear, but it is quite possible that activation of hepatic stellate and Kupffer cells could play a role.

### Exposure to trace metals and chemicals

Prolonged ingestion of arsenic has been incriminated in the causation of NCPF. Liver histology in patients with chronic arsenic ingestion reveals periportal fibrosis, incomplete septal fibrosis, with or without development of neovascularization within the expanded portal zones suggestive of NCPF or IPH. Previous exposure to arsenic as Fowler's solution for the treatment of psoriasis was reported in one study. These patients had florid skin stigmata of arsenicosis, something not commonly experienced in the Indian population. Furthermore, patients diagnosed with NCPF did not have elevated arsenic content in the liver, as assayed by electron probe microanalysis. In animal studies, arsenic was found to induce a 4–14-fold increase in hepatic hydroxyproline and hepatic collagen content compared with control mice, but features of NCPF or PHT did not develop in any animal. These observations suggest that although arsenic ingestion might induce hepatic fibrogenesis, it cannot be directly incriminated in the causation of most cases of NCPF.

In patients from Iran, a history of pica was obtained in nearly half the patients. A histologic picture resembling NCPF has also been observed following chronic exposure to vinyl chloride monomers, copper sulfate (vineyard sprayers), protracted treatment with methotrexate, hypervitaminosis A and in renal allograft recipients receiving treatment with 6-mercaptopurine or azathioprine, and corticosteroids.

### Immunological alterations

Evidence supporting immunological derangements as an etiology includes:

- a reduction in the suppressor/cytotoxic T lymphocytes (T8) in NCPF patients and a decreased T4/T8 lymphocyte ratio;
- a reduction in the cell-mediated immune status in NCPF patients; and,
- a poor autologous mixed lymphocyte reaction (MLR).



In Japan, IPH is frequently associated with autoimmune disorders including systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), thyroiditis and mixed connective tissue disease (MCTD). Nearly two-thirds of Japanese female patients with IPH test positive for anti-double-stranded DNA and one-quarter test positive for antinuclear antibody. Such a high prevalence of associated autoimmune conditions has not been the experience in the Indian subcontinent. However, familial aggregation and a high frequency of HLA-DR3 have been found in these patients.

### Experimental studies

Repeated injections of splenic extract, Freund's adjuvant and egg albumin have been shown to produce splenomegaly and histologic lesions similar to IPH in rabbits. Injection of splenic extract in sensitized rabbits resulted in the development of an NCPF-like picture, with splenomegaly and an increase in portal pressure with negligible parenchymal injury.

### Procoagulant factors

There is limited and controversial data on the role of procoagulant factors in the causation of thrombosis of the portal vein branches. Deficiency of protein C and S has been proposed along with mutations in the factor V Leiden in small series of patients. However, a cause and effect remains to be confirmed.

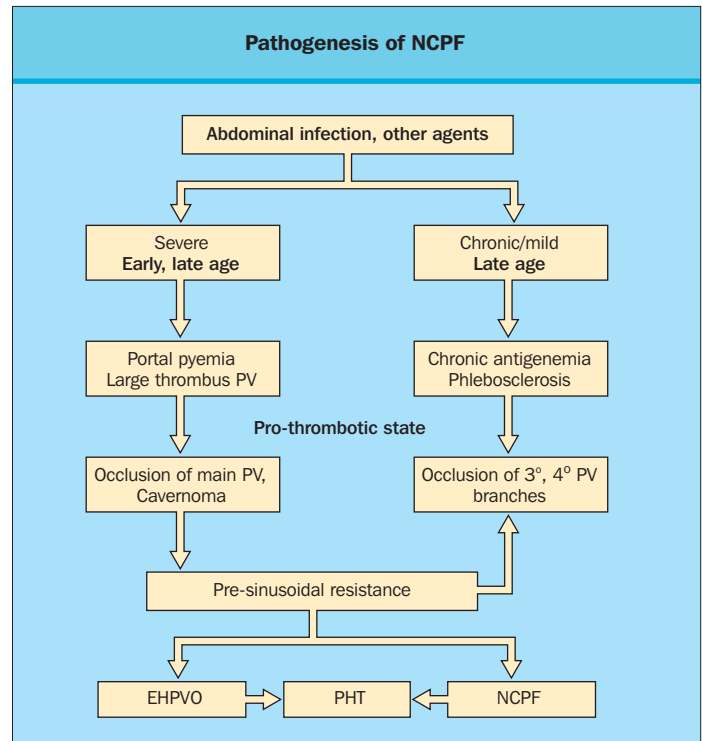
### Proposed hypothesis for the pathogenesis of NCPF

Based on the available information, a hypothesis for the development of NCPF and even possibly extrahepatic portal vein obstruction has been proposed. Both of these venous inflow tract diseases could develop if portal pyemia or endotoxemia develops in a subject born with an underlying procoagulant state. This could precipitate thrombosis in the portal vein or its radicals. If it is a major thrombotic event, occurring at an early age in life, the main portal vein becomes occluded, leading to the development of EHPVO (Fig. 35.2). However, if the event is minor but repeated, only small or medium size branches of the portal vein could develop microthrombi. Since this process may have many repeated episodes, the syndrome of NCPF would develop at a later age e.g. in a young adult.

### Pathology

When NCPF was first recognized, the salient histologic features characteristic of this condition were described. In fact, NCPF was considered a distinct clinicopathologic entity. The initial histologic descriptions were based on the systematic study of wedge hepatic specimens, obtained from patients in surgery. In patients with NCPF, the liver is usually normal grossly, but may be irregular (Fig. 35.3). This irregularity, only seen in 10–15% of all cases of NCPF, is confined to the liver surface. The portal vein and its branches are prominent and have sclerotic walls. Autopsy series commonly show thrombosis in the medium and small (diameter <300  $\mu$ m) portal vein branches. In the early stages of disease, the sclerosis may be less marked and thrombi may not be evident.

Histologic changes in NCPF were aptly coined as 'obliterative portovenopathy of liver'. Portal fibrosis is common in NCPF (Fig. 35.4). There is a marked, but patchy, segmental sub-endothelial thickening of the large and medium-sized branches of the portal vein. These changes are summarized in an excellent



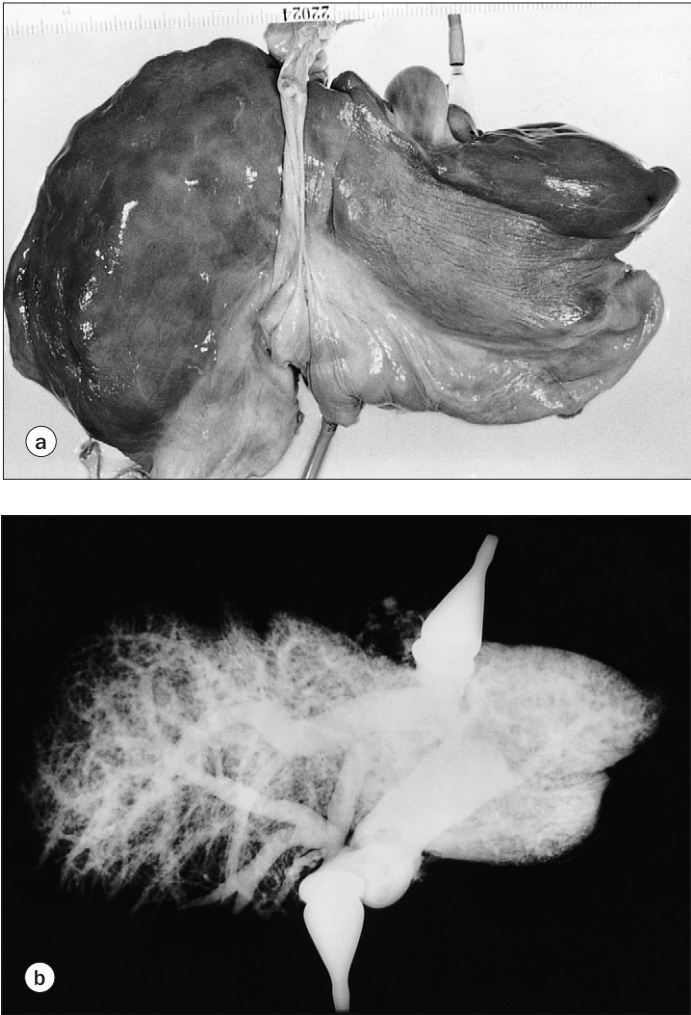
**Figure 35.2 A proposed hypothesis for the pathogenesis of non-cirrhotic portal fibrosis (NCPF) and extrahepatic portal vein obstruction (EHPVO).** MPV, main portal vein.

review (Table 35.3). The intimal thickening of intrahepatic portal venous channels, associated with obliteration of small portal venules and emergence of new aberrant portal channels, is characteristic of NCPF. Evidence of previous phlebothrombosis is suggested by the presence of old mural thrombi incorporated into the wall, and mural thickening of the extrahepatic portal vein. Coexistence of lesions characteristic of NCPF and EHPVO may occur in the same patient. While many investigators believe that thrombosis is an initial event leading to sclerosis, the Japanese investigators consider thrombosis to be secondary to a primary sclerosis of the portal vein branches. In wedge biopsy specimens from Japan, thrombi in the peripheral portal vein branches have not been found.

A widening of the space of Disse with haphazardly arranged collagen bands in the perisinusoidal space leading to capillarization of sinusoids may also be seen. Whether these electron microscopic changes correlate with the severity of PHT or occurrence of ascites, which is seen in a small proportion of NCPF patients, has not been determined.

### Hemodynamics

In patients with NCPF, the WHVP is generally normal but may be slightly elevated in up to half the patients. However, the intrasplenic and portal vein pressures are markedly elevated. Two sites of increased resistance to flow (Fig. 35.5a) have been identified: a distinct pressure gradient between the spleen (intrasplenic pressure; ISP) and the liver (intrahepatic pressure; IHP) and another between the liver (IHP) and the WHVP (Fig. 35.5b). Variceal pressure measurements in these patients are comparable to that in cirrhotic PHT (Fig. 35.6). Splenic and portal vein



**Figure 35.3 Idiopathic portal hypertension.** (a) The liver in idiopathic portal hypertension. The surface is smoothly irregular but has no diffuse nodules. (b) Postmortem portography and venography of a liver with idiopathic portal hypertension. The portal branches are seen all the way toward the periphery with no sign of thrombosis. [(a) and (b), Courtesy of Prof. M. Okudaira.]

blood flow are both markedly increased in IPH patients from Japan, even more than that seen in patients with cirrhosis and portal hypertension suggestive of a hyperdynamic circulatory state. Pulmonary hemodynamics in patients with NCPF has also been reported and pulmonary hypertension has been observed. Doppler ultrasound studies have shown that the hepatic and splenic blood flow is increased in the IPH patients.

Clinical profile

Over a 12-year period (1983–1995), our institutional experience of PHT was 2137 patients, 1133 of whom had gastrointestinal bleeding. NCPH was the cause of PHT in 35.4% of all bleeders but only 4.1% of non-bleeders. NCPF was diagnosed in 207 patients, while EHPVO was diagnosed in 236 patients. The clinical and laboratory profiles of these patients are shown in Table 35.4.

NCPF occurs predominantly in young patients with slight male preponderance. Patients may present with one or more well-tolerated episodes of gastrointestinal hemorrhage, splenomegaly

Histologic findings in idiopathic portal hypertension		
	Frequency (%)	
	Mild	Moderate to severe
Dense portal fibrosis and portal venous obliteration	48	52
Portal inflammation	47	
Irregular intimal thickening of portal veins	75–100*	
Organizing thrombotic and/or recanalization of portal veins	20–100*	
Nodular hyperplasia of the parenchyma	40	
Abnormal blood vessels in the lobules	75	
Intralobular fibrous septa	95	
Subcapsular atrophy	70	
Periductal fibrosis of interlobular bile ducts	50	
Modified from Nakanuma et al (89) by permission of Acta Pathol Jpn based on a study of 66 patients with idopathic portal hypertension. *100% abnormalities were observed only in autopsy specimens.		

**Table 35.3 Histologic findings in idiopathic portal hypertension.**

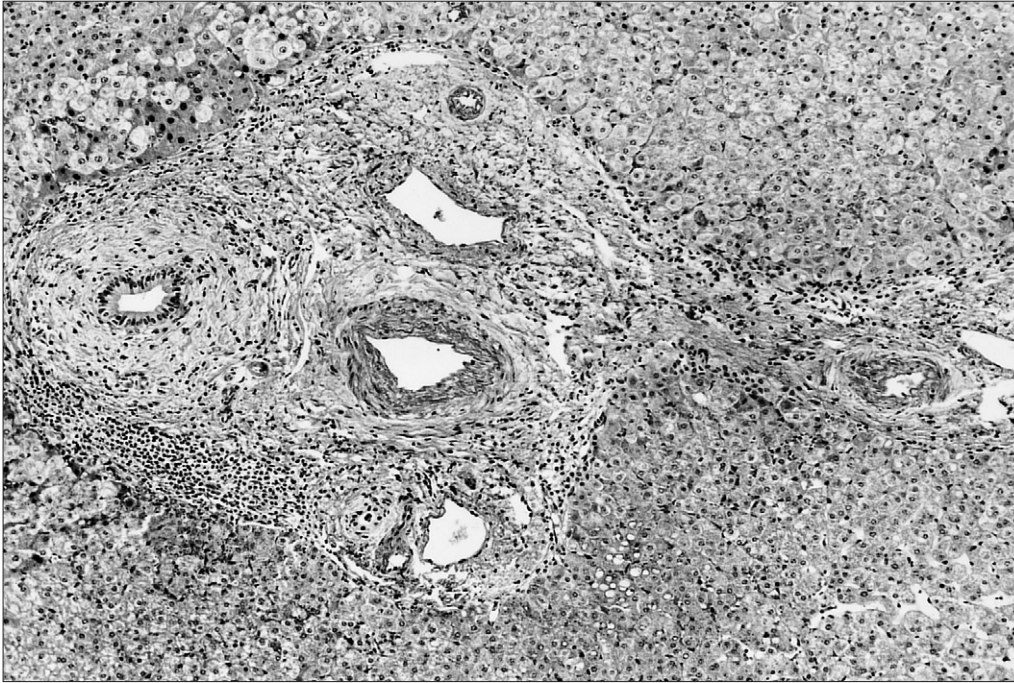
or consequences of hypersplenism. Development of ascites, jaundice and hepatic encephalopathy are uncommon and are only seen after an episode of gastrointestinal hemorrhage. The patients have normal growth and physical development. As compared to other causes of PHT, the spleen is disproportionately large, particularly so in patients with NCPF. It is not uncommon to see patients presenting with repeated attacks of left upper quadrant pain due to perisplenitis and splenic infarction. The clinical presentation of NCPF is distinct from IPH in several regards (Table 35.2). Like cirrhosis, NCPF may present occasionally with unusual disease associations such as glomerulonephritis or hypoxemia secondary to hepatopulmonary syndrome. Cardiac dysfunction has been described in both patients with NCPF and EHPVO.

Laboratory features

Conventional tests of liver function are, by and large, normal in NCPF patients. In addition, semi-quantitative tests of liver function, such as monoethylglycinylydide (MEGX) extraction are also comparable to healthy individuals. The frequency of hepatitis B and C in non-transfused NCPF patients is comparable to that found in the general population excluding a role for these viruses in the causation of NCPF.

Anemia is found in the majority of NCPF patients. This may be microcytic, hypochromic (due to gastrointestinal blood loss), normocytic or normochromic (hypersplenism). Leukopenia (<4000/mm<sup>3</sup> leukocytes) and thrombocytopenia (platelets <50 000 /mm<sup>3</sup>) may also be present. The bone marrow is hypercellular. Coagulation and platelet function anomalies have been





**Figure 35.4 Portal fibrosis in idiopathic portal hypertension.** The portal tract is markedly fibrosed with no evidence of cirrhosis. The size of the portal vein is about the same as an artery within the same portal area; normally, it should be five times the artery in diameter. The venous wall is also thickened.

observed in NCPH patients (**Table 35.5**). A state of mild compensated disseminated intravascular coagulation secondary to endotoxemia or portosystemic collaterals has been reported in a fair proportion of these patients.

Gastroesophageal varices are present in 85–95% of patients with NCPH (**Fig. 35.7**). In a series from North India, large varices were far more common in NCPH (90%) than in patients with cirrhosis (70%). Similarly, anorectal varices were also more commonly reported in NCPH than in cirrhosis (90 versus 56%), and were likely to be larger in those with NCPH. Variceal pressures (using pressure gauge) are similar in NCPH and cirrhosis, but patients with cirrhosis are more likely to bleed for any given variceal pressure. They found the prevalence of red signs and the Northern Italian Endoscopy Club (NIEC) index to be similar in these two groups of patients. Portal hypertensive gastropathy is relatively uncommon in NCPH patients and is a usual cause of upper gastrointestinal bleeding at presentation.

### Imaging

Splenoportovenography, often used in the past, shows a dilated and thickened vein with venous collaterals. There are areas of poor opacification of peripheral portal vein branches suggesting narrowing or occlusion of the second and third order branches. By ultrasonography (**Fig. 35.8**), the portosplenic axis is seen to be dilated and patent. Occasionally, thrombus is seen in an intra-hepatic branch of the portal vein. The wall thickness (echogenic boundary of portal vein) of the main portal vein and its branches has been found to be significantly greater in NCPH than for other etiologies of PHT. Spontaneous splenorenal shunts are also seen more frequently in NCPH patients than in patients with cirrhosis.

Hepatic venography and radionuclide scintigraphy (using  $^{99m}\text{Tc}$  phytate or sulfur colloid) have been used to distinguish between NCPH and cirrhosis. High marrow uptake on scintigraphy is

almost pathognomonic of cirrhosis. Computed tomography (CT) portography and CT hepatic angiography have been used to show aberrant vessels in the periphery of hepatic parenchyma in these patients (**Figs 35.9 and 35.10**). Magnetic resonance (MR) angiography is also a very useful technique to assess the patency of the portal and hepatic veins and the presence of spontaneous shunts (**Fig. 35.11**).

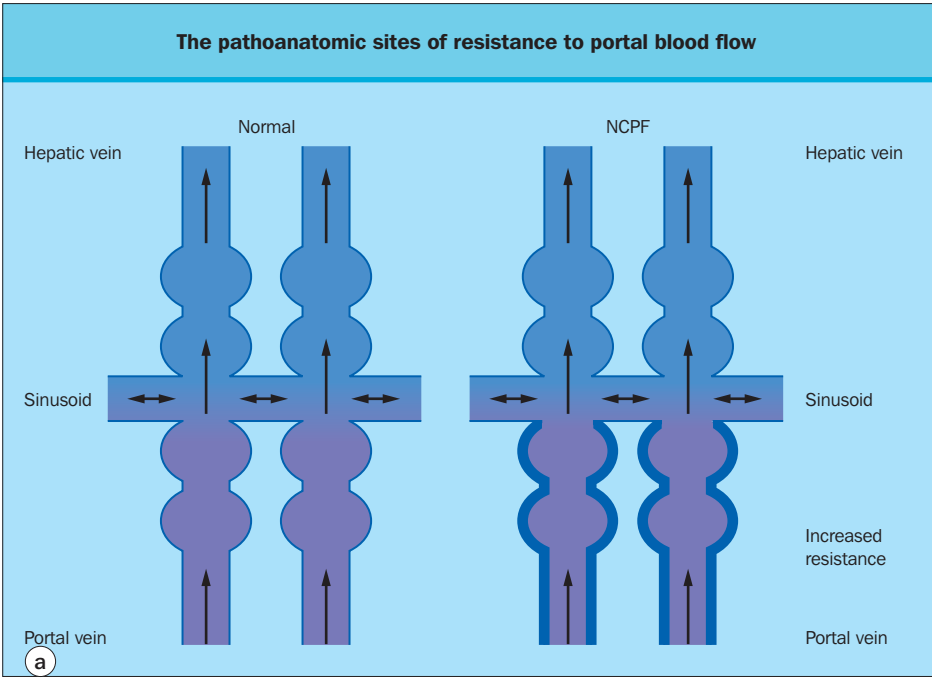
Portal cholangiopathy, defined as anomalies of the biliary system and gallbladder in patients with PHT, is commonly seen in patients with EHPVO. It is mild and less common in NCPH patients and in general is of little clinical consequence.

### Differential diagnosis

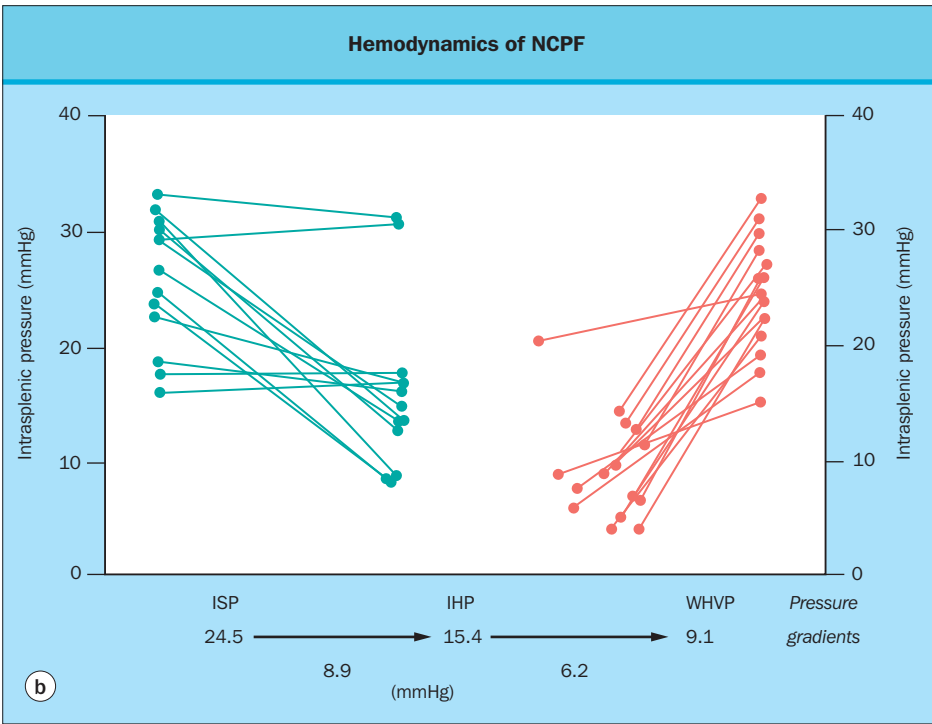
Non-cirrhotic portal fibrosis can easily be differentiated from acute or chronic portal vein thrombosis (**Table 35.6**). NCPH generally develops insidiously, while acute portal vein thrombosis often has an acute presentation (upper gastrointestinal bleeding, abdominal pain and ascites). In chronic portal vein thrombosis, there is cavernous transformation of the portal vein. NCPH, however, can not be excluded if there is only thrombosis in one branch of the portal vein.

Child Class A cirrhosis may clinically mimic NCPH, but liver histology (lobular disarray, pseudolobule formation) will distinguish between the two. Moreover, a disproportionately large spleen with a dilated and thickened portal vein favors the diagnosis of NCPH. Tropical splenomegaly syndrome (TSS) is another condition, sometimes seen in the tropics, which presents with massive splenomegaly. However, PHT is uncommon in patients with TSS and the WHVP is within normal limits. Elevated serum immunoglobulin M (IgM) levels and high malarial antibody titers are also common in TSS patients. Histologically, NCPH needs to be distinguished from conditions like nodular regenerative hyperplasia (**Fig. 35.12**), partial nodular transformation and incomplete septal cirrhosis.

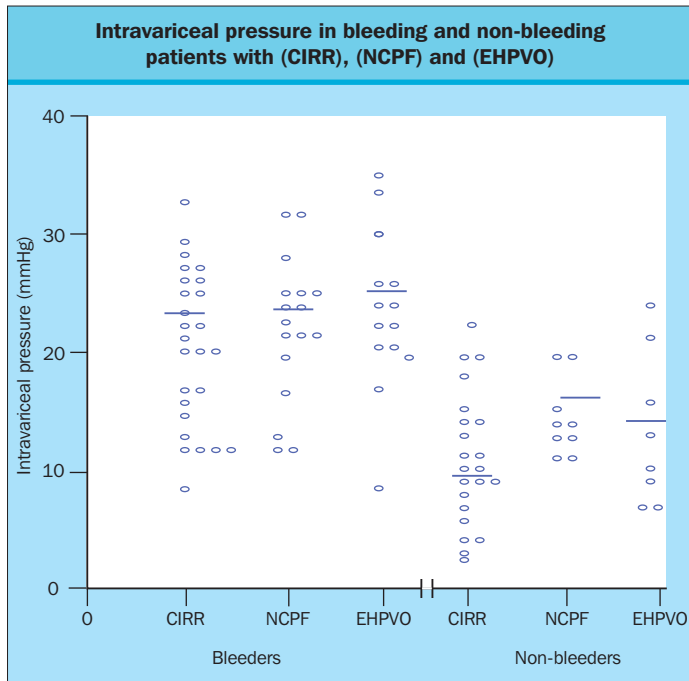




**Figure 35.5a The pathoanatomic sites of resistance to portal blood flow.** Sites of resistance in patients with non-cirrhotic portal fibrosis (NCPF), namely presinusoidal and perisinusoidal, caused by thickening and obstruction to the medium-sized and small branches of portal veins and the collagenization of the space of Disse, respectively.



**Figure 35.5b Pressure gradients in patients with non-cirrhotic portal fibrosis (NCPF).** Two pressure gradients, between the spleen and liver [intrasplenic pressure (ISP) – intrahepatic pressure (IHP)] and between the liver and the wedged hepatic venous pressure (IHP–WHVP) are observed in patients with NCPF. In effect, they represent presinusoidal and perisinusoidal resistance to portal blood flow.



**Figure 35.6 Intravariceal pressure.** Intravariceal pressure measured by direct needle puncture in bleeding and non-bleeding patients with cirrhosis (CIRR), non-cirrhotic portal fibrosis (NCPF) and extrahepatic portal vein obstruction (EHPVO).

Profile of non-cirrhotic portal fibrosis and extrahepatic portal vein obstruction patients		
	NCPF (n = 207)	EHPVO (n = 236)
Mean age (years)	30.7	13.9
Sex (males : females)	117 : 90	168 : 68
Hemetemesis/melena (%)	84.5	94.5
Mass in LUQ (%)	13.5	3.5
Ascites (transient) (%)	10	12.7
Jaundice (%)	–	12.7
Liver function tests	Near normal	Near normal
Esophageal varices (%)	92	94
Gastric varices (%)	22.3	40.7
<i>Portal gastropathy</i>		
Presclerotherapy (%)	1.6	0.5
Postsclerotherapy (%)	17	15
Portal biliopathy (%)	40	90
Portal colopathy (%)	40	44
Data are based on the experience at the GB Pant Hospital, New Delhi, India (unpublished data). NCPF, non-cirrhotic portal fibrosis; EHPVO, extrahepatic portal vein obstruction.		

**Table 35.4 Profile of non-cirrhotic portal fibrosis and extrahepatic portal vein obstruction patients.**

#### Coagulation and platelet function in non-cirrhotic portal hypertension

Parameter (normal range)	NCPF (n = 18)	EHPVO (n = 18)
INR	1.8 ± 0.68*	1.7 ± 0.4*
PTT (28–31 s)	29 ± 4.2	30 ± 4.2*
Fibrinogen (250–350 mg%)	196 ± 57**	199 ± 61**
Fibrinogen degradation products (<8 µg/mL)	<8	>8**
Platelet aggregation (40–60%)	33 ± 16.5	22 ± 11.3**
Platelet MDA (6–12 nmol/mL)	9.0 ± 3.6	9.6 ± 3.8
*P < 0.05, **P < 0.001 compared with controls. EHPVO, extrahepatic portal vein obstruction; INR, international normalized ratio; MDA, malondialdehyde; NCPF, non-cirrhotic portal fibrosis; PTT, partial thromboplastin time.		

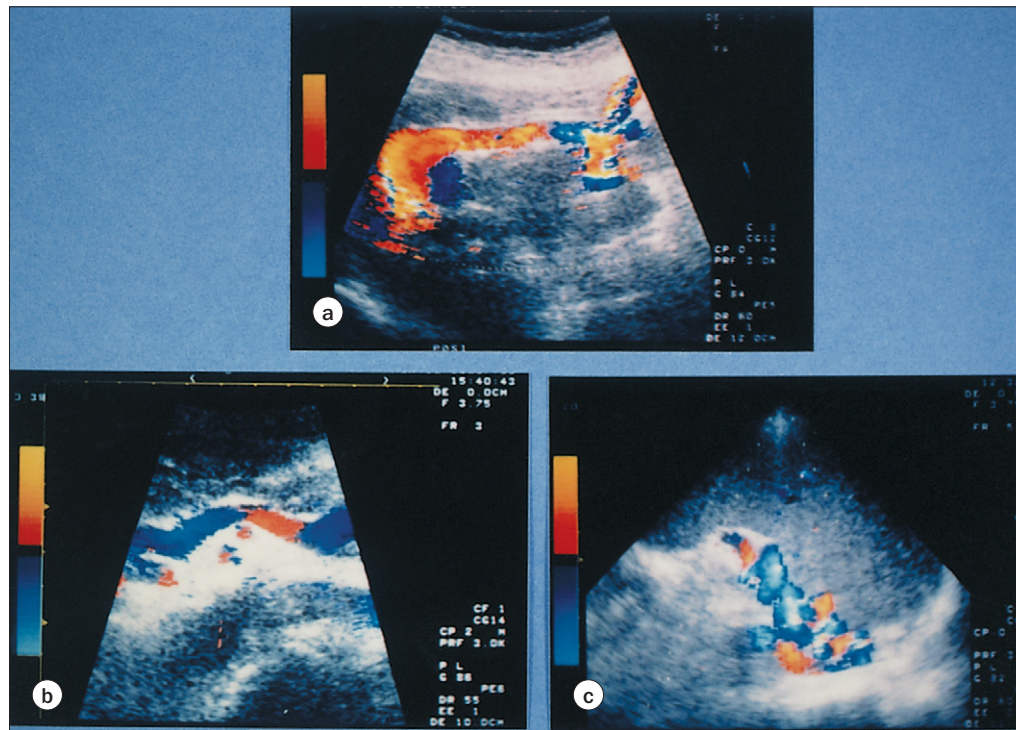
**Table 35.5 Coagulation and platelet function in non-cirrhotic portal hypertension.**



**Figure 35.7 Idiopathic portal hypertension.** An early case of idiopathic portal hypertension. Percutaneous transhepatic portogram shows a dilated splenic vein and portal trunk and unobstructed portal vein branches. A large spleen is indirectly recognized from the size of the splenic vein in its hilum.

#### Management

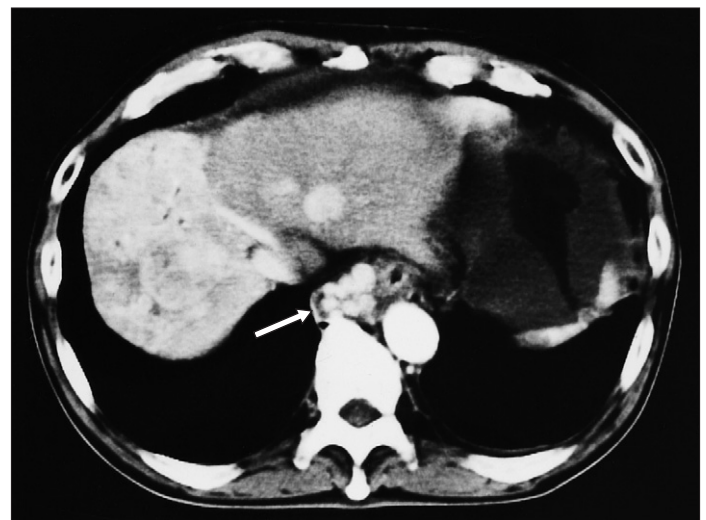
Gastrointestinal hemorrhage is the major concern in the management of NCPF. For acutely bleeding patients, endoscopic variceal ligation (EVL) and endoscopic sclerotherapy (EST) are equally efficacious (95% success in the control of an acute bleed). Emergency shunt surgery is required in less than 5% of cases. If required, selective shunting (e.g. distal splenorenal shunt), is preferred because of a lower incidence of post-shunt encephalopathy. The number of sessions of banding or sclerotherapy required for obliteration of varices is similar in patients with cirrhosis and NCPH. Variceal recurrence has been reported in approximately 20% of cases, but recurrent bleeding is quite



**Figure 35.8 Color Doppler ultrasonography for the diagnosis of portal hypertension.** (a) Markedly enlarged paraumbilical vein with flow within it. (b) An enlarged left gastric vein flowing into the esophageal varices. (c) Splenorenal shunt at the splenic hilum. Doppler signal (colors) indicates winding blood channels.



**Figure 35.9 Computed tomography of a large paraumbilical vein.** Paraumbilical vein (arrow) in the midline of the abdomen is obvious in this contrast-enhanced computed tomography scan.



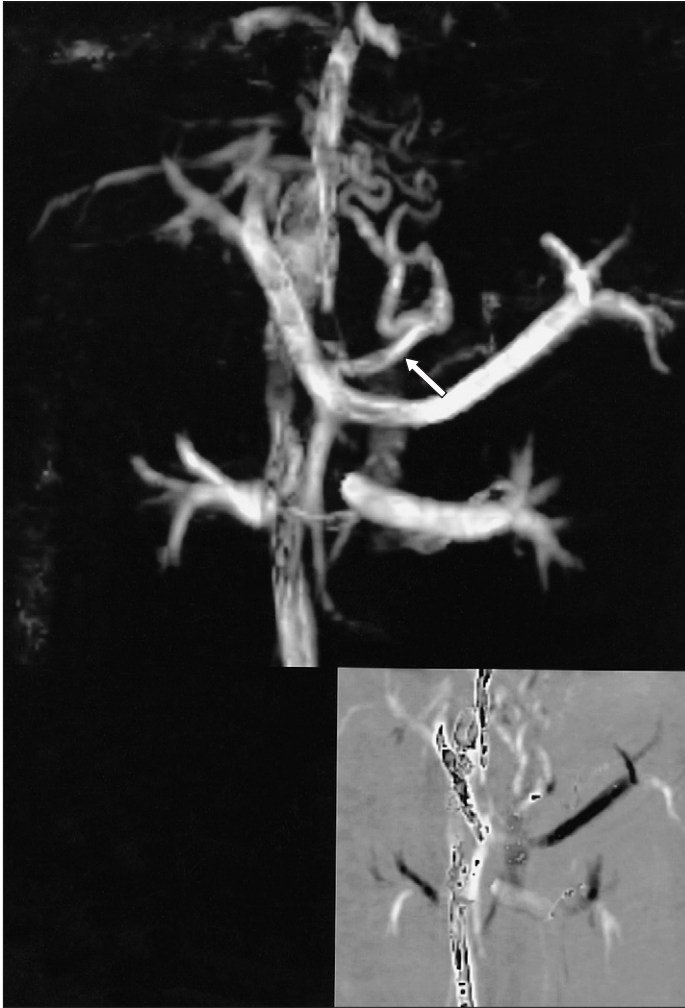
**Figure 35.10 Paraesophageal varices.** A widened paraesophageal structure was identified as paraesophageal varices (arrow) upon injection of contrast medium. The lobar attenuation difference is due to portal invasion of hepatocellular carcinoma.

uncommon (approximately 3%) once obliteration is achieved. Primary prophylaxis using  $\beta$  blockers and EVL were found to be comparable and efficacious, even in non-cirrhotic patients. There have been no large randomized controlled trials of pharmacologic versus endoscopic therapies in the primary prophylaxis of NCPF. Gastric varices are seen in nearly one-quarter of NCPF patients, more often than that seen in cirrhotic patients. These varices can be managed with cyanoacrylate glue injection and rarely require surgical intervention.

A number of interventional radiologic procedures have been reported to be effective in IPH patients. These include splenic embolization, percutaneous transhepatic obliteration and trans-

jugular intrahepatic portosystemic shunts (TIPS). Whether patency rates of TIPS in patients with NCPF are similar to those with cirrhosis is unclear. Shunt surgery has been reported to be an effective back-up in patients who fail to respond to endoscopic therapy. Surgery may also be indicated for patients with symptomatic hypersplenism, spontaneous bleeding episodes or severe anemia requiring transfusion or repeated splenic infarcts. Elective shunt surgery is also a reasonable option for patients who live remote from good medical facilities or who prefer a single therapeutic intervention. The most important cause of





**Figure 35.11 Magnetic resonance angiography of the hepatofugal left gastric vein.** Magnetic resonance angiography by the phase-contrast technique (without contrast medium) clearly demonstrates the hepatofugal left gastric vein (arrow). The black and white colors in the inset indicate the flow direction. The left gastric vein is flowing cephalad, away from the liver. (Courtesy of H. Abe.)

death in NCPF or IPH patients is exsanguination. Certain Japanese workers have advocated prophylactic devascularization.

Surgical mortality after emergency shunts is approximately 10%. Shunt occlusion, chronic portosystemic encephalopathy and rebleeding after elective shunt surgery are seen in approximately 10% of patients. The morbidity and mortality after shunt surgery and the limited expertise available to perform these procedures have restricted the use of surgery in the management of patients with NCPF. Recently, the development of membranoproliferative glomerulonephritis and other renal anomalies have been reported following shunt surgery in NCPF patients.

## EXTRAHEPATIC PORTAL VEIN OBSTRUCTION

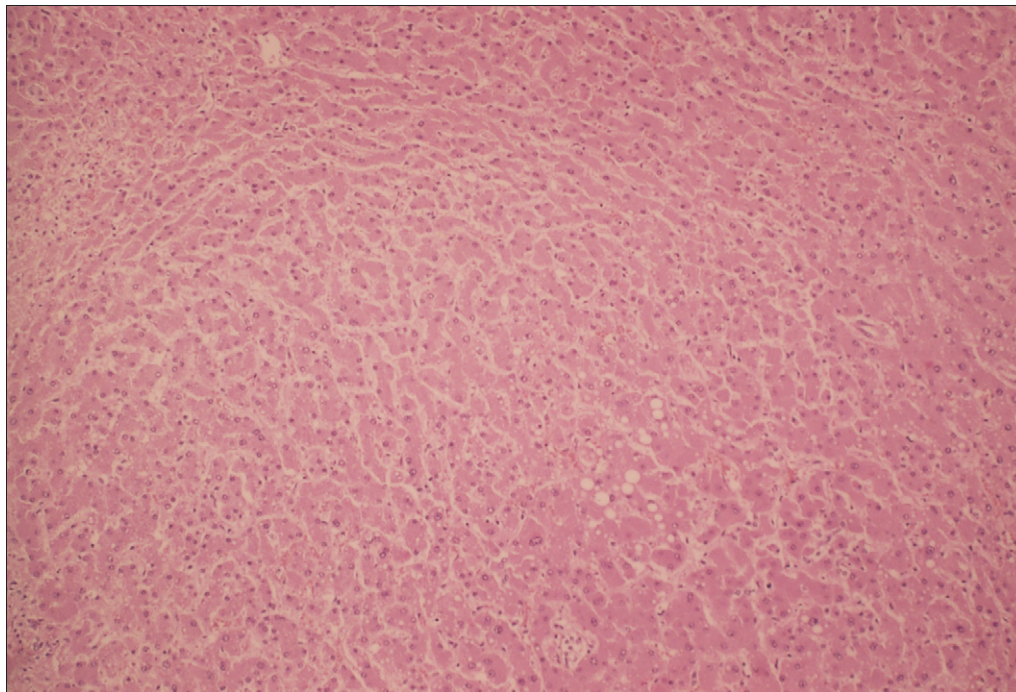
### Definition

Extrahepatic portal vein obstruction (EHPVO) is a common cause of portal hypertension in the developing countries but less so in the West. It is characterized by obstruction in the pre-hepatic portion of the portal vein. This may be accompanied by thrombosis of the splenic or superior mesenteric veins. Portal vein thrombosis is also a known complication of liver cirrhosis. However, the term EHPVO should be restricted to a group of patients with portal hypertension, who have obstruction of the main portal vein in the absence of cirrhosis of the liver (Fig. 35.13).

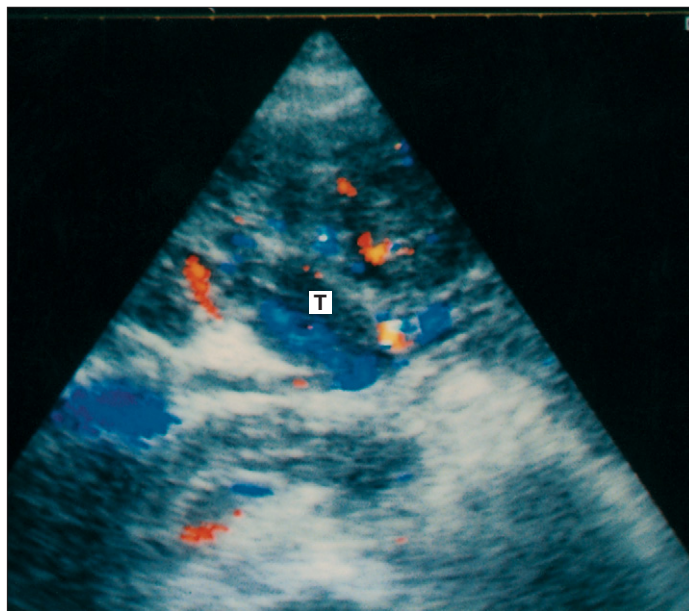
EHPVO is the most common cause of major upper gastrointestinal bleeding in children. In children, EHPVO is usually an isolated condition and is only recognized when the child develops symptoms. In adults, the diagnosis is made earlier because the patients are already under observation for another disease. After cirrhosis, this is the second commonest cause of PHT, even in the West. The most common site of blockage is at the site of portal vein formation (90%) and total blockage of splenoportal axis is seen in only 10%.

Differentiating features between extrahepatic portal vein obstruction, non-cirrhotic portal fibrosis and cirrhosis of liver			
Parameter	EHPVO	NCPF	Cirrhosis
Mean age (years)	10	28	40
Ascites after bleed	Absent/transient	Absent/transient	+ to +++
Encephalopathy	–	–	+
Jaundice/signs of liver failure	–	–	+
Liver function tests	Normal*	Normal	Deranged
<i>Liver</i>			
Gross	Normal/small	Normal, rarely irregular	Shrunken, nodular
Microscopic	Normal	Normal/portal fibrosis	Necrosis, regeneration
Splenoporto-venography/US	Portal/splenic vein block	Normal splenoportal axis, 'withered tree' appearance, periportal fibrosis	Dilated, patent portal and splenic vein
* Occasional impairment may be observed. EHPVO, extrahepatic portal vein obstruction; NCPF, non-cirrhotic portal fibrosis; US, ultrasound.			

**Table 35.6 Differentiating features between extrahepatic portal vein obstruction, non-cirrhotic portal fibrosis and cirrhosis of liver.**



**Figure 35.12 Nodular regenerative hyperplasia.** A regenerative nodule is seen compressing the surrounding hepatocytes.



**Figure 35.13 Color Doppler scan of a portal thrombus (T).** A hepatopetal blood flow along the thrombus is recognized.

### Etiology

The etiology of EHPVO in children is unknown, although various hypotheses have been proposed. The proposed mechanisms could be categorized as: direct causes, which lead to portal vein injury and subsequent obstruction, such as neonatal peritonitis, abdominal trauma, iatrogenic operative trauma to the portal vein, and cysts and tumors encroaching upon the portal vein within the porta hepatis. Next are congenital abnormalities such as

portal vein stenosis, and portal vein atresia or agenesis. Third are factors that are indirectly associated with portal vein thrombosis such as neonatal systemic sepsis from non-intra-abdominal sources, dehydration, multiple exchange transfusions and hypercoagulable states. The latter includes myeloproliferative disorders such as polycythemia vera, inherited deficiencies of natural anticoagulants such as antithrombin III, deficient protein C and protein S deficiency, and factor V Leiden mutation.

The most common predisposing condition is neonatal umbilical sepsis. In this condition, the inflammatory process begins in the umbilical stump before normal obliteration of vein and proceeds proximally to involve the portal venous system. Several investigators believe that umbilical vein cannulation and umbilical sepsis are responsible for portal vein thrombosis in a significant proportion of patients. Others, however, disagree with these observations (Table 35.7). The discrepancies between these observations are probably because the earlier studies have been retrospective and clinical. In a review of 11 major studies, a positive history of umbilical vein catheterization was found in 9% and umbilical sepsis in another 9% of patients with EHPVO. These findings suggest some role for these two factors, but the association is possibly weak. Umbilical vein catheterization was complicated by portal vein thrombosis in 40% after 25–48 h and 100% after 3 days, but was not associated with long-term occlusion. It is possible that the untreated and presumably more severe umbilical sepsis, which may result after deliveries conducted at home or in unhygienic conditions, could still result in portal thrombosis.

Some studies suggest that EHPVO is a developmental abnormality of the portal venous system. Obstruction can occur anywhere along the line of left and right vitelline veins from which the portal vein develops. Other congenital defects, usually of the cardiovascular system, have also been reported in associ-

Etiology of extrahepatic portal vein obstruction										
Etiology	Children Shalaons Sherlock (1962)	Maddrey et al (1968)	Lindsay et al (1979)	Housham et al (1983)	Carlism et al (1992)	Orazoo et al (1994)	Stringer et al (1994)	Children Boles et al (1986)	Adults Vlegger (1997)	Crloff (1994)
Number of patients	160	37	97	32	132	38	53	43	21	162
Umbilical sepsis	9	5	12	0	23	1	1	8	2	12%
Intra-abdominal sepsis	1	–	26	7	24	0	10	1	1	6%
Umbilical catheterization	1	–	6	2	0	0	8	3	0	8%
Prothrombotic disorders	–	–	3	0	7	7	5	0	6	2%
Pancreatitis	–	2	0		3	2	0	–	0	–
Trauma	–	1	2		12	2	4	–	1	4%
Idiopathic	5	29	48 (51%)	17 (59%)	54 (41%)	17 (45%)	26 (49%)	31	11	68%

**Table 35.7 Etiology of extrahepatic portal vein obstruction.**

ation with EHPVO. Congenital defects are common in patients with extrahepatic portal obstruction of unknown cause.

As the occurrence of venous thrombosis of the splenoportal axis is the predominant pathology, the presence of a prothrombotic state has been proposed. An animal model for EHPVO has not yet been produced to support the contention that a prothrombotic state is the initiating event. Myeloproliferative disorders, overt or occult, are an important cause of EHPVO in adults. It has been suggested that the progression of the underlying hematologic disease with 'ongoing thrombosis' might influence the course of bleeding in these patients, particularly in the long term, and, therefore, must be looked for in every patient. In one series, a latent myeloproliferative disorder in 58% was detected in patients with EHPVO of unknown etiology and 57% of these developed an overt myeloproliferative disorder during follow-up. One or more prothrombotic disorder could be detected in 72% adult patients with portal vein thrombosis in another study. Primary myeloproliferative disorders were the leading causal factor (30% cases); primary deficiencies in natural coagulation inhibitors (protein C, protein S, antithrombin III), prothrombin gene mutation (G-20210A), methylene tetrahydrofolate reductase gene mutation (C677T) and factor V gene (Leiden) mutation accounting for the rest. The etiology of blocked portal vein is obscure in ~ 50% of patients.

### Pathology

The macroscopic appearance of the liver varies from smooth to finely granular. The architectural pattern of liver is preserved. Mild or sometimes moderate hepatic fibrosis is seen in up to 40% of the adult patients, partly as a result of parenchymal extinction. Such periportal fibrosis could arise from non-specific or specific inflammation or from chemical irritation as a result of hepatocellular breakdown products. Vascular lesions have been demonstrated in approximately 48% of cases in the form of multiple portal vein channels in small portal tracts in addition to portal fibrosis of a mild degree in the majority. Phlebotrombosis of intrahepatic portal vein branches was seen in a

few patients though much less commonly than in NCPF cases and it was suggested that phlebotrombosis is a common pathogenic denominator in NCPF and EHPVO.

While the pathology of the liver is not very characteristic in EHPVO, the pathology of the portal vein is typical and is termed cavernous malformation of the portal vein. It is made up of a cluster of variable-sized vessels arranged haphazardly within a connective tissue support and the original portal vein cannot be identified. It is usually located at the hilum of the liver and can extend for a variable length inside and outside the liver. Although, hamartomatous and neoplastic theories have been proposed, most authors feel that these features are an end result of thrombosis of the portal vein.

### Pathophysiology of EHPVO

The prehepatic block of EHPVO with normal hepatic sinusoidal pressure and a high pressure in the obstructed splanchnic bed, results in the formation of multiple hepatopetal thin winding collaterals. These collaterals are seen on angiography as 'cavernous transformation' and have been shown by ultrasound Doppler studies to provide a significant contribution to the total hepatic blood flow in these patients. These collaterals join the intrahepatic portal vein branches at various levels. This is a kind of neovascularization to compensate for the portal vein block.

### Functional status of liver

Impairment of the hepatic storage capacity and transport maximum for bromsulfathaline has been documented. It has been suggested that periportal fibrosis prevents normal portal venous blood flow, thus making the liver in EHPVO more dependent upon hepatic arterial blood and these pathologic changes may lead to a decreased functional status of the liver.

### Clinical presentation (Table 35.8)

EHPVO can present as early as 6 weeks after birth or may not become manifest until adulthood. The typical presenting symptoms in infancy are variceal bleeding, ascites and growth failure. Later in childhood and early adult life, variceal bleeding



Clinical presentations of extrahepatic portal venous obstruction								
	Maddrey (1968)	Lindsay (1979)	Househam (1983)	Cardin (1992) Adults	Orozco (1994) Adults	Mikkelsen (1965)	Stringer	Boles
Age at presentation (years)	19.8	20.9				38		
Bleed	32/37	53/97	13/32					
Splenomegaly	5/37	23/97	11/32					
Ascites		7/97	5/32	23/32	–	5/13		
Ascites (post bleed)	(5/37)	6/97	7/32	23/32	–	5/13		
<i>Etiology</i>								
Umbilical sepsis	5/37	12	–	23/32	1/38		1/53	8/43
Pancreatitis	2	–	–	3	2		0	–
Trauma abdomen	1	2	–	12	2		4	–
Splenomegaly	100%	–	100%					
Hepatomegaly	8/37	–						
Site of block		–						
Hilar PV	17	–						
PV formation	13							
Splenic vein	7	–						
Cavernomatous transformation	21	–						
Intra-abdominal infection	–	26	7	24	–		10/53	1
Exchange transfusion	–	6	2	–	–		8	3
Idiopathic	29/37	51%	176(59%)	54 (41%)	17 (45%)		26 (49%)	31
PV, portal vein								

Table 35.8 Clinical presentations of extrahepatic portal venous obstruction.

and hypersplenism are the main presenting clinical problems. Abdominal pain and thrombocytopenia due to splenomegaly may occur as well. Rarer problems seen in adults with portal venous thrombosis (PVT) include venous infarction of the bowel, hemobilia and pulmonary emboli.

The most usual presentation for patients with portal venous obstruction is sudden, unexpected, and often massive hematemesis. The patients withstand the hemorrhages remarkably well and hepatocellular failure is not a problem. Ascites develops during the course of the disease in up to 20% of patients, but it is generally transient following hemorrhage or surgery. Ascites is more frequent in adult patients than in children suggesting that the duration of portal hypertension could determine whether ascites develops. It also has been suggested that ascites may be related to the deterioration of liver function and fall in serum albumin with increasing age.

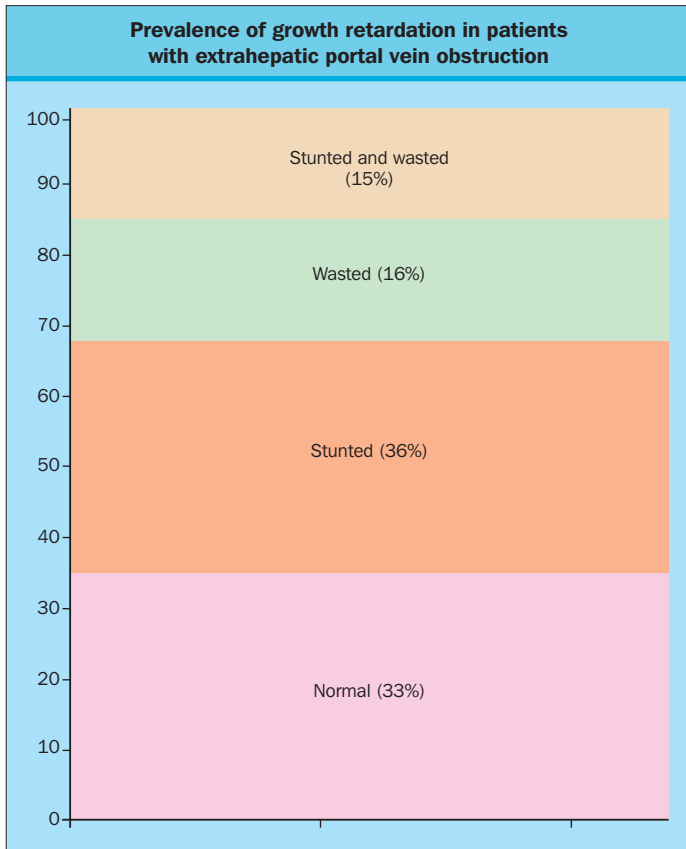
Jaundice may also be a presenting feature of portal vein occlusion. Jaundice may result from venous collaterals running in the vicinity of common bile duct and compressing the bile duct. A rise in serum bilirubin has also been attributed to acceleration in the normal aging process of the liver as a result

of impaired blood supply. Hypoxemia has also been documented in case reports in patients with EHPVO, as a result of intra-pulmonary vascular dilatation.

The clinical course is usually one of recurrent episodes of gastrointestinal hemorrhage from esophageal or gastric varices, but it is not possible to predict which patients will bleed repeatedly. The splenic size and portal pressure do not correlate with the incidence or severity of hematemesis. Except for occasional reports, there is no definitive data to suggest that the frequency of variceal bleeding decreases after puberty.

### Growth

The hepatotrophic effect of portal blood has been well established in several experimental studies. It has been shown that young rats undergoing portal vein ligation or portosystemic shunt surgery gain significantly less body weight compared with sham-operated control animals. Growth patterns in patients with EHPVO indicate that nearly one-third of patients are short in stature and have significantly diminished growth velocity (Fig. 35.14). Growth spurts have been observed after shunt surgery in patients with EHPVO.



**Figure 35.14** The prevalence of growth retardation in patients with extrahepatic portal vein obstruction (EHPVO).

### Portal biliopathy

The term 'portal biliopathy' refers to abnormalities of the extrahepatic and intrahepatic bile ducts with or without anomalies of the gallbladder wall in patients with portal hypertension. Biliary tract abnormalities have been reported in 80–100% of patients with EHPVO by endoscopic retrograde cholangiopancreatography (ERCP). The changes include indentations of paracholedochal collaterals on bile duct, localized strictures, angulation of ducts, displacement of ducts and stones in the common bile duct and focal narrowing, dilatations, irregular walls and clustering of intrahepatic branches in the hepatic ducts (Fig. 35.15). Gall bladder varices are quite common and carry significant amount of blood around the gall bladder. These do not affect gall bladder contractility. The frequency of gallstones has been reported to be higher in EHPVO patients.

The biliary abnormalities may be explained either by compression of bile ducts by prominent paracholedochal and epicholedochal collaterals (indentations and wall irregularities) or ischemic injury of the bile ducts as a result of thrombosis of veins draining the bile duct (stricture formation). These two mechanisms may not be mutually exclusive as some changes like indentations; smooth strictures and caliber irregularity disappear after shunt surgery due to disappearance of paracholedochal collaterals while others like angulation and ectasia persist suggesting fixed obstruction due to ischemia.

Although biliary abnormalities have been reported in 80–100% of cases, few are symptomatic. There are case reports

of jaundice caused by the presence of cavernous transformation of the portal vein. Symptomatic patients are usually adult, indicating that portal biliopathy is a progressive disease.

### Hemodynamic studies

WHVP within normal limits and significantly elevated intra-splenic pressure indicates the presinusoidal nature of the block. Total hepatic blood flow is normal or decreased. However hepatic arterial flow is increased after portal venous obstruction. Systemic vascular resistance is significantly lower and cardiac output is significantly higher in patients with EHPVO, indicating a hyperdynamic circulatory state, similar to that found in patients with cirrhosis.

### Autonomic dysfunction

Impaired cardiovascular autonomic reflexes occur in subjects with portal hypertension due to EHPVO. The role of autonomic nervous dysfunction in the pathogenesis of characteristic hemodynamic disturbances of portal hypertension is controversial. It has been suggested that disturbances in autonomic cardiovascular reflexes may contribute to the hemodynamic abnormalities in cirrhosis but only to a small extent in EHPVO patients.

### Coagulation abnormalities

Coagulation abnormalities have been variably reported in patients with EHPVO. While one study found normal coagulation studies, others have reported abnormal prothrombin time, partial thromboplastin time and platelet function. It has been suggested that abnormal coagulation in patients with EHPVO results from mild compensated disseminated intravascular coagulation secondary to portosystemic shunting.

### Immune status

The humoral immunity is normal in patients with EHPVO when compared to patients with cirrhosis in whom it is grossly abnormal. The cell-mediated immunity shows qualitatively similar defects in patients with EHPVO and chronic liver disease. The defects in cell-mediated immunity result, in part, from sequestration of T cells by the spleen and partly from the presence in serum of factors that influence the kinetics of lymphocyte response.

### Diagnosis

The presence of esophageal varices in a child with normal or near-normal liver function tests should alert the clinician to the possibility of EHPVO. In an adult, compensated cirrhosis may produce a similar picture. A number of radiologic techniques have been successfully used to study the portal venous system including splenoportography, celiac angiography, percutaneous transhepatic portography, duplex sonography, color Doppler flow imaging (CDFI), arterial portography, CT arterial portography, CT percutaneous transsplenic portography and MR angiography.

Splenoportography or arterial portography (selective celiac or superior mesenteric angiography) provide images of the portal venous system of sufficiently high quality to permit identification of the site of obstruction (Fig. 35.16). However, large collateral flow rate can often divert the contrast and prevent adequate visualization of patent intrahepatic radicles.



**Figure 35.15 ERCP picture showing intrahepatic and extrahepatic bile duct strictures and stones.**

Ultrasound is a reliable non-invasive alternative with high accuracy (sensitivity 94–100% and specificity 96%), particularly if cavernous transformation of the portal vein has occurred. Cavernous transformation produces a distinctive tangle of tortuous vessels in the porta hepatis and represents collateral flow around the portal vein (Fig. 35.16). Two potential errors are noteworthy: first, a new thrombus that is virtually anechoic may not be detected if color Doppler is not used; and second in patients with portal hypertension, low velocity or ‘to-and-fro’ portal flow may be very difficult to detect, causing a false impression of occlusion.

### Management

The management of EHPVO depends very much upon the age of the patient, the site of obstruction and the clinical manifestations. Variceal hemorrhage is the major complication requiring therapy. Patients with portal hypertension who have not bled from varices can be observed as bleeding may not occur for many years. In general, hypersplenism is not an indication for surgical intervention, but in an occasional patient, profound thrombocytopenia with bleeding, repeated infections or physical discomfort caused by massive splenomegaly may be sufficiently severe to merit splenectomy.

### Treatment of variceal bleeding

Since the EHPVO patients have essentially normal livers and tolerate bleeding well, therapy should be individualized. If the obstruction is confined to splenic vein, splenectomy alone is curative. The problem is not so straightforward when the portal vein is involved.

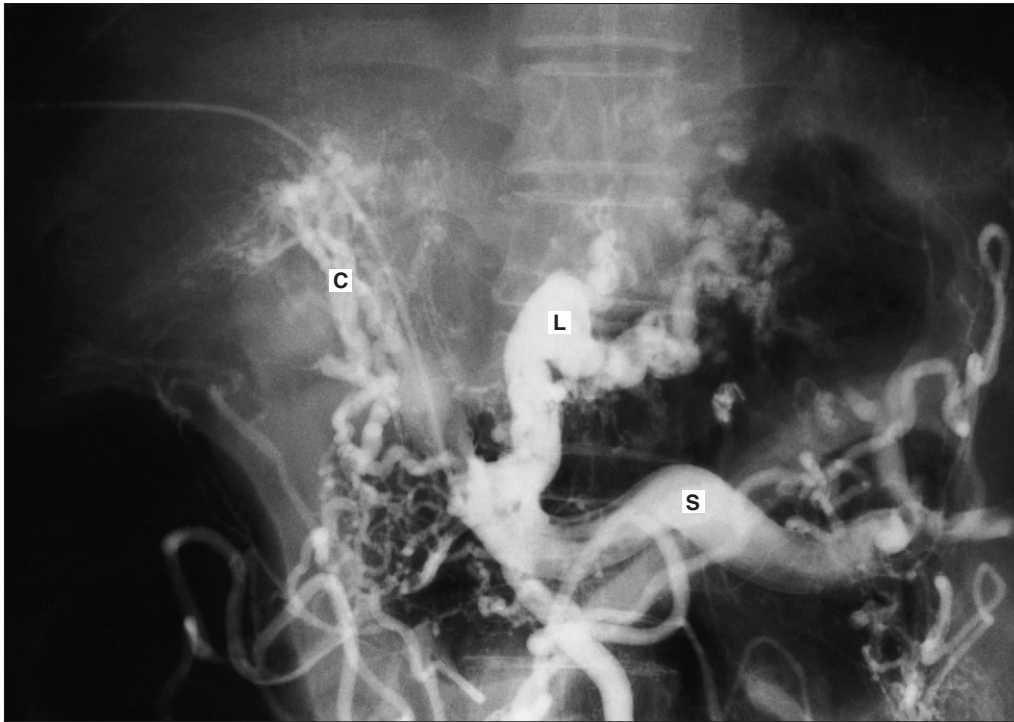
### Endoscopic variceal eradication

Both endoscopic sclerotherapy and band ligation have been found to be effective. With sclerotherapy, the risk of spread of the thrombus is not real. Long-term follow-up studies have found that sclerotherapy prevented bleeding in 88% patients after variceal eradication. In general, EVL has yielded even better results with fewer complications than sclerotherapy, particularly in smaller children. However, variceal eradication alone without alleviation of portal hypertension cannot lead to normal growth of these patients and also does not prevent the development of portal biliopathy and formation of gastric and ectopic varices.

### Shunt surgery

A variety of shunt procedures are available for those who fail endoscopy therapy. These include non-selective shunts including a central splenorenal shunt with splenectomy, mesocaval,





**Figure 35.16 Catheter penetration of a soft thrombus.** The catheter passed percutaneously through the liver penetrated a soft thrombus in the portal vein. Injected contrast medium opacified an enlarged left gastric (L), splenic vein (S), and cavernous transformation (C).

portacaval and 'makeshift' shunts. Initial rebleeding rates, although better than with ablative procedures, are still high. More recent series have shown improved patency rates even in small children. A 10% rebleeding rate was noted in children less than 6 years old who had undergone non-selective shunting with an average vein size of 6.5 mm. Others have since reported rebleeding rates with central splenorenal shunts and mesocaval shunts ranging from 4 to 33%.

The selective shunts, designed to decompress gastroesophageal varices without compromising portal blood flow, include distal splenorenal shunts (DSRS), distal splenocaval shunts, gastro-epiploic to left renal vein shunts and left gastric vein to left renal vein shunts. An overall patency rate of 92% at 5 years was reported with selective shunts and resulted in low rebleeding rates. However, selective shunts cannot be performed in patients who have thrombosis of the splenic vein, which is common in EHPVO, or who have had a previous splenectomy and only around a third of patients prove to be suitable candidates for this intervention. In addition, hypercoagulable disorders are frequently associated with EHPVO, with an increased consequent risk of thrombosis in any kind of shunt.

Other approaches that directly treat the gastroesophageal varices include transesophageal ligation of varices, esophago-gastrectomy and gastric devascularization with splenic artery ligation and splenopneumopexy. In general, these procedures have extremely high rates of rebleeding, approaching 70–80%. Therefore, they are not recommended as primary procedures especially when surgical shunting is an option. In patients with failed shunts or in patients where shunting is not possible, these ablative procedures may be considered.

### Hypersplenism

Since splenomegaly, thrombocytopenia and leukopenia are common in EHPVO patients, the question often arises whether

splenectomy should be performed, even prior to variceal bleeding. In general, splenectomy should not be performed, as it has no permanent effect on portal hypertension. Moreover, removal of the spleen can lead to thrombosis of the splenic vein making future shunt operations more difficult.

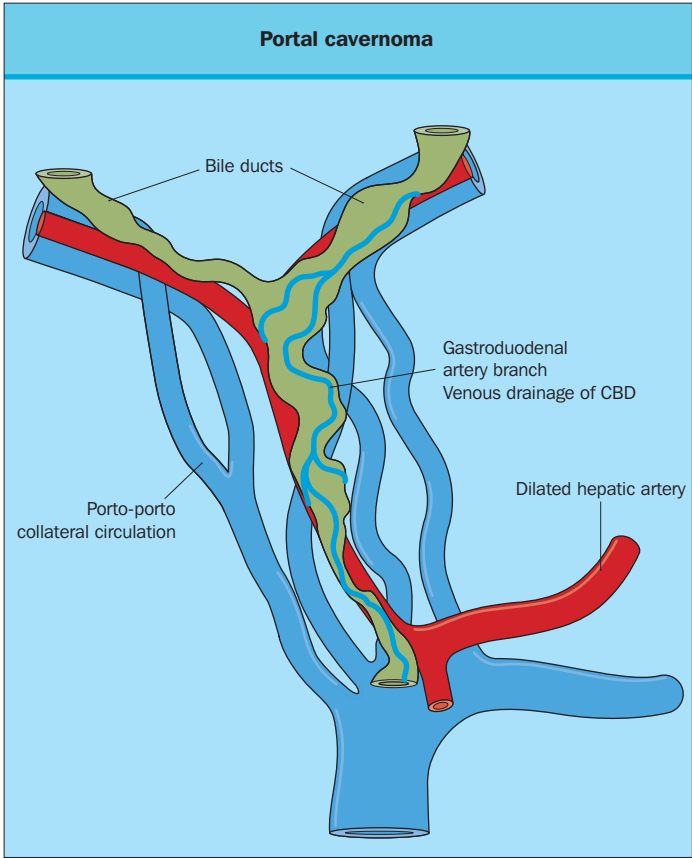
Thrombocytopenia, often below  $50\,000/\text{mm}^3$ , is more alarming to the physician than to the patient. Although reduced in number, these platelets function normally. Symptomatic hypersplenism is seen in only around 5% of patients with EHPVO. Distal splenorenal shunt will relieve splenic congestion, resulting in a reduction in the spleen volume and a modest increase in the platelet count in some patients.

### Portal biliopathy

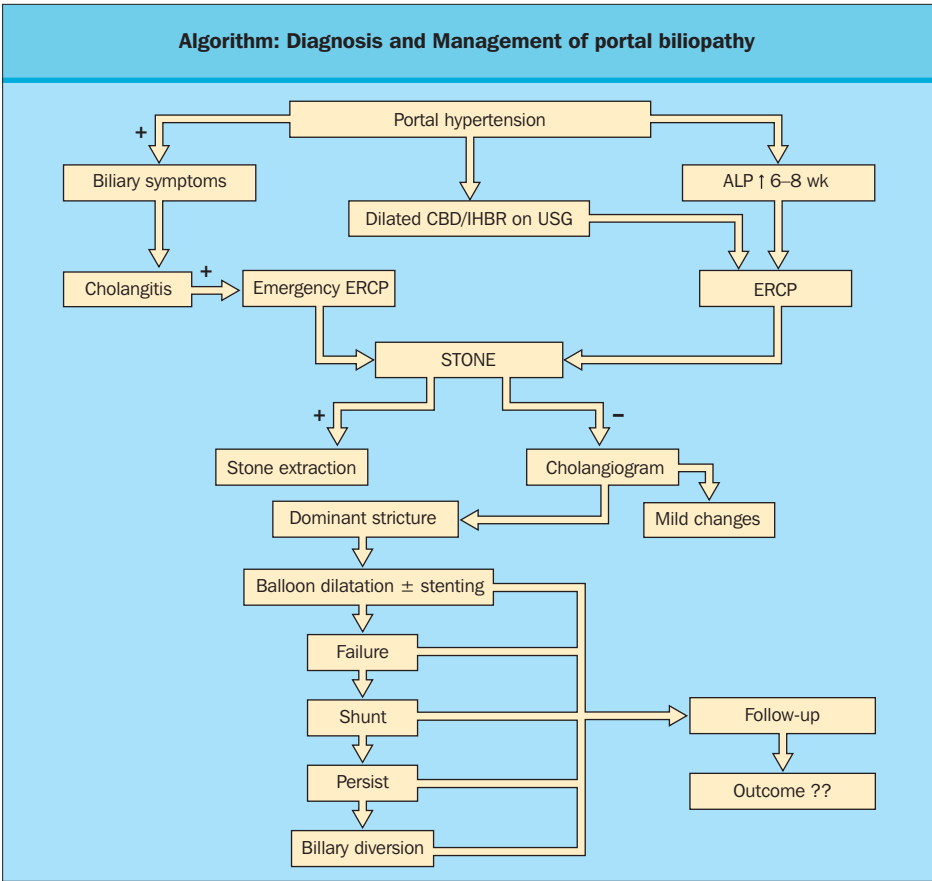
Symptomatic portal biliopathy is predominantly due to compression of the bile ducts (**Fig. 35.17**) and is indication for intervention. Although choledocholithiasis can be managed by endoscopic sphincterotomy, the biliary obstruction persists and the risk of recurrent stone disease remains high. For those with biliary obstruction, portosystemic shunting usually leads to amelioration of biliary obstruction and prevents further stone formation. In patients with persistent obstruction, hepatico-jejunostomy may be needed to treat the biliary obstruction, with access to the region being made possible by an initial portosystemic shunt. Symptomatic portal biliopathy is one of the few indications for portosystemic shunting in the absence of variceal bleeding. An algorithmic approach to such patients is helpful (**Fig. 35.18**).

### Anticoagulant therapy

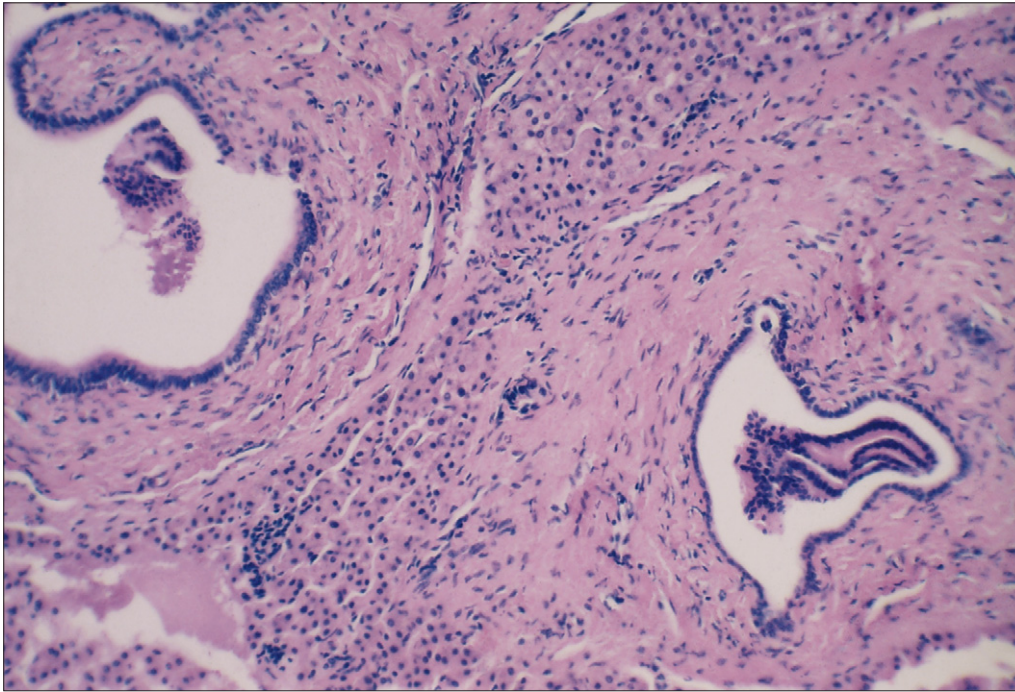
It was suggested that long-term anticoagulation might prevent both extension of thrombosis to the splanchnic veins and thrombosis in other deep veins once the role of prothrombotic disorders was recognized in the pathogenesis of PVT. The avail-



**Figure 35.17 Portal cavernoma.** A schematic diagram showing the portal cavernoma, the collaterals joining the extrahepatic to the small intrahepatic branches of portal vein and the pressure on the bile duct radicals (Modified from Lebrec D.).



**Figure 35.18 Algorithm to manage patients with portal cholangiopathy.** ALP, alkaline phosphatase; CBD, common bile duct; ERCP, endoscopic retrograde cholangiopancreatography; IHBR, intrahepatic biliary radicles; USG, ultrasound.



**Figure 35.19 Congenital hepatic fibrosis containing dilated bile ducts.**  
The liver parenchyma is sandwiched by thick fibrous bands in the portal tracts.

able preliminary data suggest a possible benefit with no evidence of an increase in either the incidence or severity of gastrointestinal bleeding. Recanalization of fresh portal vein thrombosis has been reported following anticoagulation. Acute PVT can occasionally be treated by thrombolysis, or by removal of the thrombus through the transjugular route.

### **NODULAR REGENERATIVE HYPERPLASIA AND PARTIAL NODULAR TRANSFORMATION**

Nodular regenerative hyperplasia (NRH) of the liver is a local hyperplastic response of hepatocytes probably due to vascular anomalies. In NRH, 1–2 mm nodules of regenerating hepatocytes occur diffusely, compressing the intervening liver parenchyma (Fig. 35.12). There is no fibrosis associated with the nodules. About 75% of these patients have antiphospholipid antibodies, especially anticardiolipin antibodies. NRH has been associated with collagen vascular disease, lympho- and myeloproliferative disorders, and other blood dyscrasias.

Partial nodular transformation on the other hand relates to the presence of a large regenerative nodule near the hepatic hilum leading to the development of portal hypertension. Such nodules are a result of portal circulation disturbances and have also been seen in IPH. Nodules form in the areas of the liver where there is adequate portal perfusion in compensation for the parenchymal atrophy caused by decreased portal perfusion in other areas as a result of vascular changes.

### **CONGENITAL HEPATIC FIBROSIS**

Congenital hepatic fibrosis (CHF) (Fig. 35.19), presents in childhood with liver enlargement and portal hypertension. The most common clinical presentation is bleeding from gastroesophageal varices. This condition is generally inherited in an autosomal recessive fashion and is often associated with polycystic kidney disease in children (Ch. 28). In this condition, the liver cells are normal ultrastructurally, and these patients have normal liver function. While there are bands of fibrous tissue that diffusely percolate through the liver, this condition is differentiated from cirrhosis by the absence of regenerative nodules. Within the bands of fibrous tissue is often striking numbers of dilated and cystic bile ductules. This condition is thought to be related to a failure of normal ductal development. Because these patients have normal liver function, the variceal bleeding tends to be well tolerated. Treatment of patients with CHF is primarily geared towards control of variceal hemorrhage. In general, the first level of treatment is endoscopic variceal band ligation or endoscopic sclerotherapy. Patients who fail endoscopic therapy are also excellent candidates for a selective shunt, because this disease is not progressive and they do not have impaired liver function. In general, these patients also do not develop ascites and rarely require liver transplantation. However, in the patients with CHF associated with polycystic kidney disease, renal failure ultimately becomes the predominant clinical problem.



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## SECTION IV: SPECIFIC DISEASES OF LIVER AND BILIARY TREE

### CHAPTER

# 36

## Hepatobiliary Surgery

Andrew M. Smith and J. Michael Henderson

### INTRODUCTION

As early as 2000–3000 BC, the Assyrian and Babylonian cultures used the livers of sacrificed animals to divine the future. Over the ensuing centuries, battle surgeons debrided small bits of liver protruding from abdominal wall stab and gunshot wounds. However, formal entry into the peritoneal cavity to control hemorrhage due to trauma or to remove tumors and drain cysts had to await the development of general anesthesia and antisepsis.

The first successful liver resection was performed in Berlin by Dr Carl von Langenbuch in 1887. He removed a pedicled tumor from the left lobe of a 30-year-old woman. Later that evening, however, the patient required re-exploration to control hemorrhage. Liver surgery entered the modern era in the 1950s, and remarkable progress has occurred over the past 50 years. The factors responsible for the progress include an understanding of the segmental anatomy of the liver as described by Couinaud in 1954, advances in the techniques of liver resection, a better understanding of liver diseases, the dramatic improvements in techniques to image the liver, and the ability to support patients metabolically both intraoperatively and postoperatively.

### HEPATIC ANATOMY AND DEFINITION OF RESECTIONS

The liver is divided into a left and right half based on the incoming vascular structures. Externally, a plane between the gallbladder fossa and the inferior vena cava (IVC) divide the right and left lobes; the falciform ligament further divides the left lobe into medial and lateral segments. The liver has segmental anatomy as defined by Couinaud (**Fig. 36.1**). Each liver segment has independent biliary drainage and vascular inflow and outflow. Consequently it is possible to remove an individual segment without disrupting the blood flow or biliary drainage of the remaining segments. Based on the Brisbane 2000 Terminology of Hepatic Anatomy and Resection, the liver can be divided into four sections: the right anterior section (segments V and VIII), right posterior section (segments VI and VII), left medial section (segment IV) and left lateral section (segments II and III). A right lobectomy or hepatectomy removes segments V–VIII; a left lobectomy excises segments II–IV. Removal of segments IV–VIII comprises an extended right lobectomy; the medial segment of the left lobe is resected in addition to the entire right lobe. Segments II–VI are excised in an extended left lobectomy; the anterior portion of the right lobe in addition to the entire left lobe. A wedge resection removes the tumor along with a 1–2 cm margin of normal parenchyma in a non-anatomic manner.

### HEPATIC SURGERY

#### Intraoperative monitoring and resuscitation

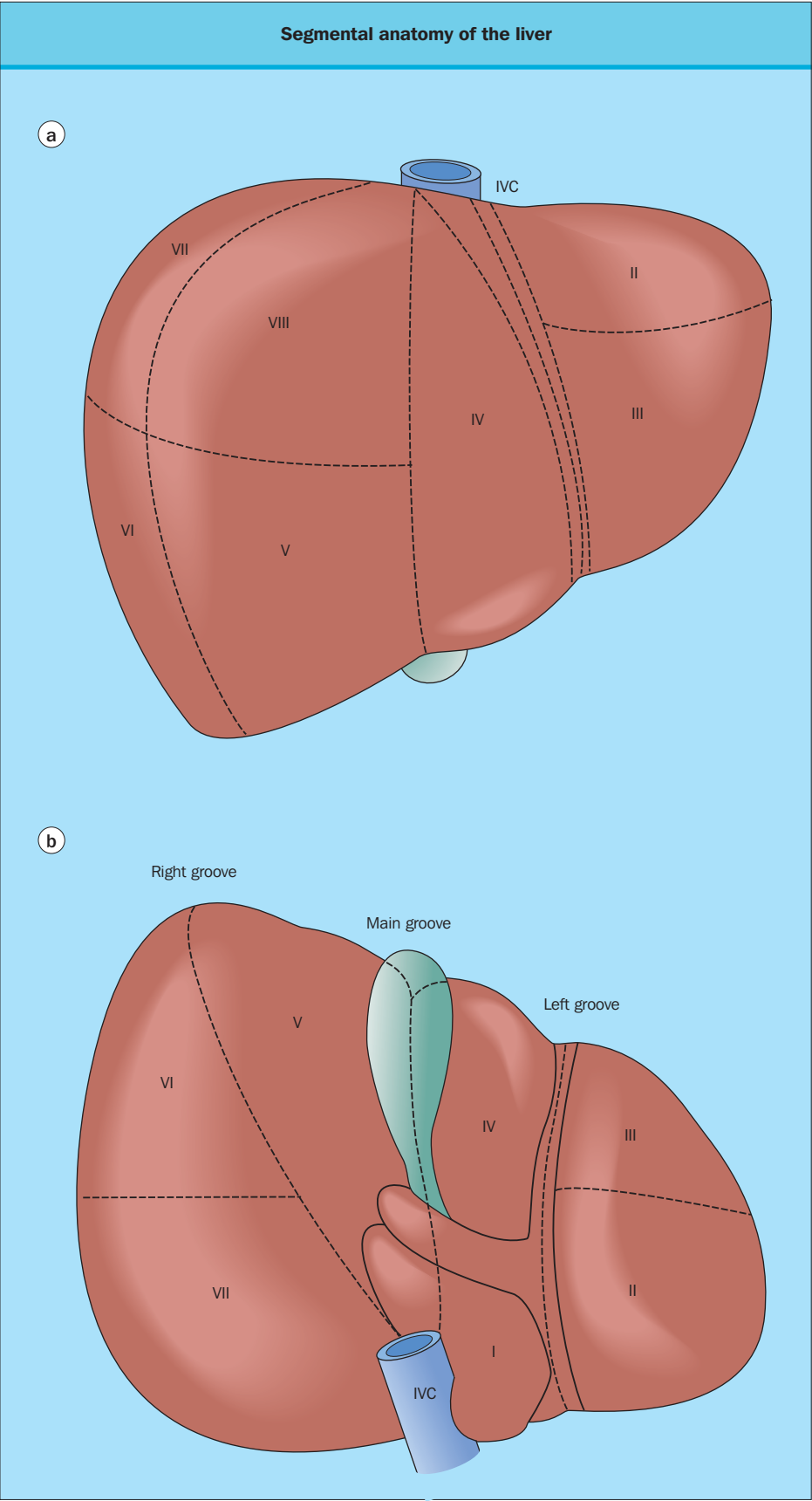
Precise intraoperative monitoring and resuscitation are crucial for the success of liver resection. Mean arterial pressure and central venous pressure are added to the routine monitoring of the heart rate, blood pressure, electrocardiogram and oxygen saturation. A Swan-Ganz catheter is reserved for patients with cardiac problems who require even more precise monitoring. A Foley catheter and nasogastric tube are placed after the induction of general anesthesia. Excessive intraoperative blood loss has been demonstrated to be the major determinant of adverse perioperative outcome. Minimizing blood loss during hepatectomy is therefore essential. Low central venous pressure anesthesia (below 5 mmHg) is fundamental, and is maintained by restricting fluid infusion and limiting intraoperative blood transfusion unless more than 25% of the blood volume is lost. Other techniques to reduce blood loss include vascular inflow and outflow control and placing the patient in the Trendelenburg position.

#### Surgical techniques

Preliminary laparoscopy may be used in some patients with primary hepatocellular carcinoma (HCC) or metastatic colorectal cancer to the liver who have not had previous upper abdominal surgery. If laparoscopy reveals metastatic disease, bilobar disease, cirrhosis or significant fatty change in the non-tumor-bearing portion of the liver, the procedure is terminated. The patient is spared a laparotomy and can be discharged within 24 h. Laparoscopy is less useful in patients who have had prior upper abdominal surgery since they have adhesions from prior surgery which will limit the visibility of laparoscopy and increase the risk of iatrogenic injury to the bowel. In patients with prior upper abdominal surgery a limited right subcostal incision is made and if extrahepatic and bilobar disease are not found, the incision is extended into a chevron with a midline extension. Once the laparotomy has been performed, the abdomen is again inspected for any extrahepatic disease.

The liver is mobilized by dividing the right and left triangular ligaments and the falciform ligament. The mobilization facilitates intraoperative ultrasound and parenchymal transactions later. There are three basic techniques for anatomic hepatectomies:

- Hepatectomy with preliminary vascular ligation was described by Lortat-Jacob in 1952. The initial maneuver is the ligation and division of the left or right side of the portal pedicle at the hilum, followed by ligation and division of the appropriate hepatic veins and finally the sectioning of the liver parenchyma. The dissection of the hepatic veins can be hazardous and control of the suprahepatic and infrahepatic inferior vena cava is



**Figure 36.1 Segmental anatomy of the liver.**  
(a) Anterior view of segments II–VIII. (b) Inferior aspect of the segmental anatomy.



recommended. This technique has two advantages: it reduces intraoperative blood loss and it produces a clear line of demarcation between the ischemic and the normally vascularized liver. The disadvantage of this technique, besides the risk of injury to major veins, is the danger of erroneous ligation of an element in the porta hepatis.

- Hepatectomy by primary parenchymal transection was described by Ton That and Nguyen-Duong-Quang. The hepatic parenchyma is divided along the line of the middle hepatic vein, and glissonian elements are ligated within the liver, without prior control. Division of the hepatic vein is performed within the liver toward the end of the procedure. The main attraction of this technique is that the risk from anatomic variants is minimized as the portal elements are approached above the hilum. The disadvantage of this technique is that bleeding can be considerable if the resection is not performed quickly, and continuous or intermittent clamping of the hepatic pedicle may be necessary.
- The final method is hepatectomy with extrahepatic vascular control and intrahepatic ligation. The hilar dissection is initially performed, gaining control of the arterial and portal elements of the side to be resected and clamping them without ligation. The appropriate side of the vena cava is freed without dissecting the hepatic vein. The liver is then divided along the main scissural line and, as in the technique of Ton That, the portal elements are located as the parenchyma is transected. Ligation of the vessels is, therefore, performed distal to the clamps. At the end of the liver transection, the hepatic vein is ligated within the liver and the clamps are removed. This technique has the advantages of minimizing blood loss by prior vascular control and of safe, accurate division and ligation of the vessels and biliary branches within the liver.

Controlling the bleeding from the parenchyma at the time of its transection remains the most challenging portion of a liver resection. Over the years, several techniques have evolved to reduce the bleeding. The 'Pringle' maneuver is performed by occluding all the vascular inflow into the liver. This is carried out by applying a non-crushing vascular clamp to the hepatoduodenal ligament. However, this maneuver does not control bleeding from the hepatic veins. Intermittent inflow occlusion – 15 min occluded, 5 min reperfusion – has gained popularity recently because of its additional benefit in reducing ischemia/reperfusion injury. The technique of total vascular exclusion (TVE) has some proponents. The dissection is identical to that performed for a total hepatectomy for a liver transplant. In addition to occluding the portal inflow, the IVC both above and below the liver are controlled. Therefore, all the vascular inflow and outflow are occluded. The technique of TVE does reduce cardiac output by 25–30%, but the majority of patients remain hemodynamically stable with additional volume (approximately 500–1000 cm<sup>3</sup>). The technique allows parenchymal transection in a virtually bloodless field. However, clamp times should be kept to less than 60 min, particularly if the remaining parenchyma has either fibrosis or fatty change.

The liver tissue may be divided by several methods, including the finger fracture technique, the Kelly clamp, and the Cavitron ultrasonic dissector (Cavitron Surgical Systems, Stamford, CT). The aim of all these methods is to divide only the parenchymal tissue, exposing the vessels and the biliary structures. The ultrasonic dissector consists of a vibrating device that oscillates at a

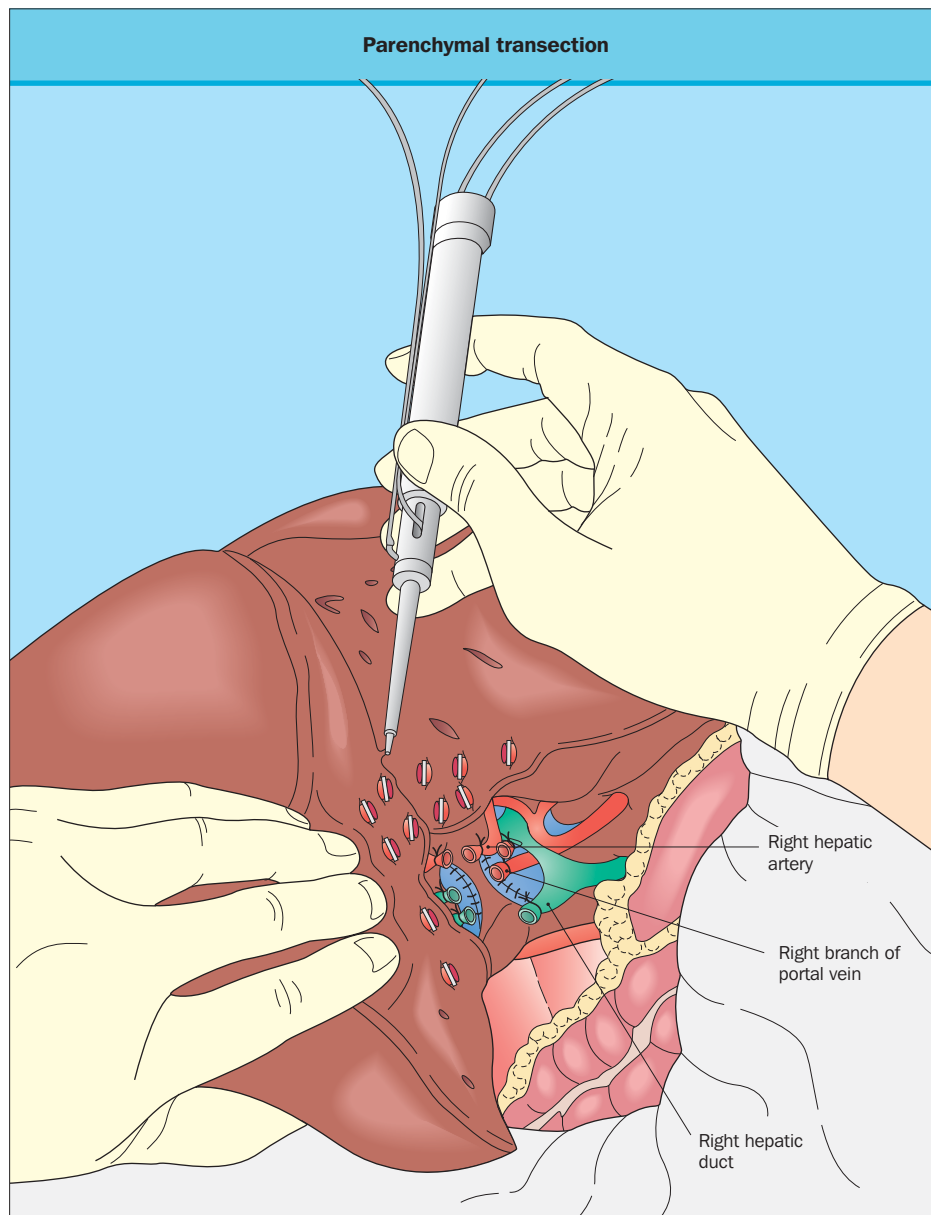
frequency of 23 kHz. It fragments preferentially the water-filled parenchymal cells that are removed by a coaxial irrigation apparatus. The collagen and elastic tissue of the vessels are preserved so that they can be dissected cleanly before accurate coagulation or ligation (Fig. 36.2). In most cases, nothing more is needed than meticulous suture or ligation of the larger vessels, and coagulation of smaller vessels as the resection proceeds. The raw surface is cauterized with an argon beam coagulator or the Tissue-Link coagulator. Both result in tissue desiccation and good hemostasis. Bile channels run with the portal pedicles and are simultaneously dealt with by suture ligation during the resection. Small bile leaks on the raw surface should be actively looked for and oversewn with fine sutures. When hemostasis has been achieved, drains are left in the subphrenic and subhepatic areas, although recent data indicates a lower morbidity if no drains are placed. The abdomen is then closed.

### Postoperative care

The majority of patients who undergo a liver resection enjoy a relatively uncomplicated postoperative course. This is particularly true if the intraoperative effort was problem free. Relatively few patients require more than an overnight stay in a monitored unit. The main points of emphasis are: absence of bleeding, control of pain, satisfactory liver function, minimizing the risk of infection, and return of gastrointestinal function. Intravenous fluids and blood products are given as needed; antibiotics are continued for the first 24 h. The nasogastric tube is removed on the first postoperative day and the diet is advanced as tolerated. The majority of patients have an epidural catheter inserted for pain control; this is removed on the fourth or fifth postoperative day, if the coagulation profile is normal. The Foley catheter is removed on the same day.

Laboratory studies, which include a complete blood count, liver and renal tests, and a prothrombin time, are obtained daily for at least the first 3 days. Initially, the aminotransferases are elevated, ranging from a few hundred U/L to more than a thousand; however, they should normalize rapidly over the next 5–7 days. The prothrombin time and serum bilirubin level may also be elevated; the former may require the administration of vitamin K or fresh frozen plasma. The severity of the derangement of the postoperative liver studies depends on the magnitude of the resection, the duration of the clamp time (warm ischemia), the integrity of the remaining parenchyma, and the intraoperative course. A difficult resection associated with hypotension and the administration of large volumes of fluid and blood products results in more postoperative liver dysfunction. The cholestatic enzymes, alkaline phosphatase, and  $\gamma$ -glutamyltranspeptidase ( $\gamma$ -GT) are initially normal and then tend to rise toward the end of the first week. Both of these tests are markers of regeneration and may stay elevated for a few weeks. The most worrisome biochemical profile 7–10 days following a major liver resection involves a rising serum bilirubin and prothrombin time, accompanied by a normal alkaline phosphatase level.

The mortality following a major liver resection in patients without cirrhosis should be <10% and in major centers is <2%. Mortality results from liver failure/severe dysfunction, infection, multisystem failure and cardiac events. The mortality in patients with cirrhosis is significantly higher. A recent series reported 14% mortality in a group of patients with Child class A cirrhosis. Morbidity ranges from 24 to 40% following liver resection and



**Figure 36.2 Parenchymal transection.** A right hepatic lobectomy is depicted. The right branches of the hepatic artery, portal vein, and bile duct have been ligated and divided. The liver parenchyma is being transected with the CUSA, which is an instrument that uses ultrasound to melt away the parenchyma and expose the vessels and bile ducts which are then clipped or ligated, depending on their size, and divided.

may be procedure-specific, or similar to that reported after any major abdominal operation, such as atelectasis, a urinary tract infection or a wound infection. Procedure-specific complications include liver dysfunction, renal dysfunction (usually as a result of the liver dysfunction), bile leaks, bleeding from the operative site, pleural effusions, ascites and subphrenic fluid collection.

### RADIOFREQUENCY ABLATION

Although surgical resection is the gold standard in the treatment of patients with primary and secondary liver tumors, most patients are not candidates for surgery because of either large tumor burden or accompanying comorbidities. Because medical therapy has limited benefit, regional treatment methods such as percutaneous ethanol injection, cryoablation, and perhaps most promising radiofrequency (RF) ablation have emerged as alternative treatment options for a large number of these patients with liver-predominant disease.

In 1990 McGahan and Rossi independently published the first descriptions of the use of RF thermal ablation for the creation of deep thermal injuries in the liver. Rapidly alternating current is applied via an uninsulated electrode tip into the liver tissue. Heat is generated when the local ionic agitation leads to localized frictional heating of the tissue surrounding the electrode. Cell death occurs in 1 s at 55°C and is instantaneous at 60°C. Heating also causes desiccation and if temperatures are high enough, carbonization. Because electrical heating occurs only very close to the electrode, the creation of high temperatures in a large sphere around the electrode is due to heat exchange. Liver and tumor tissue has high thermal conductivity. However blood vessels act as a heat sink by convective cooling, moving heat away from the intended target. This can seriously affect the zone of ablation. However it protects the blood vessel; unfortunately bile ducts do not have such protection.

RF ablation can be performed at open laparotomy, at laparoscopy or by percutaneous means. It is clear that open RF ablation

is more effective than the percutaneous method. Around 35% of patients examined by conventional imaging are found to have unsuspected disease at the time of open surgery. The electrode is passed into the center of the lesion under ultrasound control. Currently lesions up to 5 cm can be ablated. The extent and completeness of RF ablation is difficult to assess intra-operatively. 'Outgassing' is the appearance of microbubbles in the tissue and this indicates heating, but there is no reliable correlation between outgassing and tissue kill. RF ablation incurs few complications. The major risk is bile duct injury from improper placement of electrodes too close to the major bile ducts.

The clinical results of RF ablation can be divided into results of treatment of colorectal cancer metastases, HCC and other tumors. Unfortunately the literature in this area includes no randomized trials and very few long-term follow-up studies. Published studies demonstrate that the procedure is safe; only 2–8% of patients have complications. Reviewing RF ablation in patients with colorectal cancer shows there is good control of disease at the RF ablation site, with a reported recurrence of 2–21%. However as expected with advanced disease, new metastases develop in 27–66% of cases. Currently RF ablation is indicated for certain unresectable liver tumors and at the present time there is little justification for using this treatment on resectable liver tumors except for those cases deemed unfit. The results of long-term studies are awaited.

## MALIGNANT NEOPLASMS

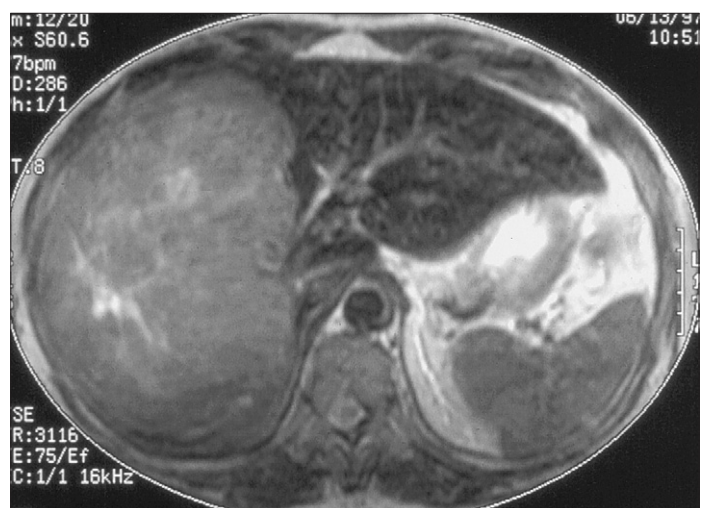
### Hepatocellular carcinoma

HCC is the fifth most common cancer worldwide, being responsible for about 1 million deaths each year. The age adjusted incidence rate of HCC varies widely between 20 and 28 cases/100 000 in East Asia and in sub-Saharan Africa. In the USA a significant increase in incidence from 1.4 to 2.4 cases/100 000 has been observed during the last two decades. Risk factors associated with the development of HCC include cirrhosis (the 5-year cumulative incidence ranges between 7 and 20%), hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, male gender, thorotrast, aflatoxin B<sub>1</sub>, parasites and certain metabolic liver diseases (e.g. hemochromatosis). The tumor normally develops within pathologically altered liver tissue. Because carcinoma itself and not the accompanying hepatic disorder accounts for disease-specific mortality, early tumor detection with an intent of curative treatment, either in terms of local resection or liver transplantation, presents the key issue in the management of the population at risk.

The clinical presentation of HCC varies considerably from an asymptomatic condition, in the case of an incidentally discovered small lesion, to general deterioration, upper abdominal pain, weight loss, jaundice, hepatic dysfunction and a palpable abdominal mass. The laboratory studies most likely to be abnormal involve alkaline phosphatase,  $\gamma$ -GT, and bilirubin.  $\alpha$ -fetoprotein (AFP) is a glycoprotein which may help in diagnosis when serum levels are greater than 500  $\mu$ g/L (normal in most laboratories is between 10 and 20  $\mu$ g/L). However, not all tumors secrete AFP, and serum concentrations are normal in up to 40% of small HCCs. Ultrasound is the most common imaging study for detecting HCC because it is often used in screening. Further diagnostic imaging usually requires computed tomography (CT) or magnetic resonance imaging (MRI). The

ability of CT to detect HCCs has improved with the development of helical CT technology. This technique involves the rapid administration of contrast material in combination with extremely fast imaging. The arterial phase of enhancement allows for detection of hypervascular HCCs as small as 3 mm. The sensitivity of helical CT for detecting HCC may be as high as 90%. On MRI, HCC appears as a high intensity pattern on T<sub>2</sub>-weighted images, and a low intensity pattern on T<sub>1</sub>-weighted images. MRI has better sensitivity and specificity compared to CT in cirrhotic patients in whom it can be difficult to distinguish an HCC from regenerative nodules. It is also more likely to reveal multicentric disease or a more extensive tumor than demonstrated by CT (Fig. 36.3). A needle biopsy, either a core or an aspiration for cytology, is often diagnostic. If a needle biopsy of the tumor is performed, a biopsy of the non-tumor bearing portion of the liver should also be carried out to rule out the presence of cirrhosis, particularly if splenomegaly is demonstrated in the imaging studies. Splenomegaly suggests the diagnosis of portal hypertension and cirrhosis. The key issue in the surgical management of HCC is to confirm the diagnosis quickly with the least invasive techniques. The debate at present is whether small HCC in good risk patients should be managed by liver transplantation or by resection, both of which can be performed safely and with a good chance of success. For more advanced HCC, other treatment modalities come into play.

Once the diagnosis of HCC has been made, further studies are necessary to rule out metastatic disease and to stage the tumor. A CT scan of the chest is required to eliminate the possibility of pulmonary spread. A bone scan may also be indicated. A number of staging systems have been proposed for HCC, none of which has been universally adopted. These schemas invariably incorporate four features that have been recognized as being important determinants of survival: the size of the tumor, the severity of underlying liver disease, extension of the tumor particularly with vascular invasion, and the presence of metastases. Clinical staging of HCC was first done using the Okuda system and, more recently refined by the CLIP (Cancer of the Liver Italian Program) and Barcelona Clinic Liver Cancer



**Figure 36.3 Hepatocellular carcinoma (HCC).** A magnetic resonance image of a large HCC that required an extended right lobectomy.



Group (BCLC) scores. These can all be applied to patients at the time of presentation and initial evaluation is made using clinical, laboratory and basic imaging data. The tumor, node, metastasis (TNM) system is of limited relevance as it only considers tumor variables for post-resection pathologic staging.

If no metastatic disease is detected, and the patient is not a transplant candidate, the patient should be further assessed for a possible resection, by excluding other comorbid conditions, such as heart or lung disease. Early detection strategies for HCC has significantly increased the number of patients undergoing liver resection for non-cirrhotic conditions, and with Child-Pugh class A liver cirrhosis with normal or moderately elevated bilirubin values ( $<1.9$  mg/dL) in the absence of portal hypertension. The resection rate remains low, around 15%, in high volume centers in the USA. Improvements in patient selection and surgical techniques have reduced the operative mortality to the range of 2–15%. Risk factors that increase operative morbidity and mortality include cirrhosis, significant fatty change, prolonged (greater than 1 h) vascular occlusion (warm ischemia), major blood loss, a major resection, coronary artery disease and an elevated preoperative bilirubin. Death is usually from liver failure or severe dysfunction and multiorgan failure. A major liver resection carries a morbidity rate of 25–40%. A recent review of hepatic resections for HCC reports 3-year and 5-year survival rates from 68 to 76% and 51 to 68%, respectively. The favorable determinants of survival by univariate analysis include no vascular invasion, asymptomatic status, a solitary tumor, a histologically negative margin, an encapsulated tumor and a tumor less than 5 cm in diameter. Multivariate analysis shows lack of vascular invasion to be the most reliable favorable factor. Preoperative portal vein embolization (PVE) is a valuable adjunct to major liver resection in the appropriate patient with the goal of hypertrophy of the future liver remnant to enable resection that would otherwise leave a remnant liver insufficient to support life following partial hepatectomy. There are three potential benefits to this technique:

- Post-resection morbidity is diminished, as evidenced by minimal changes in post-resection liver function and decreased length of hospitalization.
- Patients who were initially unresectable because of insufficient remaining normal hepatic parenchyma can undergo resection for potential cure.
- Subclinical disease or rapid progression may be detected prior to definitive surgery on post embolization imaging studies, thus sparing the patient an unnecessary operation.

The role of preoperative hepatic artery chemoembolization (HAC) in the treatment of HCC remains unclear. The impetus to evaluate neoadjuvant therapies arises from the belief that recurrences occur not because of inadequate surgical resection, but because of pre-existing clinically occult microscopic tumor foci. HAC has been used in the following three settings:

- treatment of large unresectable HCC;
- prior to resection; and
- prior to transplant.

When used as a primary treatment for large unresectable HCC, approximately 35% have an objective antitumor response. However its role prior to resection is not defined. One randomized controlled study has shown that HAC prior to surgical resection may be associated with increased mortality.

Fibrolamellar HCC (FLHCC) is a rare variant of hepatoma that was first described in 1956. In contrast to the more common type of HCC, FLHCC is found in young patients (mean age 23 years), and is not associated with either chronic hepatitis or cirrhosis. It usually occurs in the left lobe. The most common presenting symptoms are a palpable mass, abdominal pain, weight loss, malaise and anorexia. The variant FLHCC is thought to be biologically relatively more indolent than HCC because it usually becomes quite large before it is detected. Neurotensin may be a serum marker.

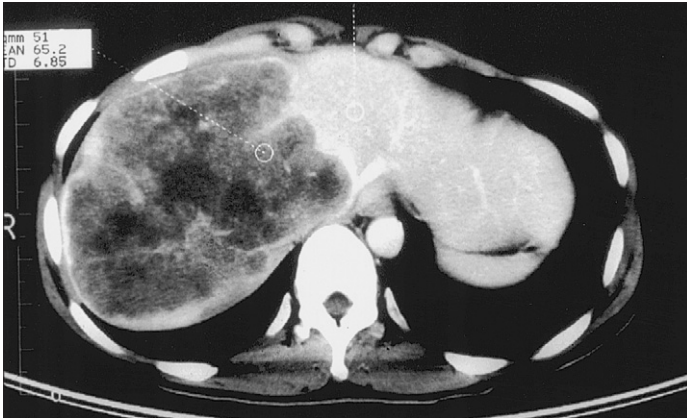
Two series reporting FLHCC have been published. In one series there were 10 patients with a mean tumors of 8 cm. The 5- and 10-year survival rates were 70%. Recurrence occurred in half of the patients and three had a repeat liver resection. Of the 20 patients in the other report, 14 were resected and six underwent liver transplantation. The 5-year survival was 36%. A review of the literature reveals a 5-year survival rate of 56%. When matched stage for stage, the survival of patients with FLHCC is no better than that for non-FLHCC patients. No patient with stage III disease survived for 5 years. Because intrahepatic recurrence is frequently observed and these tumors are slow growing, transplantation has been proposed. In two series, the results of resection and transplantation have been compared. In these studies the long-term results are comparable suggesting resection when possible is preferred to transplantation.

## METASTATIC TUMORS

The most frequently encountered liver tumors are metastatic, commonly from colorectal or neuroendocrine primary sites. Other tumors that are seen include breast, melanoma, genitourinary and sarcoma, but the results of resection have been poor with these tumors. The majority of liver resections in the USA are performed for metastatic colorectal carcinoma. Approximately 150 000 new cases of colorectal carcinoma occur in the USA annually. Of these, about one-third will develop liver metastases, 20% of which will have metastatic disease confined to the liver. Approximately a quarter of this 20% (about 2000 patients) may be candidates for liver resection.

The only curative option for patients with liver-isolated colorectal metastases is surgical resection. In patients with four or fewer isolated hepatic lesions, resection may be curative, with 5-year relapse-free survival rates ranging from 24 to 38%. However, no more than 10% of patients with isolated hepatic metastases are amenable to potentially curative resection. The majority are not candidates for resection because of tumor size, location, multifocality or inadequate hepatic reserve. Nevertheless, because of its impact on survival, surgical resection is the treatment of choice when feasible. For surgical resection to be considered, there must be no evidence of involvement of the hepatic artery, major bile ducts, or main portal vein, and there must be adequate functional hepatic reserve post-resection. Furthermore, occult extrahepatic metastatic disease must be excluded.

The optimal selection of patients who appear to have isolated liver disease for resection is evolving. In the past, contraindications to liver resection were defined by retrospective series. As a result of these analyses, surgeons have been reluctant to offer resection to patients with more than three lesions, those with bilobar distribution, and those in whom it was not possible



**Figure 36.4 Metastatic colorectal carcinoma.** Axial computed tomography image of a large metastatic lesion that was resected by an extended right lobectomy.

to achieve 1 cm margins (**Fig. 36.4**). However, these relative contraindications have been challenged. In one report of 165 patients, of whom 44 underwent complex resections (multiple tumors in both lobes), 5-year survival (37%) was similar to those with fewer or unilobar lesions. In a second report of 155 patients who underwent resection of more than four hepatic metastases, the 5-year survival was 23% overall, and 14% for those with 9–20 lesions resected. The operative mortality ranged from 3 to 8%.

Approximately 10–15% of patients are candidates for a second liver resection for recurrent disease. Almost 65–85% of patients who have a liver resection for metastatic colorectal carcinoma will develop a recurrence. About 20–30% of these patients will have a recurrence in the liver alone and 10% may be candidates for another resection. The preoperative evaluation and the operative morbidity and mortality are the same as before the first resection. The operative blood loss is higher because of adhesions from the initial surgery. The prognostic factors are also the same: obtaining a 1 cm tumor free margin and a solitary lesion. The survival is also equal to that obtained from the first resection (**Table 36.1**). Patients with a metastatic neuroendocrine tumor, such as carcinoid or an islet cell tumor of the pancreas, may also be candidates for liver resection. The patients most likely to benefit from resection are those who have symptoms from a functioning tumor that is overproducing a biologically active hormone, such as those associated with the carcinoid syndrome or a gastrinoma. A series from the Mayo Clinic included 74 patients, 50 with carcinoid and 23 with islet cell tumors. The operative morbidity and mortality were 24 and

2.7%, respectively. Over 90% experienced symptomatic relief, and the 4-year survival rate exceeded 70%. In selected patients, liver resection is safe and effective palliation for patients with metastatic neuroendocrine tumors. A small percentage of these patients may also gain a survival advantage from liver resection.

## BENIGN TUMORS

These lesions are reviewed in detail in Chapter 27. In this section we will describe the surgical indications for removal and techniques.

### Cavernous hemangiomas

Cavernous hemangiomas are the most common benign mesenchymal hepatic tumors. Around 60–80% of cases are diagnosed in patients who are between the ages of 30 and 50 years. Hemangiomas are often solitary, but multiple lesions may be present in both the right and left lobe of the liver in up to 40% of patients. The majority are small (<5 cm). Most patients with hepatic hemangiomas are asymptomatic, have an excellent prognosis and require no intervention. The main indication for investigation is to differentiate the hemangioma from other focal liver lesions.

Only symptomatic hemangiomas need to be excised, regardless of their size. Symptoms however are more likely with larger hemangiomas, presenting with upper abdominal fullness, discomfort or early satiety. Non-specific abdominal pain and the presence of a hemangioma is not an indication for surgery. However, patients who return on more than one occasion because of upper abdominal pain for which no other cause is uncovered may be candidates for excision. Massive lesions that are clearly responsible for pain should also be excised. Size itself is not an indication for surgery; a series from the Mayo Clinic which included 36 patients with hemangiomas with an average size of 8 cm followed for a mean of 12 years reported no spontaneous bleeding. The Kasabach-Merritt syndrome, which is a low-grade local consumptive coagulopathy, is also another indication.

Enucleation is the procedure of choice for removing a cavernous hemangioma (**Fig. 36.5**). A ‘friendly plane’ can be developed between the hemangioma and the normal liver parenchyma. Blood loss can be minimized if the enucleation procedure is combined with control of vascular inflow (Pringle maneuver), or if the hemangioma is massive, with total vascular exclusion (TVE). Enucleation is associated with a 50% reduction of blood loss when compared with a formal resection. Resection is indicated only if the diagnosis is in doubt and there is concern that the lesion is a hemangiosarcoma. Long-term survival after removal of a cavernous hemangioma is excellent.

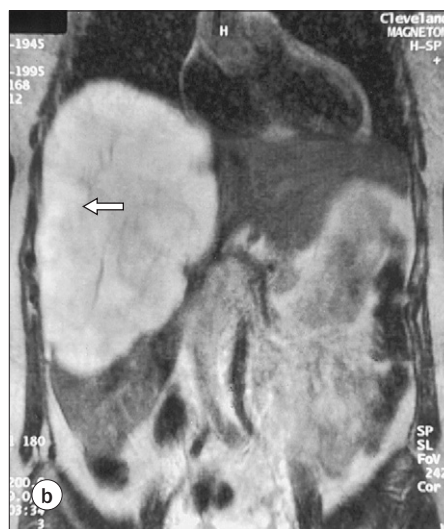
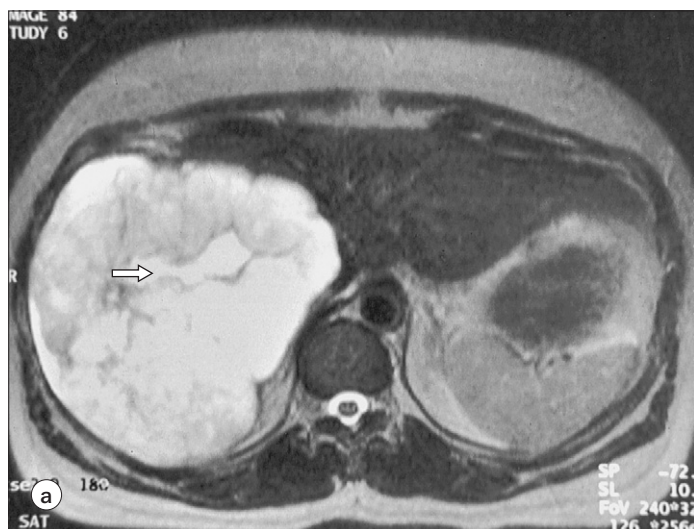
### Hepatic cell adenoma

Hepatic adenomas are uncommon benign epithelial liver tumors that develop in a liver with an otherwise normal appearance. They are seen predominantly in young women (20–44 years-old), are frequently located in the right hepatic lobe, and are typically solitary (70–80%), although multiple adenomas have been described in patients with glycogen storage diseases (GSD) and hepatic adenomatosis. Adenomas range in size from 1 to 30 cm.

**Survival after repeat liver resection for colorectal metastases**

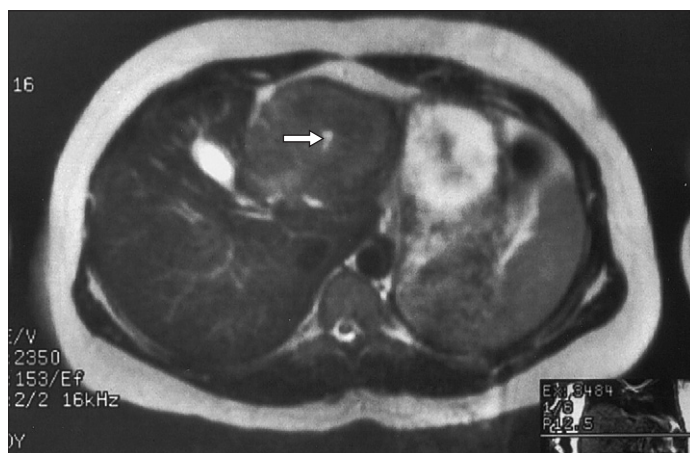
Resection	1 Year (%)	3 Years (%)	5 Years (%)	10 Years (%)
First	78	30	16	16
Second	85	39	25	11

**Table 36.1 Metastatic tumors.** A repeat liver resection for colorectal metastases has a survival comparable with that of the initial resection.



**Figure 36.5 Cavemous hemangioma.** (a) An axial magnetic resonance image showing a large, symptomatic hemangioma (white area; arrow). (b) A coronal view of the same lesion. The hemangioma was enucleated.

Patients with adenomas can present with abdominal pain (50%) and as many as 30% may present acutely with bleeding, either into the tumor or freely into the peritoneal cavity. Malignant degeneration has also been reported with hepatic adenomas. All symptomatic hepatic cell adenomas and those greater than 5 cm require resection because of the risk of hemorrhage and malignant degeneration. Asymptomatic lesions that are less than 5 cm may be observed by serial scans if the patient stops taking oral contraceptives. However, a low threshold to resect the adenoma must be maintained if the lesion does not significantly reduce in size within a few months. Because of the possibility of malignancy, a liver resection with a 1–2 cm margin of normal parenchyma is required for adequate treatment. The overall mortality associated with hepatic cell adenomas is 2–3%, which is virtually limited to those women who present with intraperitoneal rupture and hemorrhage; 20% of these women die.

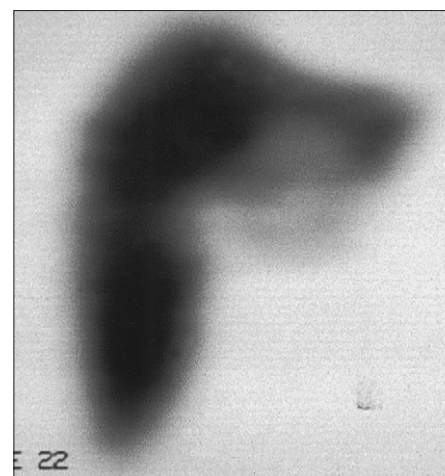


**Figure 36.6 Magnetic resonance image (MRI) of focal nodular hyperplasia.** An axial MRI picture of the characteristic central scar (arrow) of focal nodular hyperplasia.

### Focal nodular hyperplasia

Focal nodular hyperplasia (FNH) is the most common non-malignant hepatic tumor that is not of vascular origin. FNH is seen in both sexes and throughout the age spectrum, although it is found predominantly in women (in a ratio of 8 or 9:1) between the ages of 20 and 50 years. FNH is most often solitary (80–95%), and usually less than 5 cm in diameter.

Laboratory studies are normal. CT, MRI and a technetium sulfur colloid scan are the most helpful imaging studies. Scanning by CT and MRI may reveal the typical 'central scar' – composed of abnormally large portal tracts including large feeding arteries, portal veins and bile ducts (Fig. 36.6). Virtually all cases (80%) of FNH should have a positive sulfur colloid scan because Kupffer cells are present as well as hepatocytes in these lesions (Fig. 36.7). A biopsy reveals all the elements of normal liver tissue, not just hepatocytes. Unlike adenomas, FNH rarely requires resection because there is little risk of either hemorrhage or malignant degeneration. Asymptomatic lesions, even very large ones, can be followed safely. Indications for resection include pain or increasing size as demonstrated by repeated imaging.



**Figure 36.7 Sulfur colloid scan of focal nodular hyperplasia (FNH).** A sulfur colloid scan on the same lesion as depicted in Figure 36.6. The FNH takes up the isotope because of the presence of Kupffer cells in the tumor.



## CYSTIC DISEASE

### Simple cysts

Simple, benign cysts of the liver occur in about 5% of the general population; however, only 5% of these patients become symptomatic. The female:male ratio is approximately 1.5:1 among those with asymptomatic simple cysts while it is 9:1 in those with symptomatic or complicated simple cysts. Although most patients have a solitary cyst, some patients may have several simple cysts. The pathogenesis of these presumed congenital, slow growing cysts is not clearly defined, but congenital lymphatic obstruction and faulty bile duct development are proposed mechanisms. The right lobe is the most frequent site of these lesions. The lesions are lined by cuboidal epithelium and most often contain clear liquid although occasionally they may contain bloody, milky or bile-stained fluid.

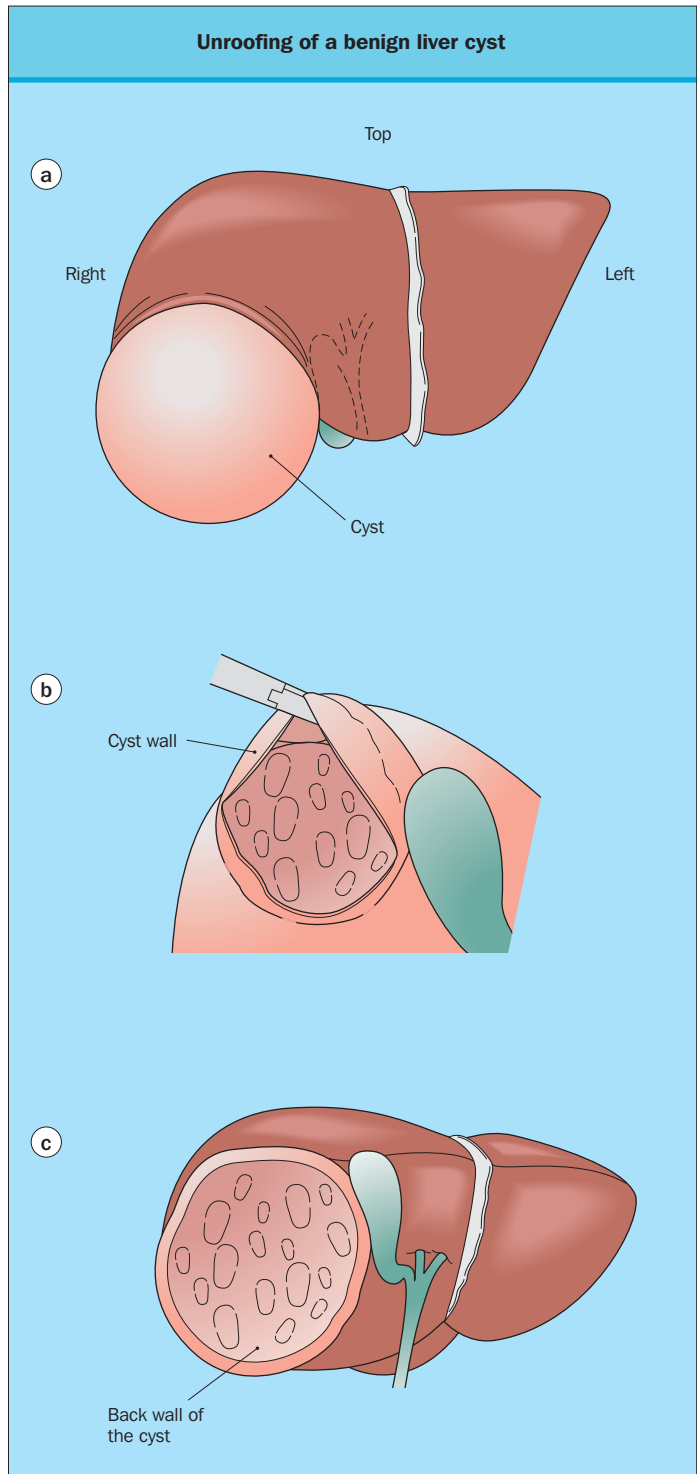
While most simple cysts are asymptomatic, when symptoms occur, they do so because of the very large size of the cyst or hemorrhage into the cyst. Physical examination may reveal a soft upper abdominal mass; a tender mass may be appreciated if hemorrhage has occurred. Liver specific tests are generally normal. Ultrasound and CT reveal a homogenous cystic lesion(s). The distinction between a simple cyst, cystadenoma, cystadenocarcinoma, and echinococcal cyst can be difficult. However, the distinction is extremely important since these lesions have different clinical significance. Ultrasonography is the most useful method for the diagnosis of a simple cyst. It appears as an anechoic unilocular fluid-filled space with imperceptible walls, and with posterior acoustic enhancement. On a CT scan, a simple cyst is defined as a well-demarcated water attenuation lesion that does not enhance following the administration of intravenous contrast. Uncomplicated simple cysts are virtually never septated. However, hemorrhage into a simple cyst can lead to confusion in the sonographic differentiation from a cystadenoma or cystadenocarcinoma.

Treatment is required only if the cyst is symptomatic or if the diagnosis is uncertain. Percutaneous aspiration alone results in prompt recurrence of the cyst. Placement of a percutaneous catheter may also result in infection of the cavity. Removing as much of the cyst wall as possible is the surgical procedure of choice. Excellent results have been obtained by excising the cyst wall laparoscopically. Several centers have reported recurrence rates ranging from 0 to 14% and morbidity rates of 0 to 15% after laparoscopic unroofing of solitary simple cysts (Fig. 36.8).

Complete excision or liver resection is not necessary and may be hazardous because of bleeding. Any fluid that is produced by the remaining cyst wall is reabsorbed by the peritoneum. If the cyst is infected, external drainage is necessary. A cyst that contains bile must communicate with the biliary tree. Formerly, the recommendation was to sew a Roux limb to the cyst. However, the sterile cyst was then converted to an abscess. The current approach is to identify the communication with the bile duct by performing an intraoperative cholangiogram and then close the fistula by suturing. The morbidity and mortality associated with procedures to treat simple liver cysts is extremely low and the long-term prognosis is excellent.

### Polycystic liver disease

Adult polycystic liver disease (APLD) is inherited as an autosomal dominant and occurs in 0.6% of the population. There is



**Figure 36.8 Unroofing of a benign liver cyst.** (a) A cyst in the right lobe. (b) The lesion is being partially excised laparoscopically; the same procedure could be performed open. (c) Final appearance after much of the cyst wall has been excised.

an increased prevalence associated with increasing age and female sex. Of patients with APLD, 50% have polycystic kidney disease as well. The hepatic cysts are grossly and histologically similar to simple cysts of the liver. However the cysts are multiple and may extensively replace both sides of the liver. In addition to macroscopic cysts, there are usually numerous microscopic

cysts and clusters of multiple bile ductules, designated as Von Meyenburg complexes. The pancreas is infrequently cystic. Hepatic cysts occurring in APLD result from a mutation in the *PRKCSH* gene that encodes a protein called hepatocystin.

In most patients the liver cysts are clinically silent. The most common symptoms, if present, are related to the massive size of the cystic liver. Abdominal distention, discomfort, early satiety, supine dyspnea, leg edema from compression of the vena cava, and hepatomegaly are the major symptoms and findings. Less frequent complications include portal hypertension and biliary obstruction. In spite of the massive cystic changes, the liver maintains its functional capability; these patients do not progress to liver failure. CT is the most helpful imaging study.

Asymptomatic patients require no treatment. Therapy for APLD is reserved for patients who have severe symptoms that are significantly affecting their quality of life. The goal of surgical therapy is to reduce the bulk of the cystic liver. Two surgical approaches that have resulted in some success include resection of the most involved portion of the liver and extensive fenestration or unroofing of as many cysts as possible. A review of 46 patients from eight series reported that combining resection and fenestration resulted in 82% of the patients becoming symptom free. The associated morbidity and mortality were 33 and 4.3%, respectively. Some authors have reported progression of the cysts in the unresected portion of parenchyma over the following several years. Liver transplantation has been successfully performed in a small number of patients. However, the technical difficulty of performing the transplant is much greater in a patient who has previously had a resection and/or fenestration. Combined kidney and liver transplantation has been advocated for patients with both APLD and polycystic kidney disease in whom renal failure seems imminent.

### Hydatid cysts

Although hydatid cysts of the liver are uncommon in the USA, they are endemic in South America, New Zealand and the Mediterranean. Approximately 95% of infections are caused by *Echinococcus granulosus*; the remaining 5% are from *E. multilocularis*, which is more virulent and difficult to control because of its diffuse nature and invasive characteristics.

Hydatid cysts are most commonly unilocular and may grow as large as 20 cm. The cyst wall is about 5 mm thick and consists of an external laminated hilar membrane and an internal enucleated germinal layer which is responsible for production of the colorless hydatid fluid, brood capsules and daughter cysts. Brood capsules are small cellular masses and together with calcareous bodies form 'hydatid sand'. About 70% of lesions are in the right lobe and 15% in the left, with both lobes involved in approximately 15% of cases.

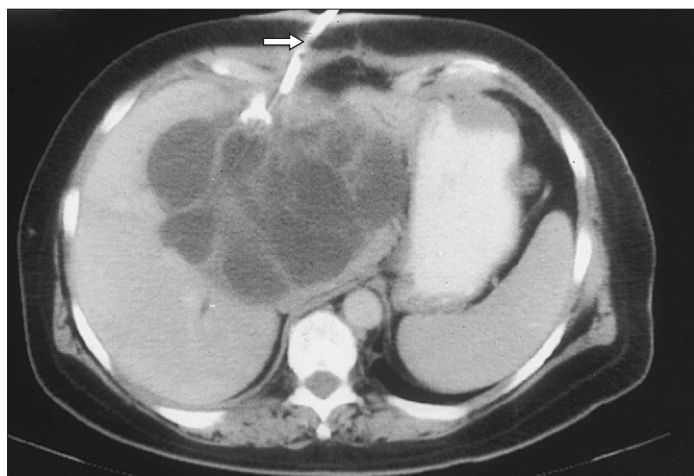
Many infections are probably contracted in childhood and remain dormant for many years. Development of symptoms is insidious. Distention of the liver capsule may produce right upper quadrant pain. Jaundice is infrequent but may be due to extrinsic biliary compression or communication with the biliary tree leading to obstruction by cystic debris. The complications associated with hydatid cysts usually result from either the cyst becoming secondarily infected with bacteria (10% of cases), or it ruptures into the peritoneal cavity, pleural space, pericardium or biliary tree. The diagnosis of hydatid disease of the liver requires a high index of suspicion, especially if the patient is

either a native of an endemic area, or has recently traveled to an endemic area. The current serum immunoelectrophoresis assay is 85–90% accurate. The most helpful study is a CT scan which demonstrates a cystic lesion in the liver that either has a calcified wall, contains daughter cysts or both.

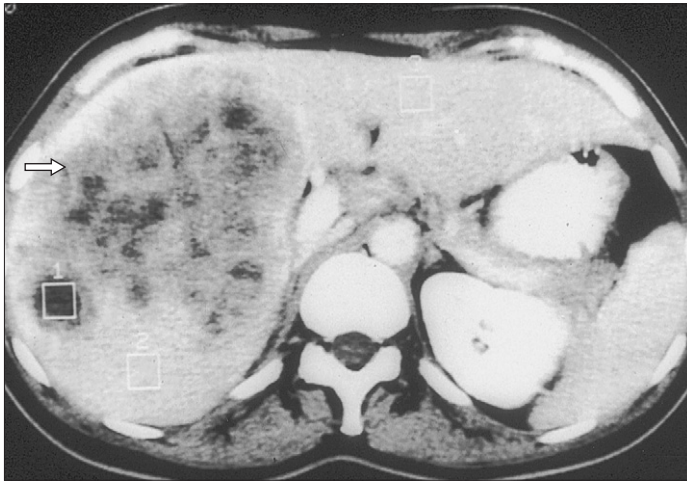
Once the diagnosis has been established, surgery is generally required as the natural history of viable hydatid cysts is one of growth and potential complications. Medical therapy includes the anthelmintics such as albendazole and mebendazole. However, these agents have limited success. They are used pre-operatively in an effort to sterilize the cyst and are reserved as definitive treatment for patients with inoperable, diffuse or recurrent disease. The principles of surgical therapy include complete evacuation of the cyst, destruction of the germinal lining, and avoiding spillage of daughter cysts. The surgical options consist of a liver resection, pericystectomy and non-resectional procedures. A liver resection is considered radical and reserved for peripheral lesions or diffuse involvement of a lobe. A pericystectomy involves removing the entire cyst; the procedure is difficult to perform and carries significant morbidity. The current preferred procedure involves complete evacuation of the cyst, instillation of 30% hypertonic saline to sterilize any remaining scolices, removing the endocyst (which contains the germinal layer), and either leaving the cavity open or packing it with omentum. With the non-resectional approach, the mortality in uncomplicated cases is 5% and the recurrence rate is 5–10%.

### Neoplastic cysts

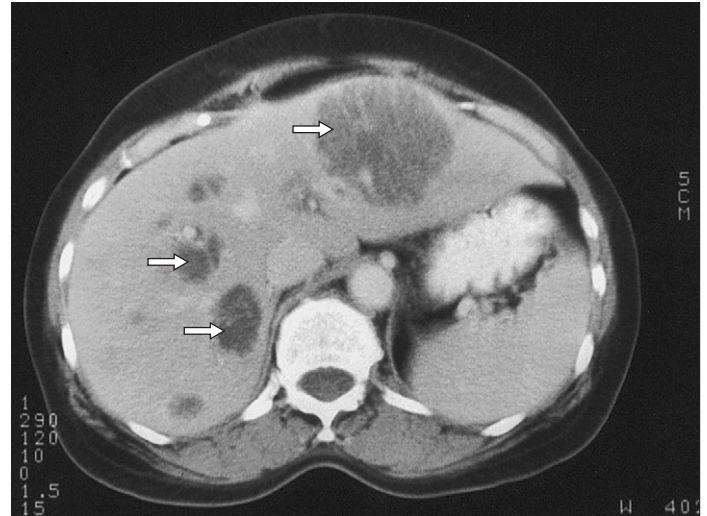
Cystadenoma and cystadenocarcinoma are rare cystic tumors of the liver. Of these tumors, 75% occur in women. Upper abdominal fullness and/or discomfort and a palpable mass are the presenting clinical manifestations. An ultrasound and/or CT scan are the most helpful imaging studies. A cystadenoma appears as a cystic mass with smooth walls and internal septa (Fig. 36.9); a cystadenocarcinoma has a similar appearance but with a solid component (Fig. 36.10). However, the distinction between a cystadenoma and cystadenocarcinoma can be difficult



**Figure 36.9 Hepatic cystadenoma.** A computed tomography scan image that shows a large, multiseptated cystic mass in the central portion of the liver. Attempts to drain (arrow) the lesion were unsuccessful. The benign lesion was excised using total vascular exclusion because of its central location.



**Figure 36.10 Hepatic cystadenocarcinoma.** A large lesion (arrow) that involves the right lobe and the medial portion of the left lobe. The patient was a Jehovah's Witness. The lesion was resected without blood products using total vascular exclusion.



**Figure 36.11 Liver abscess.** An axial image from a computed tomography scan that demonstrates multiple septated liver abscesses (arrows). This patient required operative drainage because of the loculated nature of the lesions.

to make based upon clinical and radiological evidence. A cystadenoma requires complete excision because of its malignant potential and recurrence rate of 100% if the entire epithelium is not excised. However, enucleation without liver resection of these lesions is sufficient. A cystadenocarcinoma, on the other hand, does require a formal resection with a margin of normal liver. The prognosis following surgical removal of either type of tumor is very good.

## PYOGENIC ABSCESS

Liver abscesses remain a significant problem and are responsible for 13–20/100 000 hospital admissions annually with an associated mortality of up to 30%. Over the past 20 years, several trends have emerged. The major source of liver abscesses is now the biliary tree rather than pyelophlebitis. Approximately 50% of patients have a malignancy, 80% of which are hepatobiliary. Other etiologies include hepatic metastases, diverticulitis, hepatic necrosis and infection as a result of chemoembolization and bacterial endocarditis. More patients, particularly those who are immunosuppressed either because of transplantation or intense therapy for malignancy, have mixed bacterial and fungal abscesses. Up to 50% of liver abscesses can now be successfully drained non-operatively with catheters placed by CT or ultrasound. The mortality associated with liver abscesses has decreased from 65 to 30%. The biggest decline has occurred in patients with multiple lesions.

The clinical features associated with liver abscesses are fever (50%), pain (50%), and weight loss (50%). An enlarged, tender liver and jaundice are present in approximately 50% of patients. Especially in elderly patients, fever of unknown origin may be the presenting complaint with the eventual diagnosis of liver abscess. Leukocytosis is found in 80% of patients. Alkaline phosphatase is elevated in the majority of patients (70–90%), with bilirubin and aspartate aminotransferase only elevated in 50%. At least half of the patients with a liver abscess have a positive blood culture.

Since the symptoms, physical findings, and laboratory studies are non-specific, a high index of suspicion and imaging studies

are the key to making the diagnosis of a liver abscess. CT is 93% accurate in confirming the presence of a liver abscess (Fig. 36.11). Although *Escherichia coli* and *Streptococcus* spp. remain the most frequent bacteria cultured from liver abscesses, *Pseudomonas* spp., probably because of long-term in-dwelling biliary stents, are increasing in incidence. Up to 25% of abscesses contain fungi as well as bacteria, generally associated with prior antibiotic use, especially in immunocompromised patients. *Bacteroides* spp. remain the most frequently cultured anaerobic bacteria.

Treatment is a combination of drainage and antibiotic therapy. Percutaneous drainage has virtually replaced open surgical drainage in the treatment of liver abscess. A catheter can be placed under either ultrasound or CT guidance. Drainage is superior to aspiration alone, which often requires multiple aspirations and is associated with a 37% recurrence rate. Surgery and percutaneous drainage should be viewed as complementary, and are not competitive means of treating liver abscesses. Surgical drainage is reserved for those cases where percutaneous drainage fails, or may not be possible because of the location of the abscess (dome of the liver) and the possibility of pleural cavity contamination. Other indications for surgery include the presence of infarcted hepatic tissue that needs to be debrided, where the source of the abscess requires concomitant surgical treatment, or failure of the percutaneous approach.

Surgical drainage is best accomplished by a transabdominal approach. The procedure tends to be bloody because of the associated inflammatory condition of the liver. Intraoperative ultrasound may be necessary to locate the abscess, which is unroofed and gently debrided. A drain, either a Penrose or closed-suction type, is left in place for several days. A CT scan is repeated after 7–10 days to assess the size of the abscess cavity. The drain can safely be removed when the drainage has stopped and either an ultrasound or CT scan has confirmed that the cavity has resolved. If the drainage persists or becomes bilious, a contrast study through the drain is necessary in an effort to document a communication with the biliary tree. If a biliary



fistula is present, an endoscopic sphincterotomy, with or without the placement of a stent, often results in more rapid healing by lowering the pressure in the biliary tree.

Although the mortality associated with liver abscesses has declined significantly over the past 20 years, it remains at about 30%. Factors predictive of a poor outcome include multiple abscesses, malignancy, malnutrition, abnormal liver function, leukocytosis, sepsis, fungal infection, advanced age and polymicrobial infections.

## LIVER TRAUMA

Many advances have been made in the past century in the management of liver trauma, particularly severe injuries. The current trends in the evaluation and management of liver trauma include CT staging of the injury, non-operative management (NOM) in stable patients, temporary packing to control bleeding, and the technique of mesh hepatorrhaphy. Hemorrhage and infection continue to be the major lethal consequences associated with liver trauma.

### Nature of the injury

The liver is partially protected from injury by the overlying ribs of the lower chest. Yet, as the largest solid organ in the abdomen, the liver is particularly vulnerable to the ability of compressive blows to rupture the relatively thin capsule. It is, therefore, not surprising that 45% of patients who suffer blunt abdominal trauma and 40% of those injured by penetrating trauma sustain a liver injury. The current grading of liver injuries as defined by the American Association for the Surgery of Trauma has been outlined in **Table 36.2**. Injuries of grade III and higher are considered severe.

### Diagnostic issues

The appropriate evaluation and management of liver injury results from an organized approach to abdominal trauma. The patient's physiologic status determines the most effective treatment; that is, hemodynamically unstable patients require immediate operative intervention. Stable patients can be evaluated more thoroughly using imaging techniques, and perhaps observed and managed non-operatively.

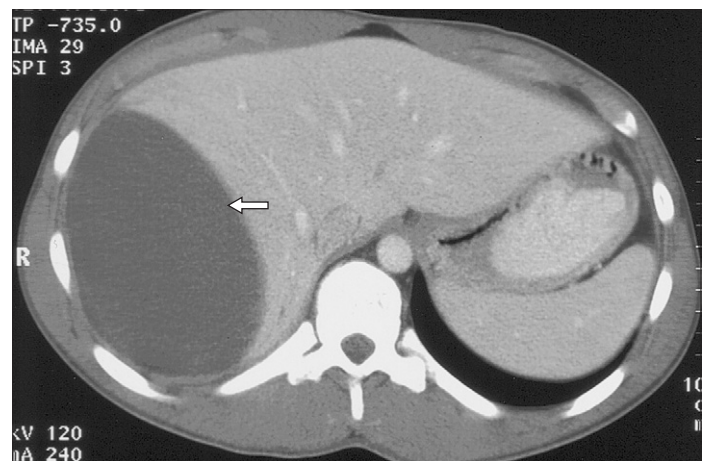
For several years, diagnostic peritoneal lavage (DPL) has been the 'gold standard' in assessing patients with blunt abdominal trauma, particularly in conjunction with a head injury. This method is also very sensitive in the detection of liver trauma, with false-negative and false-positive incidences of 1.2% and 0.2%, respectively. CT scanning has now become the preferred method to evaluate a patient who has sustained blunt abdominal trauma. The CT scan is particularly useful in delineating the nature and severity of the liver injury.

### Management

The majority of grade I and II hemodynamically stable liver injuries can be managed non-operatively provided the patient has no other source of bleeding or hollow viscus injury. **Figure 36.12** and **Figure 36.13** show the marked decrease in size of a subcapsular hematoma over the course of several weeks in a teenage boy who sustained an injury while playing soccer. Liver trauma patients managed non-operatively should be hemodynamically stable, have no associated abdominal injuries and a

Current grading of liver injuries as defined by the American Association for the Surgery of Trauma		
Grade		Description
I	Hematoma	Subcapsular <10% surface area
	Laceration	Capsular tear, <1 cm parenchymal depth
II	Hematoma	Subcapsular, 10–50% surface area, intraparenchymal, <1 cm in diameter
	Laceration	1–3 cm parenchymal depth, <10 cm in length
III	Hematoma	Subcapsular, >50% surface area or expanding; ruptures subcapsular or parenchymal hematoma Intraparenchymal hematoma >10 cm or expanding
	Laceration	>3 cm parenchymal depth
IV	Laceration	Parenchymal disruption involving 25–75% of hepatic lobe or 1–3 Couinaud segments within a single lobe
	Laceration	Parenchymal disruption involving > 75% of hepatic lobe or >3 Couinaud segments within a single lobe
V	Laceration	Parenchymal disruption involving > 75% of hepatic lobe or >3 Couinaud segments within a single lobe
	Vascular	Juxtahepatic venous injuries; i.e. retrohepatic vena cava/central major hepatic veins
VI	Vascular	Hepatic avulsions

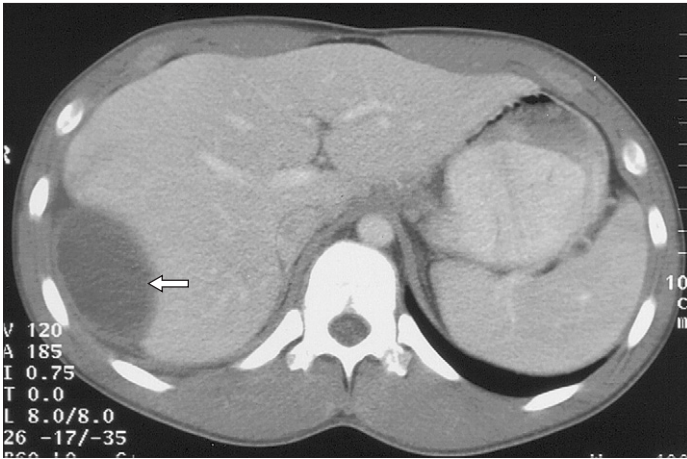
**Table 36.2** Current grading of liver injuries as defined by the American Association for the Surgery of Trauma.



**Figure 36.12** Liver trauma. A computed tomography scan demonstrating a subcapsular hematoma (arrow) of the liver caused by a soccer injury in a teenage boy. The injury was treated non-operatively.

normal neurologic examination and intensive care unit monitoring and a 24-hour operating room should be available.

It is the grade III and more severe injuries that are problematic and controversial in the current surgical literature. Hemodynamically unstable patients, those with associated injuries and those who deteriorate require immediate operative intervention. The two major goals of surgery are to control hemorrhage and prevent infection. The hemorrhage associated



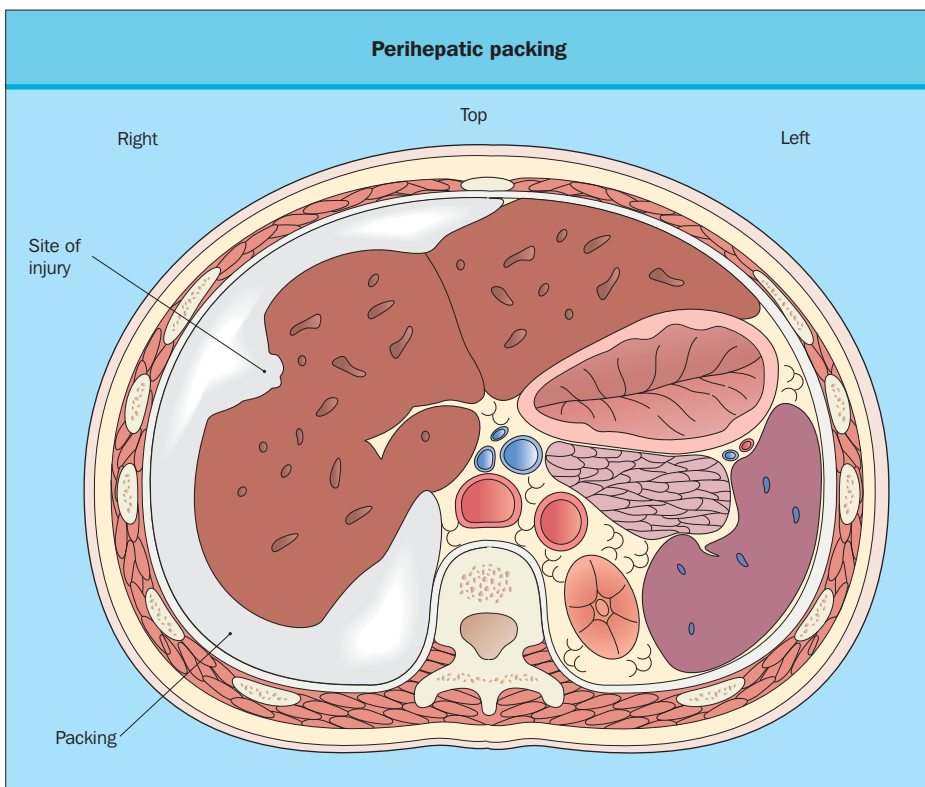
**Figure 36.13 Liver trauma after non-operative management.** A repeat computed tomography scan performed several weeks later in the same patient as shown in Figure 36.12; the hematoma (arrow) is significantly smaller.

with liver injuries can be massive, and is the most frequent cause of death. Injuries that are no longer bleeding at the time of laparotomy require no treatment. On opening the abdomen, the four quadrants should be packed. This allows the anesthesiologist to resuscitate the patient. First, removal of the lower packs allows rapid control of fecal contamination. The left upper quadrant pack is then removed and if the spleen is also injured it is removed. On removal of the pack around the liver, if there is hemorrhage, control can be improved by a 'Pringle' maneuver, which consists of temporarily occluding the hepatic artery and portal vein in the hepatoduodenal ligament. The maneuver can be performed by either digital compression, Rummel tourniquet,

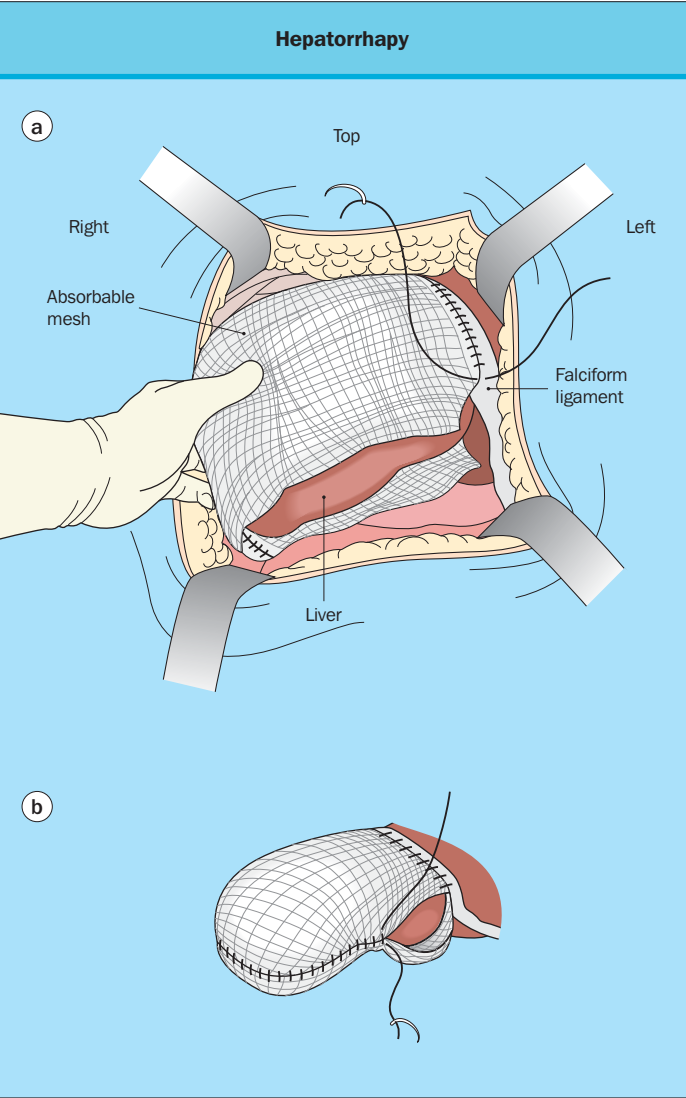
or by a non-crushing vascular clamp. This temporary occlusion can be applied safely for up to 1 h in a stable patient with normal liver parenchyma. However, since the most troublesome bleeding associated with liver injuries is venous, the 'Pringle' maneuver is often relatively ineffective. Once the injury is defined, the surgeon can either elect to simply pack the liver or follow a more aggressive approach including resection.

Packing is performed in a perihepatic, rather than in an intra-hepatic fashion (**Fig. 36.14**). The rationale is to provide adequate compression to control hemorrhage in an expedient manner, particularly in a hypothermic and acidotic patient. The packs are then removed 48 h later after further resuscitation, with some risk of recurrent bleeding. Liver injury that is severe enough to require packing to control hemorrhage carries a 30% mortality. Packing to control bleeding is associated with a significant morbidity (early or late hemorrhage, biliary fistula, arteriovenous fistula, liver abscess and necrosis). Mesh hepatorrhaphy is an alternative to packing first reported in the early 1990s. This technique uses absorbable mesh to sufficiently compress the parenchyma to control hemorrhage without causing ischemic injury. The 'Pita Pocket' technique is best suited to injuries that involve the major portion of an anatomic lobe, such as a large stellate lesion (**Fig. 36.15**). Unlike packing, mesh hepatorrhaphy does not require a second laparotomy to remove the mesh, and there is less pulmonary compromise because of less intra-abdominal pressure. However, the technique cannot be performed quickly and therefore may not be the best approach in a cold, coagulopathic patient. The associated mortality is similar to packing, about 30–40%.

Aggressive management, including lobectomy should always be considered. Series from major trauma centers report a mortality of 8–14% and a liver-related morbidity of around 20%



**Figure 36.14 Perihepatic packing.** An axial view of perihepatic packing to control hemorrhage.



**Figure 36.15 Hepatorrhaphy.** Performing a hepatorrhaphy to control bleeding from a major injury to the right lobe. (a) Demonstration of how the absorbable mesh is anchored to the retroperitoneum and the falciform ligament. (b) Almost complete wrapping is shown.

for this approach. Under inflow control the liver wound is explored, necrotic liver debrided, and individual vessels ligated (Fig. 36.16). Liver injuries associated with retrohepatic vena cava and hepatic vein injuries can still thwart surgical efforts to control hemorrhage. Even the results of using shunts introduced into the right atrium, as described by Schrock, are disappointing. Total vascular exclusion (TVE) in conjunction with a venovenous bypass, as is used in liver transplantation, is another technique that may have some promise; it is a technique of occluding the vena cava both above and below the liver in addition to controlling the hepatic artery and portal vein. This approach is limited in patients who are in shock as the venous return is already compromised. The mortality rate reported with these challenging injuries approaches 80%.

**BILIARY RECONSTRUCTION**

The early efforts to bypass or reconstruct the biliary tree were most often to deal with bile duct stones. In 1880, Alexander von

Winiwarter of Belgium performed the first successful cholecystoenterostomy for persistent symptoms following aspiration of the gallbladder. The first choledochoduodenostomy was achieved in 1891 by Oskar Sprengel in Germany following a cholecystectomy and common bile duct exploration that was unsuccessful in clearing the duct of stones. Halsted reconstructed the biliary tree with a choledochoduodenostomy in 1898 after locally excising an ampullary tumor. An important innovation was introduced by Robert Dahl of Sweden in 1909: the Roux-en-Y hepaticojejunostomy. Since those early years, choledochoduodenostomy and Roux-en-Y drainage have become the most frequently used procedures for biliary reconstruction or bypass.

**Indications for biliary reconstruction**

Table 36.3 lists the indications for biliary reconstruction or bypass. An iatrogenic injury resulting from a cholecystectomy is the leading cause of benign strictures. Other benign problems include recurrent or retained bile duct stones and chronic pancreatitis. However, the highly successful endoscopic and radiographic approaches to bile duct stones have virtually eliminated the need for surgical intervention. Biliary obstruction as a result of either a cholangiocarcinoma or a periampullary malignancy is the other major indication for biliary reconstruction. Unresectable periampullary lesions may be amenable to a biliary-enteric bypass, thus eliminating the need for indwelling stents that require changing on a regular basis to prevent cholangitis.

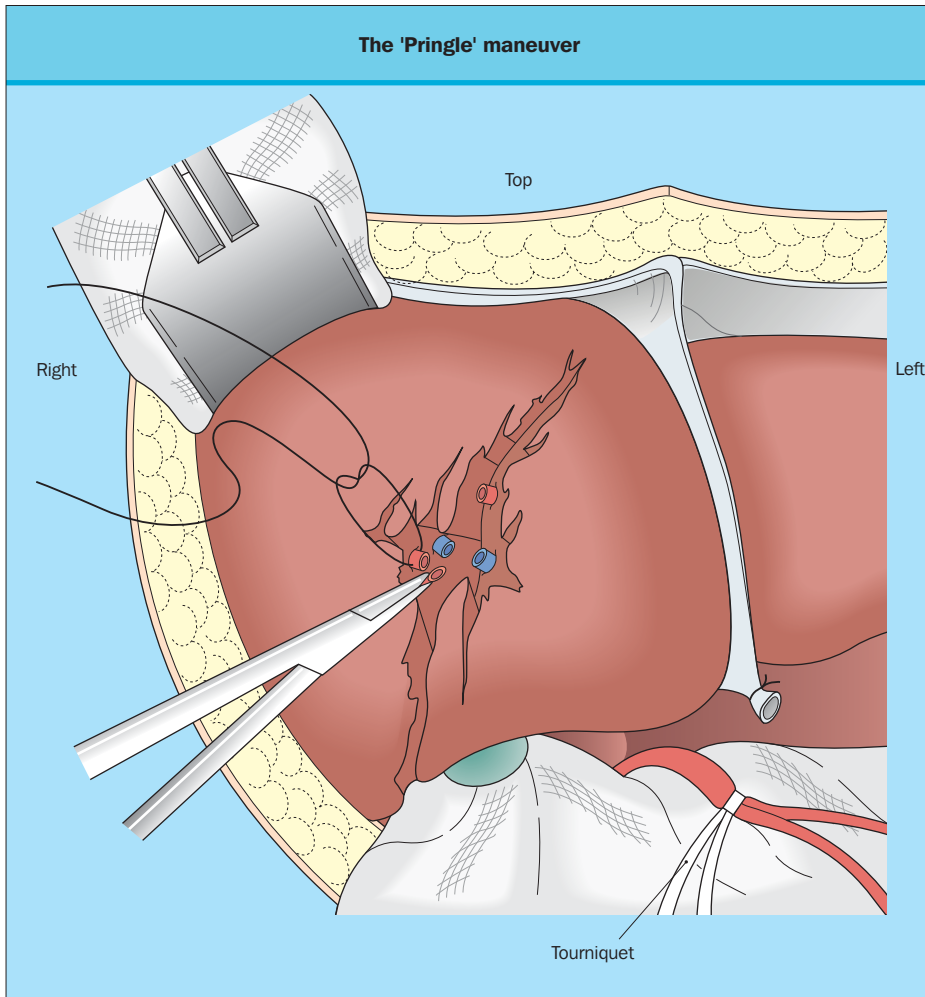
**Operative techniques**

Choledochoduodenostomy and Roux-en-Y biliary reconstruction are the methods used most frequently to reconstruct or bypass the biliary tree. Primary duct-to-duct repairs, even if performed at the time of the cholecystectomy and bile duct injury, are associated with a high rate of subsequent stricture and therefore should be avoided. The high incidence of stricture formation results from bile duct ischemia due to the tenuous nature of the

Indications for biliary reconstruction
<b>Benign conditions</b>
Strictures/injuries:
Iatrogenic
Ampullary stenosis secondary to stone disease
Chronic pancreatitis
<b>Stone disease</b>
Recurrent or persistent stone disease not amenable to endoscopic or radiographic intervention
<b>Malignant disease</b>
Unresectable periampullary carcinomas
Cholangiocarcinoma
Proximal lesions
Mid-duct lesions

**Table 36.3 Biliary reconstruction.** Indications for reconstruction, including benign and malignant conditions.



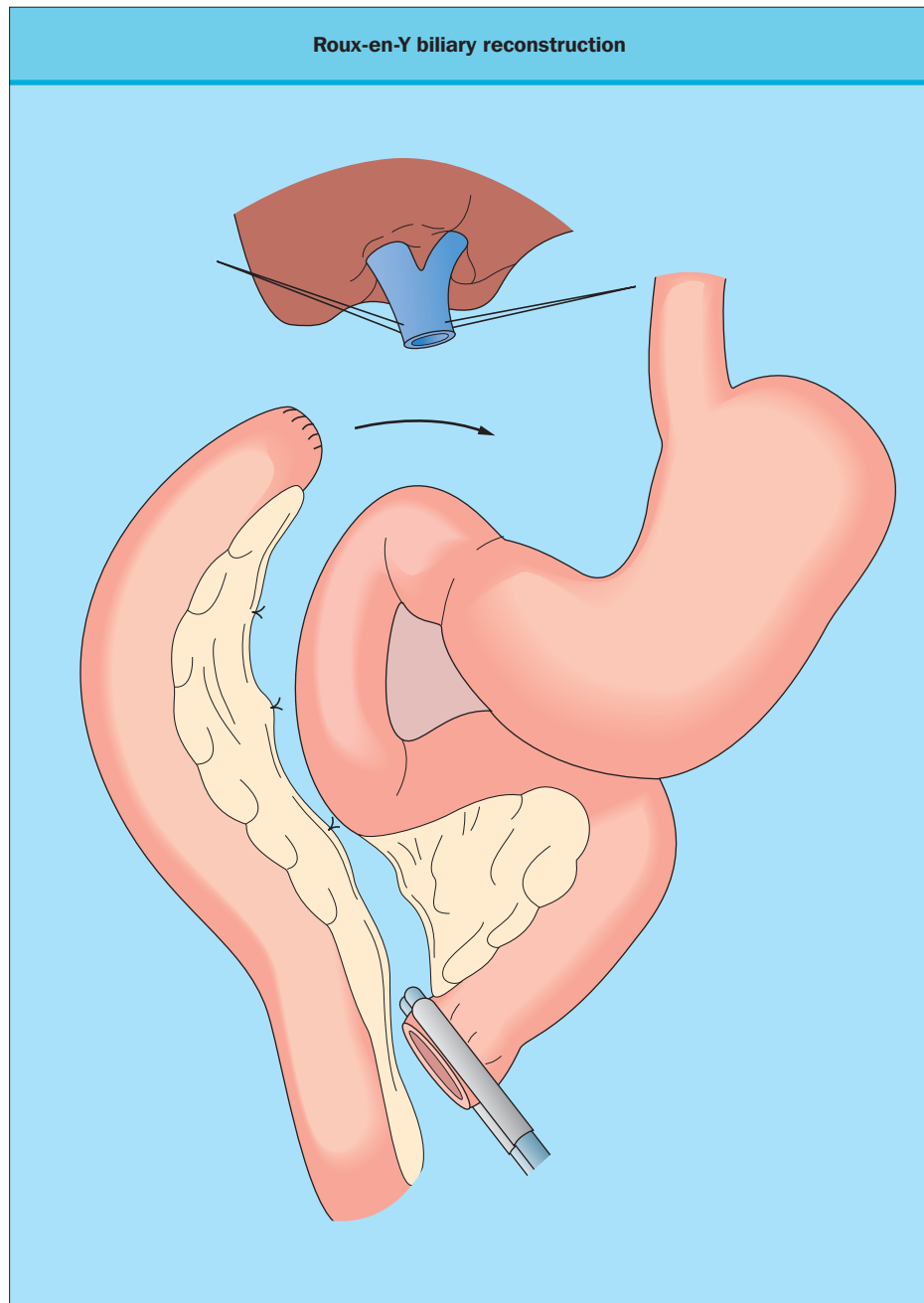


**Figure 36.16 The 'Pringle' maneuver.** A large, deep laceration in the right lobe of the liver which requires control of individual vessels to arrest bleeding. The tourniquet is occluding the vascular inflow (hepatic artery and portal vein); the 'Pringle' maneuver.

arterial blood supply to the extrahepatic biliary tree, and the tension on the anastomosis.

Choledochoduodenostomy is an excellent bypass procedure for unresectable periampullary malignancies. The procedure is relatively easy to perform and requires only one anastomosis. The anastomosis is within easy reach of the endoscope therefore allowing both diagnostic and therapeutic endoscopic intervention. Benign, iatrogenic strictures are not usually amenable to a choledochoduodenostomy because of the high location of the obstruction and the associated scarring in the area of the duodenum. Ascending cholangitis was cited as a complication of choledochoduodenostomy. However, an anastomosis 2.5 cm in length will prevent this problem. Other disadvantages include the sump syndrome which results from inadequate drainage of the distal duct. This complication can also be prevented by performing a large anastomosis. An endoscopic sphincterotomy usually eliminates the sump syndrome.

Construction of a Roux limb provides an excellent conduit for biliary reconstruction. The limb may be sewn to any accessible portion of the biliary tree, particularly the common bile duct (choledochojejunostomy) or the hepatic duct (hepaticojejunostomy). A Roux limb is the best option for either an immediately recognized bile duct injury or a subsequent benign stricture because it will easily reach the hepatic bifurcation and the anastomosis is tension free (**Fig. 36.17**). An anastomotic stricture results in cholangitis and occurs in 15–20% of patients. Many of these recurrent strictures can be successfully treated by radiographic dilatation and stenting. If stenting is not successful, the biliary-enteric anastomosis can be revised with a good long-term result in 90% of patients. Roux limb reconstruction is also the procedure of choice to restore biliary drainage following the resection of a proximal cholangiocarcinoma.



**Figure 36.17 Roux-en-Y biliary reconstruction.** Anastomosis of the bile duct to a Roux limb of jejunum provides the best method for biliary reconstruction.

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## SECTION V: LIVER TRANSPLANTATION

### CHAPTER

# 37

## Indications and Patient Selection

John R. Lake

### INTRODUCTION

Liver transplantation is the treatment of choice for those suffering from end-stage liver disease. The development and growth of liver transplantation has revolutionized the field of hepatology and greatly improved the outlook of patients suffering from various liver diseases. This procedure is now applied world wide as treatment for a large number of irreversible acute and chronic liver diseases for which there were previously no other treatment options (Table 37.1). Over the past decade there has been an enormous increase in the number of liver transplant operations performed (Figs 37.1 and 37.2).

The primary goals of liver transplantation are to prolong life and to improve the quality of life. Thus, it is essential to optimize patient selection and ideally time the transplant procedure so as to gain the maximum benefit. Unfortunately, because of the extreme shortage of organs for transplantation, optimal timing using deceased donor organs is not always possible. This shortage of organs has spawned great interest in new techniques for liver transplantation such as live donor transplants and splitting one liver for two recipients.

In the USA, 5330 liver transplant operations were carried out in 2002, while the equivalent figure for Europe was 4857. However, there are clear signs that the rate of expansion is slowing on both sides of the Atlantic. By contrast, the growth of liver transplantation in Asia has been explosive.

The outcomes of liver transplantation are currently excellent and much improved as compared to 20 years ago due to advances in operative technique, a better understanding of the course and prognosis of several liver diseases, and more effective post-operative care including immunosuppression. However, there are areas where we have made little progress, particularly in the management of post-transplant hepatitis C.

In recent years, there has also been a significant change in the indications for liver transplantation, so that nowadays the procedure is being offered in a wider range of more complicated conditions as well as to older patients. The most common indication is end-stage chronic liver disease in adults, which accounts for 69 and 70% of liver transplant activity in the USA and Europe, respectively (Fig. 37.3).

The main differences between the USA and Europe relate to the higher proportion of patients who have acute liver failure in Europe and until recently, a lower proportion of patients who have malignant disease receiving transplants in the USA (see Fig. 37.3). However, the proportion of patients receiving liver transplants for malignant disease in Europe is now decreasing and in the USA is increasing (Fig. 37.4).

Among patients who have cirrhosis, chronic viral hepatitis is currently the most common indication for orthotopic liver

disease (OLT). In particular, chronic hepatitis C has continued to increase as an indication in both Europe and the USA over the past decade, while on both continents, the percentage of patients transplanted for chronic cholestatic liver disease is decreasing (Fig. 37.5).

The expansion in the indications for transplantation contrasts with the shortage of liver donors. This continues to put pressure on the field to continue to refine the selection for, and timing of, transplantation to obtain the best and most cost-effective outcomes.

### TIMING OF LIVER TRANSPLANTATION

The success and increase in the number of liver transplants as a treatment option for end-stage liver disease has led to a marked shortage in donor organs and has greatly lengthened the waiting times for this procedure. As the waiting list grows, there has been an increase in the number of patients dying while awaiting the procedure. In the USA, there was an almost twofold increase in the number of patients dying while listed for liver transplantation between 1997 and 2001, with the number rising from 1198 to 2066. In the USA, this has led to a radical change in how patients are prioritized on the waiting list, making certain that organs are allocated to the seriously ill patients. This has already resulted in significantly fewer deaths in those on the waiting list, i.e. only 1814 waiting list deaths in 2003.

The timing of transplantation is necessarily influenced by access to donor organs. The current system of organ allocation in the USA, and a few other countries, is based on the prioritization of patients based on what is termed the MELD (model for end-stage liver disease) score. This prognostic score was originally developed to predict the short-term mortality risk for patients undergoing a transjugular intrahepatic portosystemic shunt (TIPS) procedure. However, it has now been well validated as an excellent predictor of 3-month mortality on the waiting list. This score utilizes three objective laboratory parameters, serum creatinine and bilirubin concentrations and international normalized ratio (INR) (Table 37.2). This organ allocation scheme has been in place in the USA for more than 3 years and has already led to a decrease in the waiting list mortality, while not compromising post-transplant outcomes (Fig. 37.6). More recently, it has also led to a reappraisal of who truly benefits from transplantation. Ideally, liver transplantation should be performed at a sufficiently late phase of the disease, where the patient's quality of life is impaired and they are at a greater risk of mortality on the waiting list than their risk of short-term mortality with transplantation. Although patients who have less advanced liver disease have excellent survival rates after the procedure, they also have been shown to survive with a

Indications for liver transplantation in adults	
Chronic liver disease	<b>Cholestatic diseases</b>
	Primary biliary cirrhosis
	Primary sclerosing cholangitis
	Secondary biliary cirrhosis
	<b>Viral-related cirrhosis</b>
	Viral B cirrhosis
	Viral B- $\delta$ cirrhosis
	Viral C cirrhosis
	Alcoholic cirrhosis
	Autoimmune cirrhosis
Parasitic diseases	Budd–Chiari syndrome
	Echinococcosis
Metabolic diseases	<b>With liver disease</b>
	Wilson disease
	Hereditary hemochromatosis
	$\alpha_1$ -antitrypsin deficiency
	Protoporphyrria
	Gaucher’s disease
	Glycogenosis type 1
	<b>Without liver disease, but secondary to a liver metabolic defect</b>
	Primary hyperoxaluria
	Essential hypercholesterolemia
	Familial amyloid polyneuropathy secondary to a variant transthyretin
	<b>Primary malignant tumor of the liver</b>
	Hepatocellular carcinoma
Various	Fibrolamellar hepatocellular carcinoma
	Epithelioid hemangioendothelioma
	Cholangiocellular carcinoma
	Adenomatosis of the liver
Acute liver failure	Polycystic liver disease
	Caroli disease
	Fulminant and subfulminant hepatitis
	Wilson disease (fulminant)
	Budd–Chiari syndrome (fulminant)
	Other causes of acute liver failure: post-trauma, postsurgery, postarterial embolization
Acute alcoholic hepatitis (?)	

Table 37.1 Indications for liver transplantation in adults.



Figure 37.1 European liver transplant activity. Liver transplantation is performed in 19 European countries with 103 transplant centers, 26 538 transplants in 23 476 patients. Modified from The European Liver Registry Report.

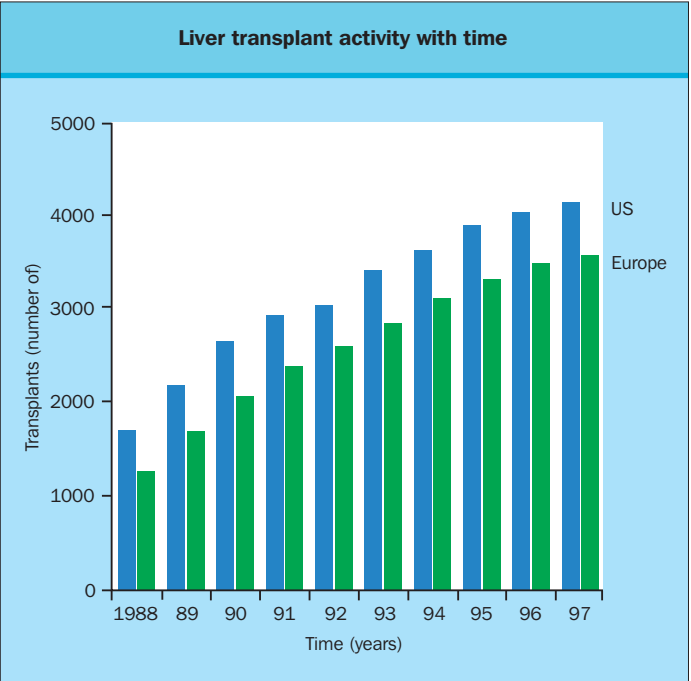
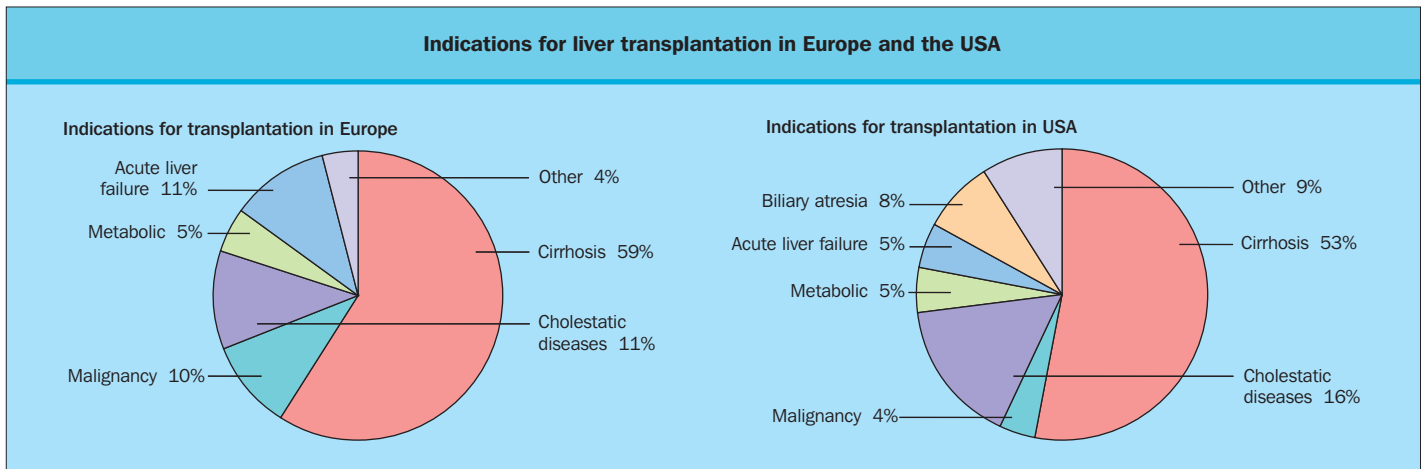
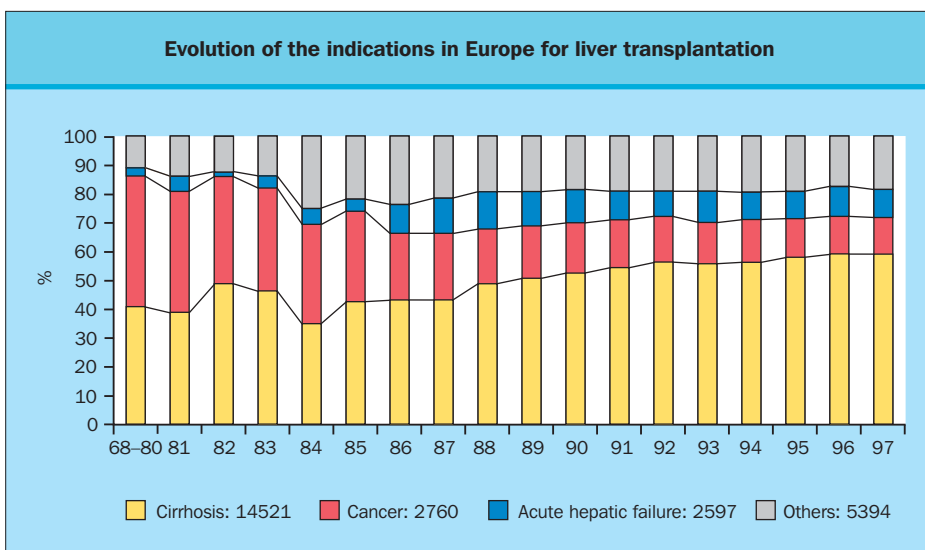


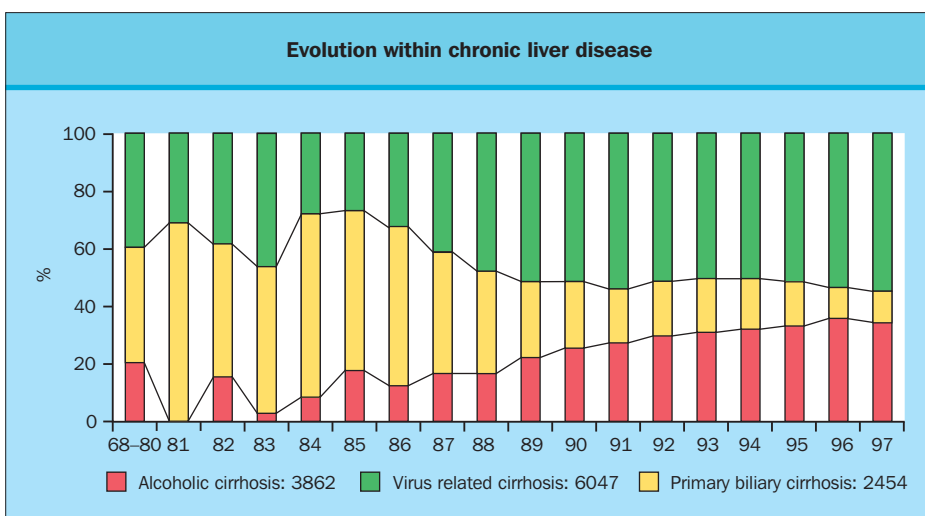
Figure 37.2 Liver transplant activity with time. Annual increases of the number of liver transplantations in Europe and the USA are decreasing as a function of the limited supply of donor organs. Modified from the United Network for Organ Sharing (UNOS) and The European Liver Registry Report.



**Figure 37.3 Indications for liver transplantation in Europe and the USA.** The main indications for liver transplantation in Europe and the USA show subtle differences relating to acute liver failure and malignant disease. Modified from the United Network for Organ Sharing (UNOS) and The European Liver Registry Report.



**Figure 37.4 Evolution of the indications in Europe for liver transplantation.** The main changes have been the progressive decrease of the patients transplanted for malignancy, the emergence of acute liver failure in the indications in 1986, and the increase of the number of patients transplanted for cirrhosis. Modified from The European Liver Registry Report.



**Figure 37.5 Evolution within chronic liver disease.** Changes in the indications among patients transplanted for primary biliary cirrhosis (PBC), alcoholic cirrhosis and viral cirrhosis. Note the progressive decline of PBC as an indication and the increase of alcoholic and viral cirrhosis, which are now the two main indications for transplantation. Modified from The European Liver Registry Report.



reasonable quality of life for extended periods without liver transplantation. However, transplantation should also be performed at a time when the patient is in a condition that does not impair the outcome after transplantation. Certainly, patients who have more advanced liver disease are more likely to develop operative complications and have longer postoperative hospitalization and rehabilitation periods. Nonetheless, there is no MELD score that identifies when transplantation is futile as even the most ill patients have a reasonable chance of post-transplant survival, and obtain a substantial survival benefit, i.e. comparing the 1-year survival with transplantation to that without (Fig. 37.7).

While in the USA one might only need to understand how MELD works and the MELD scores at which your local patients achieve priority for a deceased donor liver, but this is not true throughout much of the world that does not use MELD. Likewise, it does not apply to live donor transplants. Thus, guidelines for referral or performance of the transplant in patients who have chronic liver disease likely vary as to where one practices and may vary as to whether an appropriate live donor is available. Survival models have been applied to liver transplantation for a number of diseases such as primary biliary cirrhosis (PBC) and alcoholic cirrhosis, and these have shown that a survival

benefit may not be apparent for up to 5 years after transplantation when the operation was performed relatively early in the disease. One model was developed at the Mayo Clinic for PBC, the liver disease with the most predictable natural history. This utilizes five independent prognostic variables predictive of survival:

- serum bilirubin;
- albumin levels;
- age;
- prothrombin time; and
- the presence or absence of peripheral edema (including response to diuretic therapy).

The equation for calculating the model of end-stage liver disease (MELD) score
• $MELD = (0.957 \times \ln(\text{creatinine}) + 0.378 \times \ln(\text{bilirubin}) + 1.12 \times \ln(\text{INR}) + 0.643) \times 10$
• Capped at 40
INR, international normalized ratio.

Table 37.2 The equation for calculating the model of end-stage liver disease (MELD) score.

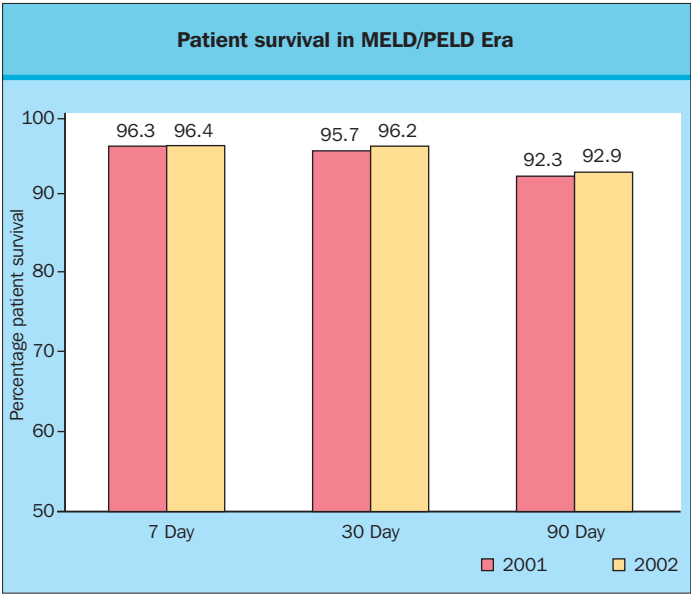


Figure 37.6 Patient survival before and after institution of the model of end-stage liver disease (MELD)

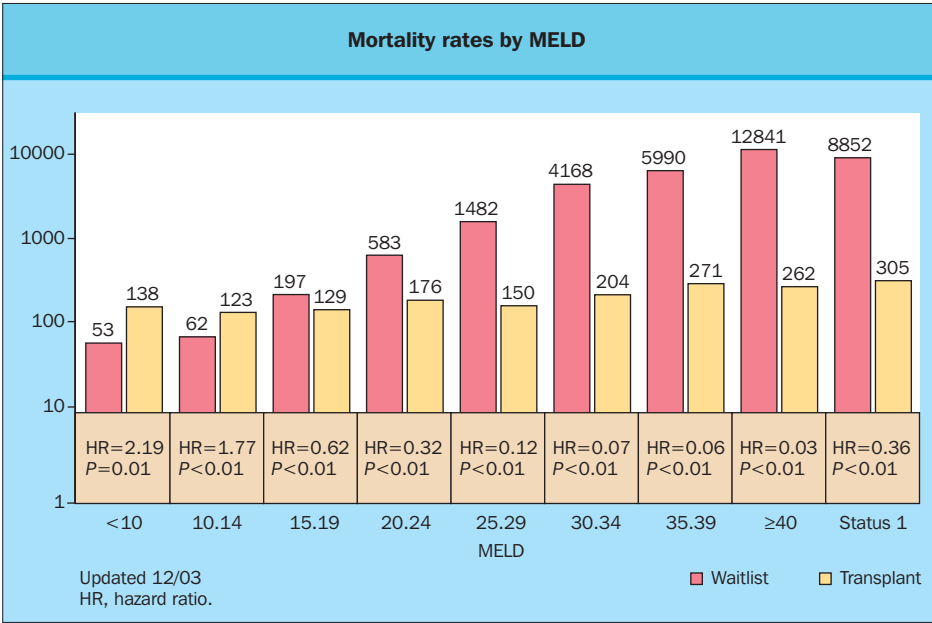
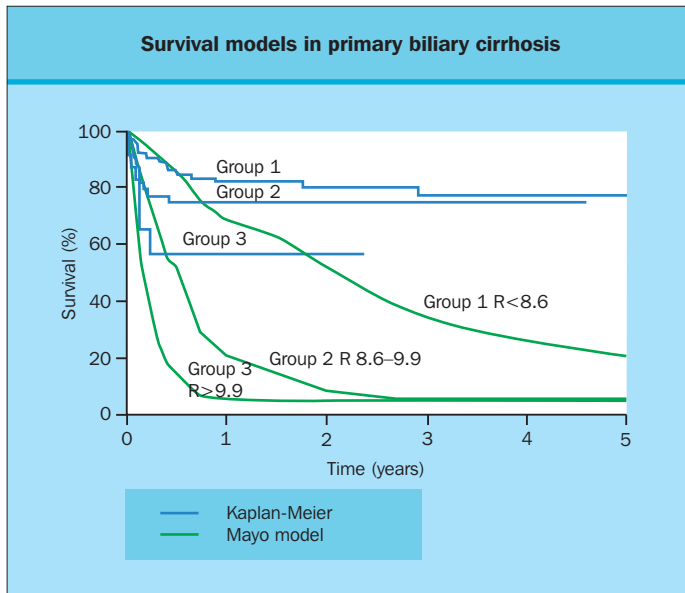


Figure 37.7 Relative risk of death with or without transplantation by model of end-stage liver disease (MELD) score.



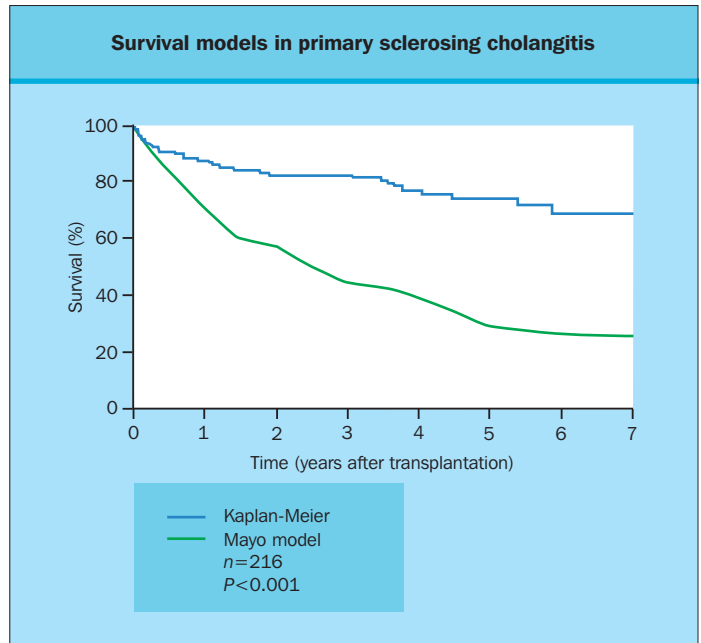
**Figure 37.8 Survival models in primary biliary cirrhosis.** Estimated Kaplan-Meier survival after liver transplantation in three groups of patients who had primary biliary cirrhosis versus estimated survival without liver transplantation as predicted by the Mayo model. Group 1, low risk; group 2, medium risk; group 3, high risk. Reproduced from Markus et al (89) by permission of the Massachusetts Medical Society.

These five variables calculate the risk score that can be translated into a survival function to estimate survival for the individual PBC patient (Fig. 37.8).

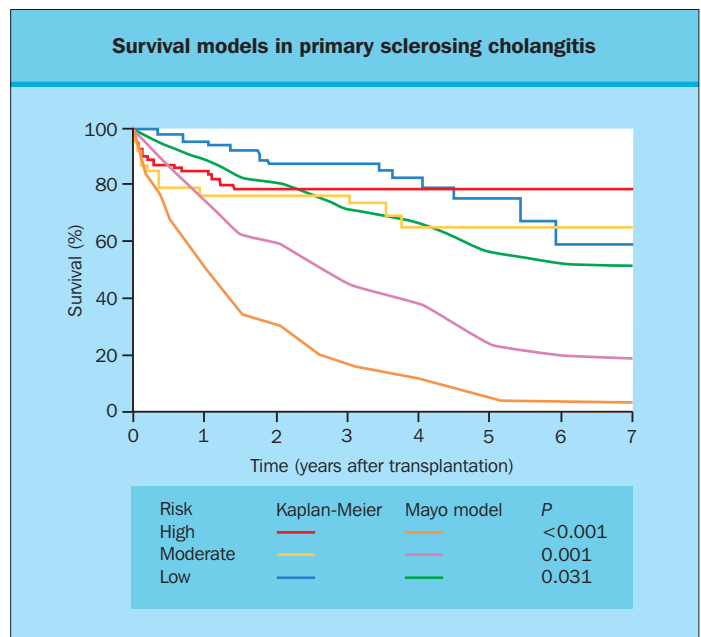
The application of this model has demonstrated that liver transplantation improves survival when compared with supportive therapy in patients who have PBC. The survival benefit with liver transplantation is greater in the highest risk group, even though the survival rate is poorer in this high risk group due to the higher postoperative mortality (see Fig. 37.8). While MELD may be a better predictor of short-term mortality in PBC patients, the Mayo model will also provide prognostic information for longer term risks.

The prognostic models have also been used to evaluate objectively the efficacy of the procedure (see Fig. 37.8). The overall benefit has been clearly demonstrated for these patients. The benefit was greater in patients with the highest risk scores, but in the highest risk group, post-transplant mortality was greater. Similar data exists for MELD where a clear-cut survival benefit from transplant is not obtained in general until the patient has a MELD score >15 (Fig. 37.7). In addition, patients with a MELD score <10 have been shown to not benefit from transplantation, at least in terms of survival. A reasonable option is to consider the patient for transplantation when the expected survival is between 1 and 2 years, i.e. MELD score >15, if a live donor is available.

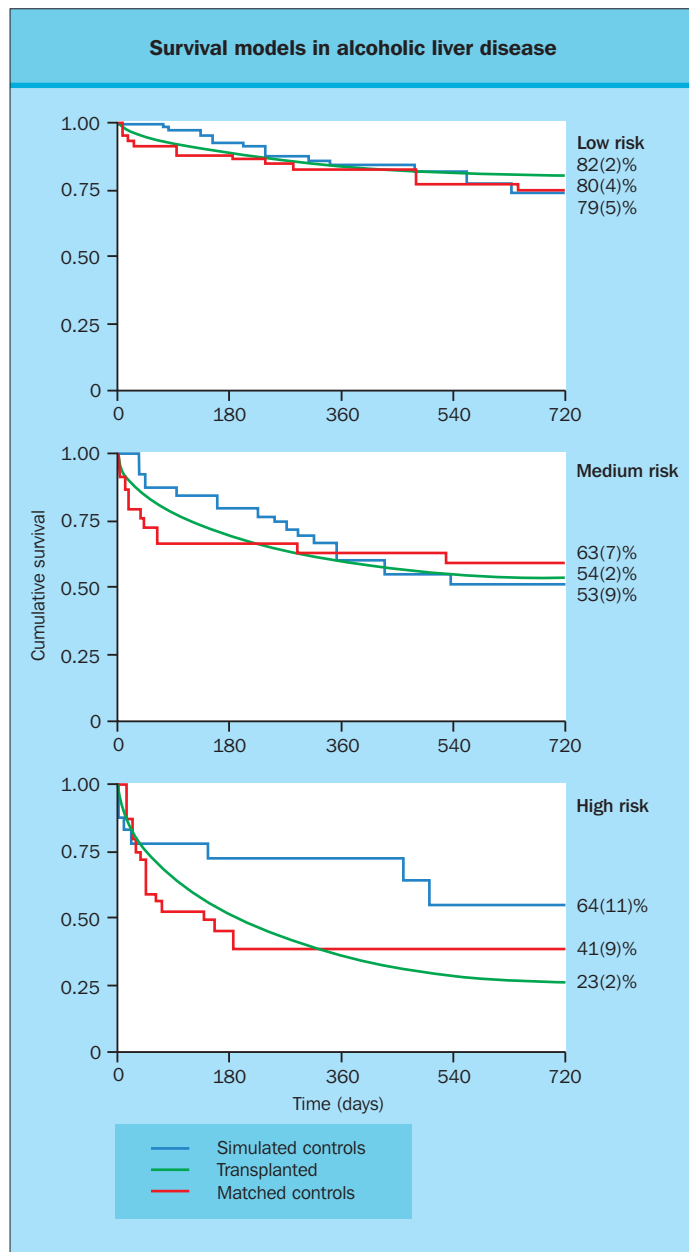
Effective prognostic models are present but less well-developed for other chronic liver diseases, including primary sclerosing cholangitis (PSC) and alcoholic cirrhosis (Figs 37.9–37.11). No model will be perfect, and they apply to a group of patients rather than an individual with that particular disease. To define the optimal timing for liver transplantation, an analysis of the natural history of the disease, together with a clinical judgment of the patient's quality of life are still the main guidelines to aid



**Figure 37.9 Survival models in primary sclerosing cholangitis.** Actual Kaplan-Meier survival after liver transplantation in patients who have primary sclerosing cholangitis and the estimated survival without transplantation as predicted by the Mayo model simulated control. Data from Wiesner et al in Maddrey & Sorrell (95).



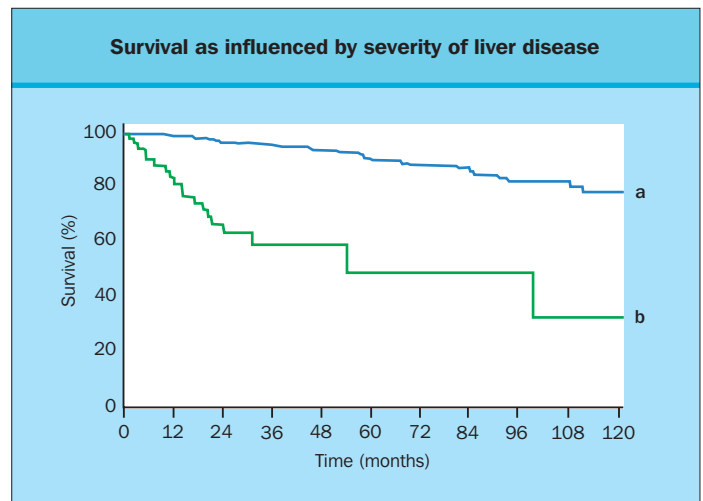
**Figure 37.10 Survival models in primary sclerosing cholangitis (PSC).** Actual Kaplan-Meier survival after liver transplantation in three risk groups of patients with PSC and their estimated survival without transplantation as predicted by the Mayo model. Data from Wiesner et al in Maddrey & Sorrell (95).



**Figure 37.11 Survival models in alcoholic liver disease.** Survival observed in patients transplanted for alcoholic cirrhosis, in matched and simulated controls, in a French multicenter trial. A higher survival after liver transplantation versus matched and simulated controls was observed only in the high risk group. Figures in brackets represent percentage of survival. Reproduced from Poynard et al (94) by permission of Elsevier.

the final decision. In addition, the clinical status of the disease should be taken into account (Fig. 37.12).

In patients who have PBC, PSC and autoimmune hepatitis, the disease process may or may not be controlled, and this will influence the decision. Likewise, in viral-related liver disease, control of viral replication is an important issue. When viral replication is controlled, e.g. using lamivudine to control hepatitis B virus (HBV) infection, the severity of the liver disease can actually improve and the clinical course either stabilizes or progresses more slowly. In contrast, if active viral replication persists and is associated already with advanced liver disease,



**Figure 37.12 Survival as influenced by severity of liver disease.** The probability of survival in patients who have compensated viral C cirrhosis (a) is greater at 91% at 5 years than after the appearance of the first major complication in patients who have decompensated viral C cirrhosis (b), which is 50% at 5 years. Reproduced from Fattovich et al (97) by permission of the American Gastroenterological Association.

liver function will deteriorate more rapidly. Sometimes, either spontaneous arrest of viral replication or spontaneous viral reactivation may occur independently of any therapeutic intervention. In the latter case, the liver disease may reactivate and the prognosis in such patients may be difficult to assess. In some cases, liver function will return to baseline values after a transient period, but in others liver function will continue to deteriorate.

In patients with alcoholic cirrhosis, the discontinuation of alcohol generally and often dramatically improves prognosis. The improvement may take several months to become apparent, and this is one of the more persuasive reasons to recommend a 6-month period of abstinence from alcohol before performing transplantation in alcoholic patients.

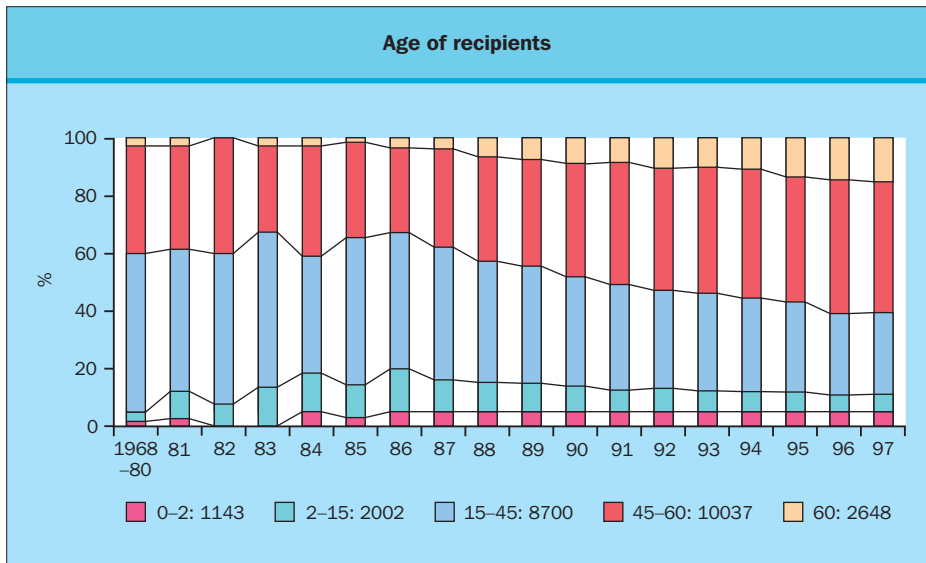
## CONTRAINDICATIONS TO LIVER TRANSPLANTATION

As more experience has been gained with liver transplantation, the number of contraindications has decreased over the years. Nowadays, there is general consensus about a number of contraindications to liver transplantation, although these are not universally agreed by all transplant centers:

- extrahepatic organ failure (heart, lungs), unless multiorgan transplantation is available;
- uncontrolled extrahepatic infection;
- extrahepatic malignant disease – the only potential exception is metastatic disease to liver from neuroendocrine tumors;
- diffuse thrombosis of the portal venous system (including the three main veins of the portal system – portal vein, superior mesenteric vein and splenic vein);
- uncontrolled immune deficiency;
- active substance abuse;
- inability to withstand the surgical procedure.

Previous contraindications no longer applicable include: an upper age limit of 55 years; previous multiple upper abdominal





**Figure 37.13 Age of recipients.** Liver transplant recipients over the age of 60 years are increasing steadily. Modified from The European Liver Registry Report.

operations or previous portocaval surgical anastomosis; isolated thrombosis of the portal venous vein; and the presence of hepatitis B surface antigen (HBsAg) in patients' serum. The ultimate decision depends mainly on the criteria developed within each transplant center. There is no absolute upper limit for age as defined by the Conference Consensus held in Paris in 1993. The proportion of recipients over the age of 60 years has been increasing steadily. However, in candidates over the age of 65 years close attention to the patients' general health and, in particular, their cardiovascular health is required (Fig. 37.13). While in general, the outcome of older patients after liver transplantation has been similar to younger patients undergoing the same procedure, the ability of older recipients to overcome postoperative complications is clearly less than that of a 20–30 year-old patient, even with the apparent absence of specific risk factors such as coronary artery disease.

## CHOLESTATIC DISEASES

### Primary biliary cirrhosis

PBC is characterized by the destruction of small interlobular bile ducts, due to an inflammatory process, that mainly affects women between 40 and 60 years of age (see Ch.19). The course of the disease comprises three periods: an asymptomatic phase, lasting up to 20 years; a symptomatic phase with mild jaundice, itching and fatigue, lasting from 1 to 20 years; and a third and final stage, lasting a few months to 2–3 years, with marked jaundice, a relentless increase in serum bilirubin levels and clinical complications of portal hypertension. The course of the disease is steadily progressive, with clinical manifestations such as fatigue, pruritus, variceal bleeding, ascites and osteoporosis. It is clear that liver transplantation has dramatically improved the prognosis of PBC.

PBC was the first disease to be universally accepted as an indication for liver transplantation, and at present it is considered that the timing of intervention should be set at the onset of the third clinical stage in parts of the world where MELD is not used. This is the point where there is no prospect of spontaneous stabilization or recovery, but sufficiently early so as

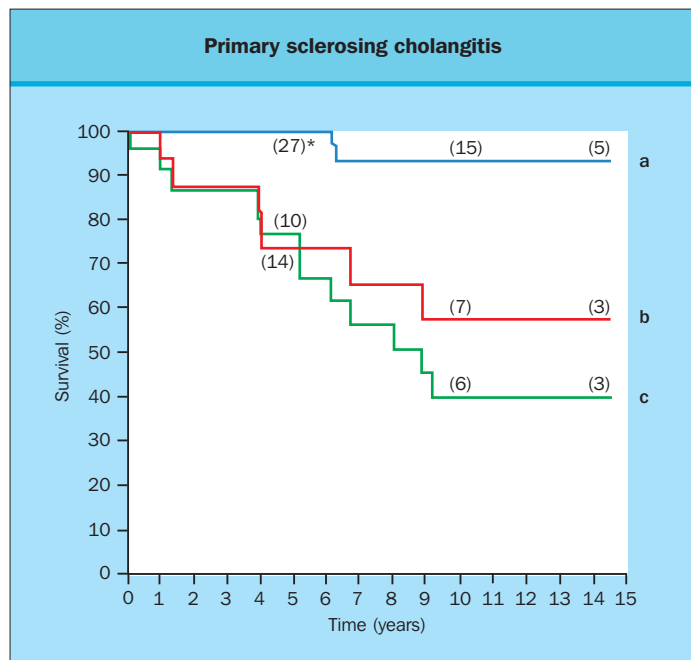
not to negatively impact the outcome of transplantation. The major indication for liver transplantation is a serum bilirubin level over 10 mg/dL ( $>170 \mu\text{mol/L}$ ). The risk of death awaiting transplantation is under 15% and the median survival when serum bilirubin level is over 6 mg/dL and 9 mg/dL is 24 and 17 months, respectively.

Other indications include variceal bleeding not controlled by endoscopic therapy or drug treatment, intractable ascites, and rarely intolerable pruritus or fatigue. With the more widespread use of ursodeoxycholic acid as a medical treatment, the indication for liver transplantation has become less frequently determined by the serum bilirubin level and more frequently by the complications of portal hypertension and by liver failure. A few patients are transplanted because their quality of life is poor despite all medical therapies. In these cases, a careful psychosocial appraisal of the quality of life and of fatigue should be performed before referral for liver transplantation.

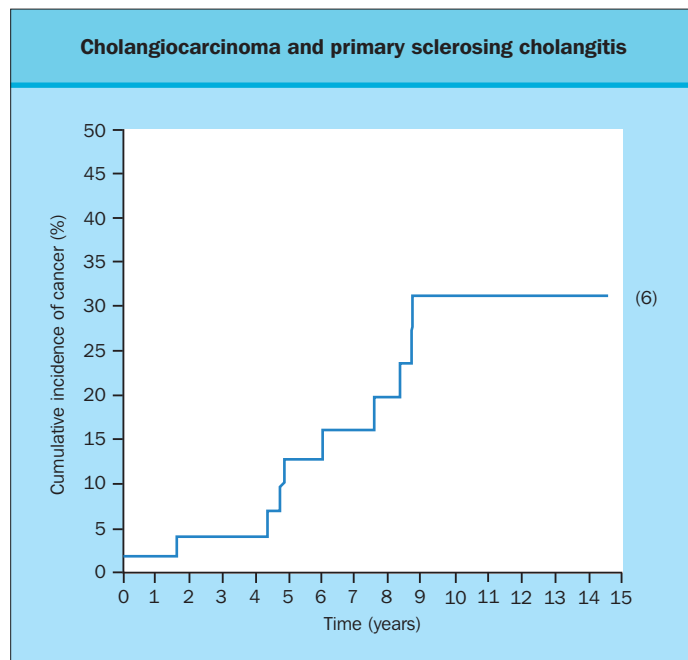
The 10-year survival rate in these patients after transplantation is 80–90%, and the risk of recurrence seems to be ~ 10–20% at 5 years, even though the immunologic manifestations of the disease persist after transplantation. Nonetheless, the impact of recurrence on liver graft function is low, and very few recipients have been retransplanted for PBC recurrence. Interestingly, pruritus disappears immediately after transplantation in 100% of cases but fatigue improves only in ~ 70% of cases. Clearly, liver transplantation increases survival, as well as quality of life.

### Primary sclerosing cholangitis

PSC is characterized by a chronic fibrosing inflammation of the bile ducts (see Ch. 20). It is commonest in men under the age of 50 years, and is associated with inflammatory bowel disease in ~ 70% of patients. It appears insidiously with pruritus, fatigue and/or jaundice initially, but later leads to biliary cirrhosis and complications of portal hypertension. There is also an increased risk of cholangiocarcinoma, with a prevalence of up to 30%. Treatment with standard doses of ursodeoxycholic acid (15 mg/kg) has not been proven to modify the course of the disease. PSC is a not uncommon indication for liver transplantation and accounts for nearly 10% of transplant activity in



**Figure 37.14 Primary sclerosing cholangitis.** Survival from the time of onset of primary sclerosing cholangitis in patients not undergoing transplantation (c); in patients not undergoing transplantation after exclusion of those who had bile duct carcinoma at time of examination (b); and in patients treated by liver transplantation (a) at the Paul Brousse Center. (\* actual patient numbers at that time point). Reproduced from Farges et al (95) by permission of Mosby.



**Figure 37.15 Cholangiocarcinoma and primary sclerosing cholangitis.** Cumulative actuarial incidence of cholangiocarcinoma from time of onset of primary sclerosing cholangitis, emphasizing the increasing risk of developing cholangiocarcinoma. (6): number of patients at 15 years. Reproduced from Farges et al (95) by permission of Mosby.

the USA. It is the fourth most common indication in Europe in adults. Currently the main indications are:

- patients who have long-standing severe jaundice [bilirubin level over  $100 \mu\text{mol/L}$  ( $6 \text{ mg/dL}$ )], cholestasis and pruritus not related to an acute episode of cholangitis;
- established cirrhosis resulting in portal hypertension (ascites/esophageal varices) and/or liver failure (prothrombin less than 50% of normal value);
- significant deterioration in the patient's quality of life; and
- recurrent episodes of cholangitis (in a few patients).

There are still some remaining questions and limitations regarding liver transplantation for PSC. The natural course of the disease and the optimal timing of liver transplantation are not totally clear. One study identified five independent clinical variables predictive of survival in PSC:

- age;
- histologic stage on liver biopsy;
- serum bilirubin level;
- hemoglobin concentration; and
- presence or absence of inflammatory bowel disease.

A recent report showed a 73% 5-year post-transplant survival rate, whereas the expected survival rate without transplantation was 28% (see Fig. 37.9). On the other hand, liver transplantation in PSC is still associated with higher morbidity and mortality due to previous surgical interventions (reported by most transplant centers), uncontrolled infection, undiagnosed cholangiocarcinoma, and, possibly, a higher risk of graft loss from chronic rejection. If the disease can be diagnosed pretransplantation, some success

has been obtained with aggressive radiotherapy and chemotherapy (see below)

Latent cholangiocarcinoma remains a problem. Cholangiocarcinoma should be in the differential diagnosis of PSC, but, in addition, the latter can be complicated by the development of cholangiocarcinoma. Progression of the tumor is often clinically silent. In addition, routine imaging studies are rarely of much benefit. Patients who otherwise would be good candidates for transplantation may have extrahepatic spread that precludes transplantation. In the explants, the incidence of cholangiocarcinoma varied from 8 to 18%, and most of these tumors were not diagnosed before transplantation. Currently, we lack the tools to recognize cholangiocarcinoma in the at-risk patient with PSC. The rapid development of persistent jaundice and pruritus, a recent clinical deterioration with weight loss, increased carcinoembryonic antigen (CEA) or CA 19-9 levels, and a long course of the disease (over 10 years) are all non-specific factors that could suggest the development of malignancy, but better methods are still needed to diagnose malignancy at an early stage (Figs 37.14 and 37.15). Patients who have coexisting inflammatory bowel disease should also have a colonoscopy at the time of assessment for transplantation to exclude colonic malignancy. Moreover, they need on-going frequent colonoscopy after liver transplantation as colorectal cancer is the most common cause of death beyond 1 year.

Since the results of liver transplantation are good and the procedure radically changes the prognosis of the disease, patients should be referred early after the onset of liver symptoms in order to reduce the operative risk and to prevent the

development of hepatobiliary malignancy. Cases of recurrence of PSC have been described, and this can occasionally affect the outcome of the graft. In contrast, PSC-like symptoms have also been observed after liver transplantation in patients transplanted for diseases unrelated to PSC, and the real significance of recurrent disease will only become apparent with long-term follow-up.

### Secondary biliary cirrhosis

The main causes of secondary biliary cirrhosis are anatomic abnormalities of the bile ducts or perioperative trauma of the extrahepatic bile ducts. Liver transplantation is indicated if surgical repair is not possible or after the development of secondary biliary cirrhosis when there is uncontrolled clinical cholestasis, jaundice, repeated episodes of cholangitis and/or portal hypertension. The timing of liver transplantation can be particularly difficult, as relief from the underlying obstruction can lead to a dramatic stabilization of the liver disease. The highest operative risk is now seen in patients who have coexisting extensive intra-abdominal adhesions and portal hypertension, and these patients are prime candidates for this unfavorable combination.

## VIRAL HEPATITIS

Chronic viral hepatitis is the most common cause of end-stage liver disease world wide, and thus it is the most frequent diagnosis in patients referred for liver transplantation. However, viral reinfection and recurrence of disease after liver transplantation is a major clinical problem and may lead to allograft failure requiring retransplantation or result in death.

### Hepatitis B

In spite of the success of vaccination against HBV, patients with cirrhosis due to HBV still constitute a sizable group of candidates for liver transplantation (5–10% of all recipients). However, the hope is that with the ever-expanding success of vaccination programs that this indication for liver transplantation will decrease over the next two decades. The current success of transplantation for hepatitis B-related disease represents one of the great triumphs of the past decade in the field of liver transplantation as the results of transplantation for hepatitis B are currently amongst the very best compared to other indications (**Fig. 37.16**). We are now able to prevent reinfection in >90% of recipients. Prior to this, transplantation for hepatitis B was marked by frequent HBV reinfection and in most patients, aggressive post-transplant hepatitis B that often led to death within a matter of months.

Although it is a progressive disease in the majority of cases, some patients present with an acute clinical deterioration at the time of viral reactivation or during the process of hepatitis B e antigen (HBeAg to anti-HBe) seroconversion. This can result in either irreversible liver failure and death, or a transient deterioration that is followed by a dramatic improvement of the liver disease. The main indications are similar to those for other liver diseases:

- recurrent ascites;
- spontaneous bacterial peritonitis;
- recurrent episodes of gastrointestinal bleeding not successfully controlled by medical treatment or ablative therapy;
- recurrent encephalopathy;

- liver failure with a low level of albumin, elevated serum bilirubin and low prothrombin level; and
- the development of hepatocellular carcinoma (HCC).

The risk of spontaneous HBV reinfection after transplantation is around 80%. The pretransplant HBV DNA status, traditionally detected using conventional hybridization techniques or now as >10<sup>5</sup> copies/mL using a quantitative polymerase chain reaction (PCR) technique, is the best predictor of HBV recurrence after transplantation. HBV reinfection is the consequence of either an immediate reinfection of the graft due to circulating HBV particles, or of a reinfection of the graft from HBV particles coming from extrahepatic sites. Reinfection of the graft in patients receiving anti-hepatitis B surface immunoglobulin (HBIG) is probably related to HBV in extrahepatic sites. It may be the consequence of HBV overproduction at these extrahepatic sites, a low protective titer of HBIG or emergence of escape mutants. This latter mechanism is probably important since mutations in the pre-S/S genome of HBV and in the 'a' determinant have been described.

HBV reinfection is characterized by the reappearance of HBsAg and HBV DNA in the serum. After HBsAg reappearance, HBV replication levels are usually high, and large quantities of HBV particles are present in the graft. Almost all patients reinfected with HBV will develop disease; most commonly an acute lobular hepatitis which evolves to chronic hepatitis. Some cases follow a particularly severe clinical course leading to acute liver failure, probably related to the high amount of HBsAg, HBeAg and hepatitis B core antigen (HBcAg) in the nuclei and cytoplasm of the hepatocytes. This form of post-transplant liver disease is termed fibrosing cholestatic hepatitis and is a unique histologic disease that is only seen in immunosuppressed patients. A similar disease has been shown to occur with hepatitis C (HCV).

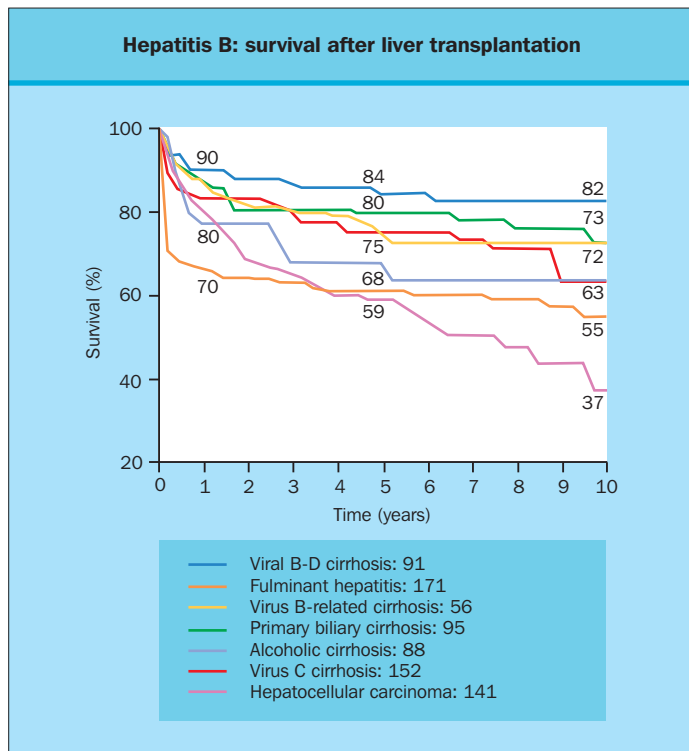
The initial strategy developed to prevent HBV reinfection of the allograft was the administration of polyclonal HBIG (see also Ch. 42). Early studies of the use of HBIG during the anhepatic phase and short-term post-transplant period yielded disappointing results as most of the patients developed HBV reinfection (**Table 37.3**).

However, subsequent studies of the long-term administration of high doses of HBIG dramatically reduced the rate of HBV reinfection and disease recurrence. In a European multicenter study, the effect of long-term administration of HBIG produced a dramatic decrease in the rate of HBV reinfection from 75% in

HBV recurrence rate in relation to initial liver disease and HBV Ig administration			
Liver disease	No Ig (%)	Short-term Ig (%)	Long-term Ig (%)
HBV cirrhosis*	78	90	56
HBV DNA, HBeAg+	75	71	70
HBV DNA, HBeAg–	66	92	37
HCV cirrhosis*	70	56	17
FHF B	56	ND	0

**Table 37.3 Hepatitis B virus recurrence rate in relation to initial liver disease and HBIG administration.** \*P < 0.001. FHF, fulminant hepatic failure B. (From Samuel et al. N Engl J Med. 1993;329:1842–7.)

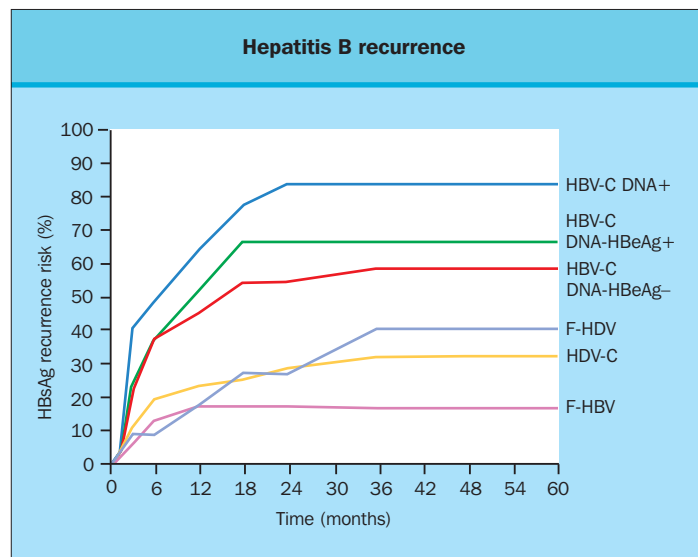




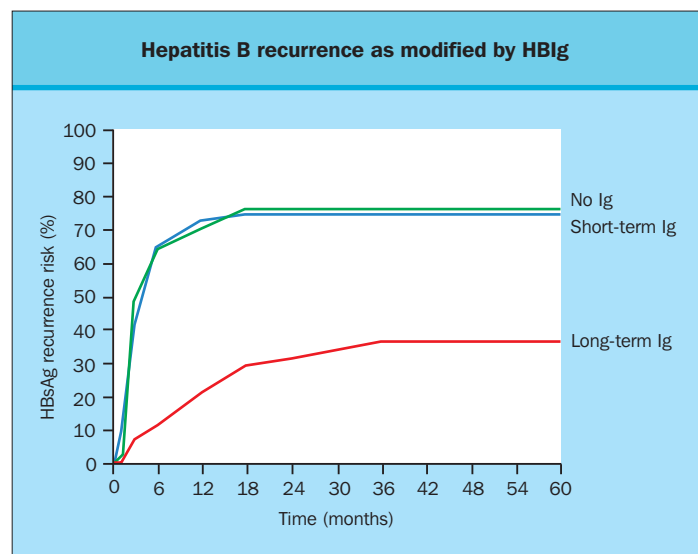
**Figure 37.16 Hepatitis B: survival after liver transplantation.** Actuarial 10-year survival after liver transplantation at Paul Brousse Hospital in 1106 patients transplanted from June 1984 to June 1997 in relation to the initial liver disease. Observe that using a policy of giving long-term administration of anti-hepatitis B surface immunoglobulin (HBIG), patients with B-D cirrhosis have the highest survival and those with B and C cirrhosis have the same survival. Patients who have hepatocellular carcinoma have the lowest survival and those with fulminant hepatitis have the lowest short-term survival due to higher postoperative mortality, but a relative stable survival curve in the long term. Numbers in key refer to number of patients in each group.

patients receiving no or short-term administration of HBIG, to 33% in those receiving long-term administration of HBIG. In the most aggressive protocols, up to 10 000 IU of HBIG was given during the anhepatic phase, and repeated daily during the first six postoperative days. The level of anti-HBs was determined weekly and repeated doses of HBIG were given when anti-HBs levels fell below 100 IU/L. This approach reduced the overall actuarial rates of reappearance of HBsAg in patients who had HBV cirrhosis to 17 and 29% at 1 and 2 years, respectively (Figs 37.16–37.19).

It is the standard to inhibit viral replication with antiviral therapy before transplantation, in order to clear HBV DNA from serum (see also Ch. 42). An ideal treatment should have a rapid and potent antiviral action without provoking a deterioration of liver function. Lamivudine, a nucleoside analog, is the most effective currently available drug to clear HBV DNA from the serum in patients who have cirrhosis awaiting liver transplantation. The drug, even when given alone, is well tolerated and is associated with a low rate of HBV reinfection. However, the major limitation to lamivudine therapy is acquired resistance, which occurs at a rate of 20–30% of patients per year. Severe disease can develop in patients developing mutations in the YMDD motif of the HBV DNA polymerase. Fortunately, new antivirals have been developed that are

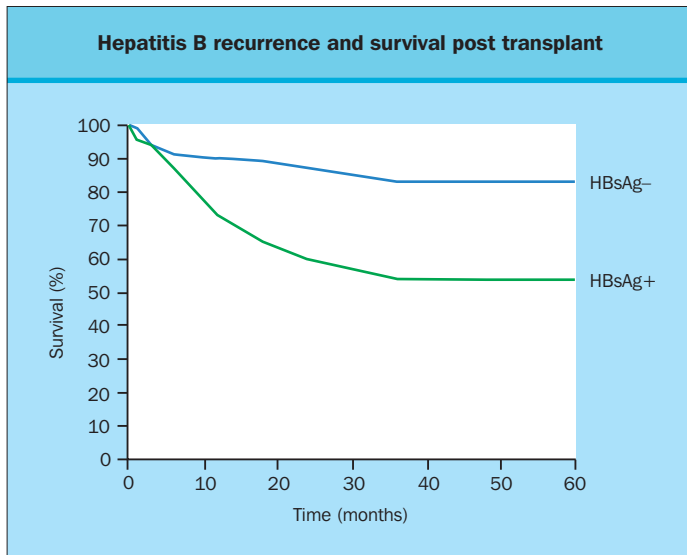


**Figure 37.17 Hepatitis B recurrence.** Hepatitis B virus (HBV) recurrence risk in relation with the initial liver disease in a multicenter European trial on 372 patients. Note that patients with fulminant hepatitis B (F-HBV) have a 17% risk, those with B-D cirrhosis [hepatitis D virus (HDV)-C] a 32% risk, those with fulminant B-D hepatitis (F-HDV) a 40% risk, and those with B cirrhosis (HBV-C) a 67% risk ( $P < 0.01$ ). Among the group of patients with HBV-C, the risk of recurrence was significantly higher in those who were HBV DNA-positive (80%), than in those who were HBV DNA and hepatitis B e antigen (HBeAg)-negative. Reproduced from Samuel et al (93) by permission of the Massachusetts Medical Society.



**Figure 37.18 Hepatitis B recurrence as modified by HBIG.** Hepatitis B virus (HBV) recurrence risk in relation to the administration of anti-hepatitis B surface immunoglobulin (HBIG) in a multicenter European trial. Note that patients receiving at least 6 months of HBIG have a significantly lower risk of HBV recurrence (32%) in contrast to those who receive either no or short-term prophylaxis (80% rate of HBV recurrence). Reproduced from Samuel et al (93) by permission of the Massachusetts Medical Society.

effective against lamivudine-induced HBV mutants and can rescue patients who develop liver disease in this setting. Adefovir dipivoxil is one such agent. It is also well tolerated but has some risk of renal toxicity and the dose must be adjusted for



**Figure 37.19 Hepatitis B recurrence and survival post-transplant.**

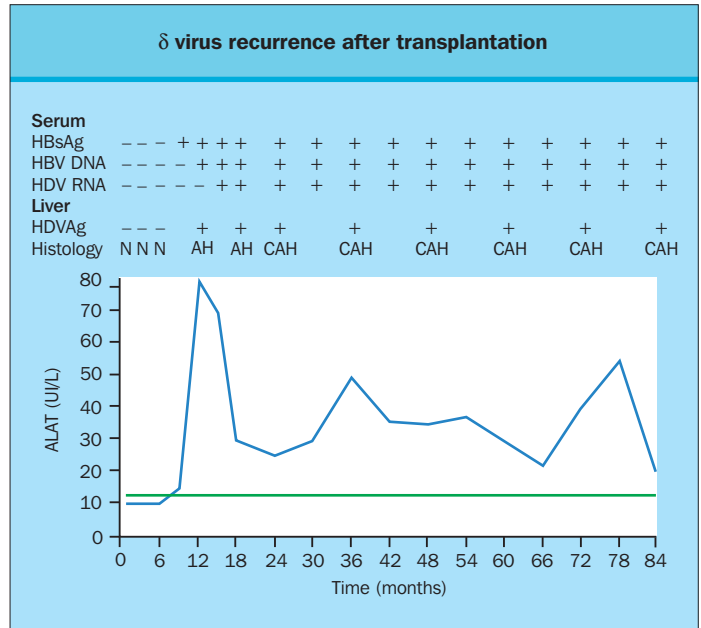
Actuarial survival in patients in relation to hepatitis B virus (HBV) recurrence in a multicenter European trial. Those HBV recurrence [hepatitis B surface antigen-positive (HBsAg+)] have a significantly lower survival rate, mostly due to HBV recurrence, than those without recurrence. This underlines the importance of prophylaxis against HBV. Reproduced from Samuel et al (93) by permission of the Massachusetts Medical Society.

renal function. The onset of action is relatively slow compared to lamivudine but the rate of resistance is much lower (1–3% per year) and it is an excellent antiviral over the long term. Several additional antivirals (e.g. tenofovir and entecavir) with similarly low rates of resistance but with a more rapid onset of action are likely to be in clinic use in the near future. The level of anti-HBs antibody that needs to be maintained in serum with HBIG monotherapy is a subject of debate. There was a general agreement that it should be at least 100 IU/L, but this level may be insufficient in patients at high risk of recurrence such as HBV DNA-positive cirrhotic patients. In this setting, the maintenance of anti-HBs antibody over 500 IU/L may be able to prevent HBV recurrence, but this approach is very expensive. It is now clear that when HBIG is combined with an antiviral, much lower doses of HBIG are required to yield extremely low rates (<5%) of HBV reinfection. For example, a protocol developed by the Australia–New Zealand Liver Transplant Study Group has shown that doses as low as 400–800 per month can be extremely effective. Moreover, there are protocols being developed that combine active vaccination with antiviral therapy to generate endogenous anti-HBs production.

Finally, it does appear that some patients on antiviral therapy can have HBIG discontinued with a low risk of reinfection. This seems certainly true for those who are HBV DNA-negative by a PCR assay. In the future, it may be that combination antiviral therapy with replace HBIG use.

### Hepatitis B and D

Patients chronically infected with HBV and hepatitis D virus (HDV) are at substantially lower risk of HBsAg reappearance than patients chronically infected with HBV alone, and if reinfection does occur, the disease is less devastating than in patients who have solitary HBV infection. The rate of HBsAg



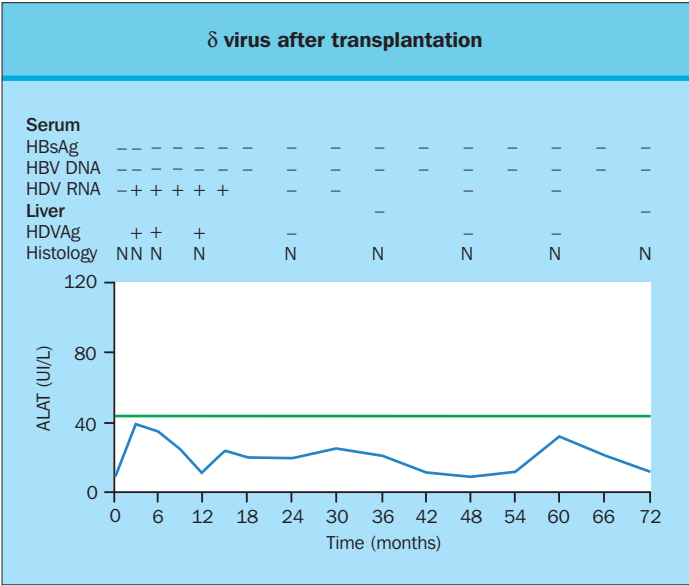
**Figure 37.20 delta virus recurrence after transplantation.** Classic course of a patient transplanted for B-D liver cirrhosis with hepatitis B virus (HBV) recurrence. Note the concomitant reactivation of B and D infection and the development of chronic B-D hepatitis on the graft. This outcome is present in 10–15% of cases when anti-hepatitis B surface immunoglobulin (HBIG) is administered after transplantation. (From Samuel personal communication.)

reappearance in patients who have hepatitis B and D-associated cirrhosis was around 50–60% in patients who did not receive long-term administration of HBIG, and 13–17% in those receiving HBIG. The overall lower HBV recurrence rate in these patients compared with HBV cirrhotic patients is probably due to the fact that almost all patients who have HDV cirrhosis are HBV DNA-negative at the time of liver transplantation and that HDV has an inhibitory effect on HBV replication.

In this group of patients, there is a risk of reinfection with either HBV or HDV or with both viruses. The course of HDV reinfection and its consequences on the graft are different depending on whether HBsAg reappears or not. In the first post-transplant months, HDV reinfection assessed by the presence of HDV RNA in the serum or in the liver was observed in 80% of cases. With HBV recurrence, there is viral reactivation of both viruses (Fig. 37.20), but HBV–HDV recurrence is in general less severe than HBV recurrence alone. In patients who remained HBsAg-negative after transplantation, HDV RNA was found transiently in serum and HDV antigen was detected in the liver. However, the amount of HDV antigen in the graft was low, the graft was histologically normal, and ultimately HDV markers progressively disappeared from the liver and serum (Fig. 37.21).

### Hepatitis C

Liver disease due to HCV is currently the most common indication for liver transplantation in both Europe and the USA. Patient selection for liver transplantation is based on the same criteria as other causes of viral cirrhosis. The major issue after liver transplantation is disease recurrence. HCV RNA is found in more than 95% of patients transplanted for HCV cirrhosis, and HCV RNA levels increase rapidly after liver transplantation,

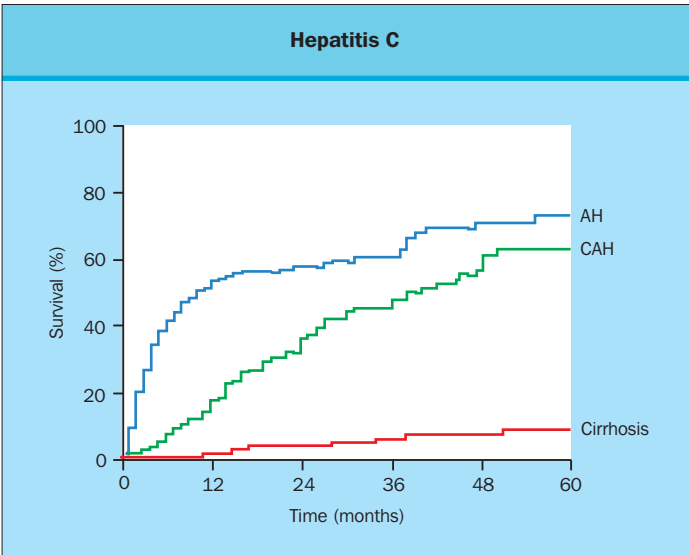


**Figure 37.21 δ virus after transplantation.** Classical course of a patient transplanted for B-D liver cirrhosis without hepatitis B virus (HBV) recurrence. Note the presences of D antigen in the liver and hepatitis D virus (HDV) RNA in serum in the first months, despite the absence of hepatitis B surface antigen (HBsAg) in serum; however there are no graft lesions and HDV markers disappeared after 2 years. This outcome is present in 85–90% of cases when hepatitis B immunoglobulin (HBIG) is administered after transplantation. (From Samuel personal communication.)

reaching pretransplant levels as early as 1 week post-transplantation. The actuarial rate of occurrence of acute lobular hepatitis is 57% at 1 year and 72% at 5 years post-transplant. The actuarial rate of occurrence of chronic hepatitis was 25% at 1 year and 60% at 4 years after the occurrence of acute lobular hepatitis; 8–20% of the patients developed liver cirrhosis within 5 years of follow-up (Fig. 37.22). Some data suggest the recurrent disease may be increasing in severity over the past decade, although this is not generally accepted. However, it should be emphasized that the majority of the reinfected patients have a benign course and 20–40% of the patients have minimal or no lesions on histologic assessment of the graft. The actuarial 5-year patient survival rate is quite good, in the order of 75–80%.

After transplantation, HCV RNA levels increase by 10- to 100-fold, a likely consequence of immunosuppressive therapy. The severity of HCV recurrence appears to be related to both the level of HCV viremia before and consequently after transplantation. Other factors associated with poorer outcomes include age, more advanced liver disease, Black race, cytomegalovirus (CMV) seropositivity and more advanced pretransplant disease.

Methods to prevent HCV infection after transplantation have yet to be identified and the long-term outcome remains unclear (see also Ch. 42). Treatment of HCV pretransplantation in patients with advanced disease using interferons and ribavirin is often challenging because of side-effects. Nonetheless, one group has obtained sustained virologic responses in around 20% of patients on waiting lists using escalating doses of both interferon and ribavirin. The likelihood of response was around four-fold greater in those with genotype 2 and 3 as compared to those with genotype 1. In those who were subsequently transplanted, 70–80% failed to develop reinfection. Unfortunately, only a minority of patients can tolerate a full course of therapy and



**Figure 37.22 Hepatitis C.** Actuarial rate of acute hepatitis (AH), chronic hepatitis (CAH), and cirrhosis in 140 patients transplanted at Paul Brousse hospital for HCV cirrhosis. Despite a 75% survival rate at 5 years, this emphasizes the need for a prevention of HCV recurrence after transplantation. Reproduced from Feray et al (94) by permission of Wiley-Liss, Inc.

thus, the search for alternative ways to prevent reinfection will continue.

### ALCOHOLIC LIVER DISEASE

While alcoholic cirrhosis is a frequent cause of liver disease it accounts for a relatively small proportion of liver transplant activity. Alcohol is the second most common indication for liver transplantation in USA and Europe after HCV related liver disease. Current data indicate that with 11 000 deaths from alcoholic cirrhosis in the USA/year, only 6% of cases are receiving liver transplants. Given the limited supply of donor organs and some reluctance to transplant patients who have a ‘self-inflicted’ illness, several centers have developed an evaluation process (based on medical and psychiatric criteria) to identify patients who would most benefit from transplantation. The number of patients from the population who have alcohol-related cirrhosis that are candidates for liver transplantation is still limited. There are several reasons for this.

- Although alcoholic disease itself is not a contraindication, many community physicians do not refer these patients for liver transplantation, and some centers are reluctant to transplant these patients, due to a concern about the ethics and legitimacy of the procedure and the risk of recidivism.
- In many patients, cirrhosis develops at a later age when transplantation is contraindicated.
- Alcohol is also the cause of extrahepatic diseases that will not be treated by transplantation. In addition, alcoholic patients appear to be at increase risk for post-transplant malignancies.
- Most centers demand a 6-month period of abstinence from alcohol before intervention.
- Some patients may show behavior patterns that suggest the compliance with long-term immunosuppressive therapy may be erratic.



- It is still difficult to determine the prognosis of patients who have alcoholic liver disease as a dramatic clinical improvement can occur after 6 or more months of abstinence from alcohol.

In this context, guidelines for patient selection are currently directed at cirrhotic patients whose liver disease remains life-threatening despite a minimal period of 6 months of abstinence. This permits a re-evaluation of the need for, and timing of, liver transplantation and gives some insight into the patient's ability to abstain from alcohol. This interval has not achieved consensus as some programs do not adhere to such requirements while others require a longer period of abstinence. Some period of sobriety is important, but it should not be used as the sole criterion for selection and should be considered along with other psychosocial predictors of abstinence. In some cases the required duration of abstinence will allow some patients to escape transplantation because of a marked improvement of liver function. Patients participating in an alcohol-counseling program with a stable and favorable psychosocial supportive environment are most often capable of avoiding recidivism and non-compliance with immunosuppressive therapy.

Acute alcoholic hepatitis is currently regarded as a contraindication in most centers because the majority of patients is actively drinking at the time of presentation and has a higher risk of recidivism. Consequently, scarce donor organs would be allocated to patients who have a predictably inferior medical and psychiatric outcome. In selected cases, in whom the 6-month period of abstinence is impossible to achieve because of the severity of the liver disease, candidacy for liver transplantation should be addressed on an individual basis. A positive selection decision needs to be supported by a self-recognition of alcoholism with a real desire for long-term cessation of alcohol consumption, in addition to a strong psychosocial and family support network and a positive appraisal by a psychiatrist.

Several reports suggest that the short-term outcome of liver transplantation in alcoholic cirrhosis is equivalent to that in non-alcoholics, although the prognosis is unclear in patients who have acute alcoholic hepatitis. The risk of recidivism of alcoholism is estimated between 15 and 40% and it seems to be related to the duration of abstinence before transplantation and the duration of follow-up after transplantation. Most patients with recidivism have a moderate drinking pattern after transplantation without consequences to the graft, but 5–10% of patients experience severe consequences of recidivism including non-compliance with medications, steatosis, and ultimately loss of the graft. The recidivism rates indicate the importance of stringent selection criteria in this context. Psychosocial support and participation in alcohol treatment after transplantation is clearly needed. Additional studies of large numbers of patients are needed to determine the long-term relapse rate with regard to alcohol consumption. An increased rate of *de novo* cancer has been observed in patients transplanted for alcoholic cirrhosis, but its impact on long-term outcomes is not well defined.

## AUTOIMMUNE HEPATITIS AND CIRRHOSIS

The clinical presentation of the autoimmune hepatitis is variable, classically presenting as chronic hepatitis, but it may also present with established cirrhosis and in a few cases as acute liver failure. The main characteristic of this disease is a good response to

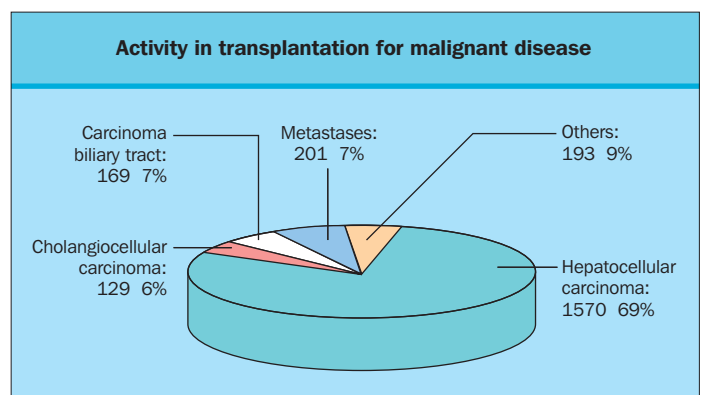
immunosuppressive treatment with steroids and azathioprine, particularly in type I patients.

Liver transplantation is indicated when there is clinical deterioration including the development of cirrhosis, esophageal varices and impaired coagulation despite long-term adequate immunosuppressive treatment. More urgent clinical conditions include resistant ascites, spontaneous bacterial peritonitis and encephalopathy. In this condition, it is essential to evaluate the severity as well as the activity of the disease. The level of liver enzymes and the histologic evaluation of the inflammatory infiltrate, together with intensity of hepatocyte necrosis and fibrosis, help to decide whether additional immunosuppressive treatment might improve the clinical condition and as a result postpone the need for transplantation. A short course of additional immunosuppressive treatment could be introduced under cautious supervision by transplant physicians, and in the absence of improvement, liver transplantation is indicated. Acute liver failure, in which immunosuppressive treatment is usually ineffective and potentially deleterious, is an indication for emergency liver transplantation.

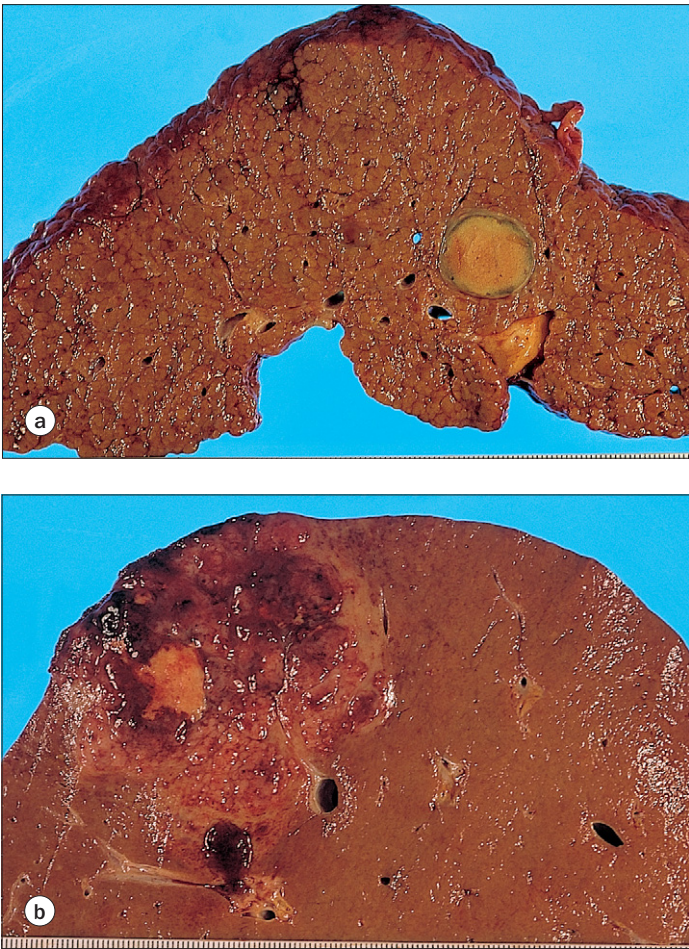
The results of liver transplantation for autoimmune hepatitis are usually excellent with a 5-year survival rate over 80%. A higher risk of rejection has been reported and the role of corticosteroid treatment before transplantation in the occurrence of rejection has been debated. Well-documented cases of recurrence have been described, but they usually respond to an increase in the corticosteroid component of the immunosuppressive regimen and rarely lead to graft failure and retransplantation of the liver.

## HEPATOBIILIARY MALIGNANCY

From the earliest days of liver transplantation, hepatobiliary malignancy was a favored indication for liver transplantation (Fig. 37.23). However, that early experience yielded results that were inferior to those transplanted for other liver diseases. The key concepts in the evaluation of the role of transplantation are the different tumor types and volumes, risk of tumor recurrence, donor organ shortage, the associated liver disease and the role of immunosuppression in promoting tumor growth. Contraindications



**Figure 37.23 Activity in transplantation for malignant disease.** The breakdown of indications shows a predominance of patients with hepatocellular carcinoma. Reproduced from The European Liver Registry Report.



**Figure 37.24 Transplantation for hepatocellular carcinoma.** (a) The small, singular, circumscribed tumor in a patient with cirrhosis is an ideal candidate for transplantation, but the large tumor arising in a non-cirrhotic liver (b) is no longer considered a good risk patient. (Courtesy of Professor B. Portmann.)

to liver transplantation are advanced primary liver tumors with any extrahepatic spread, hemangiosarcoma and liver metastases from non-neuroendocrine primary tumors.

**Hepatocellular carcinoma**

HCC, when left untreated, has an extremely poor prognosis with only 1–24 months survival from the time of diagnosis. Early enthusiasm for this indication for transplantation was frustrated by the high incidence of tumor recurrence, in excess of 50%, over 1–2 years of follow-up. Although there is some variability, most centers currently practice a restrictive policy for transplantation for HCC (Fig. 37.24).

Improved patient selection criteria have led to better results. The so-called Milan Criteria are those most commonly used which include patients with a single lesion <5 cm in diameter, who have three or fewer nodules that are smaller than 3 cm in diameter, in the absence of lymph node metastases or vascular invasion (Table 37.4). While the Milan Criteria are most widely accepted, some groups have expanded the criteria to single lesions of up to 6.5 cm or three or less lesions each up to 5 cm in diameter. However, it is important to note that these studies are retrospective analysis based on pathologic data. The Milan Criteria, by contrast, have stood the test of time for clinical staging. Moreover, many of the programs which advocate expanding the HCC criteria used the Milan Criteria to select patients. Unfortunately this demonstrates that clinical staging can both underestimate, as well as overestimate, the amount of tumor present. Until prospective studies can show that using the expanded criteria as clinical staging still yields good results, it is hard to recommend revision of the selection criteria. From a practical standpoint, in the USA in the MELD era, patients with HCC would require more MELD points than they would receive based on liver function to achieve sufficient priority for deceased donor transplantation. The criteria for awarding additional MELD points are based on the Milan Criteria.

Overall 3-year survival rate and survival without recurrence in patients with hepatocellular carcinoma				
Liver disease	Survival (%)	Resection (n=60)	Transplantation (n=60)	
		Recurrence free survival (%)	Survival (%)	Recurrence free survival (%)
All patients	52	27	49	46
Size				
<3 cm	39	18	60	56*
>3 cm	56	32	43	39*
No. nodules				
1	53	28	46	20
>1	46	20	51	49
Combination				
< 3 cm and 1–2 nodules	41	18	83	83
> 3 cm and > 2 nodules			46	44

**Table 37.4 Overall 3-year survival rate and survival without recurrence in patients with hepatocellular carcinoma treated by resection or transplantation in relation to the size and the number of nodules.** Note the excellent survival in those transplanted with less than three nodules of <3 cm. \*P <0.05. (From Bismuth et al. Ann Surg. 1993;218:145–51.)

Tumor invasion of the portal vein leading to intrahepatic metastasis is the most common means of HCC spread. A relationship between the risk of tumor recurrence and the presence of portal vein involvement has been established. The risk of recurrence was 100% when the tumor involved the main portal vein or the two main intrahepatic portal branches. Although the recurrence rate was lower in cases of involvement of a smaller portal branch, the risk remained significant and this should be considered as a negative prognostic factor. For this reason, the possible involvement of the portal vein or its branches with a tumor needs to be carefully evaluated in potential transplant candidates.

Preoperative evaluation must include at least a computed tomography (CT) scan of the abdomen and chest, and perhaps a bone scan. In the USA, patients are restaged at 3-month intervals after registering on the United Network for Organ Sharing (UNOS) waiting list in order to continue to receive additional MELD points. Some centers perform an exploratory laparotomy before transplantation, in order to confirm the absence of extrahepatic spread. As soon as the transplant procedure begins, it is essential to look for any sign of extrahepatic disease and to carefully inspect the hepatic pedicle for the presence of pathologic lymph nodes. A perioperative histologic analysis of suspicious tissue is mandatory, and the procedure should be abandoned before the hepatectomy in cases of confirmed extrahepatic spread. Despite the uncertain long-term benefit in some cases, and the need to compare carefully the results obtained from liver transplantation with those of other methods (e.g. early resection, alcohol injection, chemoembolization, radiofrequency ablation), it is clear that liver transplantation is the only possible curative option in many cases of HCC associated with cirrhosis. The procedure in patients who have early disease is associated with a recurrence rate of 10% and a 5-year survival rate of 75%, which is similar to that achieved for other indications for liver transplantation.

### **Fibrolamellar carcinoma**

This is a particular histologic variant of HCC that is more common in young women who do not have underlying liver disease. The treatment of choice is liver resection, where one can be quite aggressive because of the absence of underlying liver disease. When a partial hepatectomy is not possible, liver transplantation is a good option, although there is a significant risk of recurrence since these tumors tend to be large at presentation and at transplantation. However, the slower tumor growth rates result in a significant period of survival for those requiring transplantation, even when the tumor eventually recurs. Whether the prognosis is better than in those with standard HCC is not clear.

### **Cholangiocarcinoma**

Cholangiocarcinoma is the second most common cancer among the primary hepatic neoplasms, accounting for 5–20% of liver malignancies. The ones that arise from the epithelium of the intrahepatic bile ducts are called ‘true’ cholangiocarcinomas as opposed to those that arise from the extrahepatic biliary tree. A clear association between cholangiocarcinoma and PSC has been established, and the occurrence of cholangiocarcinoma seems to correlate with the duration of the latter disease. Nowadays there is considerable debate about the indications for, and results of, transplantation for this kind of tumor. Liver transplantation

does not offer either a cure or a significant duration of palliation in the majority of patients. This may be due to the fact that many of these tumors are diagnosed at an advanced stage, when the disease has metastasized to the lymph nodes. In the absence of lymph node disease, 1-year, 2-year and 3-year survival rates of 77, 64 and 51% were observed, respectively. On the other hand, with lymph node disease the 1-year survival rate dropped to 14%, and the 2-year survival rate was 0%.

Recently, the Mayo Clinic has reported very good results in a select group of patients with cholangiocarcinoma using aggressive external beam and intraductal radiation therapy, with chemotherapy in patients without evidence of extraductal disease. After this therapy, patients are explored and only those in whom no residual disease can be demonstrated are put on a waiting list and are granted additional MELD points. With this approach, they have attained medium-term survival rates of 80–85%. However, it must be emphasized that a very small minority of patients with cholangiocarcinoma will qualify for transplantation with this approach.

### **Epithelioid hemangioendothelioma**

Epithelioid hemangioendothelioma is a rare tumor of the liver that is generally multifocal and has an endothelial origin. It is more common in younger adults and is usually quite indolent in nature. In the absence of extrahepatic dissemination, liver transplantation should be considered. In an experience of 10 patients, the 3-month survival rate was 89%, with 3-year survival rates of 76%. The recurrence rate was 30%, although little is known about the factors affecting survival and recurrence following liver transplantation. It should be noted that metastatic spread at the time of surgery does not appear to be an absolute contraindication to transplantation, and this is distinctly different from the situation in patients who have HCC or cholangiocarcinoma. Given that these tumors have a relatively benign natural history, even when untreated, the major selection issue is to identify the few individuals who will experience a survival benefit.

### **Metastatic liver disease**

Classically, metastatic tumors of the liver have been considered a poor indication for liver transplantation, although some centers have performed this procedure in conjunction with other therapies, such as chemotherapy and radiotherapy particularly in individuals with neuroendocrine tumors. Most series have demonstrated an unacceptably high recurrence rate with non-endocrine metastases that did not justify the cost of the procedure and utilization of scarce donor organs. On the other hand, in metastases from neuroendocrine tumors, liver transplantation could be indicated for patients who have symptoms related to massive hepatomegaly or syndromes related to hormone production. Effective therapeutic alternatives are usually not available and the typical candidate for transplantation has diffuse metastases of the liver, slow-growing tumors and no extrahepatic disease. The main advantages of transplantation are a significant improvement of the quality of life in many patients and a possible cure in some of them. However, an inadequate knowledge of specific biologic and pathologic prognosis factors, the possibility of tumor growth as a consequence of immunosuppression, and the shortage of donor organs are key issues that counterbalance the argument in favor of transplantation. While the results to date have been mixed, it is believed that long-term follow-up



will determine the utility of liver transplantation for neuroendocrine liver metastases.

### **BUDD-CHIARI SYNDROME**

The Budd-Chiari syndrome is a clinical disorder with a broad clinical spectrum resulting from the occlusion of the main hepatic veins at their junction with the inferior vena cava. It is similar clinically to veno-occlusive disease of the liver, although the latter most commonly occurs in the setting of bone marrow transplantation or occasionally due to certain hepatotoxins. The diagnosis is suggested by the absence of flow in the hepatic veins on ultrasonography and confirmed by the demonstration of thrombus on retrograde hepatic venography, together with changes in liver morphology.

A surgical portosystemic shunt, principally TIPS or a mesocaval shunt, is the most commonly used treatment in all acute and subacute forms that have not responded to other treatments. The effect of portosystemic shunting is to reduce portal pressure and induce reversal of the flow in the portal vein. The liver congestion is relieved, preserving hepatocytes and allowing liver regeneration. This ultimately leads to a reduction in ascites formation.

The first liver transplantation for Budd-Chiari syndrome was performed in 1974, and the European Liver Transplant Registry recorded 224 liver transplantations for this disease from January 1988 to June 1997. The indications for liver transplantation are the fulminant form of the disease associated with liver failure, which usually does not respond to mesocaval shunt surgery, and the chronic stage with established cirrhosis and its complications despite shunt procedures.

Although the choice of transplantation is appropriate for end-stage cirrhotic disease, it is difficult to ignore the efficacy of the long-term results of portosystemic shunting in Budd-Chiari syndrome. Conclusions on the benefits of one treatment over another might be difficult due to the rarity of the disease, the wide variety of precipitating diseases and the diversity of the clinical conditions. The final decision should be made taking into account the causative disease, a careful assessment of the degree of hepatic venous occlusion, and a precise grading of the clinical form of the disease and the hepatic dysfunction. The same is true for veno-occlusive disease. If liver transplantation is performed, it is essential to identify and treat associated prothrombotic disease and adhere to a rigid protocol for long-term anticoagulant therapy.

### **CYSTIC FIBROSIS**

Cystic fibrosis is a multisystemic genetic disease with a gene carrier rate of 5% in the population. In spite of new medical therapies, life expectancy is approximately 50% at 30 years. Cirrhosis occurs in 5–15% of patients who have cystic fibrosis, with an onset in the first decade of life and later progression to portal hypertension, hypersplenism and variceal bleeding. For these patients, liver transplantation, isolated or in combination with lung or heart-lung transplantation, is the only effective treatment option to prolong survival and the quality of life. Questions have been raised regarding the suitability of such candidates for liver transplantation, because these patients are often in a poor nutritional condition as a result of severe intestinal

malabsorption and chronic infection with highly resistant bacteria. However, postoperative management does not differ significantly from that of other patients undergoing liver transplantation, except for special attention to pulmonary care, nutritional management and the risk of malabsorption of immunosuppressive drugs.

### **CAROLI DISEASE**

Caroli disease is an uncommon congenital disorder of the intrahepatic biliary tree. It is characterized by multiple and segmental dilatations of the bile ducts. The clinical course is often complicated by recurrent episodes of cholangitis that seriously impair the patient's quality of life. Abdominal pain may be quite severe in some cases. Patients may also develop progressive fibrosis and portal hypertension. Treatment is difficult and depends on the location and extent of the disease. Medical treatment is worthless because bile duct dilatation and gallstone formation favor bacterial persistence after the initial colonization of the biliary tree. Antimicrobial agents provide temporary improvement, but recurrence is the rule after cessation of this therapy. Bacterial cholangitis is the most frequent and life-threatening complication of the disease. Consequently, due to poor results of both medical and surgical therapies, liver transplantation has been proposed in complicated Caroli disease that is diffuse within the liver. However, only a relatively small number of cases have been reported.

### **METABOLIC DISEASES**

#### **Wilson disease**

In this disease, there is copper deposition in the liver, the central nervous system and the kidneys. This autosomal recessive hereditary disease is secondary to a gene mutation on chromosome 13 and results in an inability to excrete copper in bile and consequently copper accumulates in the liver. There are five main clinical presentations of liver disease:

- chronic hepatitis with a moderate increase in aminotransferases;
- decompensated liver cirrhosis with ascites and liver failure;
- acute liver failure with impaired coagulation, hemolytic anemia and encephalopathy;
- absence of clinical liver disease with a neurologic presentation or other manifestations; and
- following the diagnosis of Wilson disease in a sibling.

Most frequently, this disease is medically treatable and the drugs of choice are D-penicillamine and trientine, which act as chelators of copper and as a detoxificant of copper overload in hepatocytes. This therapy may arrest further progression of the disease and promote possible reversal of the clinical manifestations. However, liver transplantation may be indicated in the following situations:

- a fulminant course that almost invariably requires urgent transplantation;
- patients who have cirrhosis who fail to respond to 2–3 months of copper chelation therapy and supportive therapy for associated complications;
- progression of the disease despite chelation therapy; and
- severe hepatic failure and hemolysis after discontinuation of chelation therapy.

Generally, after liver transplantation, the copper balance is restored and there might be an improvement in both the neurologic and renal complications of the disease, although this is not a certainty. The prognosis is good with no evidence of reaccumulation of hepatic copper.

### **$\alpha_1$ -Antitrypsin deficiency**

The diagnosis is made by the finding of a significant reduction of serum  $\alpha_1$ -antitrypsin levels, a positive Pi ZZ phenotype, and a diagnostic liver biopsy with periodic acid-Schiff-positive diastase-resistant cytoplasmic globules. One-quarter of children who have the Pi ZZ phenotype will develop neonatal cholestasis and among this group, 50% will develop progressive liver failure at some time during childhood or adolescence. More than 50% of adults have coexistent pulmonary disease associated with  $\alpha_1$ -antitrypsin deficiency, but the lung disease is usually mild. Clinical signs of neonatal cholestasis appear during the first days of life and are usually evident before 10 weeks of age. As there is no effective replacement therapy, liver transplantation is indicated in patients who have cirrhosis and progressive hepatic decompensation. After liver transplantation, the  $\alpha_1$ -antitrypsin level returns to normal and the 5-year actuarial survival rate after transplantation is higher than 80%.

### **Hereditary tyrosinemia**

This disease is characterized by progressive hepatocellular damage, renal tubular dysfunction, hypophosphatemia, and urinary excretion at concentrations of more than 100 times the normal rate of the tyrosine metabolite succinylacetone. Hepatic fibrosis develops with prominent regeneration nodules, leading to cirrhosis. Survival beyond the age of 10 years is rare. Up to 37% of children over the age of 2 years will develop HCC.

Medical treatment has not proved to be effective. Liver transplantation corrects the biochemical abnormalities and allows patients to have a normal protein intake. In patients presenting with the disease in early infancy, transplantation should be postponed for as long as possible to allow the baby to grow. In those who have a later onset of symptoms, the timing of grafting is difficult as delay may increase the risk of developing HCC. Prospective screening with ultrasound examination of the liver and serum  $\alpha$ -fetoprotein estimates may be useful to detect tumors early enough to allow liver transplantation.

### **Crigler-Najjar syndrome**

This is an inherited disorder that occurs in two forms (type I and II), characterized by severe, life-long, non-hemolytic, unconjugated hyperbilirubinemia. Liver transplantation is not generally indicated in the type II syndrome, which can be treated with continuous phenobarbital therapy. In type I syndrome, the serum bilirubin concentration does not decrease when phenobarbital is administered. The majority of infants will develop kernicterus and brain damage in the first 18 months of life, but the onset of kernicterus and progressive brain damage may be delayed until puberty or the early twenties. Liver transplantation may be indicated when there is failure to prevent the kernicterus despite extensive phototherapy, or in cases because of poor quality of life due the need for prolonged phototherapy. Auxiliary partial

OLT has also been performed successfully. As irreversible brain damage has been described, the optimal timing for liver transplantation is important, in order to reduce neurologic sequelae and improve survival and quality of life after transplantation.

### **Hereditary hemochromatosis**

Hereditary hemochromatosis is a recessive autosomal disorder leading to iron overload with a disease frequency of 5/1000 population. The disease is secondary in 95% of cases to a point mutation (C282Y) in the HFE gene located on chromosome 6. It is characterized by increased intestinal iron absorption with deposition of parenchymal iron in the liver, heart, pancreas, pituitary and joints, and a potential risk for developing cirrhosis and HCC. Liver transplantation is indicated for hemochromatosis complicated by liver failure or the presence of HCC not treatable by resection. However, the survival of patients undergoing liver transplantation for hemochromatosis is less favorable, due to cardiac involvement and the frequent coexistence of HCC. Pretransplant phlebotomy to reduce cardiac complications needs to be carefully evaluated, to determine whether it can improve survival after liver transplantation. The risk of re-appearance of iron deposits in the liver is debatable.

### **Protoporphyrria**

This is an autosomal dominant disorder characterized by photosensitivity and elevated levels of protoporphyrin in erythrocytes, plasma and feces. It is secondary to a deficiency in an enzyme responsible for heme synthesis called heme synthase or ferrochelatase. The pathogenesis of liver disease is still unclear, but is probably the consequence of accumulation of protoporphyrin pigments in the liver. Liver transplantation should be considered when jaundice, recurrent cholangitis or liver failure occurs. After transplantation a dramatic, but partial, decrease in plasma and erythrocyte levels of protoporphyrins is obtained, and this leads to a partial or complete resolution of photosensitivity. Some patients may develop recurrent disease in the graft, but long-term survival without recurrence has also been described.

### **Primary hyperoxaluria**

Primary hyperoxaluria is a rare autosomal recessive disorder characterized by hyperoxaluria, calcium oxalate urinary lithiasis, nephrocalcinosis and consequent renal failure. There are at least two types, and type 1 is much more common and well-studied. Type 1 primary hyperoxaluria is secondary to a hepatic deficit in alanine-glyoxylate aminotransferase deficiency. Once renal failure occurs there is a dramatic acceleration in the rate of oxalate accumulation. Renal transplantation in isolation results in almost universal recurrence of oxalosis in the kidney graft and a return to hemodialysis after varying intervals. Combined liver and kidney transplantation treats both the renal failure and cures the cause of the disease, and this approach may avoid recurrence of oxalosis in the kidney graft. However, this presumes the kidney graft functions sufficiently well to clear the excess pool of calcium oxalate, but this does not necessarily happen before further damage to the graft occurs. Combined liver and kidney transplantation should be performed before the occurrence of severe complications and the ideal timing is just before or immediately after the patients are initiated on hemodialysis. Long periods of hemodialysis will significantly increase

Indications for liver transplantation in children	
Chronic liver disease	Cholestatic diseases
	Extrahepatic biliary atresia
	Byler disease
	Ductopenia (nonsyndromic)
	Alagille syndrome
	Primary sclerosing cholangitis
	Autoimmune chronic hepatitis
	Viral B cirrhosis
	Budd–Chiari syndrome
Metabolic liver disease	$\alpha_1$ -antitrypsin deficiency
	Hereditary tyrosinemia
	Crigler–Najjar type 1
	Protoporphyrria
	Type 1 and 4 glycogenosis
	Galactosemia
Primary malignant tumor of the liver	Hepatocellular carcinoma
	Hepatoblastoma
	Cholangiocellular carcinoma
Acute liver failure	Fulminant and subfulminant hepatitis
	Wilson disease (fulminant)
	Budd–Chiari syndrome (fulminant)

Table 37.5 Indications for liver transplantation in children.

the pool of calcium oxalate in the body. Some authors have advocated an isolated liver transplantation early in the course of the disease before the occurrence of renal failure, but this preemptive therapeutic approach remains controversial.

**Familial amyloidosis with polyneuropathy**

This is an autosomal dominant systemic disease usually predominantly involving the peripheral nervous system. The commonest genetic defect is a single mutation located on chromosome 18, leading to the synthesis of a variant transthyretin (also called prealbumin) that is found in plasma. It then forms amyloid plaques that are deposited in neural and visceral tissues. The disease onset is usually between 30 and 40 years of age. There is no medical treatment and patients usually die at a median of 10 years after the onset of symptoms. The principle of liver transplantation as a treatment for familial amyloid polyneuropathy is to suppress the production of the transthyretin variant, thus stopping the evolution of the disease leading to clinical improvement. The main indication is when the disease has progressed to clinically significant neuropathy but before extensive cardiac involvement has occurred. This generally happens when autonomic neuropathy leads to weight loss with changes in

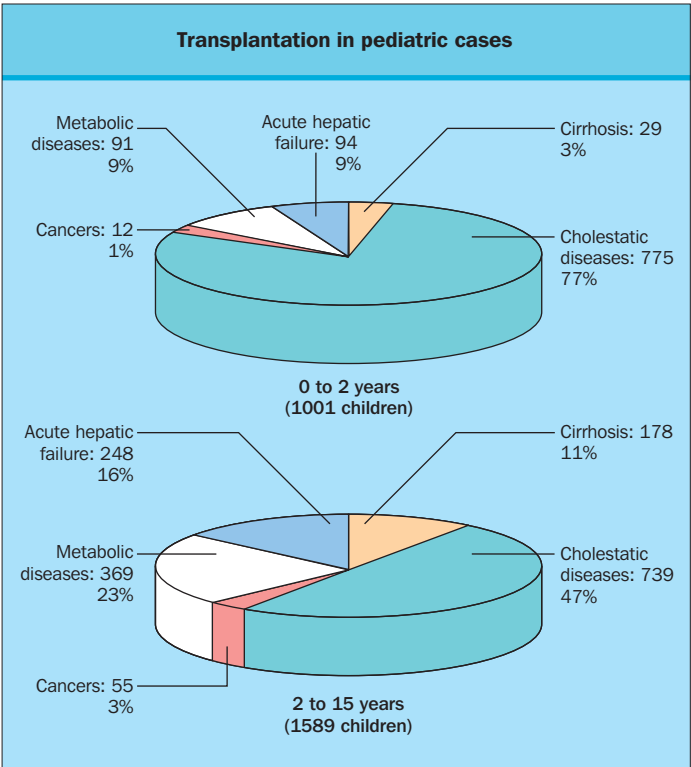


Figure 37.25 Transplantation in pediatric cases. The indications for liver transplantation up to the age of 2 years and in older children. Modified from The European Liver Registry Report.

bowel habit. Liver transplantation is not indicated in asymptomatic patients or advanced cases.

**PEDIATRIC LIVER TRANSPLANTATION**

Liver transplantation is a well-accepted treatment for children with end-stage liver disease (Table 37.5). Evaluation and appropriate patient selection for transplantation are becoming increasingly important issues as more and more children are submitted to the procedure (Fig. 37.25). The main selection criteria for liver transplantation are:

- severe jaundice;
- intractable variceal hemorrhage;
- liver failure manifested as vitamin K-resistant coagulopathy, malnutrition and hypoalbuminemia;
- hepatic encephalopathy; and
- severe malaise, weakness, and failure to grow.

The long-term results of transplantation in children are generally better than those in adults, with a 1-year survival rate of over 90% and with little further change in up to 5-years of follow-up because of the lack of recurrent disease. In addition, liver transplantation results in a dramatic improvement in quality of life, resumption of daily activities including education, psychologic transformation and a reintegration into society. In some critical clinical situations, such as age under 1 year, congenital anatomic abnormalities, multiple organ injuries and malnutrition, the outcome is not as good.



### The equation for calculating the pediatric end-stage liver disease (PELD) score

- $PELD = (0.436 \times \text{age}^*) - (0.687 \times \ln(\text{albumin})) + (0.480 \times \ln(\text{bilirubin})) + (1.857 \times \ln(\text{INR})) + (0.667 \times \text{growth failure}^\dagger) \times 10$
- No cap

Notes: \* Age <1 year = 1, Age >1 year = 0; † growth failure = 1, no growth failure = 0; INR, international normalized ratio.

**Table 37.6** The equation for calculating the pediatric end-stage liver disease (PELD) score.

The small number of pediatric donors available has necessitated the use of partial or reduced adult grafts or grafts from living related donors. There is a higher incidence of hepatic artery thrombosis after liver transplantation, and this is the main difference between pediatric and adult transplantation. The exact timing of the procedure may be difficult, but if possible it should be performed in children at least 1 year old and over 10 kg in weight. On the other hand, delayed intervention can be associated with progressive complications and clinical deterioration of the patient.

With the institution of MELD, a separate, but parallel system for allocating pediatric organs was developed. A scoring system by which pediatric patients are prioritized on the waiting list goes by PELD, and includes total bilirubin concentration, INR and albumin, in the equation (Table 37.6). In addition, children less than 1 year of age are given some additional points as are children who experience growth failure. In contrast to MELD, which is capped at 40, there is no cap for PELD. The institution of PELD as a system for priority allocation has had a similar effect to MELD, in that it has decreased waiting list mortality for pediatric patients. It has also now been shown on retrospective analysis to be a very potent predictor of short-term mortality on the waiting list. While initially PELD was applied to all patients less than 18 years of age, it has recently been shown that MELD is a better predictor of short-term mortality on the waiting list for those pediatric patients between the ages of 12 and 17 years. Similar to MELD, the institution of PELD has had no significant impact on post-transplant mortality.

### Extrahepatic biliary atresia

Biliary atresia is the most frequent cause of chronic cholestasis in infants, affecting 1:8000 to 1:12 000 live births. It is a pan-biliary disease of both the intrahepatic and extrahepatic biliary tree, probably the end result of a destructive inflammatory process leading to fibrosis and obliteration of the biliary tract. When left untreated, this condition leads to death from liver failure in early childhood, with an average survival of 12–18 months. Fewer than 10% of patients not undergoing biliary bypass surgery survive beyond 3 years.

The best primary treatment of biliary atresia remains controversial because of the limited efficacy of Kasai portoenterostomy, the success of liver transplantation, and the greater technical difficulty of transplantation in patients who have had multiple previous surgical procedures. Currently, liver transplantation for biliary atresia represents 50% of the cases of pediatric liver transplantation and between 5 and 10% of both

pediatric and adult procedures. The first-line treatment should be a Kasai portoenterostomy before the age of 8–10 weeks by an experienced pediatric surgical team. Generally speaking, 25–30% of children who undergo a Kasai procedure have restoration of bile flow, with resolution of cholestasis and no progression to cirrhosis. The remaining 70–75% are potential candidates for liver transplantation because adequate restoration of bile flow was not obtained.

The current criteria for liver transplantation are an immediate failure of the Kasai procedure (30–40% of the total), leading to transplantation before 3 years of age or secondary failure (30–35% of the total) with recurrence of jaundice, leading to transplantation at a variable age. These patients develop portal hypertension and uncontrolled bleeding, uncontrolled cholangitis, ascites, intolerable pruritus and liver failure.

The timing of liver transplantation is based on the patient's clinical condition. The procedure must be performed before the onset of growth failure, irreversible complications and deteriorating nutritional status. If possible, it is preferable to postpone the procedure until the infant reaches 1 year of age or weighs 10 kg, because the survival rates increase from 50–60% to 80% if the transplant is performed after 1 year of age. Recurrence of the disease after liver transplantation has not been observed. It has become clear that the Kasai procedure and liver transplantation are complementary treatments for biliary atresia and that transplantation should be considered for cases of early or late failure of portoenterostomy. The ultimate aims of liver transplantation in these patients are the return to full quality of life and normal development.

### Interlobular bile duct paucity

Two types of paucity of interlobular bile ducts are described: a syndromic type, the most common being called Alagille syndrome, with the presence of at least two major extrahepatic associated features; and a non-syndromic type that is secondary to multiple viral or metabolic insults to the liver. As liver failure is rarely responsible for death in the syndromic type, liver transplantation is rarely indicated. On the other hand, there is progressive biliary cirrhosis and liver failure in the non-syndromic type and liver transplantation is the only available therapy, being indicated at any age in which liver failure becomes clinically manifest.

### Byler disease

This is a poorly understood disease for which the biochemical basis is still unknown. Clinically, it is characterized by hepatocyte failure to excrete bile, without any anatomic or histologic biliary abnormalities. Recently the use of ursodeoxycholic acid has been shown to improve cholestasis and liver function in some patients. When secondary biliary cirrhosis and liver failure develop, liver transplantation should be considered.

### RETRANSPLANTATION

Due to the shortage of organ donors, the indications for retransplantation are controversial. Survival after retransplantation is lower than that after primary transplantation at around 50% at 1 year. Emergency retransplantation during the first week should be differentiated from later retransplantation. The indications for the former are in general primary graft non-function, thrombosis

of the hepatic artery and, more rarely, acute rejection. The causes of later retransplantation are chronic rejection, long-term biliary consequences of arterial thrombosis, and hepatitis C or B recurrence in the graft. In these cases, survival is more dependent on the condition of the patient at the time of retransplantation, rather than on the indication for retransplantation.

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## SECTION V: LIVER TRANSPLANTATION

### CHAPTER

# 38

## The Transplant Operation: What the Hepatologist Should Know

Michael Lewis Schilsky, Milan Kinkhabwala and Jean Crawford Emond

### BACKGROUND

The liver transplant operation has evolved over the past 40 years by overcoming a spectrum of physiologic and technical problems to become the accepted treatment for end-stage liver disease and liver failure. The first description of experimental liver transplantation is attributed to CS Welch, who performed transplantation of the whole canine liver by placing the graft next to the native liver in an auxiliary position. These initial experimental efforts were based on the assumption that total removal of the native liver would be a prohibitive undertaking. The general procedure of orthotopic liver replacement in humans was defined by Starzl in 1963, but acceptance of the procedure was associated with continued technical modifications that led to more dependable outcomes.

Modifications in liver transplantation technique continue to this day. Among the most important developments was the introduction of venous bypass in 1983, in response to the significant hemodynamic instability that was common during the anhepatic phase of the transplant procedure. While some centers no longer use routine venous bypass, its widespread use in the 1980s contributed to a reduction in operative mortality. In addition to venous bypass, other key developments in liver transplantation included simplification of the multiorgan retrieval operation in the donor, and the introduction of the University of Wisconsin preservation solution. These advances collectively mitigated the logistic constraints of the transplant procedure and facilitated training of new transplant surgeons.

A major surgical triumph was the development of partial transplantation in the 1980s, driven by a scarcity of donors for small children. As a result living donor and split liver transplantation are now accepted worldwide; in many centers they account for the majority of liver transplants performed in children. More recently these techniques in partial liver transplantation have been extended to adults, similarly driven by a scarcity of donor organs. Living donation liver transplant (LDLT) introduces an enormous challenge for the transplant hepatologist. An understanding of the techniques and outcomes is necessary to properly select and counsel patients and their donors. In addition, a new array of complications is possible, increasing the complexity of postoperative management. Auxiliary liver transplantation, in which part or all of the native liver is preserved, has also been rediscovered in recent years with several potential applications. These diverse procedures are reviewed in this chapter, displaying the range of possibilities available to the transplant surgeon.

While knowledge of all of the technical aspects of the organ retrieval and performance of the transplant operation are requisite for the transplant surgeon, an understanding of the technical needs and limitations of the procedure also enhances the practice of

the hepatologist, clinical coordinators and other members of the transplant team. These transplant team members can apply this knowledge to the transplant evaluation, patient selection and understanding of the potential post-transplant complications often managed by these individuals.

### ORGANIZATION OF THE OPERATING ROOM FOR LIVER TRANSPLANTATION

Because of the unpredictable and often urgent nature of liver transplantation, most transplant centers utilize dedicated liver transplant operating rooms with surgical teams available around the clock. The complexity of the procedure demands a complete understanding of the operation and its potential complications by specialized nursing and anesthetic teams. While costly in terms of personnel, the expense is well justified, because even small errors in functional efficiency can lead to disastrous outcomes. The consistently high survival rates in current practice have been achieved by widespread adherence to these standards. Nowhere is this high standard in the operating room more dramatic than in the role of the liver transplant anesthetist, whose interventions must address profound alterations in homeostasis caused by the failing liver, support the circulation during periods of massive blood loss, and overcome the occasional bouts of cardiac dysfunction and dysrhythmias associated with reperfusion.

Immediate access to support services for the liver transplant operating room is essential. These services include clinical laboratory, blood bank, telephone links for in-hospital and out-of-hospital calls and access to advanced monitoring devices such as transesophageal echocardiography. The operating room itself should be large enough to accommodate all of the equipment, instrumentation and personnel that are present during the transplant operation. The patient table must be electrically controlled in order to facilitate rotation of the patient during the operation. Two modern electrosurgical units and an argon beam coagulator should be available and functional at all times. A rapid infusion system permits rapid correction of volume losses, and when bypass is used the bypass pump can also serve as a reservoir for rapid infusion of blood products. To reduce blood product requirements, we also utilize a cell saver system. A sterile slush machine is required, particularly if extensive ex vivo surgery is required to prepare the allograft. A variety of cannulas and other supplies for cardiovascular procedures are required when venous bypass is used.

The instrument trays should include both vascular and general surgical instruments: these trays should be open on the field for every case. Vascular trays should include instruments for fine anastomoses such as the portal vein and artery, as well as larger instruments for the vena cava. We have added a microvascular



instrument set to our instrument list, which has been particularly useful for small hepatic arterial and biliary anastomoses. Several sets of mechanical retractors should be available in the inventory so that there are no delays for sterilization when multiple cases are performed. For pediatric cases, an operating microscope should be available, as it has become standard for arterial anastomoses in many centers, particularly for living donor transplants.

## DECEASED DONOR HEPATECTOMY

Most organs for transplantation are now procured from multiple-organ donors. The donor operation has evolved greatly from the classical method, in which most of the dissection was performed in situ ('warm') before cooling and removal of the organs. Today most donor surgeons utilize variations of the rapid flush/en-bloc retrieval technique, in which cold perfusion of the organs with preservation solution is performed first, followed by dissection and removal of the organs under asanguinous conditions. Additionally, organs are retrieved 'en bloc', in groups of grape-like clusters that are based on common vascular pedicles. The individual organs can then be separated on the back table under controlled conditions, greatly simplifying the abdominal donor operation.

In performing donor hepatectomy, the surgeon is faced with two general tasks: first, direct assessment of the donor liver for suitability; second, removal of the liver and its hilar structures without injury. Ischemic injury due to donor hemodynamic instability, macrovesicular steatosis, cirrhosis and malignancy are potential conditions for which the surgeon may choose to discard the liver during direct assessment. When additional information is required, an intraoperative frozen section biopsy may be obtained to investigate suspected liver pathology. Donor selection is evolving as the growing crisis of the waiting list has led to re-evaluation of selection and the successful use of a scale of grafts not meeting traditional criteria; informally termed 'marginal livers', but more correctly identified as 'extended criteria donors' (ECD).

Donor hepatectomy is performed through a long midline abdominal incision and median sternotomy (Fig. 38.1). The pericardium and right pleural spaces are opened for later exsanguination through the vena cava. The abdomen is explored to exclude incidental malignancy. The suprahepatic vena cava is exposed by division of the falciform and triangular ligaments. The left lateral segment is mobilized, exposing the gastrohepatic ligament. An accessory left hepatic artery, if present, is carefully protected. Limited dissection of the porta hepatis is then performed in order to expose the common bile duct, which is divided distally. The extrahepatic biliary tree is flushed with saline through an incision in the gallbladder, in order to prevent bile-induced autolysis of the biliary epithelium during storage (Fig. 38.2).

The terminal ileum, right colon and duodenum are mobilized medially by sharp dissection along the peritoneal reflection. This maneuver affords complete exposure of the retroperitoneum. The origins of the renal veins are identified on the vena cava, and the distal aorta is encircled with a tape for later cannulation. In conventional retrieval, precooling of the liver with cold crystalloid solution is accomplished by introduction of a small catheter in the portal circulation (Fig. 38.3). This same catheter

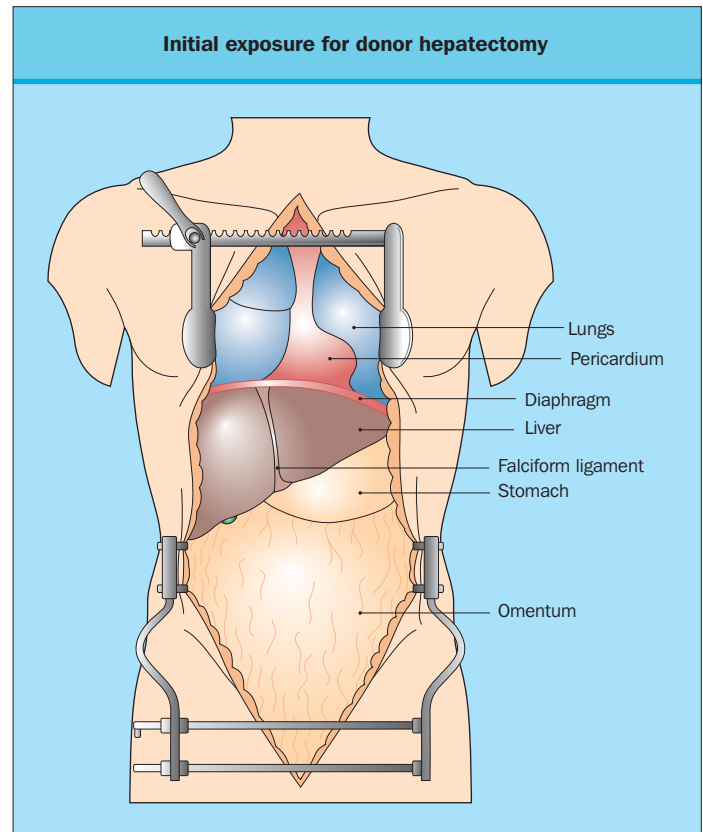


Figure 38.1 Initial exposure for donor hepatectomy.

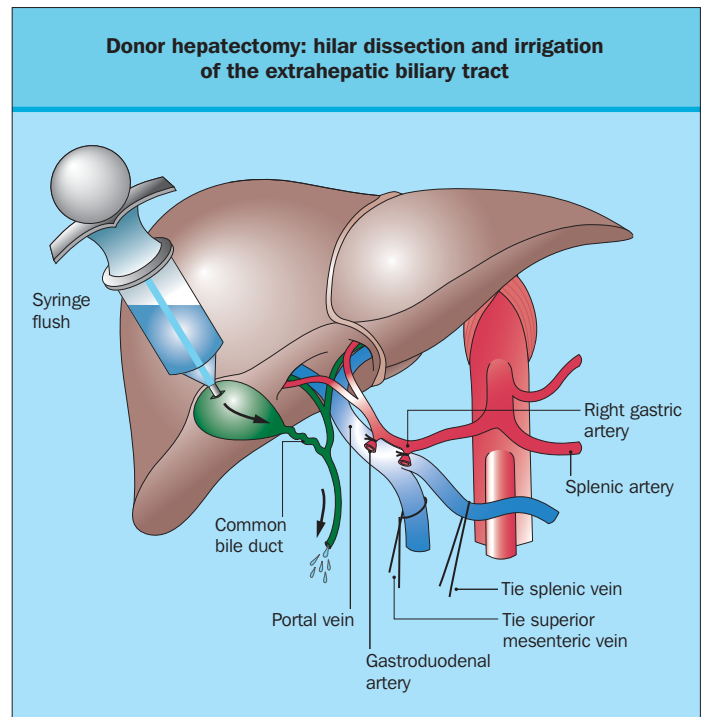
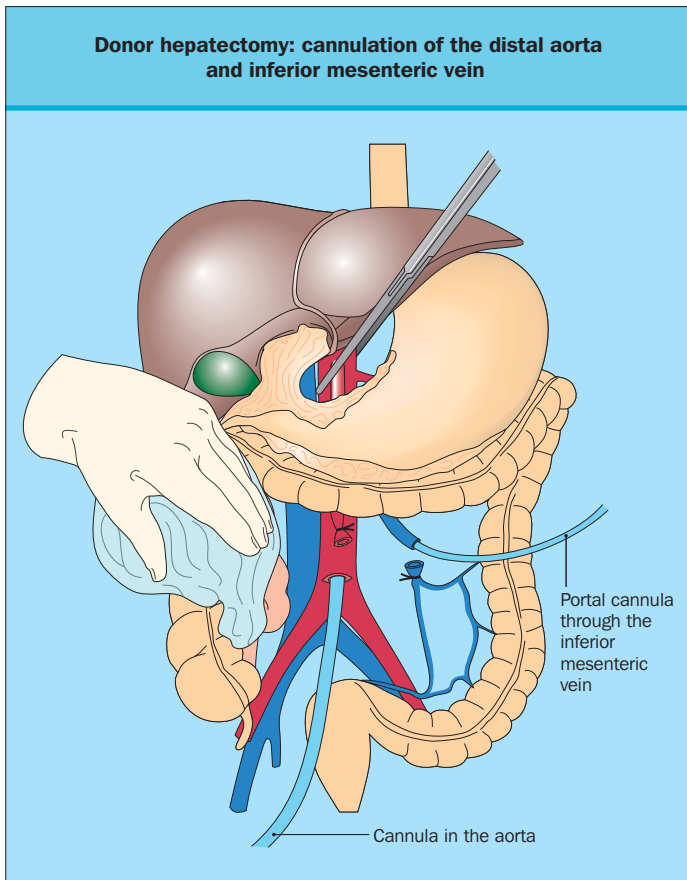


Figure 38.2 Donor hepatectomy: hilar dissection and irrigation of the extrahepatic biliary tract.



**Figure 38.3 Donor hepatectomy: cannulation of the distal aorta and inferior mesenteric vein in preparation for in situ flush with preservation solution.**

is used later in the procurement as a conduit to flush the portal vein with preservation solution. Some centers, including our own, perform neither 'precool' nor in situ portal flush routinely during en-bloc liver/pancreas procurement. In this modification the in situ flush is performed only through the arterial circulation, with the portal flush performed on the back table immediately after retrieval. In the final step before in situ flush, the supraceliac aorta is encircled with a tape at the level of diaphragmatic crus. The donor is then fully heparinized and a large-bore cannula is inserted into the distal aorta (see Fig. 38.3). The donor is exsanguinated through a large venting incision in the vena cava, while the supraceliac aorta is simultaneously clamped and cold preservation solution is flushed through the aortic and portal cannulae, allowing rapid core cooling of the abdominal organs. Concomitantly, topical cooling is accomplished by instilling ice slush into the peritoneum. Once the caval effluent has cleared, the organs can be removed sequentially: thoracic organs first, liver/pancreas second, and kidneys last. The liver is removed by first dividing the diaphragm circumferentially, encompassing the suprahepatic vena cava and the dome of the liver. The circular cuff of diaphragm is removed from the liver and cava during back-table preparation of the liver. The common hepatic artery is identified along the superior border of the pancreas and dissected proximally to the celiac trunk, sequentially ligating the principal branches: gastroduodenal, left gastric and splenic arteries. The origin of the celiac trunk on the aorta is

identified and a circular patch of aorta is fashioned around the origin ('Carrell patch'). Knowledge of variations in hepatic arterial anatomy is crucial to safe hepatectomy (Fig. 38.4).

The portal vein is divided at its origin behind the pancreas, ensuring adequate length for the recipient operation. The remainder of the porta hepatis is then divided, with careful attention to the identification and preservation of an accessory right hepatic artery. If an accessory right hepatic artery is identified, the entire length of the vessel together with a trunk of the superior mesenteric artery can be procured in order to facilitate back-table reconstruction.

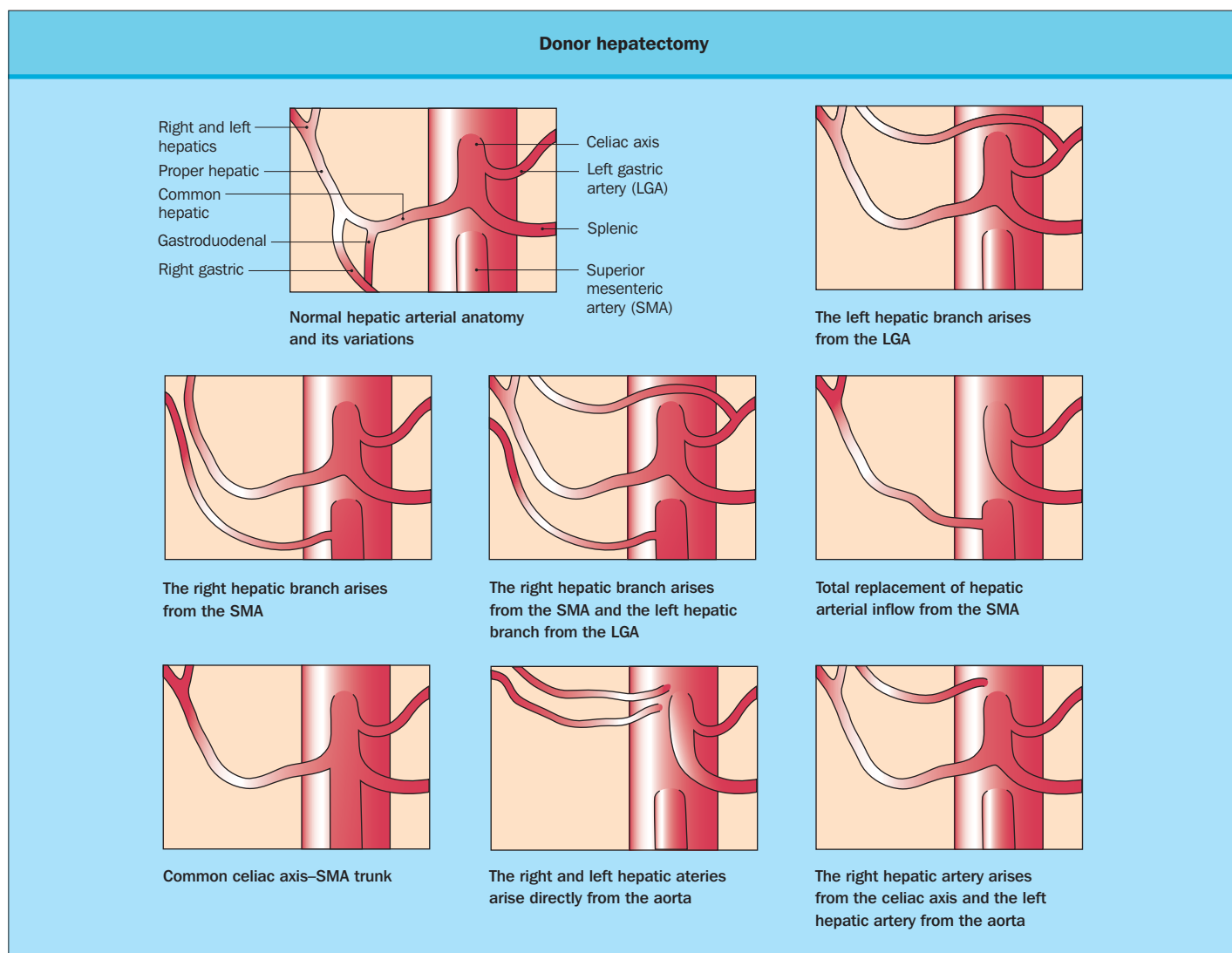
In the final step, the infrahepatic vena cava is divided above the renal veins. The liver is then removed and placed in storage containers for transport. Before closing the abdomen, lengths of iliac artery and vein are procured for potential use as vascular conduits in the recipient.

### ORTHOTOPIC LIVER TRANSPLANTATION

Like most difficult operations technical success requires mastery of anatomy, efficient surgical technique that minimizes unnecessary movement, and seamless communication between team members. Refinements in technique continue to this day, although events can be identified that have had a broad impact in shaping the liver transplant operation. For example, the technique of dissection has evolved with the introduction of improved electrocautery units and the argon beam coagulator, devices that have reduced operative time and facilitated hemostasis. It has also become standard practice to utilize mechanical retractors for exposure during liver transplantation, facilitating anatomic exposure during the liver transplant operation.

The transplant operation must also be optimized for the individual recipient. For each of the phases of the operation, knowledge of the physiologic condition of the patient, underlying comorbid disease, prior abdominal operation, presence or absence of hepatocellular carcinoma (HCC), degree of portal hypertension and coagulopathy, vascular anomalies and vascular patency, all may influence the transplant operation and perioperative patient management.

The size of the donor liver selected must take into account the recipient's size and physiologic condition. Livers that may be inadequate for sicker individuals may have predictably good success in relatively healthier recipients. Formulas that predict the normal expected liver volume still utilize height and weight as variables but do not incorporate the metabolic needs of a particular recipient based on their severity of illness. While there must be adequate hepatic mass to avoid poor function in the recipient, transplantation of an organ that is too large can lead to organ ischemia and difficulties with wound closure. Fascial closure in the presence of a large graft and significant generalized edema can result in both ventilatory and hemodynamic compromise, and rarely even abdominal compartment syndrome with failure of the graft. Post-transplant abdominal compartment syndrome is characterized by a sudden and unexpected change in hemodynamics, primary non-function of the graft, tense abdomen on examination and oliguria in an otherwise uncomplicated transplant. This is an emergency condition in which immediate re-exploration and abdominal closure with temporary mesh is indicated. At the time of re-exploration, the hepatic artery and portal vein need to be investigated for patency.



**Figure 38.4 Donor hepatectomy: variations in hepatic arterial anatomy.**

Primary abdominal closure should not be reattempted until the graft function has normalized, renal and pulmonary function have been restored, and nutritional deficits addressed. Methods of addressing organ size mismatches include graft size reduction, splitting and use of prosthetic materials for closure.

There are several factors that can increase the technical difficulty of the transplant operation. The presence of prior abdominal surgery in patients often leads to adhesions and a technically more difficult hepatectomy in the recipient, particularly in recipients with severe portal hypertension. Prolonged operative time and increased blood product requirement can be anticipated. The increased use of blood products increases the possibility of life-threatening electrolyte disturbances associated with large volume transfusion, especially in recipients with underlying renal dysfunction. At our center, we make several modifications to routine practice for patients with renal dysfunction. First, arrangements are made preoperatively for intraoperative renal replacement [continuous venovenous hemofiltration (CVVH)], and lines for dialysis are placed prior to beginning the operation. Secondly, units of packed red blood cells (PRBCs) are washed to prevent life-threatening hyper-

kalemia upon reperfusion. Third, we vent 500 cc of blood through the graft upon reperfusion rather than reperfuse the initial blood directly into circulation. Finally, in patients at high risk for bleeding and without a prior history of thrombosis or hypercoagulable state, low dose aprotinin infusion can be utilized during the hepatectomy to reduce blood loss, although randomized controlled trials have not shown a clear benefit to routine aprotinin administration in all cases. Factor VIIa has also recently become available to correct coagulopathy, although at considerable expense. The role of this novel agent in liver transplantation remains unclear and awaits further study.

Patients with sclerosing cholangitis or very small bile ducts require reconstruction using a Roux-en-Y choledochojejunostomy (biliary enteric anastomosis to a defunctionalized limb of intestine) rather than primary choledochocholedochostomy (duct to duct anastomosis), which can also lengthen the operative procedure. For patients with a history of thrombosis or those with known coagulation disorders, the use of anticoagulation with heparin and antiplatelet aggregation agents such as aspirin should be considered after reperfusion of the new graft. This is especially important in patients with a positive

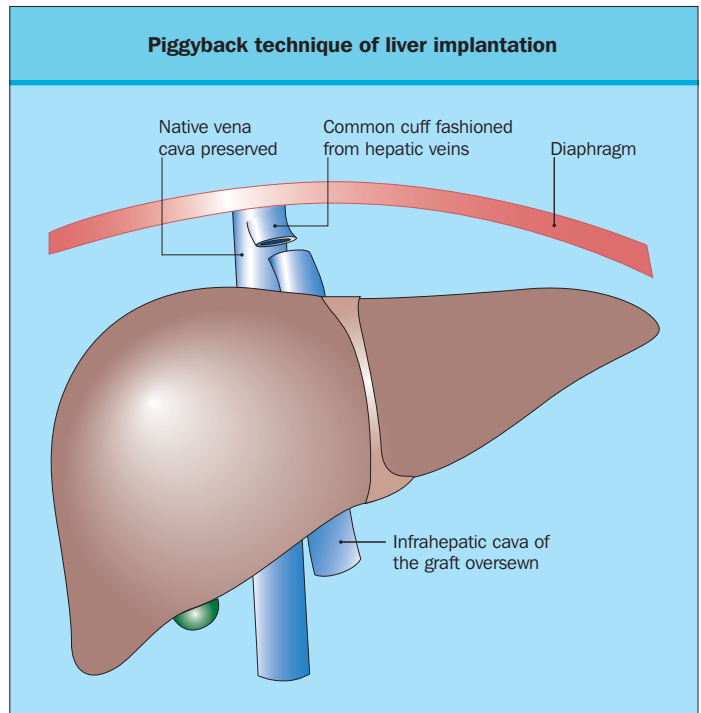


lupus anticoagulant or Factor V Leiden deficiency. The known presence of HCC in the liver prior to transplant should engender a careful search for evidence for any metastatic disease prior to, but also at the time of transplant. In those with larger or multiple tumors, it is advisable to have a backup recipient candidate available in case evidence of extrahepatic tumor is discovered during the abdominal exploration. Discovery of extrahepatic malignancy is a contraindication to completion of transplantation. Patients with known portal vein thrombosis may undergo thrombectomy at the time of the transplant, or may require venous bypass. When portal venous thrombosis is known to be present prior to transplant, veins for grafting should be available in the event that thrombectomy is unsuccessful. For some patients with transjugular intrahepatic portosystemic shunts (TIPS) placed prior to their transplant surgery, careful dissection of the stent from the vena cava may be necessary. Other devices that may appear near the operative field are vena cava filters for prevention of pulmonary embolism.

There may also be other specific recipient issues that should prompt preoperative consultations. The anesthetic team should be aware of patients with known cervical disc problems or airway problems that may require special care for intubation and airway monitoring. Patients with portopulmonary syndrome and elevated pulmonary artery pressures are at increased risk for intra- and perioperative hemodynamic instability, and cardiac or pulmonary specialists should be available to work with the anesthetic team for intra- and perioperative management of these individuals, including access to intraoperative transesophageal echocardiography. Ideally, management of patients with mild to moderate cardiopulmonary disease is carefully planned prior to transplantation, as patients with severe disease are generally not candidates for transplantation. However, occasionally underlying cardiopulmonary disease may not be evident until induction of anesthesia and placement of a pulmonary artery catheter. If significant cardiopulmonary disease is discovered only at operation, a decision to proceed must be made in consultation with the surgeon, anesthesiologist and consultants from cardiology and pulmonary medicine. Transplantation in the face of severe pulmonary hypertension is generally futile. Patients with functioning neuroendocrine tumors require special management with somatostatin infusion and  $\beta$  blockers to prevent release of hormonal factors that could result in severe hemodynamic instability. Patients with known red-cell antibodies may require extra time for cross matching by the blood bank, which must be alerted as soon as a potential liver is available. If cross match negative blood is not available, the operation may need to be delayed or cancelled altogether.

### General approaches to the operation

Three general operative strategies are available to the surgeon performing orthotopic liver transplantation: the traditional dissection without venovenous bypass, the traditional dissection with bypass and 'piggyback' placement of the liver. Each offers advantages. Traditional dissection without bypass is the most challenging, requiring both an experienced surgeon and an anesthetic team. The principal advantages are simplicity and short anhepatic times, as little as 30 min. In addition, complications associated with bypass, such as morbidity related to the cannulation sites, are avoided. Hemodynamic instability can be more severe, however, increasing demands on the anesthetic



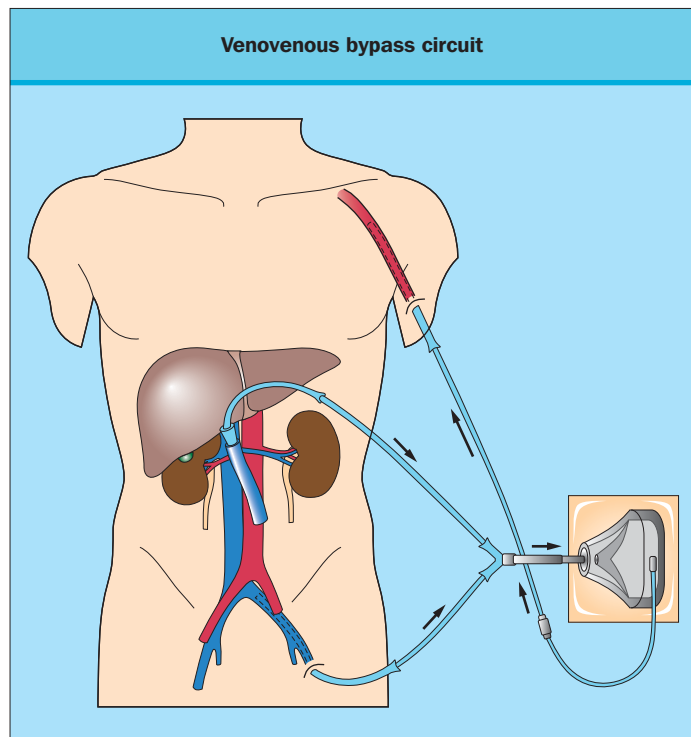
**Figure 38.5 Piggyback technique of liver implantation.** The native vena cava is preserved.

team during the anhepatic and post-reperfusion phase. Addition of bypass prolongs the operation and the anhepatic time, but mitigates the volume-associated hemodynamic changes associated with portal and vena caval clamping.

The piggyback technique is designed to circumvent the hemodynamic risks of traditional dissection without bypass by removing the native liver with preservation of the vena cava, avoiding the need to cross-clamp the vena cava during the anhepatic phase, and thereby preserving venous return to the heart (Fig. 38.5). This technique adds additional time and complexity during the hepatectomy and introduces an increased risk of hepatic outflow complications related to the single hepatic venous anastomosis. However, advocates of the piggyback technique have been pleased with a decrease in volume-related instability during the anhepatic phase. The piggyback technique may be most appropriate in patients with compromised cardiovascular reserve as an alternative to bypass. We have utilized the piggyback technique when recipients are unable to tolerate test clamping of the vena cava and portal vein after a period of resuscitation by the anesthesiologist. The piggyback technique is also useful when the surgeon is confronted with a large donor liver in a small recipient, because piggyback is performed without an infrahepatic caval anastomosis, which may be difficult in the presence of a significant size mismatch.

### Three phases of orthotopic liver transplantation

The liver transplant operation is described in three phases: hepatectomy, anhepatic phase and reperfusion phase. The patient is prepared for operation by positioning in the supine position with the right arm tucked to facilitate placement of an assistant. Upon induction of anesthesia, vascular access lines are placed by



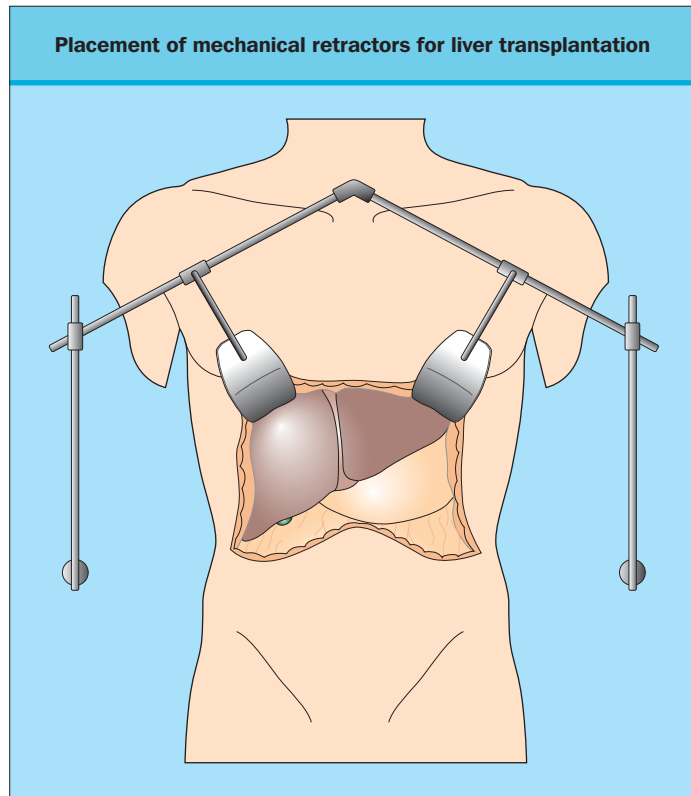
**Figure 38.6 Venovenous bypass circuit.**

the anesthetist. At least one large sheath introducer is placed in the central venous circulation, which serves as a large volume access and as a portal for a pulmonary artery catheter. An additional large volume access, usually a sheath introducer, is placed either centrally or peripherally. When venovenous bypass is used, the bypass cannulae are placed surgically after preparing and draping the entire chest, abdomen and groins. A femoral cannula is placed by cut-down on the saphenous vein. The axillary cannula, through which blood is returned to the central circulation, can be placed either percutaneously or by open cut-down on the axillary vein (Fig. 38.6). All of these steps are associated with postoperative morbidity, nerve injuries from positioning, vascular complications from venous or arterial lines and local wound complications.

### Hepatectomy

The hepatectomy is performed through a bilateral subcostal incision with extension in the midline to the xiphoid process, which is often excised. The incision should be carried as far laterally to the right as possible in order to allow a complete and unobstructed view of the liver, especially the retrohepatic and suprahepatic vena cava. Mechanical retraction under both costal margins elevates and spreads the aperture of the rib cage in order to create a horizontal rather than convex costal margin, thereby maximizing access to the suprahepatic vena cava (Fig. 38.7). It should be remembered that the cirrhotic liver is often extremely small and difficult to access in its location high above the costal margin. The exposure is completed by inferior traction on the viscera, thereby clearly exposing the porta hepatis for dissection.

The technique of dissection is unique to liver transplantation and differs from traditional general surgical operations in that

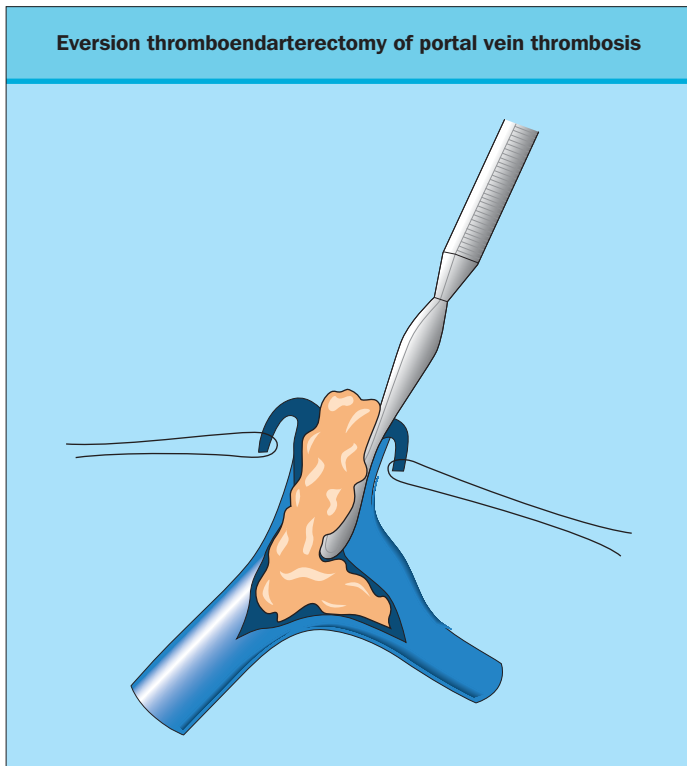


**Figure 38.7 Placement of mechanical retractors for liver transplantation.**

sharp (rather than blunt) dissection is emphasized, and the electrocautery probe is the principal dissecting tool. This unique technique was developed specifically to address the challenges of portal hypertension with engorged intra-abdominal varices, coagulopathy and thrombocytopenia, and an extensive dissection that would require several hours of work (and several hours of additional bleeding) if performed using more conventional surgical techniques. In most uncomplicated transplants, an experienced surgeon is typically able to complete the hepatectomy phase of the operation in 1–2 h using the sharp dissection/cautery technique.

The liver is mobilized completely from its ligamentous attachments (often vascularized in the presence of cirrhosis) and from the right hemidiaphragm. After the ligaments and diaphragm are completely freed, the liver can be easily elevated to expose the retrohepatic vena cava. The hilar dissection is performed after the porta hepatis is exposed by an assistant, who reflects the liver edge upward while another assistant (or mechanical retractor) holds the intestines and duodenum caudally. The goal of the hilar dissection is identification and ligation of the structures in the porta hepatis (hepatic artery, bile duct and portal vein). The general principle is that portal structures must be ligated as high in the hilum as possible in order to preserve length, which may be important during implantation of the liver allograft.

In patients previous hepatobiliary surgery (most commonly cholecystectomy), densely vascularized adhesions of the colon, stomach, duodenum and omentum present an obstacle to exposure of the hepatic hilum. In such cases, the adhesions and adherent structures must be 'shaved' off the liver surface using



**Figure 38.8** Eversion thromboendarterectomy of portal vein thrombosis.

cautery. Some surgeons delay ligation of the hepatic artery until mobilization is complete, since it is thought that the end-stage liver is predominantly dependent upon the arterial supply and the patient becomes functionally anhepatic after arterial ligation. In addition, prolonged ligation of the hepatic artery before reconstruction in the new liver may predispose to intimal injury, dissection and thrombosis. The portal vein is skeletonized in its entirety from the border of the pancreas to the bifurcation. If a TIPS is found in the extrahepatic portal vein, the first choice in strategy is to clamp proximal to the TIPS and divide the portal vein in a location that is free of stent material. Sometimes this is not possible and a clamp has to be placed across a long retropancreatic TIPS, in which case the TIPS is transected along with the portal vein in the mid portion of the porta hepatis. The wires of the transected metal stent are then individually removed, taking care not to shred the wall of the vein. If the portal vein is thrombosed, it is usually possible to re-establish flow by transecting it and removing the organized thrombus using a combination of sharp separation of the thrombus from the vein wall together with balloon thrombectomy, in a procedure that is similar to endarterectomy for atherosclerosis (Fig. 38.8). This dissection can be carried proximally to the junction of the mesenteric and splenic veins since this confluence is nearly always patent. If portal revascularization is not possible, a conduit of cadaveric iliac vein is placed on the superior mesenteric vein and tunneled through the root of the transverse mesocolon, to be used as an interposition graft to the portal vein of the new liver. Preoperative imaging of the portal vein using magnetic resonance angiography or high quality multiple phase computed tomography (CT) permits the surgeon to have a fairly accurate map of the portal circulation prior to transplantation.

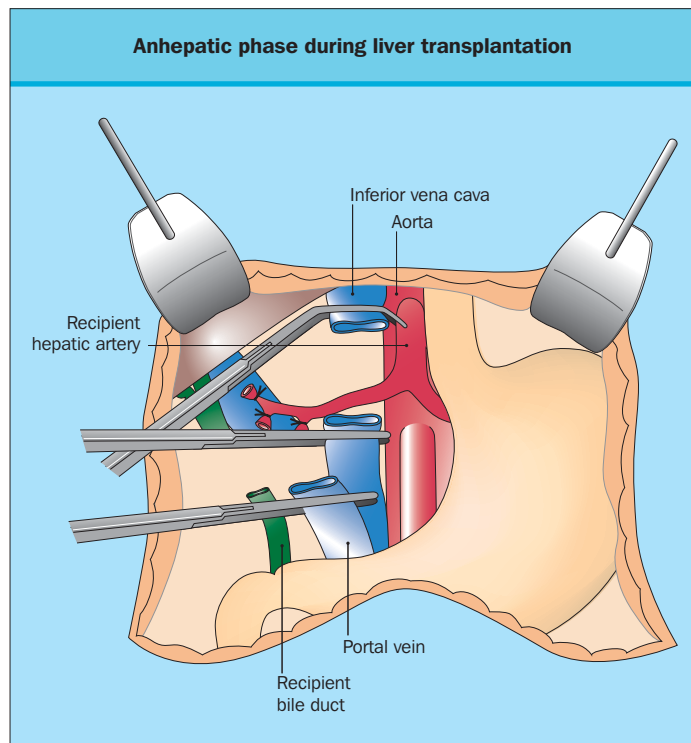
The presence of a spontaneous or surgically constructed spleno-renal shunt, for example, can be addressed by ligation of the left renal vein. A surgical mesocaval shunt can be addressed by ligation of the mesocaval conduit, provided the native portal vein is patent and of adequate caliber. When the portal vein is unusable and a prior mesocaval shunt is present, we have, for example, even utilized the shunt for inflow to the new graft. Ultimately the surgeon must be resourceful in dealing with likely unexpected findings. After completion of the hilar dissection, the retrohepatic cava is fully dissected on the right side from the infrahepatic cava to the right hepatic vein by division of the flimsy lateral and posterior attachments with cautery. The adrenal vein is encountered in this plane and must be suture ligated. In the piggyback technique the liver is separated from the vena cava by ligation of all the small hepatic vein branches to the right lobe and the caudate until the liver is completely freed with the exception of the three main hepatic veins. This permits application of a partial occlusion clamp to preserve caval blood flow during implantation of the new liver. The left side of the retrohepatic cava is exposed by anterior reflection of the left lateral segment and retraction of the caudate lobe with a narrow handheld malleable retractor, allowing cautery dissection of the left side of the retrohepatic cava.

If bypass is to be used, the portal bypass cannula can be placed as the final step in the hepatectomy, after high ligation of the portal vein close to or above its bifurcation in the hilum (see Fig. 38.6). When the portal vein cannot safely be dissected because of dense scar tissue, or when portal vein thrombosis is present, it may be preferable to gain access for portal bypass using the inferior mesenteric vein. When no bypass is used, the portal vein is simply clamped proximally, near the pancreatic border. Before explanting the diseased liver, however, we perform a test clamp of the infrahepatic vena cava and portal vein to determine whether the patient will tolerate the anhepatic phase. If the blood pressure falls, typically below a mean arterial blood pressure of 60–70, then additional measures are required before repeating the test clamp, including volume loading and initiation of baseline pressor support. Finally, the infra- and suprahepatic vena cava are clamped in traditional techniques (Fig. 38.9). Correct placement of these clamps is important in facilitating subsequent caval anastomoses; the suprahepatic clamp should be placed as close to the diaphragm as possible in order to gain as much cuff length as possible for use in the suprahepatic caval anastomosis. We secure the suprahepatic clamp with a vascular tape in order to minimize accidental release. The infrahepatic caval clamp should be placed as distally as possible, without occlusion of the renal veins. We often transect the hepatic veins within the liver substance, in order to gain as much suprahepatic caval length as possible. Similarly, the infrahepatic cava is transected high into the liver, in order to preserve length, and the liver is removed.

### **Anhepatic phase**

After the liver is removed, hemostasis is achieved by suture ligation and cautery of bleeding tissues in the retroperitoneum (see Fig. 38.9). The vascular anastomoses are then performed in the following order: suprahepatic cava, infrahepatic cava and portal vein. The hepatic arterial anastomosis is usually performed after portal reperfusion. The duration of the anhepatic phase, ending in portal venous reperfusion, is approximately





**Figure 38.9 Anhepatic phase during liver transplantation.**

30–45 min. The physiologic consequences of the anhepatic phase include progressive coagulopathy, fibrinolysis and acidosis.

When portal bypass is not used, there is also progressive bowel edema and venous engorgement. Before reperfusion, preservation solution in the liver graft must be removed to prevent arrhythmias associated with high potassium preservation solutions. We accomplish this task by flushing the portal vein of the graft on the back table with cold colloid solution (hetastarch), although this can also be performed during implantation as well. Of the three venous anastomoses performed, all have different challenges. The suprahepatic caval anastomosis has been viewed as the most difficult, because of limited exposure, the relatively short length of the cuffs available for anastomosis, and the difficulty in revising the anastomosis after completion. The surgeon therefore has to visually assess the relative diameter and lengths of the cuffs between native cava and graft, and adjust while performing the anastomosis to achieve the following goals: hemostatic suture line; intimal approximation to minimize nidus for thrombosis; and correct anterior–posterior orientation to avoid torsion and outflow obstruction. The last goal is especially important when there is substantial size mismatch between the graft and patient.

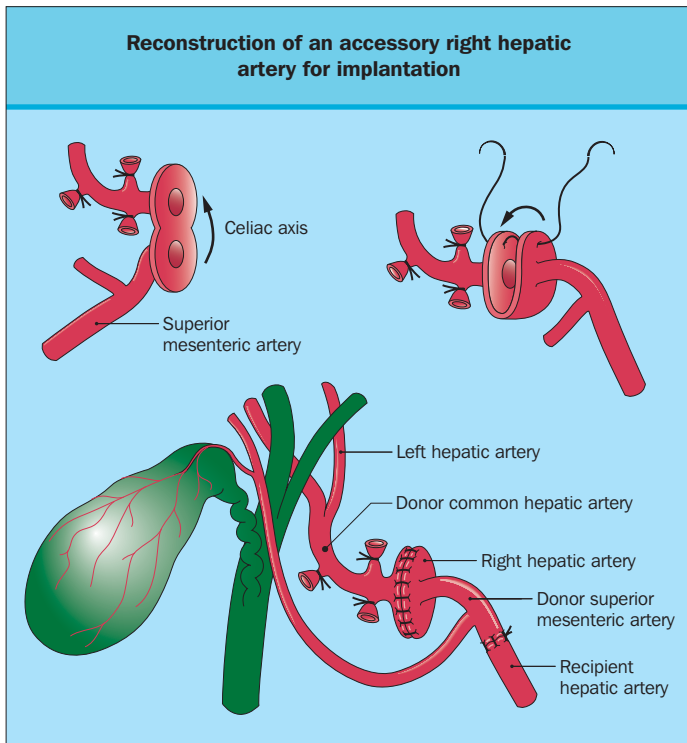
During the suprahepatic caval anastomosis, the liver graft is parachuted into the hepatic fossa and the posterior (back wall) aspect of the anastomosis is performed first, through the inside of the cava. The infrahepatic caval anastomosis is performed in a similar fashion. Assessment of the proper length of the infrahepatic cava and maintenance of the alignment of the cava on its axis are important considerations in performing the infracaval anastomosis. In the piggyback technique, the suprahepatic cava of the graft is anastomosed end to side to the confluence of the recipient hepatic veins or to a long anterior incision on the

recipient vena cava, utilizing either partial or total occlusion of the recipient vena cava. The infrahepatic vena cava of the graft is then simply oversewn. The portal vein anastomosis is performed after the caval anastomoses. A crucial aspect of the portal anastomosis is estimation of the proper donor portal vein length that allows a straight anastomosis. Care is taken to avoid constriction at the suture line. Fine sutures and delicate, meticulous technique are important during the portal anastomosis. Some surgeons intentionally tie the sutures loosely, incorporating a ‘growth factor’ that allows the vein to distend after reperfusion without a waist at the anastomosis.

### Reperfusion phase

Byproducts of metabolism accumulate in both the graft liver and the gut during implantation of the liver, especially when the portal vein has been clamped without bypass. Reperfusion is sometimes associated with significant hemodynamic instability (‘reperfusion syndrome’) due to the cold, hyperkalemic and acidotic effluent that is flushed directly into the right heart upon revascularization. Transient hypocontractility is common. The anesthetist must therefore be prepared to administer hemodynamic support, including catecholamines, bicarbonate and calcium on reperfusion. The technique of reperfusion varies between centers, but most surgeons reperfuse the liver by sequential removal of the suprahepatic clamp, infrahepatic clamp and finally the portal venous clamp. After caval clamp removals any obvious suture line leaks can be oversewn and the graft can be ‘prewarmed’ by backbleeding caval blood and with heated irrigation before releasing the portal clamp. Upon releasing the portal vein clamp, some blood may be discarded (‘vented’) to eliminate some of the detrimental substances which accumulate in the mesenteric circulation during the cross-clamp. It is not clear that venting reduces the reperfusion syndrome, except in the circumstance where the patient is known to be hyperkalemic before reperfusion. We also discard initial blood perfused through the graft when there is significant graft steatosis, because of the concern of flushing lipid particles directly into the lungs. Even with preimplantation flushing of the graft with colloid solution, there is often still enough residual preservation solution and heparin within the liver graft to cause transient hyperkalemia and systemic heparin-induced anticoagulation. When T wave elevations are seen immediately after reperfusion of the portal vein, the surgeon can intermittently occlude the portal inflow manually to permit successive washes of potassium to be absorbed and dealt with pharmacologically.

The appearance of the liver upon reperfusion may often give an early indication of graft function. Severe reperfusion injury may be manifest by a bright red color and a firm, swollen appearance. The liver with good initial function and minimal reperfusion injury will rapidly and homogeneously fill with blood upon portal reperfusion and appear a dark salmon color. To palpation, the liver will remain soft and retain the sharpness of its edges. In a well-functioning liver, bile may be seen at the common duct even before arterial revascularization. Finally, the functioning liver will readily clear lactic acid and plasminogen activators, resulting in a correction of acid–base abnormalities and resolution of the complex coagulopathy that is due to a combination of fibrinolysis and dilution of platelets and coagulation factors. In livers prone to poor initial function, it is often advisable to maintain as low a central venous pressure as possible

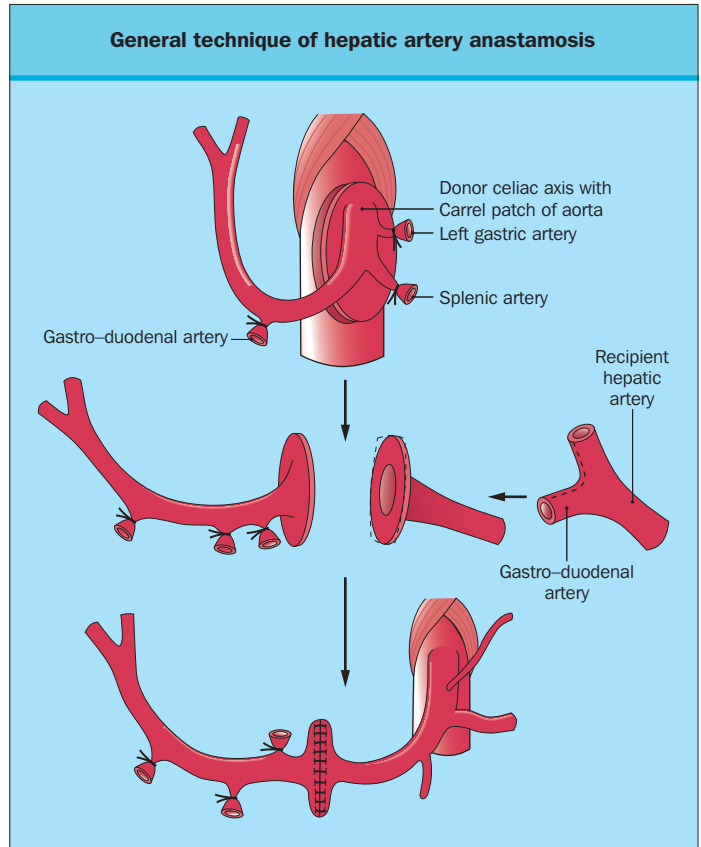


**Figure 38.10** Reconstruction of an accessory right hepatic artery for implantation.

to minimize the deleterious effects of graft engorgement in a liver with other risk factors. Volume overload is more common when no bypass is used, because of the obligatory volume loading that must occur to permit safe cross clamping. After reperfusion this excess intravascular volume can become a liability for graft function as well as oxygenation and pulmonary compliance, and constant communication between the surgeon and anesthesiologist after reperfusion is essential. Measures to reduce graft swelling can include the use of nitroglycerine and diuretics. Crystalloid volume is minimized and only blood products are administered, according to the degree of bleeding assessed by the surgeon inspecting the operative field.

#### Arterial anastomoses

After portal reperfusion the vascular suture lines are rapidly inspected for hemostasis before beginning the hepatic arterial anastomosis. If an accessory right hepatic artery is present in the liver graft, reconstruction of this vessel is performed *ex vivo* on the back table before implantation. The most common technique for hepatic arterial anastomosis is an end-to-end anastomosis of the donor aortic Carrel patch to the recipient hepatic artery (Fig. 38.10). The hepatic arterial anastomosis can be facilitated by preparation of the recipient hepatic artery as a 'branch patch', in which a common cuff is fashioned from a bifurcation, usually the hepatic–gastroduodenal bifurcation (Fig. 38.11). The recipient hepatic artery tends to be fragile and is prone to intimal dissection with even small trauma. If the recipient hepatic artery is unsatisfactory the splenic artery can be used as an alternative, or an interposition graft can be taken directly off the aorta. Before the introduction of microvascular techniques, direct



**Figure 38.11** General technique of hepatic arterial anastomosis utilizing a donor aortic Carrel patch to the recipient hepatic artery branch patch.

aortic implantation was in fact the most common approach in pediatric recipients.

While there has been some debate about the etiology of hepatic artery thrombosis in the past, it is now clear that precision in fashioning the arterial anastomosis is the most important factor in decreasing the risk of this complication. Fine atraumatic clamps and fine monofilament sutures (7-0) are used in the anastomosis, and growth factors are occasionally used to prevent constriction. High-power magnification has clearly been shown to decrease the thrombosis rate in pediatric liver transplantation, in which reconstruction of 2 or 3 mm arteries may be required. After releasing the arterial clamp, adequacy of flow is assessed by palpation, Doppler examination, and, in some cases, direct measurement with a flow probe. Causes of inadequate arterial flow include technical flaws in the anastomosis, clamp injury to the intima of the artery proximal to the anastomosis, or inadequate inflow due to alterations in the recipient, such as celiac arterial disease or constriction by the arcuate ligament of the diaphragm. When arterial flow seems inadequate in the absence of any clear anastomotic problem or anatomic injury to the artery, the common hepatic artery can be dissected proximally to the splenic artery. In some cases splenic artery ligation will increase inflow to the liver. We have also observed occasional respiratory variation in hepatic arterial inflow caused by occlusion of the celiac artery by the arcuate ligament of the diaphragm. In such cases, inflow can be restored by further proximal dissection and division of the diaphragmatic muscular

fibers surrounding the origin of the celiac trunk off the aorta. When all these measures fail to achieve adequate arterial pulsation, the artery should be immediately revised using an aortic conduit for inflow.

### Biliary anastomosis

The bile duct anastomosis is performed last. While there have been many variations in bile duct reconstruction that have been described, the two most commonly performed reconstructions are simple end-to-end choledochcholedochostomy and biliary–enteric drainage into a Roux-en-Y (defunctionalized) limb of intestine. End-to-end reconstruction is preferable because it avoids the need for an enteric anastomosis. In either case, cholecystectomy is performed first and the donor bile duct is prepared for anastomosis, usually above the cystic duct junction. End-to-end anastomosis is performed using fine monofilament absorbable suture. Size mismatches may be corrected by ductoplasty with a running suture, or by spatulation of the smaller duct. In the past, a choledochal drainage tube (T-tube) was always left in the bile duct, with one limb of the tube stenting the anastomosis. The tube allowed collection of bile during the postoperative period, which facilitated clinical management of dysfunctional livers and allowed cholangiography. However, T-tubes are associated with complications in up to one-third of patients, most commonly bile peritonitis associated with tube removal several months after transplantation. This often requires hospitalization, endoscopic placement of biliary stents, and in some cases, operation. As part of a general trend toward greater operative simplicity, most centers have abandoned the routine use of T-tubes.

Hepatojejunostomy is preferred when primary end-to-end anastomosis is not possible, as in sclerosing cholangitis or when the recipient bile duct is too small to allow effective biliary drainage (Fig. 38.12). Hepatojejunostomy is also performed in almost all pediatric patients who may already have an existing Roux-en-Y limb from a Kasai procedure for biliary atresia. The original Roux limb can be revised for use in the hepatoenteric anastomosis. When a new Roux-en-Y must be constructed, the proximal jejunum is divided 20–30 cm distal to the ligament of Treitz with a stapler. The distal intestine is then used to construct a 40 cm defunctionalized limb. Bowel continuity is restored with a jejunojunctionostomy. The jejunojunctionostomy suture line is occasionally the source of gastrointestinal bleeding several days after transplantation, although these events are usually self limited. The Roux can be brought up to the donor liver through either an antecolic or retrocolic approach. The biliary–enteric anastomosis is performed with a single interrupted layer of absorbable suture to reapproximate mucosa to mucosa. If desired, a small stent can be left across the anastomosis, usually an infant feeding tube. Small stents almost invariably fall out if not secured with an absorbable suture. One end of the stent can also be brought out to the skin through a second enterotomy, allowing access for postoperative imaging. Before closure a final hemostatic survey is performed (Fig. 38.13). When the liver graft is functioning well, hemostasis is generally accomplished without much difficulty, but in cases of poor initial graft function the surgical surfaces and suture lines can bleed profusely from fibrinolysis, coagulopathy and hypothermia. Coagulopathy is treated with fresh frozen plasma, cryoprecipitate and platelets. Epsilon aminocaproic acid can be used to treat

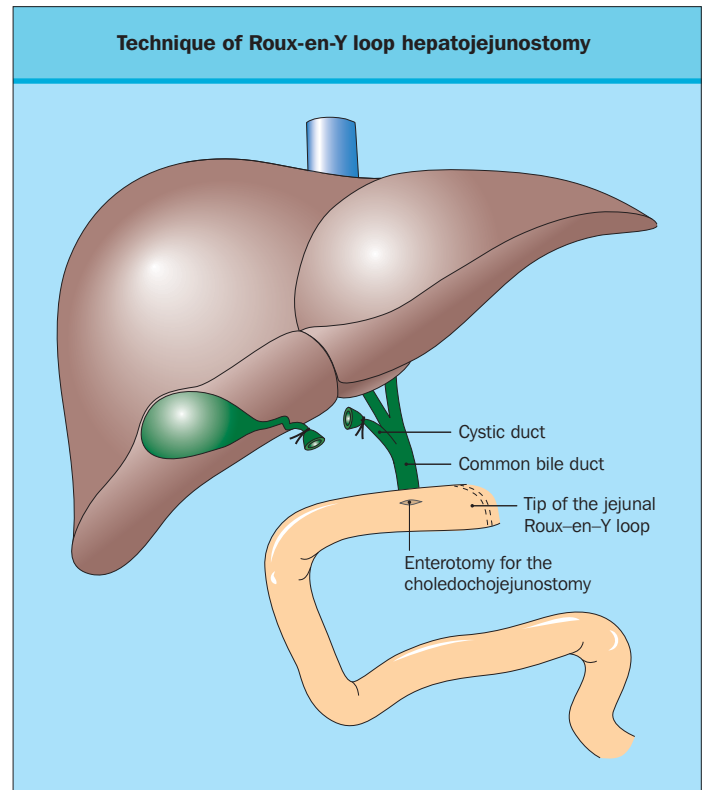


Figure 38.12 Technique of Roux-en-Y hepatojejunostomy.

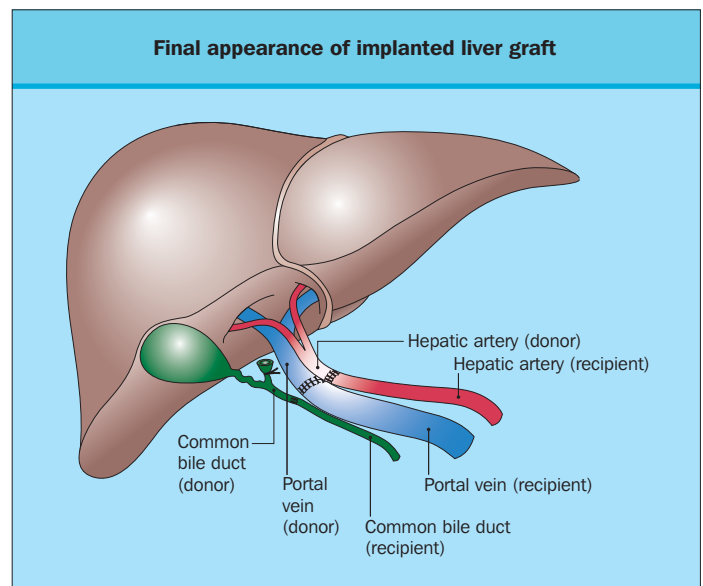
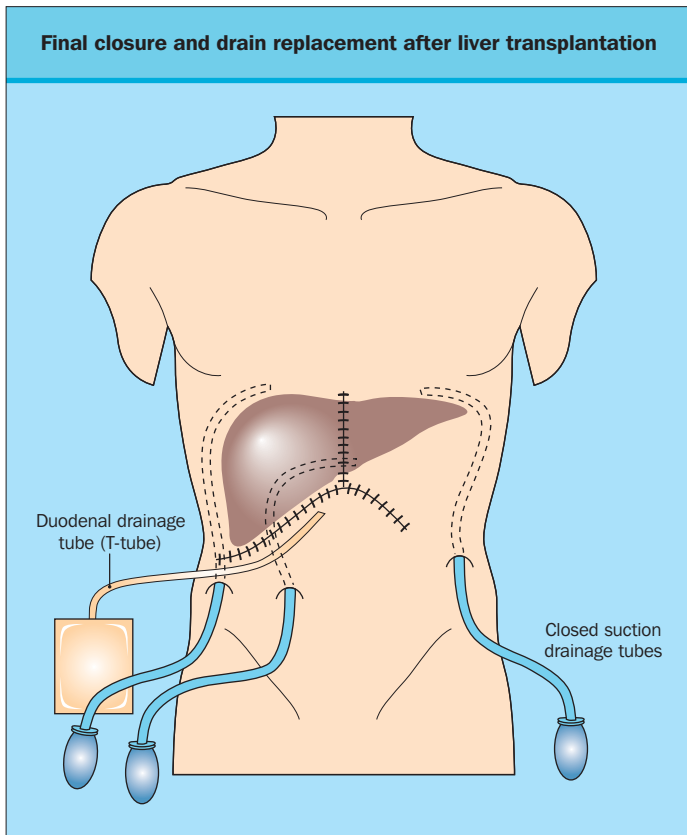


Figure 38.13 Final appearance of implanted liver graft.

fibrinolysis. When hemostasis has been accomplished, closed suction drains are left in the peritoneum (Fig. 38.14). We generally use one or two drains, which are useful in evacuating postoperative serosanguinous fluid and facilitating early diagnosis of a bile leak, but they are not reliable indicators of intra-peritoneal hemorrhage as their small size usually results in early occlusion by a clot.





**Figure 38.14** Final closure and drain placement after liver transplantation.

### **SPECIAL OPERATIVE CONSIDERATIONS: PEDIATRIC AND REDUCED-SIZE LIVER TRANSPLANTATION**

Standard cadaveric transplantation in children is quite similar to the adult transplant, utilizing smaller instruments and sutures. Bypass is not used in children, and hepatojejunostomy is the preferred method of biliary reconstruction in all except larger teenage children. Experienced anesthesiology is of paramount importance in successful pediatric transplantation. Because of the difficulty of establishing large volume access in infants, central venous and arterial lines may sometimes require open surgical placement before beginning the transplant operation.

Adult-sized children should be managed intraoperatively in the same way as adult transplant recipients, including availability of larger volume access and rapid transfusion devices. In this decade, techniques of reduced-size liver transplantation have become routine in the transplantation of children. The incentive for development of these techniques was the shortage of small donors, which leads to a waiting list mortality for children approaching 50%. Initially, when the adult waiting list was small in the 1980s, livers from adult donors could be readily diverted to children. A small liver was fashioned *ex vivo* on the back table, usually from the left lateral segment, while the remainder was discarded. Shortly thereafter, the technique was extended to create two grafts from a single liver, the 'split liver' procedure. The appeal of splitting livers was the possibility of doubling the organ supply, but initial experiences with split livers were fraught

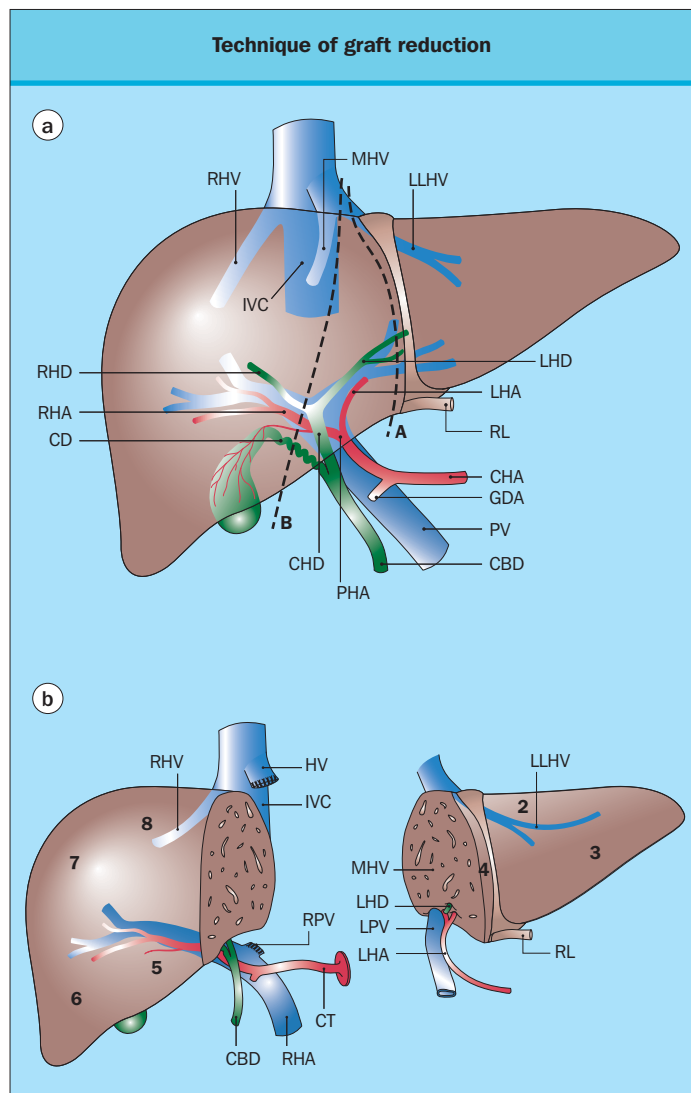
with technical complications, thereby limiting application of this technique. More recently, there has been a renewed interest in split livers with the introduction of several innovations that have improved the results, most importantly the development of techniques to perform the dissection *in vivo* (in situ), borrowing directly from the experience with living donor liver transplantation.

We introduced living donors in children in the 1980s, with rapidly improving results throughout the 1990s. Living donation has now become routine in pediatrics, although with improvements in split liver transplantation more options are available to pediatric transplant surgeons than in the past. Widespread application of split liver transplantation as a replacement for living donor transplantation has been limited, however, by a lack of sharing between centers. Currently, in areas with multiple transplant centers there is little incentive on the part of non-pediatric centers to share their full size organs for splitting. Equitable resolution of this issue must be achieved before splitting can achieve its full potential, which could largely eliminate the need to perform living donor transplants in children. For the present time, however, we continue to rely on living donor transplantation to achieve optimal results for our pediatric patients.

More recently, increasing concern over liver transplant waiting time-related morbidity has fostered increased interest in the further development of living donation for adults, which requires a greater hepatic resection from the donor than living donation in pediatrics. Ethical issues over indications for adult living donation, patient selection, techniques and outcomes dominate debate in liver transplantation forums, although the overall role of this modality in improving outcomes remains controversial.

### **Technique of graft size reduction**

Standard donor hepatectomy is performed in the cadaveric donor. The graft reduction (or splitting) is performed *ex vivo* (Fig. 38.15), or more recently, *in situ*, in which the liver is divided in the heart-beating cadaver donor to minimize ischemic time for the split liver. There are potential advantages to both *ex vivo* and *in situ* splitting, and the transplant surgeon should be prepared and comfortable with either approach. The size of liver required will determine the plane of the dissection. For a very small infant (with a donor to recipient size disparity of 10 to 1), the left lateral segment (Couinaud segments II and III) is used; whereas, if the disparity is less, the entire left lobe can be used. If the right lobe is to be discarded, the common structures of the porta hepatis are kept with the graft. When the liver is to be split into two grafts, the recipient team receiving the primary offer has the option of keeping the full length of portal structures with their graft. In practice, however, it is more optimal for the team receiving the extended right lobe (the larger portion of the liver) to receive the full length of the porta hepatis structures and vena cava, with the team receiving the left lateral segment receiving only the left portal vein, left bile duct, and left hepatic artery (same as in a live donor graft). In *ex vivo* splitting, the hilar structures and parenchymal division is performed on the back table, with the liver kept cold using ice slush. When the split is performed *in vivo*, usually in ideal hemodynamically stable donors, the hilar dissection and parenchymal division is performed like a living donor. The parenchymal division can be terminated at any point if the donor



**Figure 38.15 Technique of graft reduction.** (a) There are two planes of potential parenchymal transection, for either a segment II/III graft (plane A) or for a full lobar split (plane B). (b) Dissected lobes. HV, hepatic vein; RHV, right hepatic vein; MHV, middle hepatic vein; LLHV, left lobe hepatic vein; IVC, inferior vena cava; CBD, common bile duct; CHD, common hepatic duct; CD, cystic duct; RHD, right hepatic duct; LHD, left hepatic duct; CHA, common hepatic artery; GDA, gastroduodenal artery; PHA, proper hepatic artery; LHA, left hepatic artery; RHA, right hepatic artery; GDA, gastroduodenal artery; CT, celiac trunk; PV, portal vein; RPV, right portal vein; LPV, left portal vein; RL, round ligament.

develops hemodynamic instability, and the liver can then be flushed and taken out whole, with the remainder of the transection continued *ex vivo*. In both techniques, the liver tissue is divided with clips or fine sutures being used to control the small vascular structures. As noted above, the hilar dissection is similar whether a left lateral or full left lobe graft is required. If the full left lobe is to be used, the liver is divided in the plane of the gallbladder fossa (Fig. 38.15a – plane B), and the parenchyma is transected to the right of the middle hepatic vein. If the lateral segment is used, the liver is divided just to the right of the round ligament (Fig. 38.15a – plane A). Superiorly, the dissection of the hepatic veins varies depending on whether the vena cava of the recipient is to be replaced by the donor vena cava. For lateral segment grafts, it is best to remove the vena

cava and the caudate lobe and position the graft directly on the recipient vena cava. If the whole left lobe is used, it is more convenient to preserve the entire vena cava and replace it in the recipient as in whole organ transplantation. At the completion of parenchymal transection, it is necessary to treat the section with a hemostatic surface. Despite meticulous preparation, the cut section will generally pose a hemostatic problem when the graft is split *ex vivo*. In situ splits are less prone to cut-section bleeding, because the vessels are identified and addressed while the liver is perfused with blood. Several strategies have been utilized, including fibrin glue, or reinforcement of the surface with a mesh, which permits direct control of the bleeding sites with suture ligatures through the mesh.

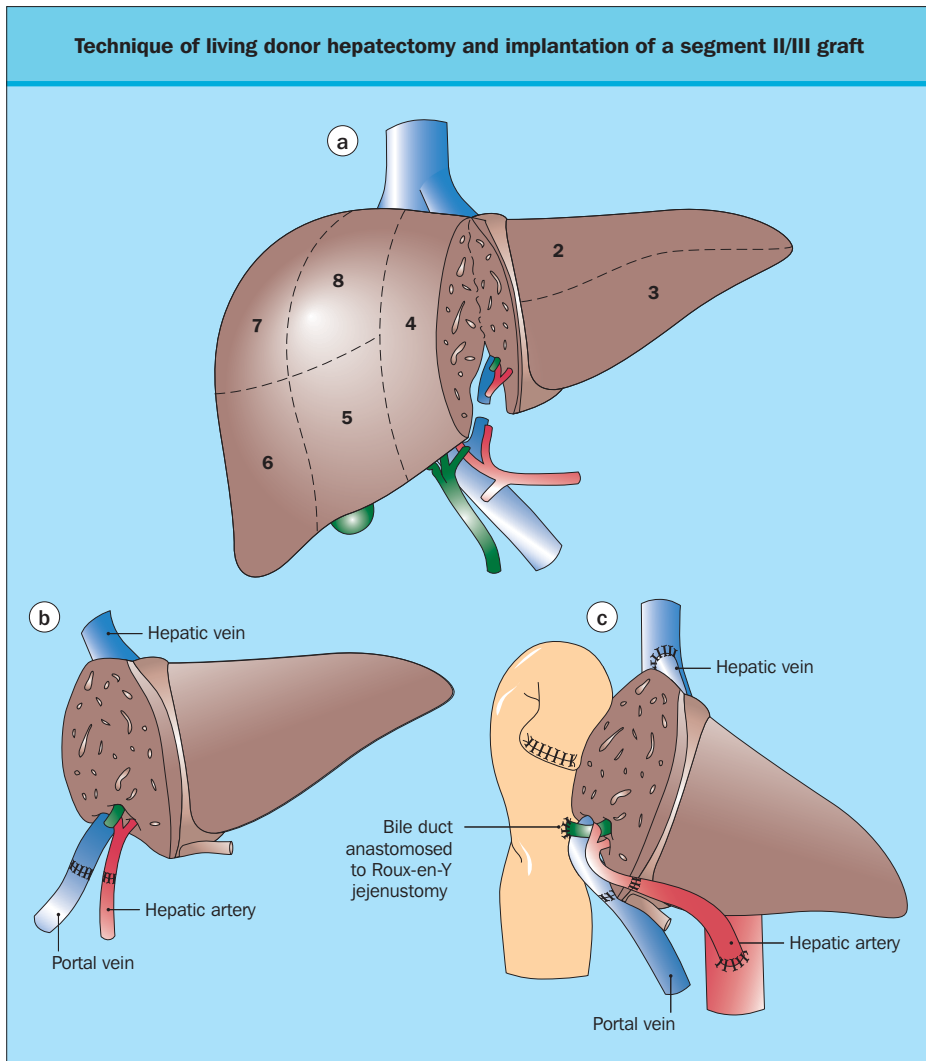
### Reduced-size liver transplantation: the recipient operation

The recipient hepatectomy for the reduced size liver is similar to orthotopic liver transplantation with exception of the native vena cava, which must therefore be preserved if there is no graft vena cava. This requires meticulous suture ligation of each hepatic vein branch to the caudate lobe during recipient hepatectomy, the goal of which is to free the entire retrohepatic vena cava, leaving the liver attached only by the three main hepatic veins. The portal vein and cava are then clamped and the liver removed. The orifices of two of the native hepatic veins are combined into a single orifice and the caval opened longitudinally to fashion a very large opening that receives the hepatic vein of the graft for a wide unobstructed anastomosis. The portal vein is anastomosed end-to-end but requires careful positioning since the course of the vessel is altered by the shape of the partial graft. The artery is then reconstructed according to the local situation. If the recipient hepatic artery is of adequate caliber, it is used for the anastomosis, using the microscope if needed. Otherwise, an interposition graft can be taken to the aorta. Reconstruction of the bile duct requires a Roux-en-Y anastomosis since the orientation of the partial graft is generally distorted to the right.

Implantation of the left lobe of a split liver graft is comparable to the technique described above. The right lobe graft usually contains the vena cava and is implanted in a fashion that is nearly identical to a standard adult liver transplant. If common structures were allocated to the left lobe, reconstruction of the artery or bile duct may be more complex and require either an interposition graft or a microvascular anastomosis. Reconstruction of the bile duct may require creation of one or more anastomoses to segmental bile ducts. Although some authors favor use of small stents for these anastomoses, we perform a direct mucosa-to-mucosa anastomosis under high magnification without intubation. Two or, occasionally, three small ducts may be anastomosed. Whenever possible, small ducts are incorporated into a single anastomosis, but if they are too far apart separate anastomoses are constructed.

### Abdominal closure

The use of reduced grafts often creates a scenario in which the graft is either too large or too small for the recipient. Both these situations can create lethal complications that must be detected and prevented at the time of abdominal closure. If the graft is too small, it can rotate on the axis of its vessels and acutely obstruct the circulation. Hepatic venous outflow is particularly prone to this complication. The graft's position must be assured



**Figure 38.16** Technique of living donor hepatectomy and implantation of a segment II/III graft.

by appropriate planning of the vascular anastomoses, and by positioning the intestines to occupy any empty space in the right upper quadrant into which the liver can rotate. Mechanical fixation of the liver is usually not helpful. If the liver is too large, closure under tension can result in a compression syndrome, leading to liver ischemia and renal and pulmonary compromise. This is managed by temporary prosthetic closure of the abdomen. The prosthetic material is subsequently removed at about 7–10 days after the transplant, a time when abdominal wall edema and ascites have abated in most patients thereby facilitating wound closure.

### Living related donor transplantation

#### Living donor hepatectomy

Living donor hepatectomy has become common in pediatrics, since the initial description of the procedure in 1989. The graft is usually segments II and III of Couinaud, comprising 15–20% of the donor's hepatic volume, generally between 200 and 250 g of liver tissue. This is ideal for infants, but as the recipient becomes larger, outcomes are compromised by the small size of the graft. In the past 5 years, donation of the full right lobe has been performed with growing frequency for use in adult recipients.

Segmentectomy of segments II and III for a pediatric recipient can be readily performed through a midline incision (Fig. 38.16a). The porta hepatis is dissected to expose the origin of the left hepatic artery. The left portal vein is then encircled, and the posterior branches to the caudate lobe are divided. The left bile duct is approached anteriorly and encircled. The left duct should be divided with a minimum of dissection to preserve the blood supply of the duct wall. Superiorly, the confluence of the left and middle hepatic veins is exposed; this is the terminal point of the dissection. The parenchyma is then divided just to the right of the falciform ligament with meticulous division of the biliary and vascular branches to segment IV. If a larger graft is to be obtained, the parenchyma can be divided in the principal fissure just to the right of the middle hepatic vein. The full left lobe graft weighs between 350 and 500 g in adults and is sufficient for a small adult or an older child. After the parenchyma is divided the graft is attached only by its vessels, which are then divided, and the liver is rapidly flushed with heparinized preservation solution.

#### Right lobe donor hepatectomy

A graft of sufficient volume for an adult recipient can be obtained by right hepatectomy. Preoperatively, donor evaluation



is generally performed independently of the recipient evaluation, with a view towards optimizing risk by careful screening of anatomic, psychosocial and general medical conditions in potential donors. Donors undergo screening angiography and cholangiography (magnetic resonance imaging is used for this purpose at our center), and occasionally liver biopsy. While the donor evaluation and patient care pathways are not yet standardized, guidelines have been developed by the American Committee on Transplantation (ACOT) and by the New York State Department of Health.

Donor right hepatectomy requires an incision similar to that used for the transplant recipient. The portal dissection requires cholecystectomy and identification of the right hepatic artery near its origin. The right border of the common hepatic duct is exposed until the right bile duct is encountered. A single common trunk of the right bile duct is absent in the majority of cases. More commonly, the duct of segments V and VIII is encountered first, and then more superiorly and posteriorly, the duct to segments VI and VII. After the ducts are transected, the right portal vein is encircled at its origin. The liver is completely mobilized, and then the small hepatic vein branches to the caudate lobe are encircled and divided to free completely the right lobe from its attachments to the vena cava. The right hepatic vein is freed and encircled. After completion of the dissection the parenchyma is divided in the plane just to the right of the middle hepatic vein. Parenchymal division in a fully vascularized liver can be both tricky and tedious. The central venous pressure is kept low, with crystalloid infusion kept to a minimum during the division. Surgeons have adopted their own unique methods of parenchymal division in live donors to minimize blood loss but avoid unnecessarily long operations. A qualified assistant, preferably an attending surgeon or senior fellow, is advantageous during the parenchymal division. Our preference, which has worked well in over 100 live donor hepatectomies, is the use of the cavitron ultrasonic dissector together with electrocautery and metal clips. Operative sonography is used during parenchymal division to localize the vessels and guide the dissection. Most surgeons performing donor right hepatectomy perform the division to the right of the middle hepatic vein, preserving the middle vein in the donor. This may lead to relative engorgement of the medial segments of the graft, which in some cases may even require venous reconstruction of the outflow to these segments. Occasionally circumstances may require incorporation of the middle vein into the graft, for example when there is a relatively small right graft with a generous left lobe in the donor. The donor surgeon, in close communication with the recipient surgeon, should be prepared to address such contingencies at the time of operation. After completion of the parenchymal transection, the vessels are clamped and divided, and the graft flushed with heparinized preservation fluid.

### Implantation of the lobe grafts

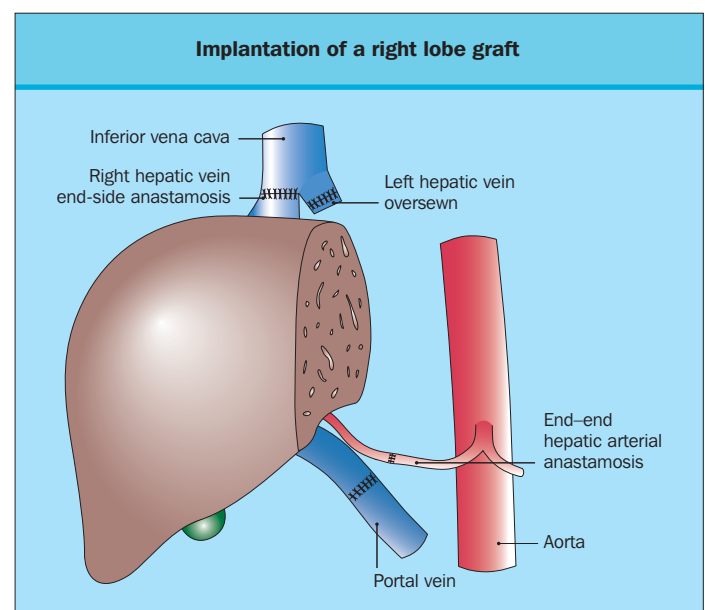
The left lobe graft is implanted as described above for reduced sized liver transplantation. (Fig. 38.16b and c).

The right lobe graft is used if the donor is the same size, or smaller than the recipient. During implantation, the central venous pressure is maintained in a low normal range to avoid engorgement of the hemigraft after reperfusion. Reduced sized grafts are especially prone to engorgement injury due to limitations

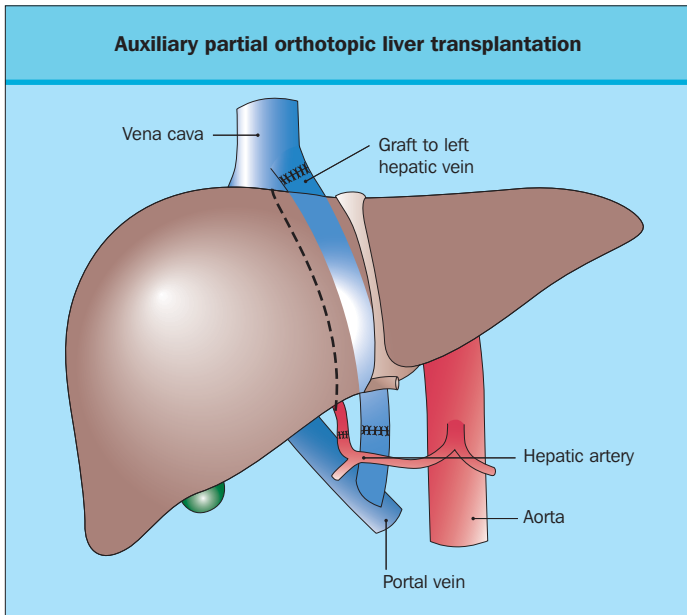
in outflow in segments of the graft. The right hepatic vein of the graft is anastomosed end-to-side to the recipient hepatic vein orifice/vena cava. The orifice is fashioned into a large longitudinal opening that lessens the risk of torsional outflow obstruction, a common cause of graft failure in the early experience with live donor grafts. The portal vein is anastomosed end-to-end with reperfusion of the liver. A microvascular technique is used to anastomose the artery, but if needed the recipient's saphenous vein can be used to create an arterial conduit for a more convenient anastomosis to a larger vessel. The biliary anastomosis is most difficult in the adult recipient, particularly if the recipient had large volume losses and resuscitation leading to bowel edema. It is extremely difficult to create a fine anastomosis between the tiny segmental ducts and massively edematous jejunum. Multiple duct anastomoses are common, and the surgeon should be prepared to utilize all of the potential conduits for biliary drainage that are available, depending on the number and location of the bile ducts. When two ducts are present, both can usually be incorporated into a single or dual biliary enteric anastomosis. When more than two ducts are present, one of the ducts can sometimes be ligated. When three major ducts are present, we have sometimes utilized both primary duct-to-duct anastomosis in conjunction with biliary enteric anastomoses to obtain complete biliary drainage. Technical complications related to biliary anastomoses and the cut surface are common in live donor transplantation. A protocol for diagnosis and management should be in place before starting a live donor program, including assurances of the skills and availability of the diagnostic and interventional radiologists who are invaluable as partners in managing these difficult postoperative problems. Fortunately, these transplants can be done electively, with careful planning to minimize these technical difficulties (Fig. 38.17).

### AUXILIARY LIVER TRANSPLANTATION

Replacement of part of the liver or the addition of a heterotopically placed partial liver graft has been used in cases of



**Figure 38.17** Implantation of a right lobe graft.

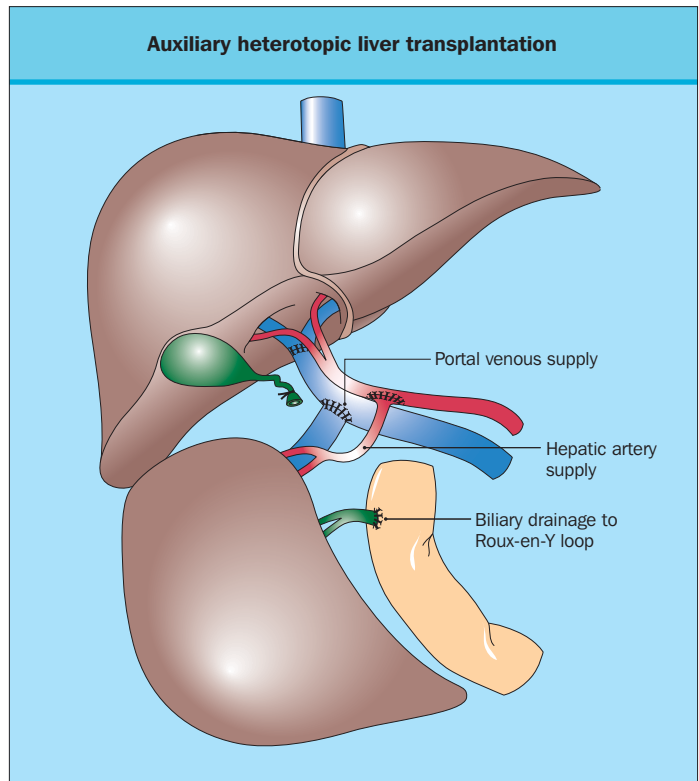


**Figure 38.18 Auxiliary partial orthotopic liver transplantation.** A left lobe graft has been implanted in the bed (orthotopically) of the resected native left lobe.

fulminant hepatic failure or metabolic liver diseases. Auxiliary liver transplantation has also been proposed for living related liver transplantation in which a small left lobe graft is implanted next to the residual functioning native liver.

For fulminant hepatic failure, the addition of the functional liver mass is used to support the recipient hepatic function until hepatic regeneration of the native liver, if possible, or the graft occurs. In those individuals in which the native graft recovers adequately, immunosuppression can be discontinued and the auxiliary graft is allowed to atrophy via rejection. For the treatment of metabolic diseases, the addition of a functional liver mass producing specific proteins (e.g. factor VIII in hemophilia) or clearing metabolites or essential minerals (e.g. bilirubin in glucuronyltransferase deficiency or urea in urea cycle defects) can sustain an almost normal phenotype.

Auxiliary liver transplantation involves a group of diverse techniques that are too complex to be covered in this section. Early experiences established the need for portal blood and adequate hepatic venous outflow for successful grafting. This is most readily accomplished by placing the liver graft in an orthotopic position, in which a specific lobe is replaced by an anatomically similar graft (Fig. 38.18). Most commonly, the left lobe of the diseased liver is replaced with a left lobe graft from a cadaveric or living donor, requiring partial hepatectomy in the recipient as the first step. The hepatic vein is reconstructed in an end-to-end fashion, as are the portal vein and hepatic artery. The bile duct generally requires hepatojejunostomy. An older technique involves placement of the graft in the hepatic fossa inferior to the native liver with attachment of the portal and hepatic veins onto the side of the recipient vessels (Fig. 38.19). The principal technical challenge involves managing the competition for portal blood between the two livers. The recipient liver tends to be favored for portal perfusion and successful grafting depends upon modifying portal perfusion to favor the



**Figure 38.19 Auxiliary heterotopic liver transplantation.**

graft, leading to relative hypertrophy of the graft. Optimal techniques for auxiliary liver transplantation remain under development as the eventual role of this technique remains uncertain.

### IMMEDIATE POST-OPERATIVE MANAGEMENT OF LIVE DONOR RECIPIENTS – SPECIAL CONSIDERATIONS

The growing use of LDLT impacts postoperative care in several ways. In the immediate postoperative period, the reduced-size graft is especially prone to engorgement compared to whole grafts. The central venous pressure should be aggressively managed within a tight range of 5–10, sometimes with pharmacologic support of the blood pressure and the use of venodilators. Although most adults are in relatively good general condition prior to LDLT, the graft function may be poor initially because of the relatively small functional size of the donor liver in adult living donors. This can lead in some cases to a syndrome of cholestasis and persistent graft dysfunction which has been termed ‘small for size syndrome’. Management of these patients must be meticulous to allow the livers to regenerate without the development of additional complications which can further injure the graft. Overall, however, the postoperative course of patients receiving LDLT has been smooth with a short intensive care unit and total length of stay and superb outcomes in experienced centers.

Vascular patency must be assessed postoperatively as well as during the transplant operation. The use of Doppler imaging is usually adequate for demonstrating hepatic artery and portal venous flow, however when there is doubt either magnetic

resonance vascular imaging or direct angiography must be performed. In recent years, as the incidence of hepatic artery thrombosis has decreased, we have nonetheless continued an aggressive policy of sonographic surveillance of the artery. Emergent angiography with arterial stenting has replaced open surgery in our practice, since precise thrombolytic therapy can be administered, intimal flaps distant from the anastomosis can be identified and treated, and occult inflow problems like celiac stenoses can be addressed.

Biliary complications are more frequent in patients receiving split liver grafts and live donor grafts though they still occasionally complicate standard transplants. Both leaks and obstructions can be managed using interventional radiologic methods. Leaks occur early after surgery and are not always detected by the surgical drains. Aggressive use of sonography and cross-sectional imaging is needed to detect fluid collections. Bile leaks may be end fistulas. These need effective external drainage and control

of sepsis, supplemented by diagnostic cholangiography and possible stenting to reduce leakage. Most biliary leaks will resolve with effective drainage, though open surgery is occasionally required as in the cases of complete disruption of the anastomosis. The anastomotic stenosis should always be suspected as part of the differential of graft dysfunction. Localized stenoses can be managed successfully with minimally invasive methods, either endoscopic or percutaneous. More complex stenoses may require a combination of interventional and open techniques.

In summary, surgical issues clearly impact hepatology practice both in selection and aftercare of the transplant patient. Our practice has evolved to full integration between hepatology and surgery for all aspects of the program. The course of the surgery, from donor selection and procurement to technical obstacles during the transplant, will determine the postoperative course. The outline presented herein attempts to describe, in a practical way, the conduct of the procedures.

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## SECTION V: LIVER TRANSPLANTATION

### CHAPTER

# 39

## Immunology and Immunosuppression

Geoffrey W. McCaughan

### INTRODUCTION

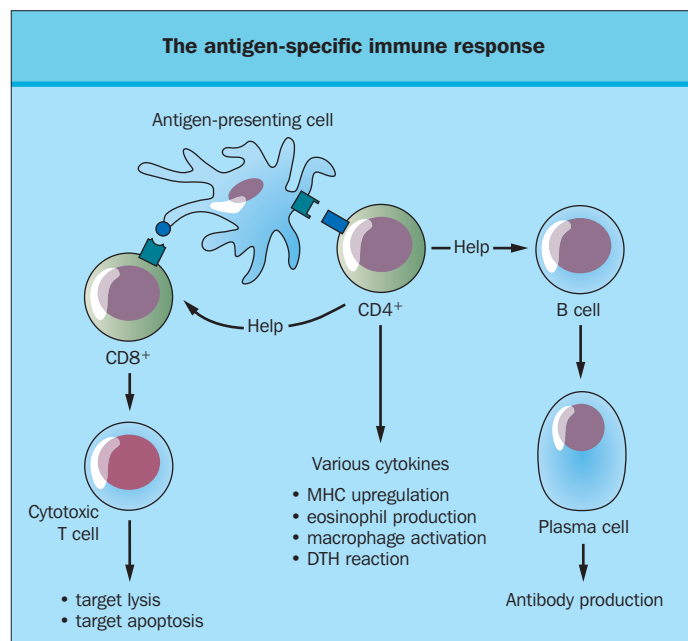
An understanding of the basic mechanisms of the immune response in relation to organ transplantation will allow the reader to appreciate the use of immunosuppressive drugs and specific aspects of allograft rejection.

### CELLULAR ASPECTS

Traditionally the immune response has been divided into antigen-specific and non-specific responses. Antigen-specific responses are also known as the adaptive immune system, while non-specific responses are known as the innate immune system.

The antigen-specific immune response is dictated by specific receptors for antigen which are restricted to T- and B-lymphocyte populations. These antigen receptors are part of a large family of molecules known as the immunoglobulin (Ig) superfamily. The T-cell receptors consist of heterodimers and are of two types ( $\alpha/\beta$  and  $\gamma/\delta$ ). These heterodimers join to form a single antigen binding site. The B-cell antigen receptor consists of two identical antigen-binding sites that are formed by the interaction of heterodimers of so-called heavy and light Ig chains. These T-cell and B-cell antigen receptors are highly polymorphic in their antigen-binding regions, as each receptor is derived from a variable set of exons in the genome. The specific immune system at the T-cell level is triggered by the presentation of antigen via cells from the innate or non-specific immune system (Fig. 39.1). The classical cell that presents antigen is the dendritic cell although other cells such as macrophages, B cells and endothelial cells may have the same capacity under some circumstances. Once T cells are triggered, they perform various functions. Cells of the CD4<sup>+</sup> phenotype provide help to B cells and produce various mediators that activate cells of the non-specific immune system such as macrophages, neutrophils and eosinophils. They also stimulate and provide help to other T cells, particularly CD8<sup>+</sup> T cells. CD8<sup>+</sup> cells are usually effector cells, particularly involved in cellular lysis and apoptosis [cytotoxic T lymphocytes (CTLs)]. Activation of these pathways results in protective immunity against specific antigens, but also may result in tissue damage via delayed-type hypersensitivity (DTH) reactions and direct cellular cytotoxicity (see below).

The non-specific immune system is characterized by immune effector cells that do not have antigen-specific receptors. These cells include immature dendritic cells, macrophages, natural killer (NK) cells, natural killer T (NKT) cells, polymorphonuclear leukocytes and the complement system. It is beyond this chapter to discuss the activation and regulation of these cells. However, it is increasingly recognized that the interaction



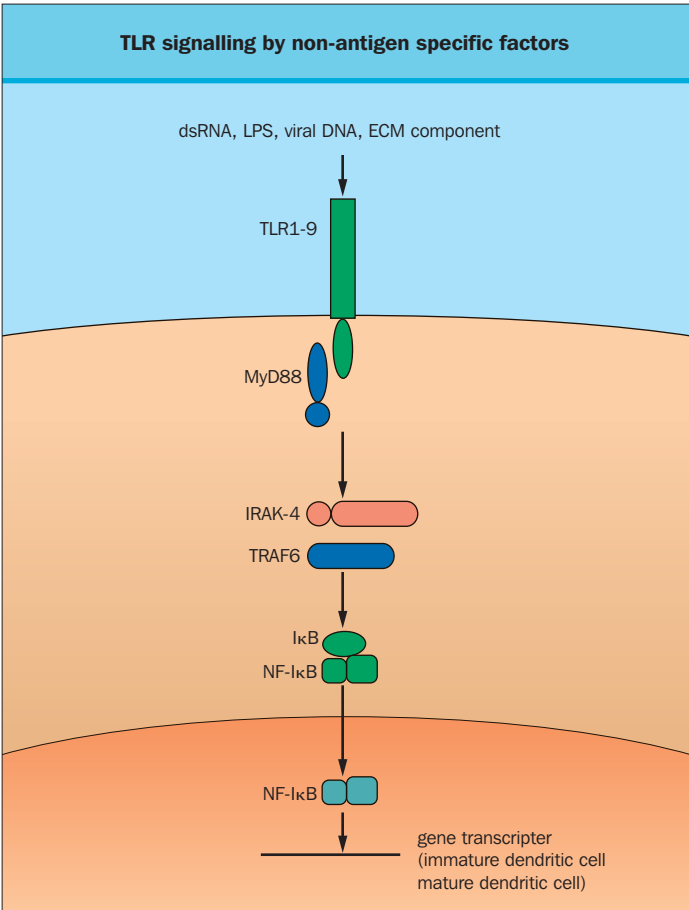
**Figure 39.1** Simplistic overview of the immune response. DTH, delayed type hypersensitivity; MHC, major histocompatibility complex.

between the antigen-specific and non-specific immune systems is blurred. Triggering of antigen-specific T cells activates cells within the innate immune system to perform effector functions whilst immature dendritic cells are triggered by agents such as endotoxin, bacterial products and viral DNA. These latter pathways are regulated by a set of molecules known as Toll receptors (Fig. 39.2). These molecules are also known as pattern recognition receptors and allow recognition of environmental pathogens in a non-specific way. Their engagement on immature dendritic cells allows cellular maturation which allows the development of antigen specific presentation to T cells.

### Cytokines

Key components in the regulation of the antigen-specific and non-specific systems are soluble mediators known as cytokines (Table 39.1).

Cytokines are relatively low molecular weight polypeptides that are secreted from cells and act upon other cells in the immune system via specific cell surface receptors. They may also act in an autocrine fashion on the cytokine-producing cell itself, in an enhancing or inhibitory way. Cytokines are pleiotropic in their action and often display redundancy for some functions. Cytokines can be grouped into various subfamilies, namely the interleukins (ILs), interferons (IFNs), tumor necrosis



**Figure 39.2** Simplistic overview of Toll-like receptor (TLR) signaling by non-antigen specific factors.

factor (TNF) family, chemokines and growth factors. The important cytokines that play a role in rejection and tolerance are discussed below.

One of the key interleukins in the immune system is IL-2. IL-2 is a 15 kDa polypeptide produced by activated T cells, and is the most important autocrine activator of T cells following initial T-cell stimulation. Its function is not only to expand T cells clonally, but to stimulate T cells to produce the release of other cytokines such as TNF- $\alpha$  and IFN- $\gamma$ , which leads to further T-cell activation. IL-2 acts via a three-chain IL-2 receptor. The high affinity receptor is an  $\alpha/\beta/\gamma$  heterotrimer, but low affinity complexes of  $\alpha/\gamma$  or  $\beta/\gamma$  exist. The cytokines IL-1 and IL-6 are produced by numerous cells, particularly macrophages. They have important functions within the liver by stimulating the acute-phase protein response, but also play a role in T- and B-cell and endothelial cell activation. IL-6 has also been shown to play an important role in liver regeneration, as IL-6 knockout mice have a markedly reduced capacity for liver regeneration. Excess IL-1 may inhibit bile flow at the level of the hepatocyte canalculus, thus playing a role in the pathogenesis of cholestasis. IL-4 is mainly produced by activated T cells, but also by mast cells and it is important in B-cell activation and antibody production, particularly of the IgE subclass. It also activates antigen-presenting cells (APCs) by increasing expression of major histocompatibility complex (MHC) class II and CD40, which are important costimulatory molecules. IL-5 is produced by a large range of cells, particularly by activated T cells. IL-5 plays a role in B-cell antibody production, particularly of the IgA isotype, and is crucial to eosinophil differentiation and growth. IL-10 has immunosuppressive properties and inhibits IFN- $\gamma$  and IL-2 production from activated T cells. It also inhibits monocytes and the expression of adhesion molecules on APCs. It is largely produced by macrophages and by T cells of the so-called Th2 type. IL-10 has antifibrotic properties. IL-12 is produced by NK and by T cells, and is important in stimulating IL-2 and IFN- $\gamma$  production during the early phases of T-cell activation. It is crucial in the induction of the Th1-type cytokine response. IL-13 shares similar properties with IL-10, in that it inhibits T-cell activation, production of IL-2 and IFN- $\gamma$ . IL-15

Key cytokines in antiallograft responses		
Cytokine	Main source	Main actions
IL-1	Many cells (macrophages)	Lymphocyte co-stimulation, acute phase protein response, fever, neutrophil/endothelial cell activation, decreased bile flow
IL-2	Activated T cells (Th1)	T-cell proliferation
IL-4	Activated T cells (Th2) Mast cells	B-cell activation and antibody production (particularly IgE)
IL-5	Activated T cells (Th2)	Eosinophil differentiation and proliferation, B-cell antibody production (IgA)
IL-6	Many cells (T-cells, macrophages)	Acute phase protein production, B-cell antibody production (IgA), liver regeneration
IL-8	Multiple	Neutrophil chemotaxis and activation
IL-10	Multiple T cells (Th2) Macrophages	B-cell activation, inhibition of DTH (Th1) responses
IL-12	Macrophages NK cells	Stimulation (T1-type cells), DTH responses and IFN- $\gamma$ production, NK stimulation
IL-13	Activated T cells	B-cell activation, inhibition of Th1 pathway, stimulation of NK cells
IFN- $\gamma$	Activated T cells NK cells	Upregulation of MHC class I and MHC class II expression, macrophage activation, stimulation of NK cells, B-cell activation, class switching, inhibits IL-4 activation, chemokine induction

**Table 39.1** Summary of major cytokines that play a role in inflammatory responses seen in allograft rejection. Ig, immunoglobulin; NK, natural killer.

has similar properties to those of IL-2 and triggers through the same receptor. IL-18 has been recently shown to have profound IFN- $\gamma$ -inducing properties, particularly in macrophages.

A key group of cytokines is the chemokine family. Chemokines are small molecular weight molecules (8–11 kDa) with specific amino acid motifs. These motifs have been used to classify these molecules into four subfamilies the largest of which are the CC and CXC chemokines based on the arrangement of conserved amino terminal cysteine (C) residues. These families contain large numbers of molecules with >28 in the CC and >16 in the CXC families. The CC chemokines are largely responsible for the chemotaxis of mononuclear cells whilst the CXC chemokines act mainly on neutrophils. The chemokines play key roles in the migration of lymphocytes in and out of organized lymphoid tissues and regulate tissue cellular migration at the site of inflammatory responses, including allograft rejection.

The interferon families consist of the  $\alpha$  and  $\beta$  subclasses, which are homologous to each other and are produced by numerous cell types following stimulation by infectious organisms or other inflammatory responses. These molecules increase the cellular expression of major histocompatibility molecules, and stimulate macrophage and NK cell activation. They are known to have antiviral properties. Interferon- $\gamma$  shares no homology with  $\alpha$ - or  $\beta$ -interferon, and is produced by T cells of the Th1 phenotype following antigen presentation via macrophages or dendritic cells. Interferon- $\gamma$  is a powerful activator of macrophages and also inhibits IL-10 and IL-4 production from other cell types. Interferon- $\gamma$  results in upregulation of MHC, particularly class II molecules. Interferon- $\alpha$  also plays a role in the innate immune response at several levels including maturation of immature dendritic cells thus enhancing antigen presentation to the specific immune system.

TNF- $\alpha$  is produced mainly by macrophages but also by other cell types, including activated T cells. The molecule exists as a homotrimer and not only exists in soluble but also in cell surface forms. It binds to two receptors, TNF receptor (TNFR) type 1 (p75) and TNFR type 2 (p55). TNF- $\alpha$  has many pleomorphic reactions, including the triggering of nitric oxide release from endothelium resulting in systemic vasodilatation, and it plays a role in septic shock. At the hepatocyte level it is crucial in priming for hepatocyte proliferation in liver regeneration, but also may trigger cell death and apoptosis via the apoptotic cell death cascade. The TNF and TNFR are foundation members of large superfamilies of molecules that are involved in cell death

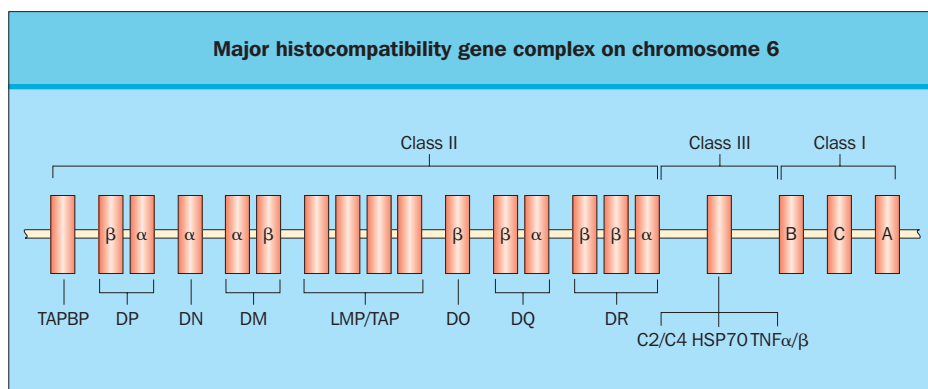
and survival signals. This includes Fas/Fas ligand and CD40/CD40 ligand (CD154).

Important growth factors in the inflammatory response include transforming growth factor (TGF)- $\beta$ . This is an important anti-inflammatory cytokine as it is a potent inhibitor of T- and B-cell responses (see later under tolerance). It also has the potential to inhibit hepatocyte proliferation. Furthermore, it is the key player in hepatic stellate cell stimulation, and in the production of extracellular matrix, which is important in tissue remodeling within the liver and the hepatic fibrosis response that occurs in chronic allograft rejection. It is produced by many cells including macrophages, hepatic stellate cells and fibroblasts.

## MAJOR HISTOCOMPATIBILITY COMPLEX

The MHC was recognized initially in mice, when it was realized that the time to rejection of experimentally transplanted organs and tumors in animals depended on the degree of genetic relationship between the donor and recipient. The closer the animals were related, the longer the graft survival. These initial experiments took place in the 1930s and 1940s, but were soon followed in the late 1940s and early 1950s through to the mid-1970s by skin transplantation in humans. In parallel with these studies it had been recognized that polymorphic antigens on leukocytes were recognized by serum from patients who had received multiple blood transfusions, or patients who had a history of multiple pregnancies. These leukocyte antigens were then used to define human subjects that underwent experimental skin transplantation, and they were linked to the survival of allograft tissue. This work led to the molecular definition of the MHC complex, particularly in humans and mice, which has now been characterized by molecular techniques.

The MHC gene complex is located on the short arm of human chromosome 6 (Fig. 39.3). The complex consists of over 50 genes spanning an area of 3500 kb. Genes in the area have been classified as class I, class II, class III and class IV loci. The MHC class I locus consists of MHC A, B, and C regions. MHC class I molecules are heterodimers. The  $\alpha$ -chain possesses three domains  $\alpha$ -1,  $\alpha$ -2 and  $\alpha$ -3, and the  $\beta$ -chain is a single domain consisting of  $\beta_2$  microglobulin. The  $\beta_2$  microglobulin and the  $\alpha$ -3 domain show very strong homology to single Ig-related domains of the immunoglobulin superfamily. The  $\alpha$ -1 and  $\alpha$ -2 domains each interact, forming two parallel  $\alpha$ -helical loops (one derived from each domain). These two helices form the sides of a cleft



**Figure 39.3 Major histocompatibility gene complex on human chromosome 6.**



that binds peptide antigens. The protein structures of each molecule are highly polymorphic, varying from individual to individual, with the main heterogeneity occurring in amino acid residues around the antigen-binding cleft.

The MHC class II region consists of four sets of molecules: DR, DQ, DP and DM. These are heterodimers of  $\alpha$ - and  $\beta$ -chains that consist of two immunoglobulin-like domains each. Similar to MHC class I molecules, two parallel  $\alpha$ -helices (one derived from each  $\alpha$ -1 and  $\beta$ -1 Ig domain) form an antigen-binding cleft. Major histocompatibility complex class II molecules are highly polymorphic, with the major amino acid heterogeneity in the vicinity of the antigen-binding cleft. The DP, DQ and DM genes are encoded by single  $\alpha$ - and single  $\beta$ -chain genes, while there are two  $\beta$ -chain genes in the DR region. The MHC class II region between the DQ and DM genes also consists of the transporters associated with antigen processing (TAP) genes and large multicatalytic protease (LMP) genes. The LMP genes encode the proteasome complex, while TAP genes encode peptide transporters. Both of these gene complexes are associated with the function of peptide ligand binding to MHC molecules. Class III genes consist of the TNF family, complement molecules and steroid hydroxylase. This region may be eventually split into class III and IV genes, with the latter including the TNF family and other inflammatory mediators.

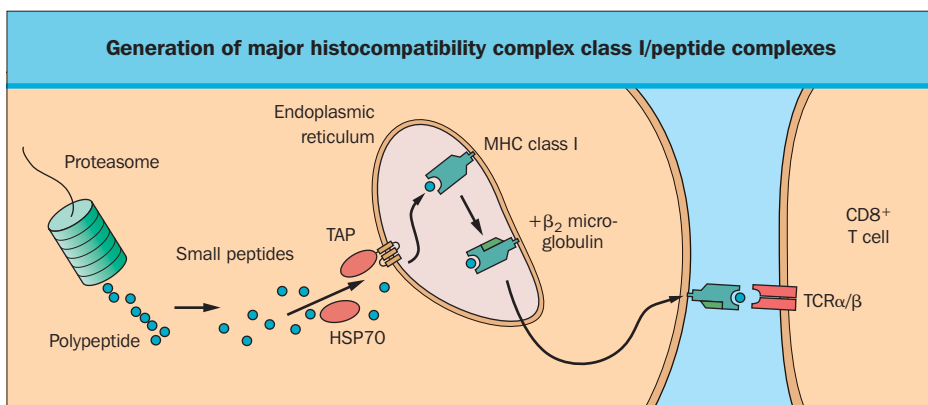
Understanding the MHC complex is important in transplantation. These molecules are crucial in regulation of antigen presentation to the immune system, and the polymorphisms are crucial in the recognition of foreign antigen during the effector phase of the immune response. Matching of polymorphisms within the MHC complex plays a role in clinical transplantation, particularly in renal and bone marrow transplantation.

## T-CELL RECOGNITION OF ANTIGEN

The recognition of antigen, including alloantigen, by the T-cell system occurs via the specific T-cell receptor (TCR) molecules on both CD4+ and CD8+ T cells. Recognition mostly occurs via the  $\alpha/\beta$  rather than the  $\gamma/\delta$  set of T-cell receptors, and is fundamentally different between CD4+ cells and CD8+ cells. CD8+ cells recognize antigen in association with MHC class I molecules, while CD4+ cells recognize antigen in association with class II molecules. In both situations small peptides, generated from whole antigens, bind to these molecular

complexes, then make their way to the cell surface and are recognized by the TCR as an MHC/peptide complex.

Peptides associated with class I molecules are usually derived from antigens that are processed intracellularly in the cytoplasmic compartment (Fig. 39.4). These antigens are largely the result of background cellular protein turnover. The source of these may largely be defective ribosomal protein products. Such antigens are degraded by a large 26S multicatalytic, 700 kDa protease known as the 26S proteasome. This is a complex, cylindrical-shaped structure consisting of outer rings of  $\alpha$  and  $\beta$  subunits. Following this interaction, peptides are generated and then transported from the cytoplasmic proteasome complex to the endoplasmic reticulum, usually in association with various chaperones of the heat shock protein (HSP) family (HSP70 and HSP90). These peptides are transported into the endoplasmic reticulum by the TAP-1/TAP-2 molecules. Transporters associated with antigen processing are a superfamily of adenosine triphosphate (ATP) multimembrane cassette transporters, and transport of peptides into the endoplasmic reticulum is ATP-dependent. The generation of peptide sequences, usually 8–11 amino acids in length, which are capable of binding to the MHC class I complex via an MHC peptide-binding groove are few (approximately 1 in 2000). There is now evidence that important proteases play a role in this generation at a post-proteasome level. The peptides are anchored at the carboxyl terminus, usually by a hydrophobic amino acid (although this may vary depending on the MHC class I allele). The loading of peptides onto the class I MHC molecules is tightly regulated. As class I molecules are synthesized they interact with a number of molecular chaperones. Peptide loading at low affinity may occur at this stage. These chaperones allow the assembly of the peptide loading complex which consists of the interaction of class I molecules with the TAP complex and other endoplasmic reticulum proteins such as tapasin. This complex then allows the replacement of low affinity suboptimal peptides with high affinity optimal peptides which stabilize the MHC class I molecule with the important chaperone  $\beta_2$  microglobulin which is necessary for the transport of the MHC/peptide complex to the cell surface through the Golgi complex. At the cell surface antigenic peptide in association with its MHC groove is recognized by the  $\alpha/\beta$  T-cell receptor on CD8+ cells. The amount of antigen presentation is not fixed and can be regulated particularly by interferon- $\gamma$  which upregulates MHC and TAP gene products. Interferon- $\gamma$  may also increase the efficiency of



**Figure 39.4 Schematic diagram of the generation of major histocompatibility complex (MHC) class I/peptide complexes.**

the proteasome itself by alteration of subunit composition thus generating an 'immunoproteasome'.

MHC class II peptide generation and binding to MHC class II molecules is more complex than described for the peptide/MHC class I pathway (Fig. 39.5). As mentioned earlier, MHC class II molecules are  $\alpha/\beta$  heterodimers. These are synthesized and form a nonameric complex with another molecule known as the invariant chain. This complex is targeted to the endocytic pathway within the transGolgi network via sorting signals that are present within the cytoplasmic tail of the invariant chain. It is here that the interaction with antigenic peptide takes place. Antigenic peptides become associated with MHC class II molecules and are usually generated by the endocytic pathway following the ingestion of extracellular antigen. The endocytic pathway where this interaction takes place has been defined as having multivesicular structures. Antigen is degraded by lysosomal type proteases into small peptides of varying lengths. At the same time, the MHC class II invariant chain complex is degraded by other proteases (including cathepsin) into single  $\alpha/\beta$  structures with a small individual peptide of the invariant chain (clip) remaining associated in the MHC class II peptide binding groove. This clip peptide is then exchanged for antigenic peptides by a process catalyzed by human leukocyte antigen-DM (HLA-DM) molecules. The peptide requirements for binding to the MHC class II groove are not as stringent as that for class I ligands. The peptides are usually longer, ranging between 14 and 18 amino acids in length (even up to 28 amino acids). These peptides extrude from the binding groove at both the amino- and carboxyl-terminal ends. The peptides are anchored in the MHC class II groove by various anchor residues, the most important one being at the beginning of the groove where the amino acid at this position is often hydrophobic in nature (but may be charged, once again depending on the MHC class II allele with which it is actually associating). Once the  $\alpha/\beta$  heterodimer and peptide complex is stabilized, this is then transported and appears on the cell surface where the antigenic determinants in the groove are recognized by the  $\alpha/\beta$  T-cell receptor on CD4+ cells.

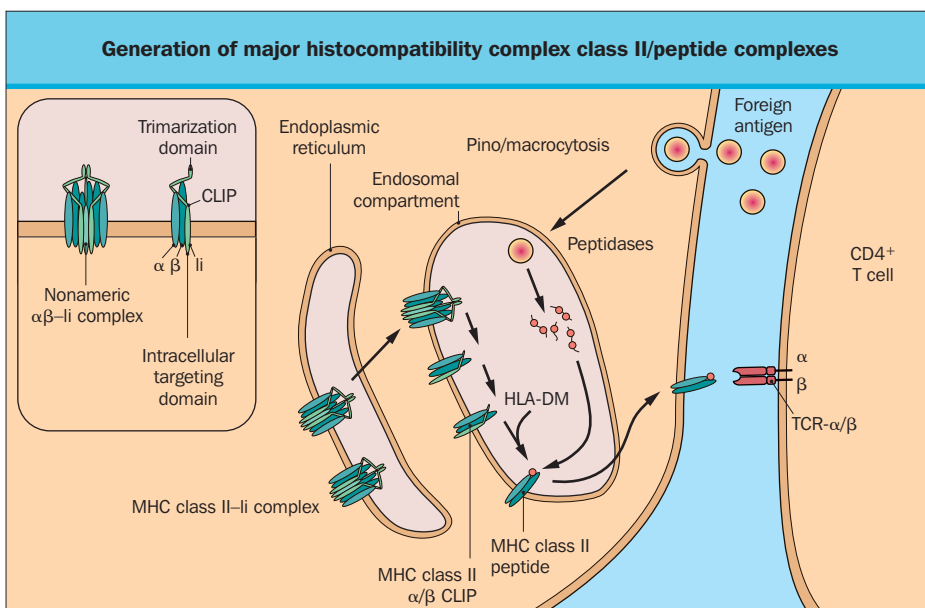
## EARLY EVENTS IN ACTIVATION OF T CELLS BY ANTIGEN (G0-G1 TRANSITION)

### Activation via the T-cell receptor

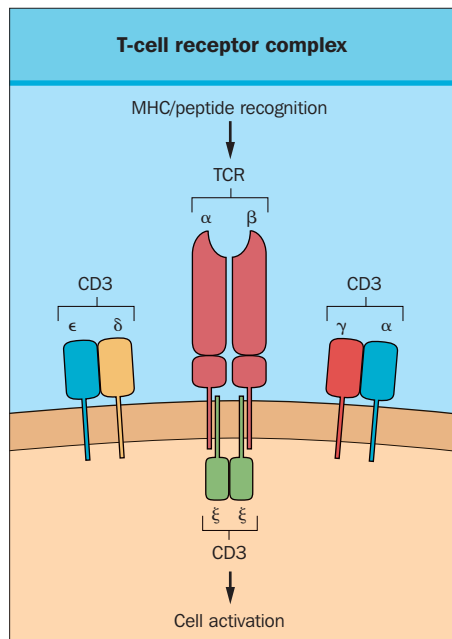
The recognition of antigen/MHC complex by the TCR occurs at what is known as the 'immunological synapse'. This consists of the central T-cell receptor, adhesion molecules, costimulation molecules, an intracellular signaling complex and cytoskeletal components. The engagement of this synapse activates complex signaling pathways that lead to the induction of lymphocyte activation, division and clonal proliferation. The T-cell receptor is associated at the cell surface with a group of molecules known as the CD3 complex (Fig. 39.6).

This complex consists of  $\epsilon/\gamma/\delta$  heterodimers and an important  $\zeta/\zeta$  homodimer. In particular, the  $\zeta/\zeta$  homodimer has a long cytoplasmic tail, part of which is a repetitive 16 amino acid motif known as the immunoreceptor tyrosine-base activation motif (ITAM). Following antigen binding to the receptor and oligomerization of the receptor and the CD3 complex, phosphorylation of the ITAM takes place by tyrosine kinase molecules associated with CD4 (lck) or the T-cell receptor itself (fym) (Fig. 39.7).

Phosphorylation of the ITAM leads to the generation of a specific binding site for other protein tyrosine kinase (PTK) molecules, in particular ZAP-70 and Syk. These molecules become activated, generating further multiple tyrosine phosphorylation sites that serve as binding sites for other proteins that regulate two major pathways of cellular activation, finally leading to transcription of IL-2 and other cytokine genes. One of the key mediators in one of the pathways is phospholipase C $\gamma$  (PLC $\gamma$ ). Following triggering of the T-cell receptor complex PLC $\gamma$  is recruited to the cell membrane, is phosphorylated and results in the hydrolysis of inositol phospholipids, generating inositol polyphosphates (IP-3) and diglycerols (DG) from the cell membrane. IP-3 stimulates the mobilization of calcium from intracellular stores. The increase in intracellular cytoplasmic calcium leads to the activation of the calmodulin-dependent serine phosphatase calcineurin. This is a



**Figure 39.5 Schematic diagram of the generation of major histocompatibility complex (MHC) class II/peptide complexes.**



**Figure 39.6** Schematic diagram of T-cell receptor complex that recognizes major histocompatibility complex (MHC)/peptide complexes resulting in T-cell activation and proliferation.

the mitogen-activated protein kinase (MAPK) cascade at the site of the JNK (JUN N-terminal kinase) molecule. The other product of phospholipid breakdown, DG, is important in the activation of another transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B).

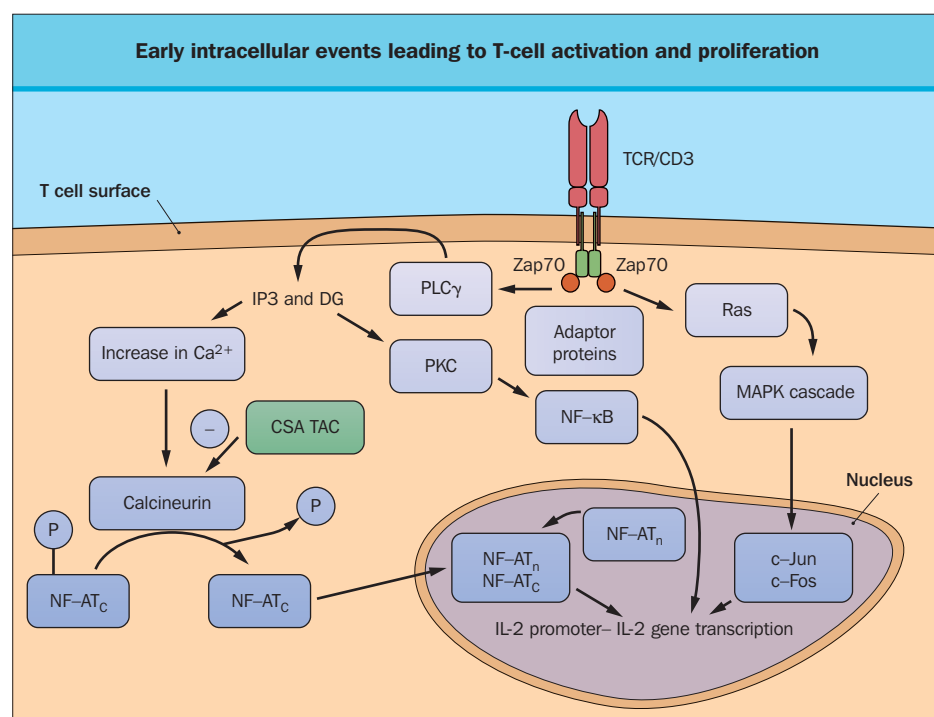
The second main pathway following the initial events at the TCR is activation of the MAPK cascade. This cascade is characterized by the successive phosphorylation of various cytoplasmic kinases which may act on themselves and finally lead to cytokine gene transcription. Mitogen-activated protein kinases phosphorylate at proline x-threonine/serine-proline sequences where x is often a leucine residue. They require dual phosphorylation at both serine/threonine and a tyrosine residue for activation. Activation through this cascade results in activation of c-JUN, c-FOS and NF- $\kappa$ B transcription factors, as well as optimally activating the NFAT transcription factor. Early activation of the MAPK cascade occurs via the RAS pathway and RAS-mediated recruitment of Raf-1 to plasma membrane. Raf-1 is one of the MAPKK (MAPK kinase) molecules that form part of the MAPK cascade itself. It is important to note that activation via both of these cascades (i.e. the PLCg/calcineurin pathway and the MAPK pathway) is needed for optimal T-cell activation.

crucial molecule in T-cell activation, and is a target for immunosuppressive drugs such as cyclosporine and tacrolimus. Activation of calcineurin has two main effects. It leads to the activation of the cytoplasmic form of nuclear factor for activated T cells (NFAT<sub>c</sub>), resulting in its transfer to the nucleus where it combines with NFAT<sub>n</sub> to form an active nuclear transcription activator which binds to various gene promoters, including the promoter for the IL-2 gene. This transcription factor acts together with other transcription factors of the AP-1 family such as FOS and JUN. Calcineurin also plays a major role in the phosphorylation status of other pathways, in particular

### Costimulatory pathways

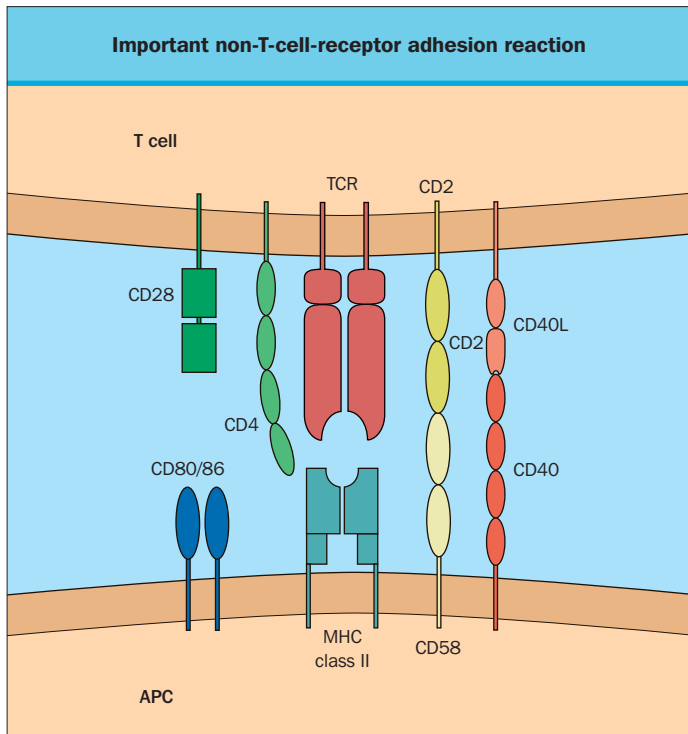
Optimal activation via the T-cell receptor requires recruitment of other cell surface molecules as part of the immunological synapse signaling complex. These include the CD8 and CD4 molecules themselves which interact with MHC class I and class II proteins respectively on the cell surface of the APC, the CD58/CD2 interaction, and the CD54/intercellular adhesion molecule (ICAM) interaction (Fig. 39.8).

However, the most important adhesion molecule is CD28, which interacts with the B7 (CD80/CD86) molecules on the APC. These molecules are both members of the immunoglobulin



**Figure 39.7** Early intracellular events leading to T-cell activation and proliferation. Sites of drug interactions outlined in the text are labeled. PKC, protein kinase C. CSA, cyclosporin; TAC, tacrolimus.





**Figure 39.8** Costimulatory molecules and their ligands.

superfamily. CD28 is a two-domain structure, while CD80/CD86 has a single immunoglobulin domain. Following T-cell receptor interaction, CD28 is rapidly expressed at increased levels on the cell surface, and ligation of CD28 with CD80/CD86 results in activation of the protein tyrosine kinase complex, including the MAPK cascade. It is at this point that the calcineurin pathway via the T-cell receptor and the activation pathway via CD28 meet. This process leads to the optimal activation of various gene transcription factors, which rapidly upregulate IL-2 itself, the IL-2 receptor, and the CD40 ligand. The pathway of CD28 signaling also leads to the upregulation of a molecule known as cytotoxic T-lymphocyte antigen (CTLA) 4. This molecule has approximately a 10- to 100-fold higher avidity for CD28 than for CD80/CD86. It has been recently recognized that CTLA4 may have an important negative influence on T-cell activation.

Another adhesion costimulatory pathway for T-cell activation is the CD40/CD40 ligand (CD40L/CD154) interaction. CD40/CD 154 is a member of the TNF cytokine family, while CD40 is a member of the TNFR family. CD154 is expressed on T cells following activation via the TCR, while CD40 is widely expressed on cell types including antigen-presenting dendritic cells. This interaction is thought to be important in upregulating CD80/86 on APCs and in enhancing APC function itself. The CD40/CD154 interaction seems to be critical in T-cell-dependent macrophage activation.

Recently novel costimulatory molecules have been identified that make the above description somewhat simplified. These new molecules are part of the B7 or TNF family of molecules on APCs and part of the CD28 or TNFR family of T cells (Fig. 39.9). They include ICOS, PD-1, CD134 and CD27 on T cells with respective ligands on APCs. These interactions deliver both positive and negative signals in the context of T-cell receptor

stimulation. It is likely that the integration of these various costimulation signals may deliver different outcomes depending on the exact in situ environment.

### LATER EVENTS IN ACTIVATION OF T CELLS BY ANTIGEN (G1-S PHASE TRANSITION)

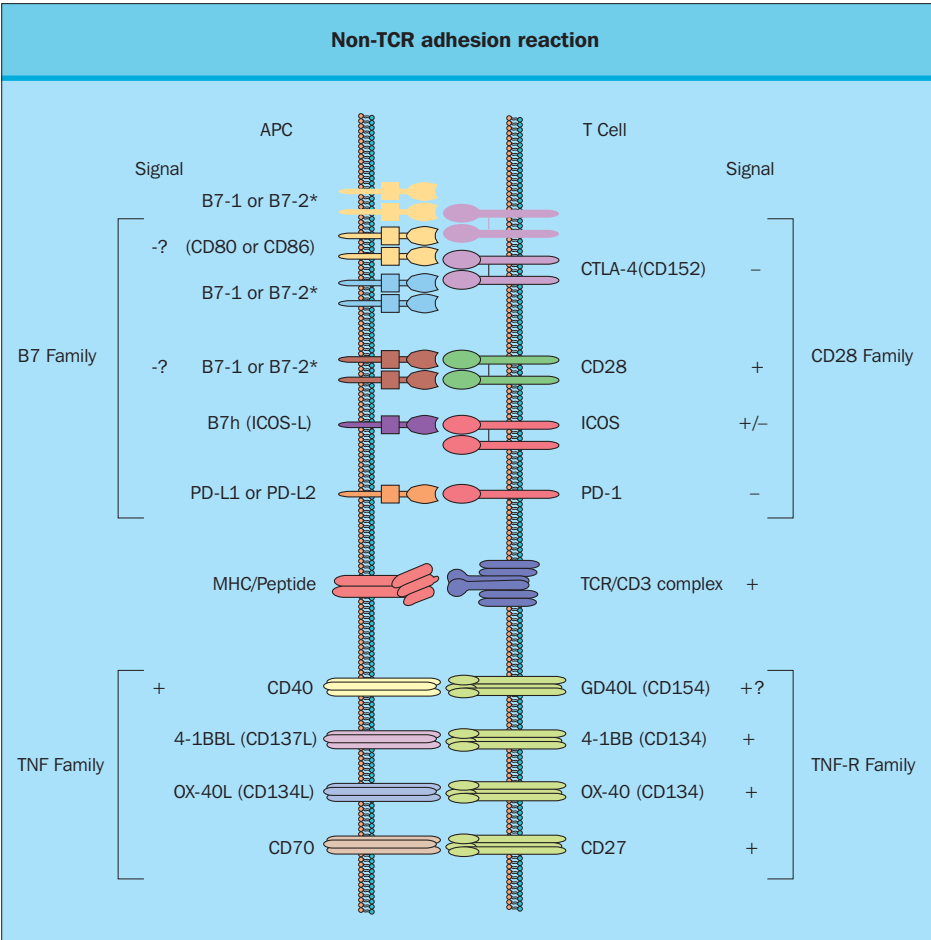
The early activation steps and production and secretion of IL-2 lead to the induction of the IL-2 receptor and subsequent signaling through the IL-2 receptor. This signaling pathway and signaling through other cytokine receptors occurs via a different set of cytoplasmic molecules known as the Janus kinase (JAK) proteins and signal transducers and activation of transcription (STAT) molecules. This results in progression of lymphocyte activation in the cell cycle from the G1 to S phase. These latter events are important as the STAT/targets of rapamycin (TOR) molecules are important targets of the rapamycin group of immunosuppressant drugs.

### HOW DO ACTIVATED CELLS ENTER TISSUES?

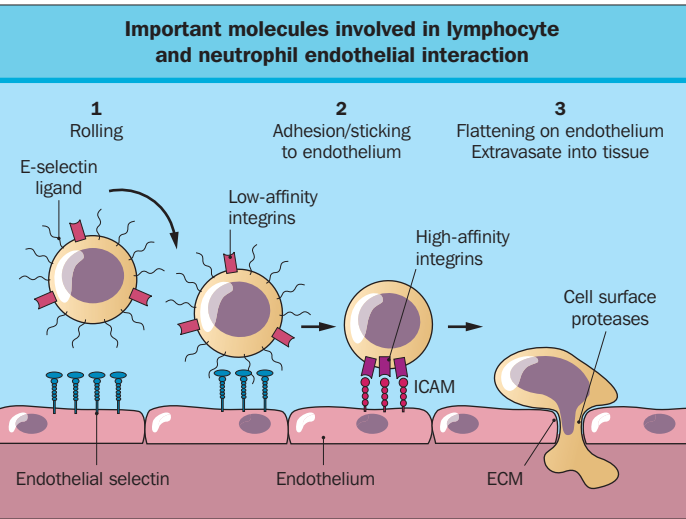
Following activation of T cells and the subsequent induction of other non-specific inflammatory cells, these cells circulate and enter tissues and, in the case of organ allografts, cause tissue damage and allograft rejection. The way in which these cells actually enter tissues has now been well defined. The process consists of three steps involving leukocyte-endothelial cell interactions. These steps are known as:

- the initial tethering and rolling step;
- step of adhesion of the leukocyte to the endothelium; and
- the extravasation of the leukocyte through the endothelium into the tissues (Fig. 39.10).

The initial rolling and tethering step is initiated by a group of molecules known as selectins. These are expressed on leukocytes themselves (L-selectin) and endothelial structures (E-selectin and P-selectin). Selectins are 'long' molecules that extend well out from the endothelial surface and have plant C-type lectin domains, which are positioned on the end of repetitive consensus repeat domains, these being homologous to the complement binding proteins. It is the presentation of this C-type lectin domain away from the cell surface that allows interaction with selectin ligands. The selectin ligands are a class of complex oligosaccharides known as lactosaminoglycans, and these contain the core tetrasaccharide structure sialyl Lewis-X (SLEX). Binding of selectins to their ligands is calcium dependent, and the initial tethering and rolling steps are relatively loose and reversible. Selectins are upregulated quite rapidly by various cytokines/chemokines which also lead to the induction of a high affinity group of molecules known as integrins, in particular lymphocyte function-associated antigen (LFA)-1, very late antigen (VLA)-4 and  $\alpha 4\beta 7$  on the leukocyte cell surface. Integrins mediate the second step of leukocyte-endothelial interaction. The integrins bind to endothelial adhesion molecules such as the ICAMs (ICAM-1/ICAM-20), vascular adhesion molecule (VCAM-1) and mucosal addressing cell adhesion molecule (MAdCAM)-1. The interactions between integrins and the integrin receptors mediate firm adhesion of leukocytes to the vascular endothelium. Another molecule, vascular adhesion protein (VAP)-1, may play an important role in leukocyte-



**Figure 39.9** Important non-TCR adhesion reaction between the T-cell and the antigen-presenting cell (APC).



**Figure 39.10** Important molecules involved in lymphocyte and neutrophil endothelial interaction resulting in cellular chemotaxis into inflamed tissues. ECM, extracellular matrix; ICAM, intercellular adhesion molecule; PCAM, platelet-cell adhesion molecule.

endothelial interactions within the liver. The second step of firm adhesion to the endothelium leads to leukocyte flattening, and the development of cell surface pseudopodia which insinuate themselves between endothelial junctions, and result in leukocyte extravasation into tissues. This last step involves the platelet

endothelial cell adhesion molecule (PECAM)-1 and leukocyte cell surface proteinases, which are involved in the breakdown of endothelial cell junctions and extracellular matrix degradation. The above processes of extravasation and chemotaxis are driven particularly by the  $\alpha$  and  $\beta$  chemokines.

### MECHANISMS OF TISSUE INJURY

Once the activated cell enters tissues further activation of antigen-specific and non-specific effector mechanisms may take place resulting in tissue damage. One of the important cell interactions involved in tissue damage involves the cytotoxic T cell. CTLs are predominantly of the CD8+ phenotype, which interact with antigen in association with class I MHC expressed on tissue cells. Cell surface MHC class I is rapidly upregulated by any inflammatory response, particularly by mediators such as TNF and IFN- $\gamma$ . This increased expression is important in clearance of viral antigen from tissues but also potentiates tissue damage in allograft rejection.

Cytotoxic T cells express important cell surface molecules such as Fas ligand, CD40 and cell-surface TNF. These molecules interact through their respective ligands such as Fas, CD154 and the TNFR and may induce cells to undergo programmed cell death or apoptosis. Activation of these pathways may also result in intracellular oxidative stress and cell necrosis. The pathways of apoptosis are similar in many cells,

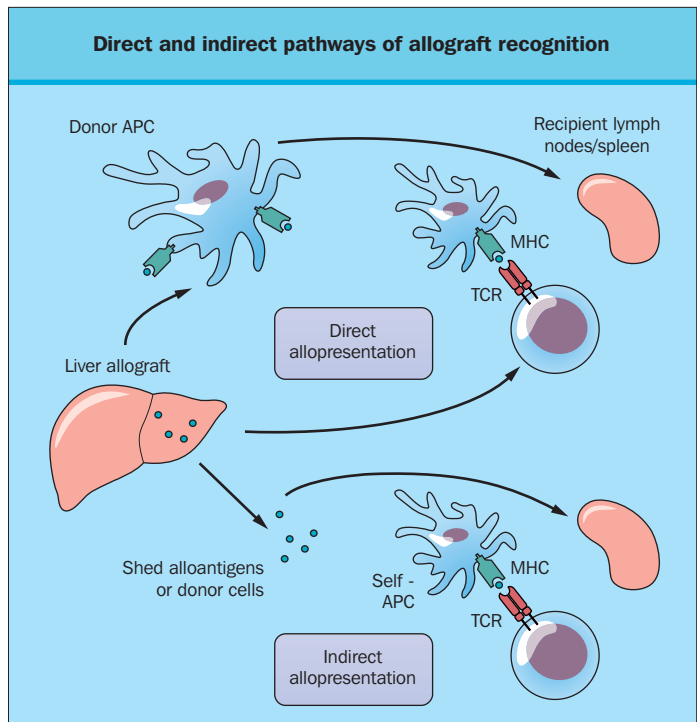
and have been described in great detail over the past 1–2 years. They are not discussed in detail here. Cellular apoptosis is triggered via a 'death domain' amino acid motif on the cytoplasmic tail of TNFRs (e.g. Fas), which are members of a larger family sharing these motifs. Cytotoxic T cells also produce various enzymes such as granzymes and perforin, which attack target cells and cause cell injury and death through apoptotic and necrotic pathways. Cells triggered in non-specific effector responses include NK cells, which were initially described in vitro and whose role in vivo has been difficult to define. They recognize and kill targets that do not express MHC class I molecules, and therefore their role in allograft rejection is probably minimal. They in fact carry receptors for MHC class I molecules, so that their activation is inhibited once the interaction between that receptor and MHC class I takes place.

Activated T cells, particularly of the CD4+ phenotype, produce significant amounts of IFN- $\gamma$  and other molecules that turn on and activate macrophages. These cells are primarily involved in the DTH reaction, and they produce numerous cytokines such as TNF- $\alpha$ , IL-1, IL-6, IL-10 and TGF- $\beta$ . All these molecules are important in cell injury via various mechanisms. The specific molecular pathways that are important in allograft rejection itself are discussed on the previous page.

Antibody production within non-lymphoid organs is probably minimal, but serum-derived antibody produced in lymph nodes and spleen may enter organs, activate complement and cause cellular injury. Such events are important in hyperacute allograft rejection and xenoallograft-associated rejection. The cellular target of serum antibody is likely to be the endothelial cell.

## Th1 VERSUS Th2 CYTOKINE RESPONSE

One of the paradigms that is important in tissue injury, inflammation and regulation of the immune response, including outcomes from allograft rejection, has been the Th1/Th2 paradigm. This refers to the existence in some experimental systems of polarized cytokine responses following T-cell activation. The Th1 cytokine response involves the production of predominantly INF- $\gamma$ , IL-2, and TNF- $\beta$  (lymphotoxin), and results in macrophage activation and DTH reactions. In contrast, the Th2 response involves the production of IL-4, IL-5, IL-10 and IL-13, is associated with strong antibody responses including IgE production, and results in cross-inhibition of the Th1 response, predominantly via IL-4 and IL-10. Although the paradigm was initially worked out on isolated CD4+ T-cell clones in vitro, it has been observed in vivo following various immunization schedules and during experimental and clinical infectious states. For example, human tuberculoid leprosy is associated with a strong Th1 response at the site of the lesion, while lepromatous leprosy is associated with Th2-type responses. In experimental leishmaniasis, the BALB/c mouse is associated with systemic infection and progressive disease and a Th2-type response, while in the CH3B6 mouse leishmania is controlled and associated with a DTH Th1-type response. It is unclear how these responses are diverted at the early stages of T-cell activation. It is thought that IL-12 production and IFN- $\alpha$  production from perhaps macrophages and NK cells are important in the induction of the Th1 response. In contrast, IL-4, either produced from macrophages, T cells themselves, or mast cells, is crucial in directing the response towards a Th2 phenotype. In some experimental



**Figure 39.11** Direct and indirect pathways of allograft recognition.

situations, the Th1 response has been associated with allograft rejection, while the Th2 response has been associated with allograft tolerance.

## ALLOANTIGEN RECOGNITION

The description of the immune response is formulated on the recognition of foreign antigen, consisting of foreign protein or infectious organisms, by the immune system. However, the response against an organ allograft is complicated by the presence of allo-MHC as well as self-MHC cell surface molecules. This has led to the concept that T-cell stimulation following allograft transplantation can occur by direct and indirect pathways (Fig. 39.11).

The direct T-cell response is defined as the stimulation of T cells by the allogeneic MHC antigen on allogeneic APCs. This response does not require intracellular processing and presentation of peptides in order to stimulate the T-cell response. Quite extraordinarily, when this response is measured in vitro, the frequency of precursor T cells for a direct alloimmune response is about 100-fold greater than that for a response to individual peptides derived from environmental antigens. The direct pathway is likely to involve two kinds of APCs. Initially, in tissues such as the liver, true APCs such as dendritic cells or macrophages are likely to be the main source of antigen presentation. Secondly, endothelial cells of the graft themselves which have been induced to express increased amounts of MHC, particularly class II and adhesion molecules, may also stimulate an allo-response. In contrast to direct T-cell activation, allostimulation may take place by normal physiological antigen processing. This is called indirect antigen presentation. In this situation, peptides derived from alloantigen combine with self- (recipient-) MHC molecules and are presented to the immune system as foreign



antigen. Indirect T-cell activation is likely to depend more on the APCs in the recipient's draining lymph nodes and spleens, being primed either by soluble antigen or cellular antigen itself, following degradation into allopeptides.

Although it is thought that the direct pathway is crucial in allograft recognition, there is evidence that indirect antigen presentation actually does occur, as it is possible to immunize with allo-MHC peptides and induce recognition responses and even accelerate experimental rejection. The process may not be as efficient as the direct pathway and may play an important role in the processes of chronic allograft rejection.

## CELLULAR AND MOLECULAR BASIS OF ACUTE AND CHRONIC ALLOGRAFT REJECTION (GENERAL)

### Acute allograft rejection

The initial target of the acute allograft rejection response in vascularized allografts is the microvasculature. The microvasculature has high constitutive expression of MHC molecules and other adhesion molecules. Furthermore, the microvasculature has a very narrow lumen. This combination predisposes to leukocyte-endothelial cell interactions. The predominant cells that mediate acute rejection are T cells, particularly the T-helper CD4+ subset. The molecular mechanisms particularly involve a DTH reaction, with macrophage activation and production of cytokines such as IL-2, INF- $\gamma$ , TNF- $\alpha$  and lymphotoxin. The T-helper subset also triggers alloreactive CTL production. Alloreactive CTLs have been isolated from allografts during rejection, and there is evidence of increased messenger RNA and protein for granzyme and perforin, major components of the CTL effector pathway. These CTLs are predominantly CD8+ and have been shown to be important, not so much in determining whether rejection occurs, but in the speed of the rejection process. As well as the recruitment of macrophages, there is evidence for the recruitment of other components of the non-specific immune system. In particular the presence of eosinophils and eosinophil protein products (eosinophilic cationic protein) and IL-5 have been correlated with acute rejection. Recently much interest has been shown in studying 'final common pathways' in tissue damage during acute rejection, particularly the role of nitric oxide and oxidative stress.

The role of antibody in causing tissue damage during acute allograft rejection has recently received more attention particularly in a subset of renal allograft recipients. This subset is identified by the deposition of the classical complement pathway component C4d. This form of rejection may be more severe and resistant to therapy than pure cellular rejection.

### Chronic allograft rejection

Chronic allograft rejection usually results from uncontrolled acute rejection episodes, but it does have some distinct pathogenic mechanisms not seen in acute rejection. The microvasculature, an initial target during acute rejection, is totally obliterated in the chronic rejection process. In conjunction with obliteration of the microvasculature, there is the development of a process known as 'transplant vasculopathy' that affects medium-sized arteries. This vasculopathy is a result of antigen-specific and

antigen non-specific damage to arterial endothelium. The initiating stimulus is a cell-mediated immune response directed against allo-MHC molecules. This results in the accumulation of CD4+- and CD8+-activated T cells within the vessel wall and the consequent activation of macrophages. The release of chemotactic factors such as membrane cofactor protein (MCP)-1, MIP-1 $\alpha$ , and MIP-1 $\beta$  seems to be crucial in the resulting macrophage activation and proliferation. The release of platelet-derived growth factor from macrophages, adherent platelets and damaged endothelium is a major stimulator for smooth muscle proliferation. Transforming growth factor- $\beta$  production by macrophages leads to the transition of smooth muscle cells to the myofibroblasts which secrete significant extracellular matrix protein resulting in vessel (subintimal) fibrosis. Similar processes also take place in the interstitium of various organs resulting in organ fibrosis.

## DRUGS THAT INHIBIT THE IMMUNE SYSTEM: PHARMACOLOGY AND MECHANISMS OF ACTION

### Corticosteroids

Corticosteroids are chemically modified derivatives of natural adrenal hormones and are used in various forms, such as prednisolone, hydrocortisone and methylprednisolone. They have similar efficacy apart from hydrocortisone, which acts at 1:5 strength compared with prednisolone and methylprednisolone. Corticosteroids have numerous mechanisms of action in inhibiting the immune system. At very high doses they cause lympholysis/apoptosis and margination of lymphocytes resulting in significant lymphopenia. They also inhibit macrophage/dendritic cell antigen-presenting function via inhibition of transcription of cytokines such as IL-1 and TNF- $\alpha$ , and by inhibiting arachidonic acid metabolism. Corticosteroids inhibit the first enzyme of the arachidonic acid metabolic pathway: phospholipase A2. This leads to blockage of the cyclo-oxygenase and 5-lipoxygenase pathways with the resulting inhibition of thromboxane and prostacyclin production. Corticosteroids also inhibit interferon-dependent adhesion molecule expression including MHC class II. Furthermore, there is some evidence that corticosteroids stimulate TGF- $\beta$  production, a known inhibitory cytokine resulting in a more Th2-type cytokine profile (anti-inflammatory) rather than Th1 cytokine production.

### Azathioprine

Azathioprine is an imidazole derivative of 6-mercaptopurine, and is well-absorbed and converted to 6-mercaptopurine and to 6-thio-inosine monophosphate in the liver. Xanthine oxidase is required for azathioprine metabolism with conversion to 6-thiouric acid, followed by excretion in the urine. These chemicals are alkaloid DNA precursors and azathioprine leads to reduced bioavailability of purine molecules by inhibition of inosine monophosphate and the de novo purine pathway. Azathioprine also blocks phosphoribosyl pyrophosphate (PRPP), thus blocking purine synthesis via the salvage pathway. The overall effect of azathioprine is to inhibit lymphocyte proliferation at the G1-S phase transition (i.e. following signaling via the T-cell receptor/adhesion molecule cascade).

### Cyclosporin

Cyclosporin was isolated from the fungus *Trichoderma polysporum*. It is a cyclic endecapeptide. It is currently available as a microemulsion (Neoral). Before this microemulsion form was available, absorption of cyclosporin (Sandimmune) showed large individual variation with an average oral bioavailability of only 30%, but varying between 5 and 90%. Cyclosporin (Sandimmune) is highly lipid-soluble, and dependent on bile salt availability in the gastrointestinal lumen. The microemulsion form (Neoral) has an improved overall bioavailability, with less individual variation and less dependence on bile salts for absorption. The 'area under the curve' profile increased by 1.5–3 fold with conversion to the microemulsified preparation of cyclosporin. Cyclosporin is highly metabolized in the gastrointestinal tract and the liver by the cytochrome P450 complex, mainly cytochrome P450 3A4. This results in numerous metabolites that are excreted in the bile. Less than 1% of cyclosporin is excreted unchanged. There is evidence of enterohepatic circulation.

Cyclosporin exerts its immunosuppressive actions by binding to a cytoplasmic protein called cyclophilin, the prototype member of the immunophilin family. Cyclophilin is a highly basic, abundant protein present in many tissues. It is an 18 kDa cis-transpeptidyl-propyl isomerase ('rotamase') which plays a role in protein folding. However, this action is not related to the drug's immunosuppressive property. Cyclosporin forms a complex with cyclophilin and this complex binds with high affinity to calcineurin (a cytoplasmic calcium-dependent phosphatase) resulting in inhibition of its phosphatase function. The phosphatase function of calcineurin is crucial in the activation of the NFAT<sub>c</sub> transcription factor resulting in its translocation into the nucleus to act as a transcription factor for the IL-2 molecule. This step is effectively blocked by the cyclosporin cyclophilin/calcineurin complex. Cyclosporin has two domains: one involved in the drug interaction with cyclophilin itself; and the effector domain that is a composite of the cyclosporin and the cyclophilin itself, which interacts with calcineurin. The interaction between cyclophilin and cyclosporin is highly dependent on the hydrophobic side chain of the 6-methyl leucine in cyclosporin. Also, it has been shown that tryptophan at position 121 in cyclophilin itself is required for calcineurin inhibition. The overall effect of cyclosporin is to block the early cytokine transcription gene and G0–G1 transition following T-cell activation.

### Tacrolimus

Tacrolimus is a cyclic macrolide antibiotic similar to erythromycin. It was derived from the soil-based micro-organism *Streptomyces tsukubaensis*. It is well absorbed from the gastrointestinal tract, not influenced greatly by the presence or absence of bile in the gastrointestinal lumen, and like cyclosporin is extensively metabolized by the cytochrome P450 system and excreted in bile. The mechanism of immunosuppressive function is very similar to that of cyclosporin. It also binds to a cytoplasmic immunophilin. This immunophilin, known as FK-binding protein (FKBP), is a 12 kDa protein consisting of many isoforms, the most important of which is FKBP-12. In mice a recent isoform FKBP-51 has been shown to be mainly restricted to T cells. Its importance is currently under study. Like cyclosporin, the FK/FKBP complex then binds to calcineurin

resulting in inhibition of its phosphatase activity, inhibition of translocation of NFAT<sub>c</sub> to the nucleus, and inhibition of early transcription of genes such as IL-2. FK/FKBP and cyclosporin/cyclophilin bind at two overlapping but distinct sites within a region on calcineurin. Therefore, they do compete with each other for immunosuppressive function and thus are unable to be used simultaneously. There are three-dimensional crystal structures of the tacrolimus/immunophilin complex available, indicating a similar formation of an immunophilin-binding domain and an effector domain, as in the cyclosporin/immunophilin complex.

### Rapamycin

Rapamycin is also a cyclic macrolide. It is also derived from a soil-based micro-organism, *Streptomyces hygroscopicus*. It is well-absorbed and metabolized by the cytochrome P450 system. Rapamycin, like cyclosporin and tacrolimus, mediates its action by binding to an immunophilin. In fact it binds to the FKBP immunophilin, particularly FKBP-12. However, it has no effect on the phosphatase activity of calcineurin. In contrast to tacrolimus, rapamycin does not undergo a dramatic conformational change when it binds to FKBP-12. Instead of binding to calcineurin, the complex of rapamycin/FKBP targets two cytoplasmic proteins associated with G1–S phase cell cycle progression. These molecules are known as targets of rapamycin TOR-1 and TOR-2. These 200 kDa proteins are highly expressed in human tissues, particularly in the testis and skeletal muscle. The two proteins have 67% identity but their exact function has not been totally defined. They seem to be cytoplasmic kinases involved in activation cascades that eventually turn on cytokine genes. The rapamycin/FKBP complex has been shown to bind to a 90 amino acid region adjacent to a lipid kinase domain within TOR-1/TOR-2. A crucial serine motif in this complex seems important in blocking TOR-1/TOR-2 function. Apart from the affect on TOR-1/TOR-2, there is evidence that rapamycin may also inhibit the promoter activity of proliferating cell nuclear antigen which is important in cell cycle progression within many cells. Apart from its effect on lymphocytes in blocking immune function, rapamycin also inhibits the proliferation of other cell types and, in terms of allograft rejection, inhibition of smooth muscle proliferation may be important in its effects on chronic rejection. Thus, rapamycin blocks the second set of phosphorylation events following the binding of IL-2 to the IL-2 receptor and the release of other cytokines, thereby acting later in the phase of lymphocyte activation than cyclosporin and tacrolimus.

### Mycophenolate mofetil

Mycophenolic acid (MPA) was originally isolated from the fungal genus *Penicillium* and has been modified by a synthetic morpholinoethyl side chain ester resulting in the production of mycophenolate mofetil (MMF). This modification significantly improved oral bioavailability of MPA. Mycophenolate is converted back to MPA and undergoes conjugation in the liver. The drug is well-absorbed and primarily (87%) eliminated in the urine. Its half-life is approximately 12 h. MPA is a non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), the key enzyme that converts inosine monophosphate (IMP) to adenosine monophosphate (AMP), thus effectively blocking the rate-limiting enzyme step in the de novo purine

synthetic pathway. The drug seems more selective for lymphocytes because the IMPDH isoform-2 is five to six times more sensitive to MPA than the IMPDH isoform-1 enzyme. The IMPDH isoform-2 is predominantly expressed in lymphocytes and monocytes and this, in conjunction with the fact that lymphocytes tend not to use the salvage pathway for purine metabolism, results in the relative selective action of MMF on lymphocyte activation. The depletion of guanosine purine-type precursors also results in the inhibition of transfer of fructose and mannose to the cell surface of various glycoproteins. This seems particularly to affect adhesion molecules. Thus a further action of mycophenolate may be to inhibit lymphocyte binding to endothelium and extravasation into inflamed tissues. MMF not only has an effect on T- and B-cell proliferation, but also inhibits smooth muscle cell proliferation and thus has potential in the treatment of chronic rejection.

### **Antibodies (ALG, OKT3, IL-2 receptor)**

Antibodies directed against antigens on human leukocytes are used as immunosuppressive therapy. Antithymocyte globulins (ATGs) or antilymphocyte globulins (ALGs) are raised in horses, goats or dogs and have been extensively used in human renal transplantation. Currently they have largely been replaced by the mouse monoclonal antibody OKT3 directed against the  $\epsilon$ -chain of the human CD3 complex and is thus specific for human T cells. OKT3 is given as a bolus intravenous injection and leads to the disappearance of all T cells in the peripheral blood within 30–60 min. This effect is due to a combination of cell apoptosis/lysis, cell marginalization, opsonization, and antigenic modulation of CD3/TCR from the cell surface. After 2–3 days of therapy, a small number of antigenically modulated cells appear in the peripheral blood, but these cells are non-functional. Other monoclonal antibodies (mAbs) against other T-cell antigens have been used experimentally, but have not met with significant clinical use.

The synthesis of 'humanized' mAb directed against the IL-2 receptor  $\alpha$ -chain has resulted in high affinity reagents with powerful immunosuppressive properties. These are currently under study and preliminary evidence suggests that they result in a significant decrease in acute rejection episodes in renal transplant recipients. These agents are being mainly used in induction regimens and with a half-life of approximately 20 days can be administered monthly.

### **Future clinical therapies**

A particularly interesting new drug is the molecule known as FTY 720. This compound acts on sphingosine-1-phosphate receptors leading to sequestration of lymphocytes to secondary lymphoid tissues and away from inflammatory organ responses such as allografts. It has been shown to be effective in experimental models of allograft rejection and in phase III trials in human non-liver transplantation. Side-effects are not prominent although some negative inotropic effects on the heart have been observed. A further new drug is on the horizon known as CP-690,550. This is a new JAK-3 inhibitor and it has been used to prolong renal allograft survival in non-human primates.

There is also considerable interest in the development of new mAbs against other adhesion molecules such as the CD28/

CD80 pathway and against molecules involved in lymphocyte endothelium interactions such as selectins. The humanized anti-CD52 mAb CAMPATH-1 is currently under study in both non-liver and liver transplantation. This mAb has profound lymphodepletion properties.

## **USE OF IMMUNOSUPPRESSIVE DRUGS TO PREVENT ACUTE REJECTION IN LIVER TRANSPLANTATION**

Currently the most common prophylactic therapeutic combination that has been used is based around initial triple immunosuppressive therapy with corticosteroids, azathioprine/mycophenolate, and tacrolimus/cyclosporin. High dose induction corticosteroid regimens usually consist of 500 mg at the time of transplant, reducing to 20–30 mg within 7–10 days. Lower dose regimens consist of just 1 mg/kg in tapering fashion. Some units withdraw corticosteroids at 3 months without any significant increase in acute rejection. Withdrawal of corticosteroids at varying time points post-transplant has received more support in recent times and is associated with improvement in side-effect profiles and little increase in frequency of allograft rejection. Corticosteroid-free protocols are being developed.

The calcineurin inhibitors, tacrolimus and cyclosporin, remain the pivotal drugs in the maintenance immunosuppression regimens. Tacrolimus usually starts at a dose of 0.1 mg/kg/day, while cyclosporin is given at a dose of 10 mg/kg/day. Absorption of tacrolimus and cyclosporin in the first 24–48 h is variable, but this does not seem to decrease their effectiveness. Tacrolimus is given in two divided doses and the dose is titrated to maintain 12-h trough levels in the 8–12 ng/mL range initially and in the 4–8 ng/mL range after 3–6 months. Drug doses are also adjusted according to side-effects and allograft rejection status. Cyclosporin is also given twice daily and doses are adjusted according to either 12-h trough (C0) or 2-h post-intake (C2) levels. The latter has recently been advocated to minimize allograft rejection and side-effects. Target C2 levels in the early post-transplant phase are approximately 1600–1800 ng/mL, whilst later are in the 800–1000 ng/mL range. Trough drug levels are usually maintained in the 100–300 ng/mL level or lower in long-term patients. Two controlled trials have compared tacrolimus to cyclosporin therapy. One study compared tacrolimus to cyclosporin with C0 monitoring and showed better outcomes for tacrolimus therapy. The other compared tacrolimus to cyclosporin with C2 monitoring and showed no difference in the frequency of allograft rejection, but more diabetes and diarrhea was seen in the tacrolimus arm.

Although azathioprine is still used on a background of corticosteroid and tacrolimus/cyclosporin therapy, many units, particularly in the USA, have replaced it with MMF. The dose recommended in liver transplantation is 2 g/day. A multicenter international trial directly comparing mycophenolate and azathioprine in liver transplantation (in conjunction with corticosteroids and cyclosporin using C0 monitoring) showed no difference in patient or allograft survival but did find a 10% reduction in frequency of acute allograft rejection.

Rapamycin has been evaluated as a part of induction immunosuppression in liver transplant patients. In a randomized controlled trial, patients receiving rapamycin had worse patient



and allograft survival, increased wound breakdown, increased infections and more vascular thromboses. Thus it is currently not recommended as part of primary induction immunosuppression in liver transplantation. It is however under study at lower doses than used in the initial trial.

The use of mAbs as part of immunosuppression induction protocols has not been common in liver transplantation. The addition of prophylactic OKT3 or ALG therapy, so-called 'quadruple therapy', did not lead to a significant decrease in rejection rates or alteration in outcomes. More recently the use of IL-2 receptor mAbs has been assessed. In a randomized controlled trial IL-2 receptor mAb induction was associated with a decrease in acute rejection in non-hepatitis C virus (HCV) infected patients but no difference in patient or allograft survival was seen.

### USE OF IMMUNOSUPPRESSIVE DRUGS TO TREAT ACUTE REJECTION

Moderate to severe acute allograft rejection is usually treated with a 3-day pulse of corticosteroid therapy, at 1 g/day then tapering back to base line levels (Fig. 39.12).

Depending on the baseline regimen, calcineurin inhibitor doses may be increased and azathioprine may be replaced by MMF. Increasingly mild episodes of acute rejection are simply treated by increasing the doses of the maintenance immunosuppressive drugs. This is particularly the case in patients on tacrolimus therapy. In some instances, a patient on cyclosporin is converted to tacrolimus without a corticosteroid pulse. Patients who do not respond to pulsed corticosteroid therapy may undergo repeat therapy or receive OKT3 as a rescue strategy.

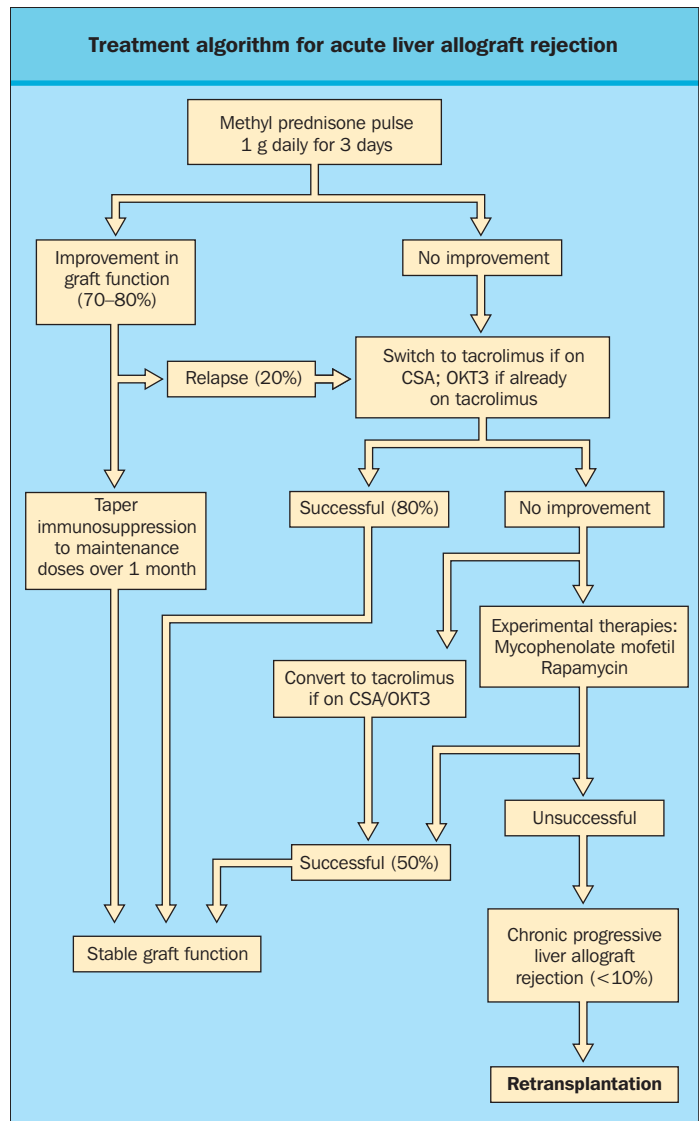
### DRUGS THAT INHIBIT THE IMMUNE SYSTEM: SIDE-EFFECTS AND DRUG INTERACTIONS

#### General side-effects of immunosuppressive agents

All immunosuppressive drugs predispose the recipient to infection and the development of neoplastic disease, and it is important to remember the close correlation between suppression of the immune system to facilitate transplantation and these side-effects. The infections predisposed to by immunosuppressive drugs include the activation of the herpes viruses [cytomegalovirus (CMV), herpes simplex virus (HSV) and herpes zoster] and other opportunistic infections. There is also a predisposition to fungal and bacterial infections. General immunosuppression leads to the development of lymphoproliferative diseases and the development of epithelial tumors. Lymphoproliferative disease occurring relatively early post-transplant in the setting of immunosuppression is often associated with Epstein-Barr virus (EBV). Apart from these general side-effects, each individual immunosuppressive drug has particular side-effects that are exacerbated or decreased by certain drug interactions (Table 39.2).

#### Corticosteroids

The side-effects of corticosteroids are numerous and are to a significant degree dose-dependent. The most common effects involve the musculoskeletal problems of osteoporosis, osteonecrosis and myopathy. Associated with these are the endocrine



**Figure 39.12** Treatment algorithm for acute liver allograft rejection.

effects of glucose intolerance with the development of overt diabetes, hypokalemia, and fluid retention and associated hypertension. There are effects on the neuropsychiatric system, such as psychosis, insomnia and depression, which are usually associated with higher doses. Cataracts commonly occur with prolonged use. The common gastrointestinal effects include dyspepsia and the predisposition to peptic ulceration. The skin bruises easily, atrophies and develops striae and acne. Corticosteroids are not contraindicated in pregnancy.

#### Azathioprine

The main side-effect of azathioprine is bone marrow depression, particularly leukopenia and thrombocytopenia. Other side-effects include mild alopecia, gastrointestinal upset and retinopathy. It should be also recognized that azathioprine may cause pancreatitis, cholestatic hepatitis and vascular abnormalities within the liver with the development of nodular regenerative hyperplasia and veno-occlusive disease. Its toxicity is increased by interaction with allopurinol, a xanthine oxidase inhibitor. Xanthine oxidase is involved in the metabolism of azathioprine

Summary of major side effects and drug interactions of immunosuppressive therapies		
Drug	Side effects	Drug interaction
Corticosteroids	Glucose intolerance, delirium, hypertension, osteoporosis, cataracts	N/A
Azathioprine	Thrombocytopenia, neutropenia 'allergic' reaction, pancreatitis	Allopurinol
Calcineurin inhibitors (cyclosporin or tacrolimus)	Hypertension, renal dysfunction, headaches, hair growth, central nervous system problems, hyperkalemia	Increased levels: diltiazem doxycycline erythromycin itraconazole omeprazole protease inhibitors (HAART)
		Decreased levels: rifampin phenytoin carbamazepine
Mycophenolate mofetil	Diarrhea, dyspepsia, neutropenia, thrombocytopenia, headache, skin rash	Not known
Rapamycin	Hyperlipidemia	Not known
OKT3/ALG	'Cytokine release' syndrome: aseptic meningitis bronchospasm fever, chills, rigors myalgias hypotension	Nil significant

**Table 39.2 Summary of major side effects and drug interaction of main immunosuppressive therapies.**

and these drugs should not be used in combination. Azathioprine may also cause an allergic reaction in <1% of individuals resulting in high fevers, arthralgia and myalgia. Re-introduction of azathioprine in these circumstances reproduces the syndrome. Azathioprine is not contraindicated in pregnancy.

### Cyclosporin

Cyclosporin has quite a narrow therapeutic window. Its most common side-effects include hypertension (over 30% of cases) and nephrotoxicity. There is an almost universal decrease of 30% in the glomerular filtration rate, and a minority of patients gets more severe renal dysfunction. The nephrotoxicity is associated with structural changes within the kidney, particularly the development of interstitial fibrosis. Cyclosporin also has a tendency to cause hyperkalemia. Its other toxicities include effects on the central and peripheral nervous systems and it commonly causes tremor, headache, seizures (early complication) and may cause peripheral neuropathy. It has been associated with leukoencephalopathy, which mainly involves the occipital cortical pathways and may result in coma and the development of cortical blindness. Hirsutism and gingival hyperplasia commonly occur and can be distressing in females and children. It may also cause breast adenofibromatosis. Respiratory side-effects include sinusitis and sinus congestion.

The side-effects of cyclosporin are largely dose related, and toxicity can be enhanced by drug interactions that result in higher cyclosporin levels, including:

- calcium channel blockers, particularly diltiazem and verapamil;
- antibiotics such as doxycycline, clarithromycin and erythromycin;
- itraconazole and ketoconazole; and
- protease inhibitors.

Apart from these direct drug interactions on levels, neurotoxicity seems to be increased with the interaction with the antibiotic imipenem, and nephrotoxicity is increased with the use of aminoglycosides, non-steroidal anti-inflammatory drugs (NSAIDs), amphotericin, and intravenous bactrim. Angiotensin-converting enzyme inhibitors, used for the treatment of cyclosporin-associated hypertension, increase the hyperkalemic effect of cyclosporin.

Decreased cyclosporin levels can also result from drug interactions and this may predispose to allograft dysfunction. Cytochrome P450 enzyme inducers, such as the anticonvulsants (phenytoin, barbiturates), the antituberculous drugs [isoniazid (INH) and rifampin (rifampicin)], octreotide and bactrim are particularly important in this regard. Cyclosporin is not contraindicated in pregnancy.

### Tacrolimus

The side-effects of tacrolimus are very similar to those of cyclosporin, apart from a few significant differences. The renal nephrotoxicity is almost identical, although the neurotoxicity seems to be worse. Tacrolimus causes more diarrhea than cyclosporin. There seems to be less hypertension, but there is

increased glucose intolerance with tacrolimus. There is certainly less gingival hyperplasia and hirsutism as compared with cyclosporin. Tacrolimus is thought to be associated with a 'lower cardiovascular risk' lipid profile than cyclosporin. The drug interactions with tacrolimus are very similar to those of cyclosporin. Concerns about the teratogenicity of tacrolimus are easing and it is now licensed for use during pregnancy in a number of countries.

### **Mycophenolate mofetil**

The main side-effect is on the gastrointestinal tract, resulting particularly in diarrhea. There may be associated nausea and vomiting. Headache seems to be a common problem. Bone marrow suppression with anemia, leukopenia and thrombocytopenia is less common than with azathioprine, but does occur. MMF is contraindicated in pregnancy.

### **Rapamycin**

The main side-effect of rapamycin is the development of hypertriglyceridemia, which may be quite profound. It also has effects on the gastrointestinal tract with nausea, vomiting and diarrhea. Rapamycin is also associated with a fibrosing pulmonary disease, despite its inhibitory effect on fibroblast proliferation that is clinically associated with impaired wound healing. It is contraindicated in pregnancy.

### **OKT3**

The main effect of this drug is the development of the cytokine release syndrome, resulting in high fevers, arthralgia and myalgia. This syndrome can be quite severe and may be associated with aseptic meningitis, coma, adult respiratory distress syndrome and systemic hypotension. Cardiac arrest has been described with the use of OKT3. It is particularly important that the patient is not fluid overloaded at the time of administration. Side-effects are modified by the use of 1 g of methylprednisolone during the first injection and the regular use of antihistamines and acetaminophen (paracetamol) with each sub-

sequent injection. The cytokine release syndrome is most severe during the first one or two injections. OKT3 causes profound immunosuppression. There is clinical reactivation of herpes viruses (HSV, CMV) in over 70% of cases. Total doses of >75 mg predispose to lymphoproliferative diseases.

## **IMMUNOSUPPRESSIVE STRATEGIES TO DECREASE CALCINEURIN INDUCED NEPHROTOXICITY**

Probably the most common serious side-effect of immunosuppressive drugs is the nephrotoxicity associated with cyclosporin and tacrolimus therapy. The frequency of this problem increases with time post-transplant. There are now several studies that have shown that calcineurin inhibitors can be withdrawn in liver transplant patients and replaced with MMF resulting in improvement in renal function in some but not all patients. In this situation, MMF is usually used in combination with a low dose of corticosteroids in order to minimize the risk of rejection. Similar but less extensive data are available for sirolimus. However, access to this drug is more restricted.

## **LIVER ALLOGRAFT REJECTION**

### **Hyperacute liver allograft rejection**

This is an uncommon event in liver transplantation with only a small number of cases reported in the literature. It may come on within the first 1–2 days after transplantation, but usually does not appear until the second week (Table 39.3).

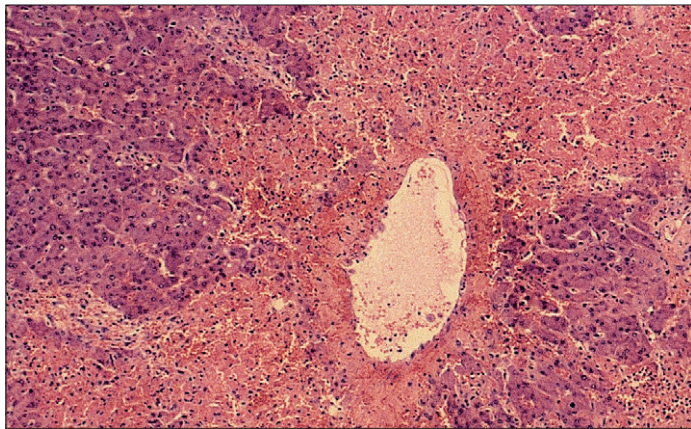
It is characterized by a rapid deterioration of graft function, often with the clinical picture of acute liver failure. There is usually a high fever, right upper quadrant pain, rapidly deteriorating liver function tests with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at levels of >1000 IU/L. The international normalized ratio (INR) becomes rapidly prolonged and there is profound thrombocytopenia and acid/base disturbance.

Hyperacute rejection is an antibody and complement mediated attack on the vascular endothelium. The antibodies that result in the hyperacute rejection syndrome are either preformed HLA

<b>Patterns of liver allograft rejection</b>				
<i>Type of rejection</i>	<i>Clinical</i>	<i>Biochemical changes</i>	<i>Biopsy features</i>	<i>Molecular/cellular pathways</i>
Hyperacute	Within 10 days of transplant, acute liver failure	AST > 1000 µ/L AST > 1000 µ/L INR prolonged hypoglycemia	Hemorrhagic necrosis	Antibody and complement
Acute	Any time, commonest days 7–9 post-transplant, nonspecific symptoms (if any)	Any pattern	Mixed portal infiltrate, endotheilitis, bile duct damage	DTH, T cells, cytokines
Chronic	Any time after the first month post-transplant, jaundice and cholestasis	AP, γ-GT markedly elevated, hyper-bilirubinemia	Loss of inter-lobular bile ducts, portal tract fibrosis, a decrease in cellular filtrate	T cells, macrophages, growth factors, stellate cells

**Table 39.3 Patterns of liver allograft rejection.**





**Figure 39.13** Liver biopsy features of hyperacute liver allograft rejection showing widespread hemorrhagic necrosis. (Hematoxylin and eosin)

antibodies against the donor MHC antigens, or against blood group antigens. The characteristic pathologic finding is that of massive hemorrhagic necrosis associated with complement, fibrin and immunoglobulin deposition in arteries, veins and sinusoids (Fig. 39.13).

There may be segmental infarction of the graft, together with necrosis of the biliary tract. The management is very difficult. Plasmapheresis, cyclophosphamide and OKT3 therapies have been attempted with little success. The only really effective management is that of urgent re-transplantation.

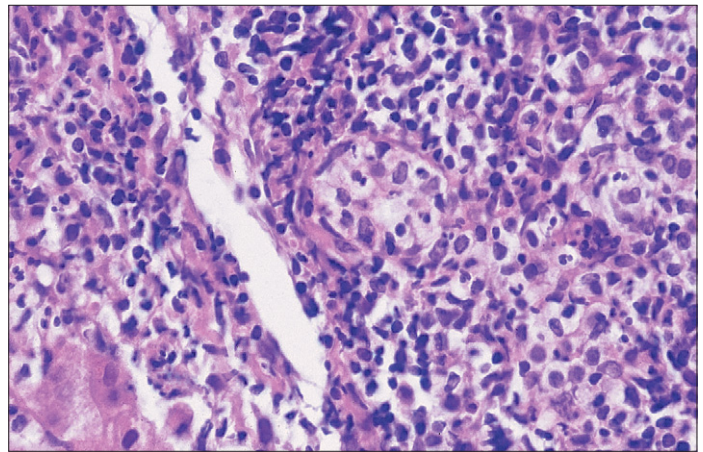
### Acute liver allograft rejection

Acute liver allograft rejection occurs in about 20–40% of patients. It occurs at a mean of 7–9 days after transplant, and is often clinically silent. Occasionally there may be right upper quadrant discomfort and fever. Biochemically there is derangement of the serum bilirubin, alkaline phosphatase (AP),  $\gamma$ -glutamyltranspeptidase ( $\gamma$ -GT), AST and ALT levels. However, these do not conform to a characteristic pattern, and cannot be used to definitively distinguish acute rejection from other causes of liver graft dysfunction. The coagulation status and platelet count is usually not disturbed.

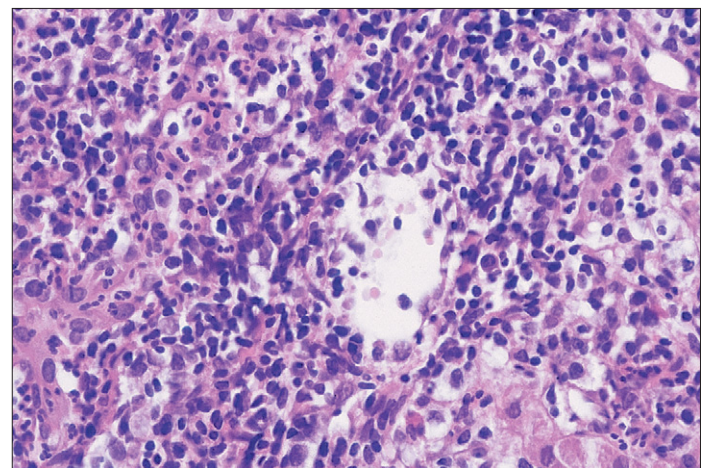
The diagnosis of acute liver allograft rejection is made on liver biopsy and has been defined by an international panel as ‘inflammation of the allograft, elicited by a genetic disparity between the donor and recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal veins and hepatic venules and occasionally the hepatic artery and its branches’. Hence, there are three predominant features:

- portal inflammation;
- bile duct damage; and
- endotheliitis.

At least two of these three features are required for a histopathologic diagnosis of acute rejection. First, a ‘mixed’ portal tract infiltrate consisting of lymphocytes, neutrophils, monocytes and eosinophils is present (Fig. 39.14). The percentage of eosinophils is usually determined by the corticosteroid dose at the time of the acute rejection. If the corticosteroid dose has been high and acute rejection occurs, then the eosinophils are not often seen.



**Figure 39.14** Liver biopsy features of acute liver allograft rejection with portal tract infiltrate and bile duct damage with invading inflammatory cells.

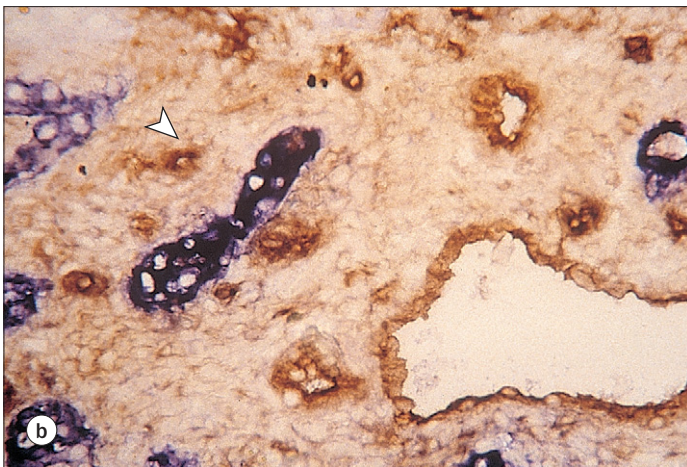
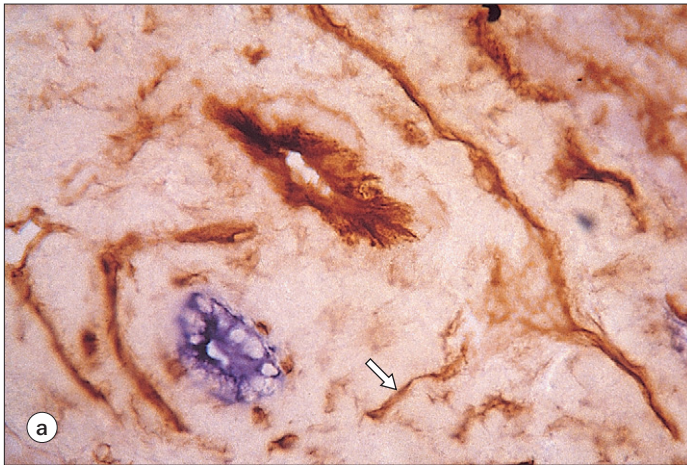


**Figure 39.15** Liver biopsy features of acute liver allograft rejection showing endotheliitis.

The second feature is that of vascular endotheliitis, particularly of the portal tract venules, but sometimes including central veins (Fig. 39.15). This is characterized by activated lymphocytes and monocytes adhering to the vascular endothelium, invading underneath and sometimes lifting off the endothelial structures.

The third feature is inflammation of the interlobular bile ducts with infiltration of the epithelium with lymphocytes and monocytes (see Fig. 39.13). In more advanced cases, there is flattening of the epithelium and an increasing eosinophilic component to the cytoplasm and loss of nuclei from individual cells. Immunohistochemical studies have shown that the lymphocytic infiltrate within portal tracts is of both the CD4+ and CD8+ subsets, while infiltration of the biliary epithelium is predominantly by CD8+ cells. The molecular pathogenesis of acute liver allograft rejection is associated with increased intrahepatic expression of IL-2, INF- $\gamma$ , and IL-5 (particularly if eosinophils are present). Studies have also indicated that there





**Figure 39.16 Identification of microvascular portal tract structures (brown staining).** (a) In normal liver and (b) their reduction during acute liver allograft rejection.

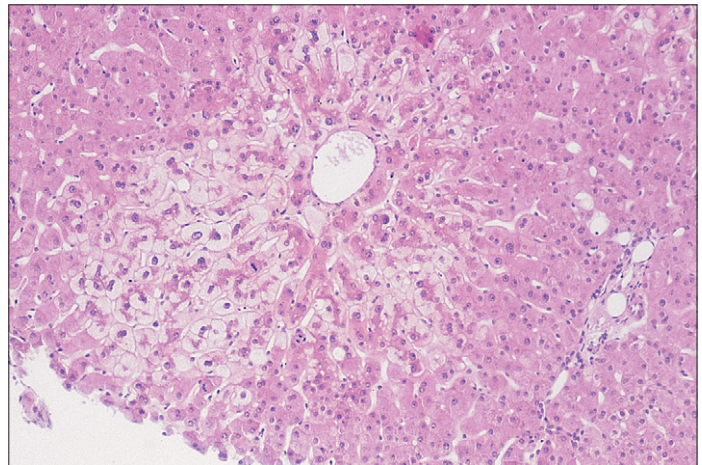
is loss of portal tract microvascular structures during acute rejection (**Fig. 39.16**).

Although the three key pathologic changes described above are the basis of diagnosis, other changes may occur. In lobular areas there may be cell necrosis/apoptosis and a lobular portal tract infiltrate consisting mainly of CD8+ cells. The presence of centrilobal ballooning and particularly fallout of hepatocytes in zone 3 of the hepatic lobule suggests that the rejection is severe and may have a vascular component (**Fig. 39.17**).

Very rarely there may be arteritis of small arterioles in portal tracts.

Grading systems for acute liver allograft rejection have been provided by a number of groups. More recently a grading system has been developed by an international panel to classify and grade liver allograft rejection (**Table 39.4**).

This system essentially grades the level of acute rejection as indeterminate, mild, moderate or severe dependent on the degree of portal tract infiltrate. As part of this, a rejection activity index (RAI) can be derived by scoring each of the three main features (i.e. portal tract infiltrate, bile duct damage and endotheliitis) on a score of 0–3 (**Table 39.5**).



**Figure 39.17 Centrilobal ballooning during acute liver allograft rejection suggesting vascular involvement and severe rejection.**

#### Grading of acute liver allograft rejection

Global assessment	Criteria
No rejection	No infiltrate present or the inflammation is related to other causes of dysfunction (viral hepatitis, biliary obstruction, etc).
Indeterminate for rejection	Rejection-type portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of rejection because of lack of direct bile duct damage, or involvement of an insufficient percentage of portal triads.
Mild rejection	Evidence of a mixed portal infiltrate (usually lymphocytic with blasts and often with eosinophils and neutrophils) in at least one-third of the triads, that is generally mild and confined within the portal spaces. Clearly defined bile duct damage is present and subendothelial inflammation of portal or central vein branches is often seen.
Moderate rejection	As above for mild, with the rejection infiltrate expanding most or all of the triads.
Severe rejection	As above for moderate, with spillover of the infiltrate into the periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with hepatocyte necrosis.

**Table 39.4 Diagnosis of acute graft rejection – Banff International Consensus Schema.** This table shows the grading of acute liver allograft rejection.

#### Acute allograft rejection and HCV infection

HCV infection in the liver allograft makes the diagnosis of acute allograft rejection somewhat more difficult. Features of mild/moderate acute rejection can be seen in HCV infection alone. These include the portal tract inflammation, some bile duct damage and interface hepatitis. A predominate allograft rejection

The acute liver allograft rejection activity index		
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 luminal disruption.	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority 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
<b>Total score</b>	(Sum of components) =	9 (max)

**Table 39.5 Diagnosis of acute graft rejection – Banff International Consensus Schema.** This table shows the rejection activity index (RAI). Criteria that can be used to score liver allograft biopsies with acute rejection, as defined by the Banff International Consensus scheme.

Risk factors for chronic rejection
Persistently low CSA levels
Gender (M/F) allograft mismatch
CMV mismatch
Multiple episodes of acute rejection
Severe acute rejection
Late acute rejection
HCV infection and possibly interferon therapy
Positive lymphocytotoxic crossmatch with donor

**Table 39.6 Risk factors for chronic rejection.**

response is favored by a mixed portal tract infiltrate of neutrophils and eosinophils together with more severe bile duct injury or centrilobular cell loss.

### Chronic liver allograft rejection

Chronic liver allograft rejection occurs at a low frequency in human liver transplantation. Currently, experience in most centers indicates a frequency of less than 3–5%. Several factors have been shown to predispose to chronic liver allograft rejection (Table 39.6). These include low cyclosporin levels, multiple episodes of acute rejection, late episodes of acute rejection, CMV infection, positive lymphotoxic antibody cross-matching,

HCV infection and gender mismatching between donor and recipient.

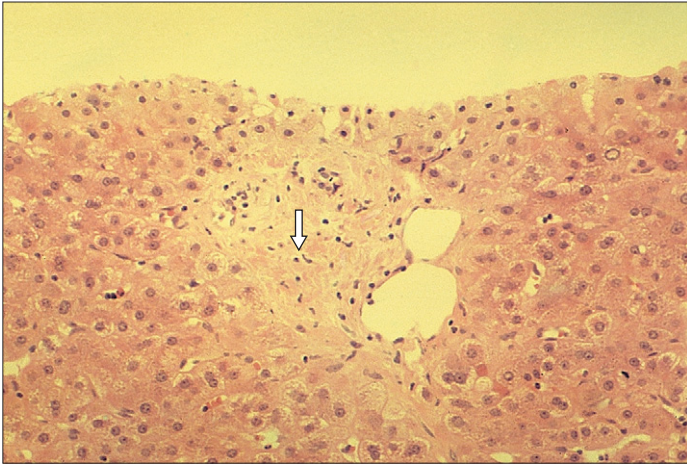
Clinically, chronic rejection is associated with progressive cholestasis, a rise in the serum bilirubin with clinical jaundice, an enlarged liver, increasing alkaline phosphatase and  $\gamma$ -GT with only moderate elevations in AST and ALT levels. The INR and the platelet count are usually normal. The diagnosis is based on liver histology and is characterized by two predominant lesions. First is the loss of interlobular bile ducts, the so-called ‘vanishing bile duct syndrome’. This is associated with loss of microvasculature and arteriolar structures within the portal tract and a decreasing rather than increasing inflammatory infiltrate. The portal tracts often become small and fibrotic (Fig. 39.18).

The second major change occurs in the hepatic lobule with progressive ballooning in zone 3 of the hepatic lobule and cellular fallout with hepatocyte necrosis (Fig. 39.19).

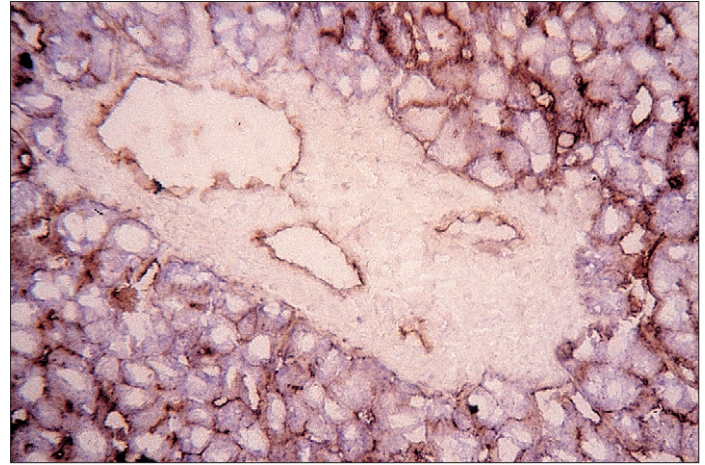
There is marked upregulation of MHC and adhesion molecules on hepatocytes and remaining bile ducts. CD8<sup>+</sup> T cells can still be found attacking bile duct remnants and in lobular zone 3 areas (Fig. 39.20).

Although often not seen on needle biopsy specimens, there is a marked transplant vasculopathy in medium-sized arteries in liver explants or at autopsy (Fig. 39.21). This consists of thickening of the arterial intima, destruction of the internal elastic lamina, and the presence of macrophages (arterial foam cells), smooth muscle cells (myofibroblasts), and thickening and fibrosis of the intima. There is also continuing infiltrate in these lesions of CD4<sup>+</sup>, and CD8<sup>+</sup> cells, as well as macrophages (Figs 39.22 and 39.23).

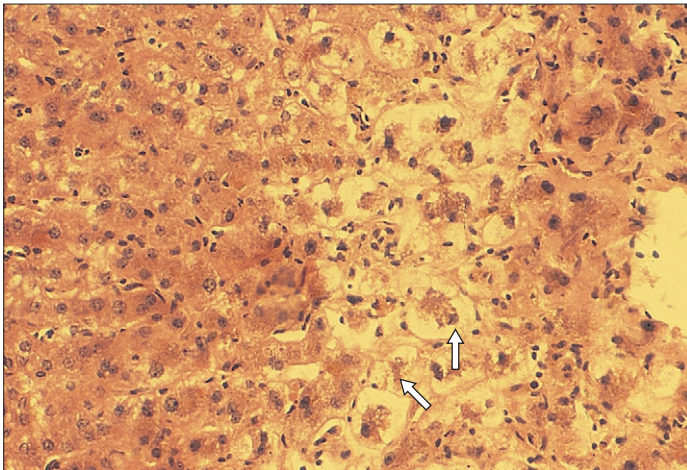




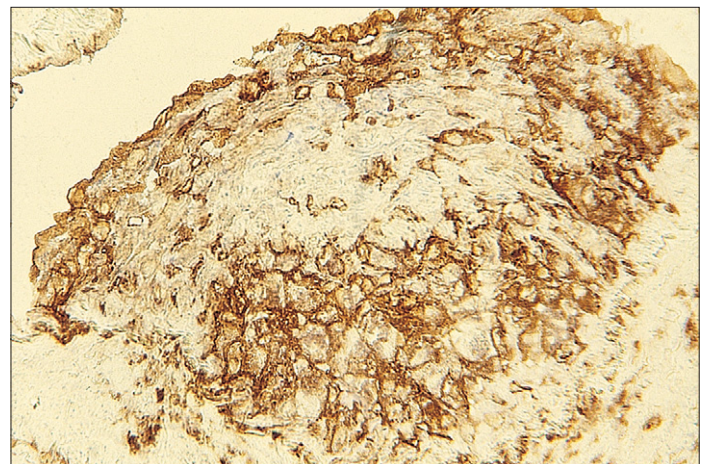
**Figure 39.18 Liver biopsy features of chronic allograft rejection.** Hematoxylin and eosin stained section of portal tract (arrow) showing lack of inflammatory infiltrate and portal tract fibrosis, severe bile duct damage, and loss of portal tract arterioles.



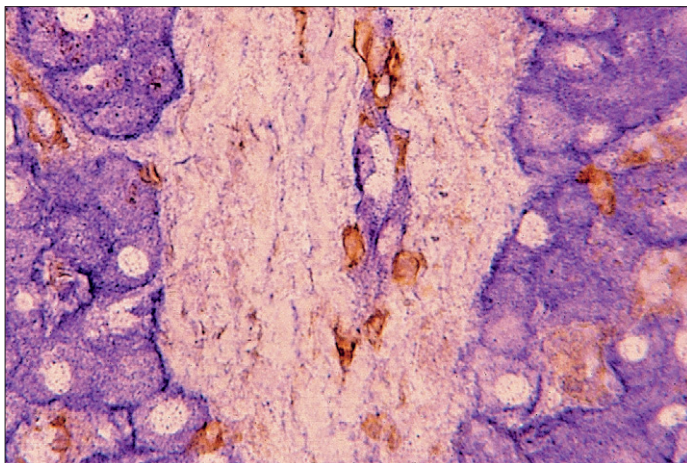
**Figure 39.21 Liver biopsy features of chronic allograft rejection.** Total loss of microvascular structures in chronic rejection.



**Figure 39.19 Liver biopsy features of chronic allograft rejection.** Hematoxylin and eosin stained section of liver lobule showing ballooning of hepatocytes in zone 3 of hepatic lobule (arrows).



**Figure 39.22 Liver biopsy features of chronic allograft rejection.** Macrophage infiltrate in allograft atherosclerosis. Reproduced from Wiesner et al (91) by permission of Wiley-Liss, Inc.

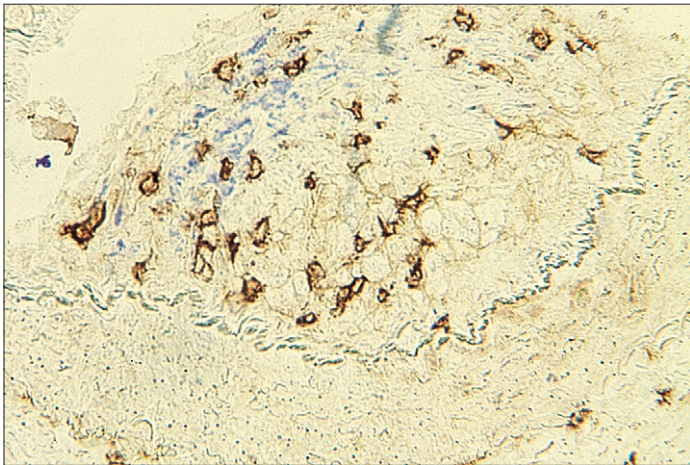


**Figure 39.20 Liver biopsy features of chronic allograft rejection.** Remnant of damaged bile duct (blue) being attacked by CD8+ T cells (pink). Reproduced from Wiesner et al (91) by permission of Wiley-Liss, Inc.

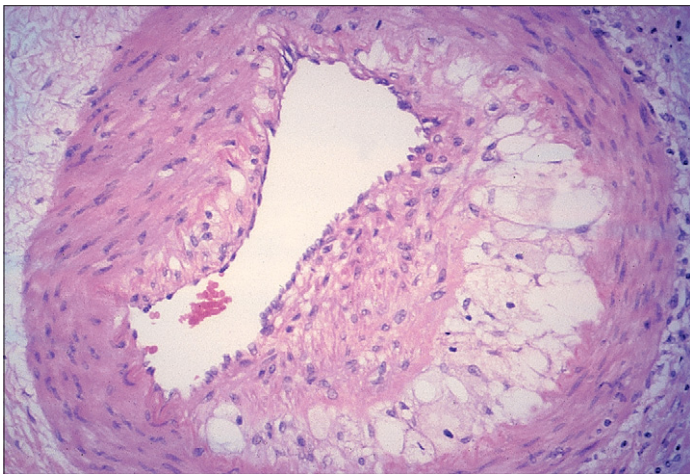
These lesions show increased expression of adhesion molecules on the endothelium. The process is not dissimilar to classic atherosclerosis, although the lesions tend to be concentric, affect medium-sized vessels, and have intact endothelium (Figs 39.24 and 39.25).

Once fully established, chronic rejection invariably progresses to graft failure. However, it is clear that there are intermediate cases where reversibility may occur. It is thought that tacrolimus therapy may be effective at early stages of chronic rejection, particularly when the serum bilirubin is  $<200 \mu\text{mol/L}$ . MMF may also be useful in this situation, although this has not yet been proven. Both these drugs inhibit smooth muscle, as well as T- and B-cell proliferation. Smooth muscle proliferation is an important feature of the chronic liver allograft response particularly at the level of the medium-sized arteries giving the transplant vasculopathy. Eventually rapamycin may also prove useful in chronic rejection.





**Figure 39.23 CD3+ T cells in allograft atherosclerosis.** Reproduced from Wiesner et al (91) by permission of Wiley-Liss, Inc.



**Figure 39.24 Liver biopsy features of chronic allograft rejection.** Evidence of 'allograft atherosclerosis'.

**XENOTRANSPLANTATION**

The possibility of xenotransplantation (i.e. the transplantation of organs from one species to another) (Table 39.7) has been an ongoing area of research for several decades. The major problem with organ xenotransplantation has been hyperacute rejection characterized by interstitial hemorrhage and widespread vascular thrombosis leading to necrosis and destruction of the organ within minutes or hours after revascularization. This process is mainly mediated by the binding of xenoreactive antibodies to recipient endothelium, leading to the activation of the complement system. Natural inhibitors of the complement system are species specific and are unable to dampen down this process.

Recent studies have identified that 85% of xenoreactive antibodies (>90% of the IgM subclass) in human serum recognize a single structure: Gala1-3Gal. The synthesis of this sugar on various glycoproteins is catalyzed by  $\alpha$ 1,3-galactosyltransferase. Approaches to overcome hyperacute xenograft rejection have involved the depletion from recipient serum of these antibodies by various columns, and this has been shown to prevent hyper-

Current approach to xenotransplantation	
Modification type	Example
Recipient	Depletion of anti-Gal $\alpha$ 1-3Gal antibodies
Donor	Knockout of $\alpha$ 1,3-galactosyltransferase gene
	Creation of human CD55 (decay accelerating factor) transgenics
	Creation of human CD59 (membrane inhibitor of reactive lysis) transgenics
	Creation of combined human CD55/CD59 double transgenics

**Table 39.7 Current approach to xenotransplantation.**

acute rejection of some xenografts. The other approach has been to produce 'knock-out' organ donors. In mice, xenografted organs in which the  $\alpha$ 1,3-galactosyltransferase gene has been ablated survive significantly longer than do 'normal' xenografts. A third approach has been the generation of transgenic xenograft donors which express a galactosyltransferase that catalyzes the addition of an extra sugar other than  $\alpha$ -galactose onto the terminus of various oligosaccharide chains. Currently all of these processes remain at the experimental level, and have not been applied to the human situation.

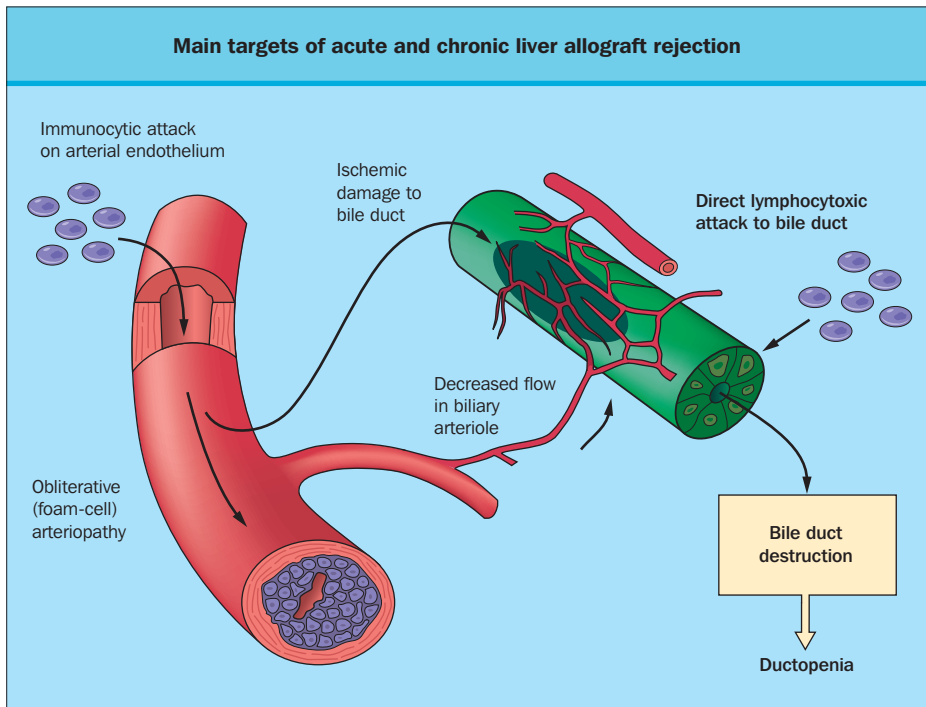
Complement activation is a critical event during hyperacute xenograft rejection. This is regulated by complementary regulatory proteins such as decay-accelerating factor (CD55) and membrane inhibitor of reactive lysis (CD59). These control proteins are species specific. For example, pig CD55 and CD59 are unable to break down human complement. Thus, when human complement is activated on pig endothelium, the process is ongoing. Approaches to overcome this have included the production of transgenic animals that express human CD55 and CD59. Such experiments have recently shown that significant delays in hyperacute rejection after xenotransplantation can be obtained.

Apart from the problem of hyperacute rejection there are other layers of acute vascular rejection that are mediated by a significant cellular component, as well as an antibody/complement component. There is increasing interest in the possible role of NK cells in acute vascular xenograft rejection. Other hurdles to be overcome include further cellular responses resulting in T-cell-mediated xenograft rejection. Finally, concerns about infectious agents and the transfer of endogenous retroviral infections from other species into the human need to be overcome before xenotransplantation becomes a clinical reality.

**LIVER ALLOGRAFT TOLERANCE**

There are four main general mechanisms of tolerance:

- anergy, where the antigen is ignored by the immune system,
- regulation away from an active immune response towards a non-active immune response involving the induction of what are now known as T regulatory (Treg) cells,
- deletion of APCs, and
- activation-associated tolerance which may or may not be related to the deletional processes.



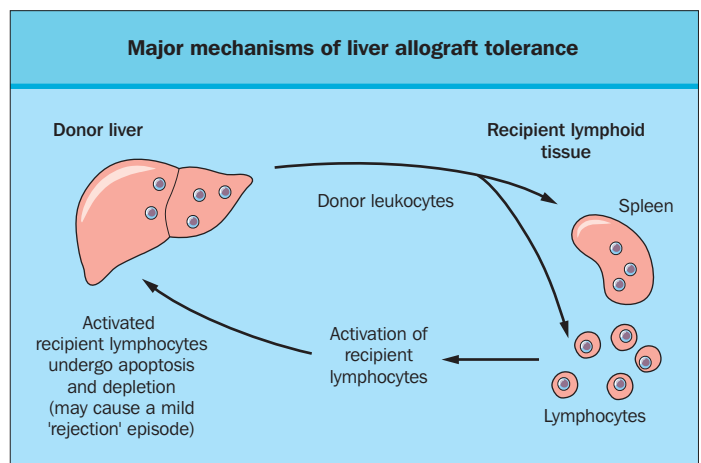
**Figure 39.25 Schematic summary of the main targets of acute and chronic liver allograft rejection.** Reproduced from Wiesner et al (91) by permission of Wiley-Liss, Inc.

It has been recognized in human clinical studies that a select group of patients can be weaned off immunosuppression without loss of their liver allograft, thus implying that a subset of humans in fact have become tolerant. The mechanisms of liver transplant tolerance have, however, been best studied in models of spontaneous tolerance, which have been identified between inbred rat strains, inbred mouse strains and some outbred pig strains. One of the important observations in clarifying the mechanisms of spontaneous liver allograft tolerance was the recognition that there was a migration of a significant number of donor leukocytes from the transplanted liver to the recipient lymph nodes and spleen. This led to the concept of the establishment of microchimerism and 'minigraft versus host disease' as a suppressor mechanism of the recipient immune response. While this process may definitely occur, it seems to evolve over a long period of time, while acceptance of livers in the experimental spontaneous tolerance systems occurs very rapidly within the first few days.

Recently, the mechanism of this early tolerance process has been defined to a significant extent. It seems that the early rapid migration of donor leukocytes from the liver allograft to recipient lymphoid tissues results in 'hyperactivation of recipient lymphocytes' (Fig. 39.26). This has been shown by the demonstration of increased messenger RNA production for IL-2 and IFN- $\gamma$  by these cells. In subsequent events, it has been shown that within the liver allograft itself there is increased apoptosis of infiltrating lymphocytes in tolerant animals compared with animals undergoing rejection. These two observations imply that spontaneous liver allograft tolerance is an activation-associated event, resulting in apoptosis and probable deletion of alloreactive cells. The above concept has important implications

for approaches designed to increase the chances of liver allograft tolerance in the clinical setting. It would imply that donor leukocytes are crucial in establishing the process and that early heavy immunosuppression at the time of transplantation or in the 24–48 h afterwards may inhibit the tolerance process, rather than enhance it. Such concepts need to be tested in the clinical setting.

The above description applies to the induction of liver transplant tolerance. In long-term patients the presence of Treg cells may be important in the maintenance of tolerance. The study of these cells and their relationship to possible immunosuppressive drug withdrawal requires further investigation.



**Figure 39.26 Major mechanism of liver allograft tolerance.**



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## SECTION V: LIVER TRANSPLANTATION

### CHAPTER

# 40

## Early Management

Federico G. Villamil and Andrés E. Ruf

### INTRODUCTION

Orthotopic liver transplantation (OLT) is the most effective therapy for adults and children with decompensated cirrhosis, acute hepatic failure or primary liver cancer. Following this complex but formidable operation, the majority of patients are able to return to a productive life and resume pretransplant activities. However, the price of the new life offered by OLT is the morbidity and mortality associated with graft dysfunction, the adverse effects of immunosuppression or recurrence of the original disease in the allograft. Virtually all livers are injured after OLT and most patients develop at least one significant postoperative complication.

### INTENSIVE CARE UNIT MANAGEMENT

Upon arrival at the intensive care unit (ICU) most patients are unconscious and intubated. Multiple interventions are usually required during the first 24 h to achieve hemodynamic, respiratory, metabolic and renal stability. However, patients with no surgical complications and adequate graft function recuperate rapidly allowing the progressive removal of intravascular lines and early extubation. Cirrhosis is associated with a hyperdynamic circulation characterized by a high cardiac output and decreased peripheral vascular resistance. Management of these pre-existing circulatory disturbances during surgery requires generous volume replacement and administration of vasoactive drugs to maintain hemodynamic stability. Volume replacement with crystalloid or colloid solutions and use of vasoactive drugs should be balanced to maintain a systolic blood pressure above 100 mmHg and a central venous pressure below 10 cm H<sub>2</sub>O to avoid graft congestion.

Hypothermia is the rule early after OLT. It is due to the combined effects of prolonged surgery, the need for multiple transfusions and grafting of an organ preserved in cold solutions. Rapid correction of hypothermia is indicated to prevent its deleterious effects on tissue perfusion, and cardiac, renal and platelet functions.

Almost all patients develop oliguria or some degree of renal dysfunction during the first 48–72 h, especially those with hepatorenal syndrome before OLT. Hypotension during the operation and the toxic effects of antimicrobials and immunosuppressive agents are the most frequent causes of renal abnormalities in the first postoperative week. Management of early renal impairment requires generous volume replacement with colloids or crystalloids to maintain a diuresis >0.5 mL/kg/h and avoidance of nephrotoxic drugs. For example, less nephrotoxic immunosuppression is recommended for patients with persistent oliguria. Diuretics and/or dopamine are indicated in patients

with persistent oliguria and normal filling pressures after volume replacement. Fortunately, renal dysfunction improves in the majority of patients within a few days.

Hyperglycemia is the most common metabolic abnormality encountered in OLT recipients and is usually related to the administration of high doses of corticosteroids or other diabetogenic drugs such as tacrolimus. Blood glucose should be frequently monitored and maintained below 200 mg/dL with regular insulin.

Patients who receive a large number of transfusions during surgery may develop citrate overload resulting in hypocalcemia. Correction of hypocalcemia is indicated to prevent complications such as myocardial dysfunction. Similarly, both citrate and the renal tubular effects of cyclosporin may cause significant hypomagnesemia which is a risk factor for seizures and other neurologic complications.

Close attention should be paid to the output and characteristics of fluids from the nasogastric, abdominal and biliary drains. The nasogastric tube allows early detection of gastrointestinal bleeding and enteral administration of drugs including immunosuppressive agents, which at present rarely require intravenous administration. The presence of blood or bile in the abdominal drains is often the first clue to the diagnosis of bleeding or bile leaks. Measurement of bilirubin levels and hematocrit in abdominal fluid samples is useful to confirm these diagnoses. Ascites occurs in almost all patients following OLT, but is greatest in those with poor graft function. It is important to adjust volume replacement to the output of ascitic fluid, minimizing the risk of hypovolemia and renal dysfunction. The quantity and quality of bile drainage through the T-tube in patients with duct-to-duct anastomosis is useful to assess graft function and to detect complications such as hemobilia.

Most patients are extubated within 48 h of surgery. Pleural effusions, especially on the right side, and atelectasis are common during the first week. For this reason, intense and frequent respiratory physiotherapy is part of the standard postoperative care.

The length of ICU stay is generally determined by the duration of mechanical ventilation and the need for vasopressors. On average, most patients are transferred out of ICU 2 or 3 days after OLT. The length of hospital stay ranges from 7 days to several weeks or even months depending on the severity of postoperative complications. However, most patients are discharged home during the second or third postoperative week.

### ASSESSMENT OF EARLY GRAFT FUNCTION

The earliest parameters of successful engraftment are production of golden brown bile after the liver is reperfused, and

Intra- and postoperative parameters of early graft function	
Intraoperative	Postoperative
Texture and color after reperfusion	Rapid awakening
Bile production	Output of golden brown bile
Hemodynamic stability	Improving coagulopathy
Acid-base status	Normalization of serum lactate
Adequate hemostasia	Increase in body temperature
Urinary output	No need for vasoactive drugs
	Progressive decrease of AST

**Figure 40.1** Intraoperative and postoperative parameters of early graft function. AST, aspartate aminotransferase.

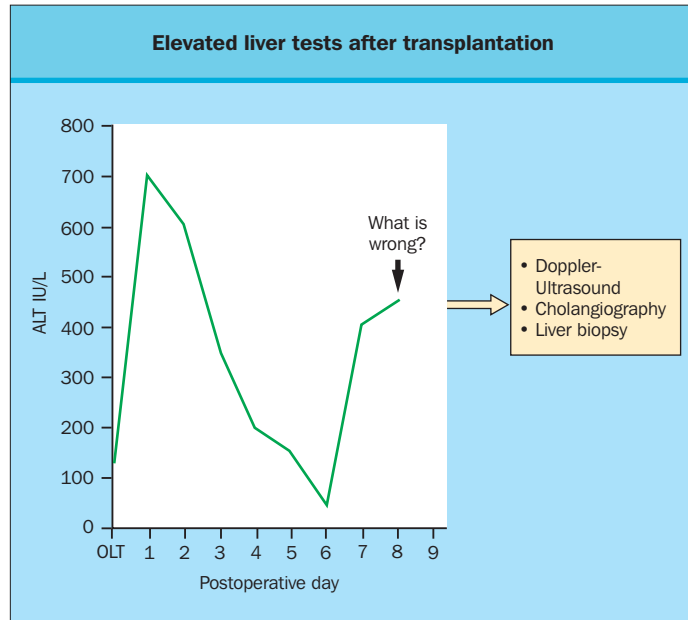
improvement in coagulopathy allowing satisfactory hemostasis (Fig. 40.1). Awakening of the patient shortly after arrival in the ICU reflects the rapid clearance of the anesthetic agents by a functioning liver. Serum lactate and international normalized ratio (INR) are the most valuable biochemical parameters to assess early graft function. Ideally, peak INR should be less than 2.0. However, the progressive improvement in coagulopathy rather than the absolute value is what indicates adequate function of the new liver. Serum lactate levels usually normalize within 12 h of OLT in patients with good allograft function (Fig. 40.1). Serum aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are indirect markers of the severity of preservation injury. In OLT recipients with good early function, AST peaks at less than 2000 IU/L and decreases rapidly over the subsequent days. Hyperbilirubinemia is not always indicative of graft dysfunction during the first days, but rather may reflect resorption of hematomas or blood in the abdomen or accelerated breakdown of transfused erythrocytes. Serum alkaline phosphatase (Alk Phos) and gamma-glutamyltransferase (GGT) usually remain within the normal range during the first days and are of little value in the differential diagnosis of early graft dysfunction.

## MONITORING AND DIAGNOSTIC WORK-UP OF EARLY GRAFT DYSFUNCTION

A variety of insults can damage the allograft following early successful engraftment. The differential diagnosis of graft dysfunction is frequently challenging, even for experienced physicians, and relies on the combination of clinical parameters, serum biochemical tests, non-invasive and invasive radiologic procedures, and liver biopsy.

### Clinical parameters

Clinical parameters are more important with respect to alerting the physician, rather than being a valuable tool for the diagnosis of a specific type of allograft dysfunction. As an example, fever, malaise, increasing jaundice and failure to thrive may be present in patients with rejection, infection with opportunistic organisms



**Figure 40.2** Elevated liver tests after the initial decline following liver transplantation. Requirement of specific studies to establish the diagnosis of graft dysfunction.

or biliary complications. In addition, a large proportion of recipients with significant graft dysfunction remain completely asymptomatic.

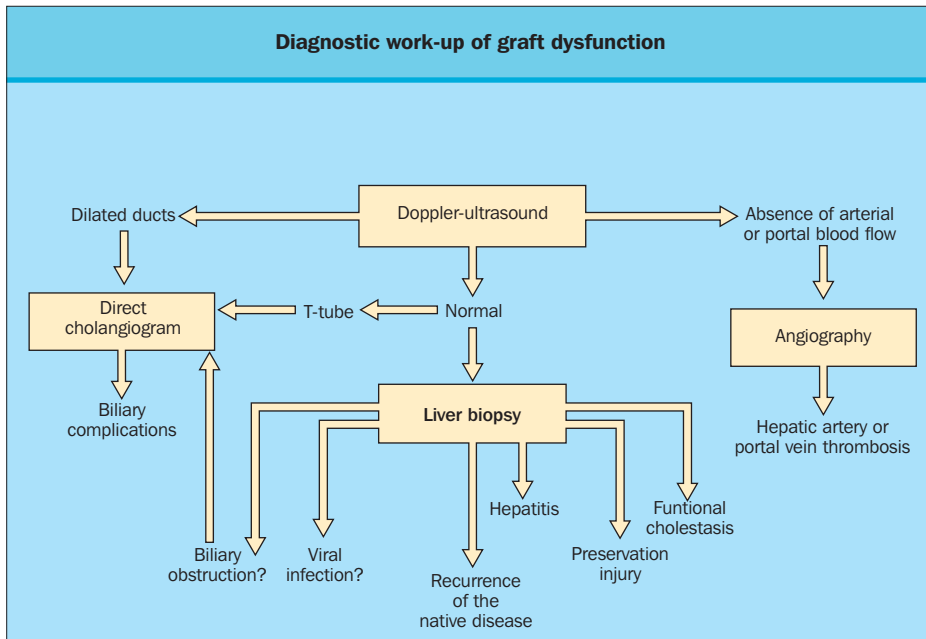
### Serum biochemical tests

After a successful transplant, serum bilirubin and liver enzymes decrease progressively over the following days and reach the normal range within 2–3 weeks. A rise in serum bilirubin, AST, ALT, Alk Phos or GGT after the initial postoperative decline is usually indicative of allograft injury. However, neither the degree of biochemical abnormalities nor the pattern of enzyme elevation is specific for any given cause of graft dysfunction. In addition, poor correlation exists between the degree of biochemical abnormalities and the severity of histologic findings. Significantly elevated liver tests are often found in patients with mild histologic disease and vice versa. Like clinical parameters, biochemical tests only alert one to allograft dysfunction while additional studies are needed to identify the specific cause of graft dysfunction (Fig. 40.2).

### Non-invasive radiologic procedures

Ultrasonography (US) with Doppler examination is the most commonly utilized non-invasive imaging study following OLT due to its accuracy, low cost and availability at the bedside. Doppler-US allows assessment of the hepatic parenchyma, abdominal cavity, biliary tree and patency of the hepatic vasculature. US has good sensitivity for the detection of abdominal fluid collections, which are commonly present during the first weeks post-OLT. However, it is not very useful in differentiating loculated ascites from other types of fluids such as blood, bile or pus. The demonstration of bile duct dilatation on US is almost diagnostic of biliary obstruction. However, the US examination is normal in as many as 50% of patients with abnormal cholangiograms.





**Figure 40.3 Diagnostic work-up of graft dysfunction.** Simplified algorithm for the differential diagnosis of graft dysfunction in liver transplant recipients.

A bedside study is indicated during the first day in the ICU to detect as early as possible vascular problems that may require prompt surgical revision. Subsequently, it is performed whenever graft dysfunction is discovered. The accuracy of Doppler-US to detect vascular thrombosis or stenosis after OLT is around 90%. A confirmatory angiogram is required only when arterial or portal blood flow is not demonstrated by Doppler-US. Even a normal study is clinically relevant because it strongly suggests a non-vascular cause of graft dysfunction.

Direct cholangiography is the gold standard for the diagnosis of biliary complications. T-tubes, in patients with duct-to-duct anastomosis, and transjejunal catheters, in those with a Roux-en-Y choledochojejunostomy, provide easy access for visualization of the biliary system. However, a large proportion of duct-to-duct biliary anastomoses are currently performed without a T-tube. In these cases, magnetic resonance (MR) cholangiography may be used to diagnose biliary complications.

### Invasive radiologic procedures

Diagnostic percutaneous transhepatic or endoscopic retrograde cholangiography may be indicated in OLT recipients without indwelling biliary catheters, if a non-invasive MR cholangiogram fails to provide an accurate diagnosis. The most appropriate technique is selected according to the type of biliary anastomosis and the suspected location of the complication. The percutaneous route is preferred in patients with a Roux-en-Y choledochojejunostomy or biliary lesions located at or above the confluence of the right and left ducts. The endoscopic route is indicated for more distal disease, i.e. at the anastomosis or below. If needed, percutaneous or endoscopic therapy can be performed at the time of the diagnostic procedure.

### Liver biopsy

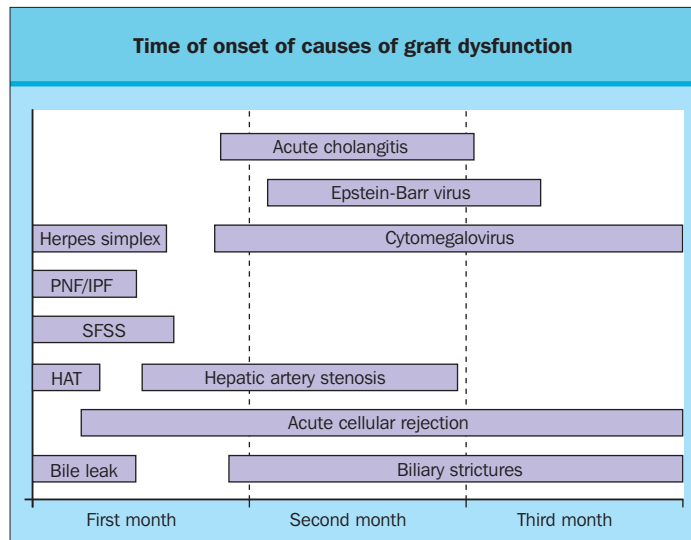
Liver biopsy is the single most valuable procedure for determining the cause of graft dysfunction and is indicated whenever graft dysfunction is suspected. Biopsies are processed using a rapid technique with a turnaround time of only 4–6 h. Most are

performed percutaneously. The transjugular technique is reserved for patients with a contraindication to percutaneous biopsy such as severe coagulopathy or tense ascites. Alternatively, a small window in the abdominal wound provides good exposure of the graft surface for biopsy and the possibility of performing direct hemostasis. Most causes of graft dysfunction can be determined with hematoxylin and eosin stained specimens. Immunohistochemical studies may be useful to confirm specific causes of allograft injury such as viral infections.

Histopathologic interpretation in OLT recipients is more difficult than in non-transplant patients because more than one process may affect the allograft simultaneously. Examples of this are the frequent coexistence of rejection with preservation injury, biliary complications, cytomegalovirus (CMV) infection, or recurrent viral hepatitis. Therefore, a combined effort is required from both the pathologist and transplant physician, not only to determine the correct cause of graft dysfunction, but to establish the clinical relevance of histologic findings in order to select the best treatment strategy. Not infrequently, the biopsy findings do not fulfill diagnostic criteria for any specific cause of graft dysfunction. When doubt exists, it is generally preferable to adopt a conservative approach to therapy. A simplified algorithm for the differential diagnosis of graft dysfunction after OLT is shown in **Figure 40.3**.

## CLASSIFICATION OF EARLY GRAFT DYSFUNCTION

The simplest and most practical classification of graft dysfunction is based on time of onset following OLT (**Fig. 40.4**). While specific complications tend to occur at different time intervals, significant overlap exists. As a general rule, preservation injury, vascular and biliary complications, acute cellular rejection, and most bacterial, fungal and viral infections are encountered within the first 3 postoperative months. In contrast, ductopenic rejection, biliary strictures, recurrent disease and de novo malignancies tend to occur later.



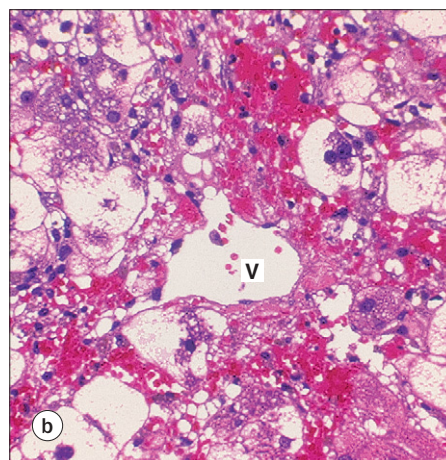
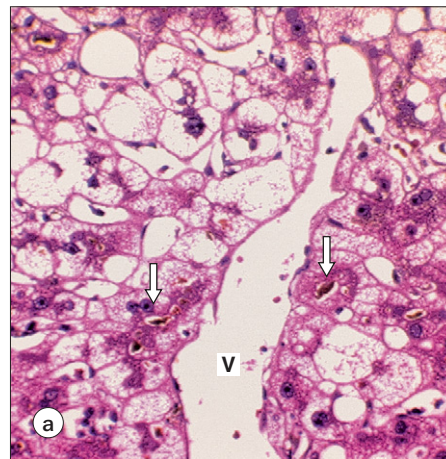
**Figure 40.4** Time of onset of causes of graft dysfunction during the first 3 months following liver transplantation. PNF, primary non-function; IPF, initial poor function; SFSS, small-for-size syndrome; HAT, hepatic artery thrombosis.

## PRESERVATION INJURY AND PRIMARY GRAFT DYSFUNCTION

The term preservation injury is used to describe the organ damage that results from the effects of cold and warm ischemia followed by reperfusion. Pre-existing donor diseases, including alcoholic steatosis, non-alcoholic steatosis or drug-induced hepatotoxicity can render the liver more susceptible to the effects of ischemia. Episodes of hypotension or hypoxia occurring after the declaration of brain death or during surgical removal of the donor organ may also result in graft injury. Cold preservation of the graft and rewarming during the interval from cold storage to resumption of hepatic circulation are the major events leading to ischemic injury. To minimize the risk of severe preservation injury, cold and warm ischemia times should be kept as short as possible. A good example of this is living-donor liver transplantation (LDLT), where cold ischemia time is minimal and primary non-function (PNF) is rare. The spectrum of preservation injury is quite variable and ranges from mild and rapidly reversible clinical and histologic abnormalities to PNF, a life-threatening complication leading to death or emergency retransplantation.

### Initial poor function/primary non-function

Severe preservation injury leading to graft failure is called primary graft dysfunction, a syndrome with two similar although distinct variants: initial poor function (IPF) and PNF. In most reported series, IPF was defined as AST greater than 2000 IU/mL, and prothrombin time greater than 20 s during the first week after OLT. Despite borderline function, grafts with IPF are able to support life, although retransplantation may become necessary later. PNF is defined as death or need for emergency retransplantation within 2 weeks of surgery in patients with IPF and is usually associated with clinical features of severe acute liver failure such as persistence of encephalopathy or slowed awakening, absence of bile flow or production of 'white' bile, hypoglycemia,



**Figure 40.5**  
**Preservation injury.** Perivenular (V) area with (a) ballooning of hepatocytes and canalicular cholestasis (arrows) and (b) congestion and hemorrhagic necrosis.

lactic acidosis, hyperkalemia and renal dysfunction. Although PNF may be diagnosed immediately following OLT, it can also occur after several days following a period of adequate initial function. The reported incidences of IPF and PNF are 15% and 5–10% respectively. A major challenge for the transplant physician is to differentiate, as early as possible, reversible from irreversible primary graft dysfunction. In this dramatic scenario, a timely decision is required to establish the need for emergency retransplantation. PNF is the principal indication for regrafting during the first 2 weeks after OLT.

### Histologic findings

Centrilobular hepatocyte ballooning and hemorrhagic necrosis are the main histologic features of preservation injury (Fig. 40.5) Submassive or massive coagulative necrosis is found in the explanted livers of patients with PNF. Portal tracts are usually unremarkable. Ductular proliferation and cholangiolitis, mimicking biliary obstruction, are frequently observed in patients who survive IPF without re-OLT. These findings are considered to be a non-specific portal reaction to the severity of parenchymal necrosis.

### Risk factors for IPF

A number of donor and recipient risk factors for IPF have been identified. Donor and perioperative factors include donor age greater than 50 years, moderate or severe steatosis, cold preservation times greater than 12 h and warm ischemia times

greater than 1 h. Retransplantation and more advanced pretransplantation disease are the only recipient factors that have been found to be associated with poor outcome. Combining risk factors such as transplanting of old, steatotic grafts into medically unstable recipients should be avoided to minimize the risk of IPF. Despite the availability of better preservation solutions and the continuous search for more accurate risk identification, PNF remains unpredictable in most cases.

Other causes of early graft failure include hepatic artery thrombosis (HAT), portal vein thrombosis (PVT) and antibody-mediated rejection. The profound alteration of the liver microcirculation after cold storage and reperfusion may render the allograft more vulnerable to vascular thrombosis and biliary complications.

Adult-to-adult LDLT is today a universally accepted treatment modality. The volume of the reduced graft per se has a significant impact on outcome. Adult recipients of small reduced livers may develop early graft dysfunction, the so-called 'small-for-size syndrome', characterized by prolonged cholestasis, persistent ascites, coagulopathy and histologic features of ischemic injury, and may require retransplantation. Persistent portal hypertension and the relatively increased portal blood flow, due to the small volume of the right lobe graft, are the main factors involved in the pathogenesis of small-for-size syndrome.

## FUNCTIONAL CHOLESTASIS

The term 'functional cholestasis' is used to describe progressive jaundice during the first weeks following OLT in the absence of biliary obstruction, rejection or viral hepatitis. Patients with this complication develop a gradual increase in serum bilirubin associated with normal or mildly elevated hepatocellular and canalicular enzymes. Histologic examination reveals prominent cellular and canalicular cholestasis without significant portal or lobular inflammation. Functional cholestasis is a benign and reversible condition of unknown cause for which there is no specific therapy.

## TECHNICAL COMPLICATIONS AFTER LIVER TRANSPLANTATION

OLT is a highly complex operation and technical complications still occur in 10–30% of patients. The most frequent complications affect the biliary tree and to a much lesser degree the vasculature.

### Postoperative hemorrhage

Severe coagulopathy and thrombocytopenia are prevalent in patients with end-stage cirrhosis and constitute the major risk factors for early postoperative hemorrhage. These patients often develop diffuse bleeding after reperfusion requiring prolonged and meticulous surgical hemostasis. The risk of hemorrhage further increases when graft function is poor due to coagulopathy. Postoperative bleeding may also result from technical problems during the harvesting procedure or the recipient's hepatectomy and grafting. Donor sources of bleeding include lacerations of the right lobe of the liver, the gallbladder bed, cystic artery or undetected small hepatic veins draining into the inferior vena cava (IVC). Injury to the adrenal gland during total hepatectomy and imperfect vascular anastomoses are the most common

surgical causes of bleeding during the recipient's operation. Finally, acute hemoperitoneum may result from the spontaneous rupture of splenic artery aneurysms or mycotic aneurysms located at the site of the hepatic arterial anastomosis. Clinical manifestations of abdominal hemorrhage after OLT range from an asymptomatic decrease in hematocrit to hypovolemic shock with hypotension and tachycardia. Patients with sustained and severe hypotension are at increased risk of ischemic damage to the allograft and renal failure, which adds significant morbidity. The presence of frank blood in the percutaneous drains or enlarging abdominal hematomas on imaging studies are sufficient to establish the diagnosis in most cases.

### Hepatic artery thrombosis

The reported incidence of HAT ranges from 2.5 to 10% in adults and from 10 to 20% in children. Risk factors for HAT include recipient age, atypical recipient and donor hepatic artery anatomy, technical factors, underlying medical conditions and poor early graft function. Pediatric recipients are at higher risk of developing vascular complications due to the small diameter of the arterial vessels. Donor and recipient aberrant arteries require careful reconstruction to prevent HAT early after OLT. The need for vascular extension grafts is the main technical risk factor that may result in inadequate arterial blood supply to the transplanted liver. Hypercoagulable syndromes frequently found in patients with Budd-Chiari or pre-existing PVT predispose to HAT, especially if other anatomic or technical factors are present. Finally, graft edema associated with IPF and requirement of high doses of vasopressors may increase vascular resistance resulting in reduced arterial inflow. The impact of HAT on the allograft depends on the ability of the parenchyma and biliary system to survive on portal flow alone. OLT recipients with sufficient portal blood flow or rearterialization from collaterals can tolerate HAT without significant parenchymal changes. In contrast, bile ducts depend primarily on arterial flow from branches of the gastroduodenal and retroportal arteries that are divided in the transplant operation. The clinical presentation of HAT is variable. Early thrombosis in grafts that are unable to survive on portal blood flow alone results in infarction of the liver parenchyma and acute liver failure. In less dramatic cases, HAT produces necrosis of the extra- or intrahepatic biliary tree presenting as anastomotic bile leaks or intrahepatic bilomas, respectively. Patients who tolerate early HAT with no major sequelae have a more insidious clinical presentation with late development of multiple intrahepatic biliary strictures. Of note, HAT may be discovered incidentally in children, and to a lesser degree in adults, who remain asymptomatic with normal graft function and no biliary complications. Doppler-US accurately identifies HAT in around 90% of cases. Angiography is indicated to confirm the diagnosis and also in patients in whom HAT is strongly suspected on clinical grounds despite a normal Doppler study.

When HAT is detected very early after OLT, acute thrombectomy with or without reconstruction of the anastomosis may save the allograft. However, the majority of patients will require retransplantation some time later. Emergency regrafting is a life-saving procedure for patients with massive necrosis of the graft. In contrast, elective retransplantation is indicated only after the resolution of septic complications in patients with HAT presenting with hepatic abscesses or diffuse biliary strictures.



### Hepatic artery stenosis

Hepatic artery stenosis is less frequent than HAT and usually occurs at the anastomotic site or within extension grafts utilized for the arterial reconstruction. Detection of high velocity flows across the anastomosis on Doppler-US is usually the first clue for the diagnosis. The clinical presentation of hepatic artery stenosis is similar to that of HAT but much less dramatic. The decreased arterial inflow affects the biliary tree more than the hepatic parenchyma. Although angioplasty is effective in some cases, surgical reconstruction of the anastomosis is the preferred therapy.

### Portal vein thrombosis and stenosis

Inadequate portal vein perfusion is most detrimental in the early postoperative period and may lead to severe allograft dysfunction. The incidence of PVT following OLT is low and ranges from 0.3 to 2.2%. Prior splenectomy or large portosystemic shunts, pre-existing PVT or hypoplasia, hypercoagulable states, and size mismatch between donor and recipient portal veins are the principal risk factors for this complication. Early and complete PVT can be a catastrophic event with rapid onset of liver failure, shock and massive ascites. In this scenario, absent portal vein flow on Doppler examination is usually diagnostic although angiographic confirmation may be desired before surgery. Incomplete thrombosis or portal vein stenosis is associated with gradual development of portal hypertension. Splenomegaly and esophageal varices are the most frequent clinical findings. Development of hepatopetal venous collaterals may result in cavernous transformation of the portal vein. Stenosis of the portal vein is suspected whenever a Doppler examination shows turbulent flow, especially at the level of the anastomosis. Therapy should be tailored to each individual case. Among patients with early and complete PVT, emergency thrombectomy may salvage the graft, although many patients may still require retransplantation. Percutaneous angioplasty with balloon dilatation, transjugular intrahepatic portosystemic shunt (TIPS) and surgical reconstruction have been shown to be effective therapies for patients with partial PVT or stenosis.

### Inferior vena cava thrombosis or stenosis

Stenosis or thrombosis of the infra- or suprahepatic IVC anastomoses occurs in only 1–2% of OLT recipients. The standard technique of liver grafting includes the removal of the intrahepatic IVC with the explant. In these cases, the superior end-to-end anastomosis is more susceptible to stenosis or thrombosis. Venous outflow problems are more frequent when the native IVC is preserved utilizing the so-called ‘piggy-back’ technique. Risk factors for venocaval obstruction or stenosis include use of large allografts for small recipients and OLT for Budd-Chiari syndrome. Clinical presentation is variable according to the location and degree of outflow obstruction. Most patients with stenosis or obstruction of the suprahepatic IVC anastomosis develop graft congestion with hepatomegaly, ascites and peripheral edema that may result in renal dysfunction. Complete obstruction is associated with severe graft failure and hemodynamic instability. In contrast, patients with stenosis or obstruction of the infrahepatic IVC anastomosis present with peripheral edema but no hepatomegaly, ascites or liver failure. Doppler-US is the most valuable screening procedure to identify decreased or absent blood flow as a result of IVC stenosis or

thrombosis. However, contrast venography may be required to establish a precise anatomic diagnosis. Although surgical reconstruction of an obstructed suprahepatic IVC anastomosis is feasible, urgent retransplantation may be required. Balloon dilatation with or without insertion of expansile stents has been found to be an effective therapy both for supra- and infrahepatic venocaval stenoses and to correct venous outflow problems resulting from the use of the ‘piggy-back’ technique. Surgical reconstruction or retransplantation is indicated when the angiographic techniques fail. The key for success in patients transplanted for Budd-Chiari syndrome is immediate anticoagulation with heparin followed by conversion to warfarin a few days later.

### Biliary complications

The biliary tract has been called the ‘Achilles heel’ of OLT. Biliary complications occur in 10–20% of patients transplanted with deceased donors. However, the incidence is significantly higher, up to 50%, in recipients of reduced-size grafts, especially in adult-to-adult LDLT. Major risk factors for biliary complications are bile duct ischemia resulting from HAT, ductopenic rejection, prolonged cold ischemia times, and use of organs from ABO incompatible donors. In general, bile leaks are more frequently observed after choledochojejunostomy and strictures after duct-to-duct anastomosis. The clinical spectrum of biliary tract complications ranges from an asymptomatic elevation of cholestatic enzymes to severe peritonitis and sepsis.

#### Bile leaks

Bile leaks in the early postoperative period are due to ischemic dehiscence of the anastomosis. In deceased-donor OLT, bile leaks may also occur from the insertion site of the T-tube, a cystic duct remnant or from an inadvertently transected aberrant bile duct. In recipients of LDLT the cut surface of the reduced graft is a frequent site of bile leakage. Bile leaks occurring several weeks post-OLT are most commonly related to T-tube removal. Bile leaks usually present as abdominal pain, jaundice, fever and leukocytosis. Detection of bile in the suction drains or abdominal fluid collections dictates the need for cholangiography to define the severity and location of the bile leak. Unclamping of the T-tube combined with percutaneous drainage of abdominal bilomas and antibiotic therapy is effective in most cases. When this strategy fails, or in patients without a T-tube, endoscopic or percutaneous biliary stenting may be necessary. Most severe anastomotic leaks require surgical revision, often with conversion of a duct-to-duct to a Roux-en-Y anastomosis.

#### Biliary strictures

Strictures are the most common cause of biliary obstruction following OLT. Aside from the surgical anastomosis itself, strictures may be located anywhere in the biliary tree from the intrahepatic ducts to the sphincter of Oddi. The reported incidence of biliary tract stenoses ranges from 3 to 20% in recipients of deceased donors. However, it is higher, 30–50%, following adult-to-adult LDLT, especially when more than one drains the right lobe graft. Most anastomotic strictures present within a few weeks of surgery, resulting from technical problems or ischemia, and are more common in patients with a duct-to-duct biliary reconstruction. Non-anastomotic strictures are strongly associated with prolonged cold ischemia or hepatic artery ischemia, and most commonly occur at the hilum. In

most cases, these present after 2 months post-OLT. Sludge or stones may form as a result of bile duct strictures. In severe cases, large intrabiliary casts are found at the time of reoperation. OLT recipients with biliary obstruction can present in a variety of ways.

When biliary obstruction is incomplete, an asymptomatic rise in liver enzymes with a predominant cholestatic pattern is often the only abnormality. Jaundice, abdominal pain and signs of cholangitis usually indicate severe impairment of bile flow. US may show bile duct dilatation, particularly in patients with longstanding or complete biliary obstruction. However, a normal study does not exclude biliary complications. Most patients will eventually require MR cholangiopancreatography (MRCP) or direct cholangiography to assess the type, severity, number and location of the biliary strictures. Histologic abnormalities such as portal edema, ductular proliferation and centrilobular cholestasis may indirectly suggest the presence of biliary complications, although these findings lack specificity.

Re-establishment of biliary drainage is the therapeutic goal and should be urgently achieved when sepsis and secondary organ dysfunction are present. Percutaneous or endoscopic procedures are indicated both for diagnosis and therapy including removal of sludge, stones and biliary casts, balloon dilatation and stenting. Endoscopic papillotomy is the treatment of choice for patients with bile duct obstruction due to stenosis of the papilla of Vater. In most centers, surgical procedures are reserved for endoscopic or radiologic treatment failures. Diffuse ischemic-type strictures involving both the intra- and extrahepatic bile duct systems usually require multiple interventional procedures, and aggressive treatment of infection. Chronic administration of ursodeoxycholic acid may be of benefit. Although this strategy may provide several months or years of a good quality of life, some patients will develop biliary cirrhosis requiring retransplantation.

## ALLOGRAFT REJECTION

Classically, allograft rejection has been classified as hyperacute, acute or chronic according to the time of onset following OLT. However, acute cellular rejection may occur at any time post-OLT, and chronic ductopenic rejection has been recognized within a few weeks following OLT.

### Antibody-mediated rejection

Antibody-mediated or humoral rejection is extremely uncommon in OLT recipients. Binding of preformed antibodies to donor antigens expressed on the vascular endothelium results in diffuse small vessel thrombosis, widespread hemorrhagic necrosis, and rapid graft failure requiring emergency regrafting. OLT performed across the ABO blood group barrier is the major risk factor. Immunohistochemical demonstration of immunoglobulins and complement deposited along the sinusoids, veins and arteries, helps to differentiate antibody-mediated rejection from PNF or other causes of early graft failure.

### Acute cellular rejection

Acute cellular rejection (ACR) is a frequent finding in OLT recipients. However, as the immunosuppressive agents and regimens have become more potent, the incidence of ACR has decreased over the last decade from 50–70% to only 20–30%, or even less. The vast majority of ACR episodes occur between

5 days and 8 weeks after surgery. Patients with ACR may be asymptomatic or present with a variety of symptoms and signs such as unexplained fever, malaise, increasing ascites or graft tenderness, associated with elevated serum bilirubin or liver enzymes.

Liver biopsy is the gold standard for the diagnosis of rejection. The histologic hallmarks of cellular rejection are portal inflammation, bile duct damage and endotheliitis (**Fig. 40.6**). Portal inflammatory infiltrates usually contain large activated or blastic lymphocytes, small lymphocytes, macrophages, neutrophils, plasma cells and a regular number of eosinophils (**Fig. 40.6a**). The mixed nature of the portal infiltrate is helpful in distinguishing rejection from recurrent or acquired viral hepatitis. Disruption of the limiting plate and bridging necrosis indicate severe cellular rejection. Non-suppurative cholangitis, also called rejection cholangitis or lymphocytic cholangitis, is characterized by infiltration of the epithelium of small interlobular bile ducts with lymphocytes or other inflammatory cells, producing degenerative changes or nuclear pyknosis (**Fig. 40.6b**). In severe cases, necrosis of epithelial cells results in bile duct loss. Endotheliitis is defined as the attachment of inflammatory cells to the endothelial surface of portal or terminal hepatic veins. Perivenular hepatocyte necrosis associated with central endotheliitis is regarded as a histologic feature of severe cellular rejection (**Fig. 40.6c and d**). Although no correlation exists between the exuberance of portal inflammation and response to therapy or outcome, severe bile duct damage or loss and centrilobular necrosis may announce the occurrence of irreversible rejection requiring re-OLT. Recent studies have shown that cellular rejection may not have a detrimental impact on overall patient survival. Moreover, mild ACR was significantly associated with increased patient survival suggesting that some level of alloreactivity may contribute to later immunologic hyporesponsiveness. These findings raise the question of whether complete elimination of all ACR should be a desirable goal in patients undergoing OLT.

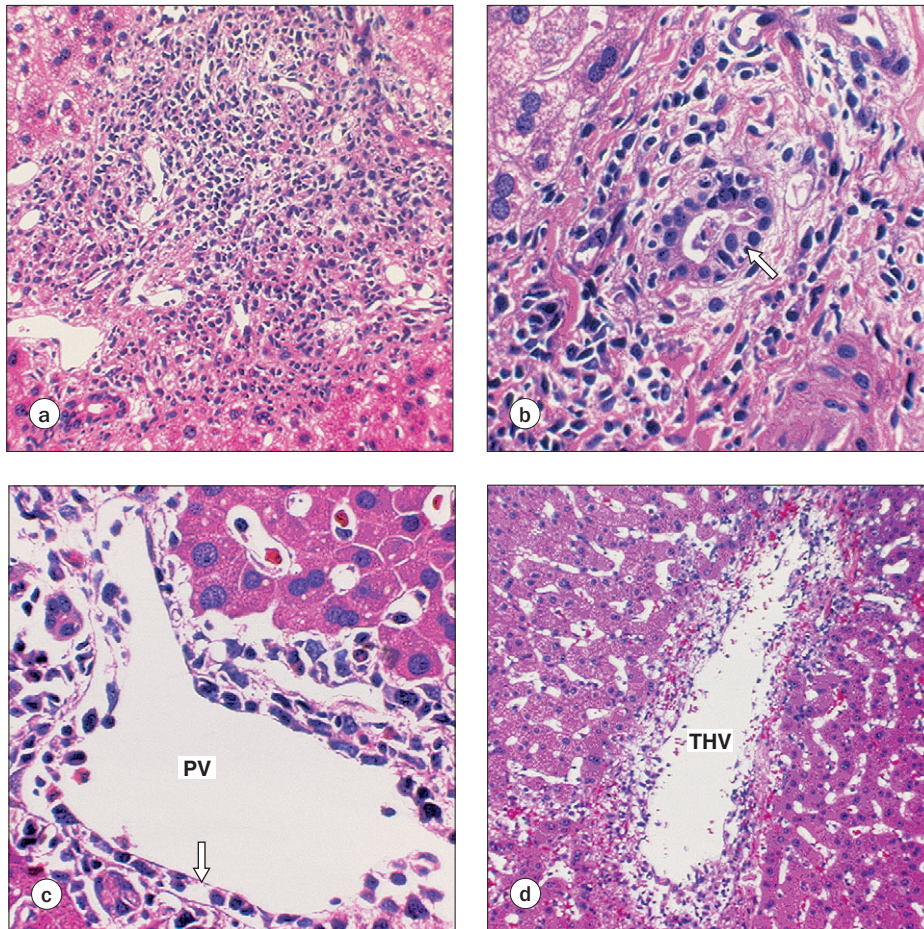
### Chronic ductopenic rejection

Early chronic ductopenic rejection (CDR), occurring weeks after OLT, usually follows episodes of severe or refractory cellular rejection. In this setting, duct loss is found in portal tracts with prominent inflammation. CDR diagnosed several months or years after OLT, one cause of vanishing bile duct syndrome, has a more indolent presentation with an asymptomatic increase in cholestatic enzymes, with or without increased serum bilirubin. Late CDR is characterized by duct loss in the absence of significant portal inflammation and is commonly associated with foam-cell arteriopathy. CDR has become increasingly uncommon occurring in only 1–2% of recipients.

## INFECTIOUS COMPLICATIONS

Despite remarkable progress in liver transplantation, infection continues to be a significant cause of morbidity and mortality. The incidence of infectious complications in OLT recipients is up to 70% higher than that observed after kidney or heart transplantation. The majority of patients experience at least one episode of infection, particularly during the initial hospitalization when immunosuppression is maximal and surgical complications prevail. Sepsis is the leading cause of death the first 3 months





**Figure 40.6 Cellular rejection.** (a) Mixed portal inflammation. (b) Non-suppurative cholangitis (arrow). (c) Portal vein (PV) phlebitis with lifting of the endothelium (arrow). (d) Severe endotheliitis of terminal hepatic venule (THV) with perivenular necrosis.

after OLT. The transplant operation per se is a risk factor for infection because it is performed in a potentially contaminated environment within the abdominal cavity. The poor pre-operative medical and nutritional conditions of recipients and the intensity of immunosuppression are additional risk factors for the development of infection. Steroid boluses and use of antilymphocyte preparations significantly increase the risk of infection. Specific infectious complications tend to appear at different times after OLT, although overlap is frequent. As a general rule, bacterial and fungal infections are more prevalent early after OLT and commonly involve extrahepatic organs, whereas viral infections occur later and frequently compromise the allograft. Infection-related mortality has decreased from 50% in the 1980s before the advent of selective immunosuppression, to around 10% at the present time. This remarkable improvement was largely due to a therapeutic shift from management of established infection to prophylaxis.

### Bacterial infections

Bacteria are the most common cause of infection in OLT recipients. The majority of episodes occurs within 2 months and originates mostly in the abdomen, especially among patients with surgical complications. Major risk factors for bacterial infection are prolonged surgery, high intraoperative transfusion requirements and the need for reoperations including retransplantation, CMV infection, renal impairment and the intensity of immunosuppression. The most common sites of infection are

the biliary tree, abdominal cavity, intravascular catheters, lungs, surgical wounds and the urinary tract. Most early bacterial infections are due to Gram-positive organisms such as *Staphylococcus* spp. and *Streptococcus* spp. and aerobic Gram-negative bacilli such as *Enterobacteriaceae* and *Pseudomonas*. The spectrum of commonly isolated bacteria following OLT varies among patients treated preoperatively with oral non-absorbable antimicrobials such as the combination of norfloxacin and nistatin or selective bowel decontamination preparations. These regimens eliminate most aerobic Gram-negative bacilli and *Candida* species while preserving the anaerobic gut flora.

Ascending cholangitis is the most frequent bacterial infection affecting the allograft. Bile leaks or biliary obstruction may result in the formation of hepatic abscesses. In addition, infected liver tissue secondary to HAT frequently become seeded with bacteria leading to hepatic gangrene. Since sterile intrahepatic or abdominal collections are frequently found after OLT, aspiration under US or computed tomography guidance and culture are required to confirm the presence of infection. Nosocomial pneumonia is another common bacterial infection after OLT, particularly when there is need for prolonged mechanical ventilation. When examination of the sputum is not diagnostic, invasive procedures such as bronchoalveolar lavage or thoracentesis are often required to differentiate pneumonia from atelectasis and sterile pleural effusions which are common findings early after surgery. Infections with *Legionella* and *Nocardia* species are not uncommon after OLT and should be



considered in patients with pulmonary infiltrates of unclear origin. Bacteremia in immunosuppressed transplant recipients is a serious infectious complication and is associated with increased mortality. Most commonly, it arises from abdominal collections or intravascular lines and to a lesser degree from wound, lung and urinary tract infections. Biliary or vascular complications and deep wound infection should always be considered whenever OLT recipients develop unexplained fever or bacteremia of unclear source. Infection with *Listeria monocytogenes* usually present as meningitis or encephalitis within 2 months of OLT. When suspected, a lumbar puncture should be performed without delay to prevent a fatal outcome. Fortunately, routine administration of trimethoprim-sulfamethoxazole to prevent *Pneumocystis carinii* is also effective against listerial and other bacterial infections.

Despite a paucity of randomized controlled trials demonstrating benefit from perioperative antibacterial prophylaxis, most centers administer intravenous antibiotics for the first 48–72 h after OLT. The antibiotics chosen are directed against organisms commonly found in the gastrointestinal flora and *Staphylococcus* such as cefoxitin, ceftizoxime, ampicillin-sulbactam, or cefotaxime plus ampicillin. The major concern about the use of antibiotic prophylaxis is selection of resistant organisms. When bacterial infection is suspected early after OLT, and before results of culture become available, empiric antimicrobial regimens are frequently used to cover the most likely pathogens according to the presumed site of infection. The susceptibility patterns of the microorganisms that prevail in each transplant center should also be considered for empiric therapy. However, once the offending organisms are identified, the antibiotics should be changed to those which have the narrowest spectrum coverage. Renal insufficiency is a frequent finding early after OLT due to the combined effects of underlying renal dysfunction, hypovolemia and nephrotoxic effects of calcineurin inhibitors. Therefore, empiric use of aminoglycosides and vancomycin should be avoided to prevent further nephrotoxicity. The most frequently used antibiotics to treat bacterial infections in OLT recipients are third or fourth generation cephalosporins,  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations and quinolones.

### Fungal infections

Fungal infections after OLT are less frequent than bacterial and viral infections but are associated with higher mortality. Major risk factors for fungal infection are similar to those described for bacterial infection and include advanced age, history of diabetes, emergency OLT, a prolonged transplant operation, transfusion of large amounts of blood products, repeated abdominal surgery, retransplantation, HAT, renal failure, CMV infection, intensity of immunosuppression and preoperative therapy with antibiotics or corticosteroids. OLT recipients are prone to develop opportunistic infections with fungi such as *Candida*, *Aspergillus* and *Cryptococcus* that rarely cause invasive disease in immunocompetent hosts. A high index of suspicion is required because the clinical presentation of fungal infections is frequently non-specific and may also be obscured by other infectious or non-infectious complications. *Candida albicans* is the most prevalent fungi encountered following OLT. Although non-invasive mucocutaneous disease is the commonest presentation, candidal infections may produce many other complications such as pneumonia, abdominal abscesses, urinary tract infection, endo-

carditis and meningitis. Bloodstream invasion from these sites, or more frequently through intravascular catheters, results in disseminated disease in around half of the patients. Invasive infection with *Aspergillus fumigatus* characteristically involves the central nervous system and associated with this the mortality approaches 100%. Dissemination occurs through hematogenous spread and may compromise almost any organ. *Aspergillus* infection should be suspected in OLT recipients with persistent fever despite antibiotic therapy and in those with progressive pulmonary infiltrates of unclear origin.

Diagnosis relies on the identification of pathogenic fungi by staining and/or culture of blood or other specimens obtained through invasive procedures such as bronchoalveolar lavage or lung biopsy. Amphotericin B is currently the drug of choice to treat invasive fungal infections, however this may be changing. The benefits of amphotericin therapy are limited by its significant bone marrow and renal toxicities. This is particularly relevant in OLT recipients because renal dysfunction and cytopenias are common adverse effects of calcineurin inhibitors and antimetabolites. Liposomal formulations of amphotericin are less nephrotoxic and allow the administration of larger amounts of the active drug. However, because of the elevated cost, liposomal amphotericin is not routinely utilized in most countries as a first-line agent, unless significant nephrotoxicity is present. Prophylactic low-dose amphotericin is utilized in many centers to reduce the incidence and severity of fungal infections among high-risk patients. Azole antifungal agents such as fluconazole and itraconazole have been increasingly utilized to treat fungal infections in OLT recipients. Fluconazole is well absorbed and has good penetration in the central nervous system. It is effective both for *Candida* and *Cryptococcus* species but much less so for *Aspergillus*. The antifungal spectrum of itraconazole covers not only *Candida* species but also *Aspergillus*. The major drawback of this drug is its erratic absorption that often results in unpredictable plasma levels. Both fluconazole and itraconazole inhibit the hepatic metabolism of calcineurin inhibitors which may result in increased blood concentrations and overimmunosuppression. Two newer antifungals appear promising. Voriconazole is a new azole that has good activity against *Candida*, *Aspergillus* and *Cryptococcus neoformans*. Caspofungin is a good novel antifungal with good activity against *Candida* and *Aspergillus*. Most importantly they appear to have relatively little toxicity as compared to other antifungals.

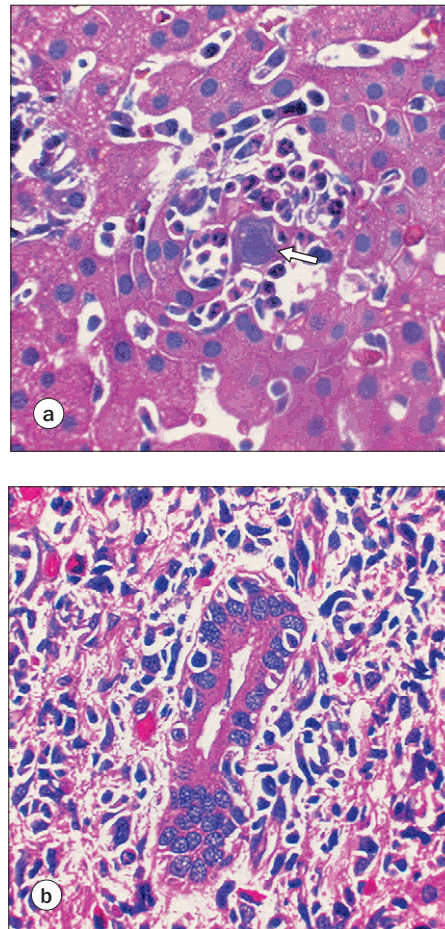
### Viral infections

Infection with opportunistic viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella-zoster virus (VZV) and adenovirus may produce graft dysfunction at variable time intervals following OLT (Fig. 40.4). Pediatric recipients without exposure to childhood illnesses before OLT have the highest risk of primary infection with opportunistic viruses. In contrast, reactivation of endogenous latent viral infections is more prevalent among adult recipients. Primary infection is usually more severe than reactivation. Diagnosis of viral infections in transplant recipients mostly relies on assays to detect viremia or antigenemia. Histologic examination of liver specimens with routine stains, immunohistochemistry, or in situ hybridization techniques may be helpful in some cases. However, serologic tests are unreliable because they lack sensitivity and specificity in immunosuppressed transplant recipients.

### Cytomegalovirus infection

CMV is the single most common viral pathogen encountered in OLT recipients. When no antiviral prophylaxis is given, the incidence of CMV infection is 50–60% and CMV disease occurs in 20–30%. Without specific therapy, CMV disease is associated with high mortality. Main sources of infection are grafts from CMV-positive donors, transfusion of leukocyte-containing blood products and endogenous reactivation of latent infection. Risk factors for infection and disease include transplantation of a CMV-seropositive donor into a CMV-seronegative recipient, OLT for fulminant hepatic failure, intense baseline immunosuppression, use of antilymphocyte preparation and retransplantation. Most CMV infections occur between 4 and 12 weeks after surgery. Patients may develop primary infection, secondary infection (reactivation) and superinfection (reinfection). Primary infection occurs when CMV-seronegative recipients receive an organ or blood products from a seropositive donor. Reactivation is observed in seropositive recipients under the effects of immunosuppression. Superinfection is defined as reactivation of latent donor CMV infection after being grafted into a CMV-seropositive recipient. Clinical presentation of CMV infection is variable and ranges from asymptomatic viral shedding to lethal systemic disease with multiorgan involvement. Low-grade fever, malaise, arthralgias, myalgias, leukopenia with atypical lymphocytes, thrombocytopenia and elevated liver tests are the most common findings. Severe CMV disease is a tissue invasive disease involving the gastrointestinal tract, liver, retina or central nervous system. CMV has the ability to depress the host's immune response exerting an additive effect to that of immunosuppressive drugs. This overimmunosuppression induced by CMV increases the risk of superinfection with opportunistic bacterial or fungal pathogens.

Serologic tests are useful to assess donor and recipient past exposure to CMV. However, immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies lack sensitivity for the diagnosis of CMV infection after OLT. A number of sensitive and rapid diagnostic assays are now available to detect the presence of replicating CMV in serum samples or body fluids. The shell vial leukocyte culture technique utilizes a labeled monoclonal antibody that binds to an early viral antigen and has a turnaround time of only 24–48 h. CMV antigenemia is more sensitive than the shell vial culture and has the advantage of being more rapid, with results available in around 5 h, semi-quantitative and of not requiring cell culture methodologies. Antigenemia assay is based on the reaction of a monoclonal antibody with a structural CMV antigen (pp65) located in the nuclei of polymorphonuclear leukocytes. Levels of antigenemia correlate with the severity of infection and decrease following effective therapy. Polymerase chain reaction (PCR) is the most sensitive diagnostic technique allowing the detection of even minimal concentrations of CMV DNA. The major drawback of qualitative PCR is the lack of specificity to differentiate CMV infection from CMV disease. Quantitative PCR is at present the diagnostic method of choice, especially to monitor the response to therapy. The demonstration of CMV viremia or antigenemia should strongly suggest active infection and the need for antiviral treatment. The modern diagnostic methodologies allow the detection of replicating virus several days before the onset of clinical disease and thus the initiation of pre-emptive therapy. Histologic diagnosis of CMV hepatitis mostly relies on the



**Figure 40.7**  
**Coexistence of**  
**cytomegalovirus**  
**(CMV) hepatitis and**  
**allograft rejection.**  
(a) Microabscess  
surrounding a necrotic  
hepatocyte with a CMV  
inclusion body (arrow).  
(b) Portal inflammation  
and rejection  
cholangitis.

presence of characteristic inclusion bodies in hepatocytes or mesenchymal cells, surrounded by clusters of neutrophils (microabscesses) or microgranulomas (Fig. 40.7a). Immunostaining of biopsy specimens with monoclonal antibodies that bind early CMV antigens or in situ hybridization with specific DNA probes are useful to confirm the diagnosis, especially when cytomegalic inclusion bodies are not present.

The advent of effective antiviral agents over the last decade has significantly decreased the CMV disease burden in OLT recipients. Ganciclovir and its analogs, foscarnet and cidofovir have excellent activity against CMV and other herpesviruses. CMV disease requires 2–3 weeks of intravenous ganciclovir, although the optimal length of therapy is still a matter of debate. The use of alternative drugs such as foscarnet and cidofovir should be restricted only to patients who develop viral resistance or fail to respond to ganciclovir. Both agents are nephrotoxic and therefore should be used with caution in OLT recipients. Intravenous or oral ganciclovir, intravenous immunoglobulin, CMV hyperimmune globulin and more recently oral valganciclovir have been successfully utilized for universal or selective prophylaxis of CMV infection. Use of valganciclovir, a valine ester prodrug of ganciclovir, appears to be practical and effective because it has a bioavailability up to 10-fold higher than that of oral ganciclovir. In most centers, ganciclovir prophylaxis is indicated in CMV-negative recipients grafted with CMV-positive donors or following administration of supplemental immunosuppression to treat cellular rejection. Another commonly used

strategy is pre-emptive therapy in which ganciclovir is initiated at the time of the virologic diagnosis of CMV infection but before the onset of disease. Cellular rejection and CMV hepatitis often coexist (Fig. 40.7b). As the virus itself decreases the immune response, reduction of the level of immunosuppression is safe, and when combined with ganciclovir therapy, results in resolution of both rejection and CMV hepatitis in most cases.

### **Epstein-Barr virus infection**

EBV produces a spectrum of abnormalities in OLT recipients ranging from asymptomatic infection to diffuse lymphomatous infiltration of multiple organs. Primary infection may be acquired from the allograft, transfusion of leukocyte-containing blood products and, less likely, from the community. Small children are at particular risk of developing primary EBV infection because around 50% lack previous exposure and are seronegative at the time of OLT. EBV infection is usually diagnosed from 2 to 6 months following OLT, but the risk of developing EBV-related post-transplant lymphoproliferative disease (PTLD) continues beyond that. Primary EBV infection often results in a mononucleosis-like syndrome with fever, malaise, circulating atypical lymphocytes and mild and reversible hepatitis. Diagnosis of EBV infection includes IgM and IgG antibodies to several viral antigens and molecular virologic tests to detect and quantitate viremia. EBV-related hepatitis is histologically characterized by polymorphous portal and sinusoidal inflammation, but with minimal hepatocellular necrosis. Endotheliitis of portal and central veins may be present, mimicking cellular rejection. The disproportion between the intensity of the portal infiltrate and mild or absent bile duct damage favors the diagnosis of EBV hepatitis over rejection. Demonstration of EBV antigens or genes in liver tissue by immunohistochemistry or in situ hybridization is useful to confirm the diagnosis in difficult cases. Uncomplicated EBV infection is self-limited and reversible unless supplemental immunosuppression is administered. EBV produces life-long latent infection, has unique tropism for B cells, and the ability to induce lymphocyte transformation and proliferation under the effects of immunosuppressive therapy. Around 2% of adults and up to 25% of EBV-seronegative children experience PTLD between 2 months and 2 years following OLT.

### **Herpes simplex virus infections**

Primary HSV infection occurs via the allograft itself or from person-to-person contact. As 75% of adults are HSV-seropositive before OLT, the vast majority of infections result from reactivation of latent virus. The most common clinical presentation is the appearance of oral mucocutaneous or anogenital lesions within 4 weeks of OLT. Bacterial superinfections of orolabial lesions and esophageal involvement are frequent findings among heavily immunosuppressed patients. HSV pneumonia and central nervous system involvement are rare in OLT recipients. Serologic diagnosis of HSV infection is based on the detection of specific IgM antibodies or a fourfold rise in the serum concentration of IgG antibodies. However, direct immunofluorescence performed on swabs taken from mucocutaneous lesions is a rapid and sensitive methodology to confirm the diagnosis. Severe reactivation of virulent strains of HSV produces a rapidly progressive and fatal hepatitis early after OLT, with or without associated mucocutaneous lesions. Histologically, HSV hepatitis is characterized by circumscribed areas of coagulative necrosis

with a non-zonal distribution and Cowdry-type nuclear inclusions in viable hepatocytes located at the periphery of necrotic areas. The presence of HSV antigens in infected hepatocytes can be confirmed by immunohistochemistry. Oral aciclovir is an effective therapy for most patients with mucocutaneous infections. Intravenous administration of aciclovir or ganciclovir is reserved for patients with disseminated disease. The routine use of prophylactic aciclovir or ganciclovir has resulted in a dramatic decrease in the incidence and severity of HSV infection after OLT over the last decade.

### **Human herpesvirus-6 and -7**

Human herpesviruses (HHV) -6 and -7 are novel members of the  $\beta$ -herpesvirus family, agents known to maintain latency in the human host after primary infection. OLT recipients with HHV-6 infection may develop fever, encephalitis or other neurologic disorders, hepatitis, skin rash, pneumonitis and myelosuppression. In contrast, the clinical significance of HHV-7 infection following OLT remains unclear. The frequent association of HHV-6 and -7 infections with allograft rejection, CMV infection and other opportunistic infections suggest that these novel agents have immunomodulatory effects and trigger viral interactions. Diagnosis of HHV-6 and -7 infections mostly rely on antigenemia assays utilizing monoclonal antibodies that bind specific viral antigens in mononuclear cells. Molecular virologic assays are available for HHV-6 (HHV-6 DNA) but not for HHV-7. Viral antigens can also be detected in tissue with immunohistochemical methods.

### **Other viral infections**

The large majority of adult patients, but not children, have antibodies to VZV before OLT. Reactivation in VZV-seropositive adults usually results in herpes zoster characterized by the appearance of painful vesicular lesions with a dermatomal distribution. In contrast, primary VZV infection occurs mostly in pediatric recipients and may cause severe illness with hemorrhagic skin lesions, pneumonia, hepatitis, encephalitis and pancreatitis leading to multiorgan failure and death. Direct immunofluorescence of swabs taken from cutaneous lesions, viral culture and Tzanc preparations are useful to certify the diagnosis. Intravenous aciclovir is the treatment of choice for VZV infections. Success of antiviral therapy largely relies on the early recognition of the disease. Varicella-zoster immunoglobulin is indicated in seronegative recipients whenever exposure to the virus is documented or strongly suspected. Vaccination pretransplant may also be effective.

Adenovirus infection is mostly restricted to pediatric recipients who usually present with fever associated with hepatitis or pneumonia within 1 month of OLT. The severity of hepatitis ranges from mild and self-limited disease to fulminant hepatic failure requiring urgent re-OLT. Liver biopsy shows areas of parenchymal necrosis with no particular zonal distribution, collections of inflammatory cells with a granulomatoid appearance, and nuclear inclusions in the periphery of necrotic areas. Immunostaining of specific viral antigens in liver specimens is required to confirm the diagnosis.

Papillomavirus infection in OLT recipients frequently causes extensive warty growths that may undergo malignant transformation or cervical lesions that may lead to squamous cell cancer.



Infection with human polyoma JC virus in immunosuppressed hosts produces multifocal leukoencephalopathy, a progressive demyelinating disease of the central nervous system.

Finally, many viruses that cause benign illness in the general population may have a serious impact in OLT recipients. Good examples of this are infections with the respiratory syncytial or influenza viruses.

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## SECTION V: LIVER TRANSPLANTATION

### CHAPTER

# 41

## Long-Term Management

James Neuberger

### LONG-TERM MANAGEMENT

With the success of liver transplantation over the last two decades, the role of long-term care of the liver allograft recipient is becoming an area of increasing importance. Although the quality of life following liver transplantation is usually excellent, it can never be considered normal and regular monitoring is a requirement to maximize the recipient's opportunity to live as fulfilling a life as possible. Immunosuppressive regimens require surveillance and adjustment to maintain good graft function whilst minimizing the adverse effects. It should be remembered that the hepatic metabolic functions and profile will reflect the donor rather than the recipient, so that diverse metabolic conditions such as Gilbert syndrome, peanut allergy, protein C deficiency and alcohol-flush syndrome may be acquired following the transplant. In addition, some infectious and malignant complications may be derived from the donor.

Graft function must be carefully tracked and abnormalities investigated so appropriate intervention can be directed at limiting damage to the graft. The care of these patients requires close collaboration and cooperation between the recipient, the transplant unit, the local clinician and the primary healthcare provider. In the transplant recipients who die more than 5 years after transplantation, complications of immunosuppression (over-immunosuppression such as infection, renal failure, de novo malignancy and underimmunosuppression such as chronic rejection) account for just over half of all deaths, vascular disease (cardiovascular and cerebrovascular disease) account for about one-quarter, and recurrent disease or technical factors (biliary or vascular complications) account for the rest.

### MAINTAINING GOOD GRAFT FUNCTION

The frequency of monitoring must balance the need for early detection and correction of abnormalities against the ambition to maximize the patient's quality of life. Most liver transplant programs monitor patients every 3 months during the second and third postoperative years and at a lower frequency thereafter. A template for follow-up is suggested in Table 41.1.

### INVESTIGATION OF ABNORMAL LIVER FUNCTION

The finding of abnormal liver tests should prompt further investigation to identify the cause and extent of the graft dysfunction (Table 41.2). The cause of the liver dysfunction may be suspected from the clinical and biochemical features, but imaging and/or histologic evaluation are usually required to establish the precise diagnosis.

#### Suggested template for assessment of liver allograft recipients more than 1 year post-transplant

Recipients should be assessed every 3–4 months or as clinically indicated

Clinical	General health Hepatic problems
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Examination	Weight Body mass index (BMI) Blood pressure Evidence of liver dysfunction Skin lesions
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Investigations	Blood counts Renal function tests Liver tests Auto-antibodies* Immunoglobulins* Urine: creatinine clearance; urine albumin/creatinine ratio* Serum lipids*
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Where appropriate	Viral hepatitis markers Bone densitometry† Liver ultrasound† Liver biopsy† Ophthalmologic examination‡
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\*Annually.

†As clinically indicated.

‡For those on corticosteroids.

**Table 41.1 Suggested template for assessment of liver allograft recipients more than 1 year post-transplant.**

### ALLOGRAFT REJECTION

Acute rejection usually occurs within the first weeks after transplantation but it may also occur at any time after transplantation. However, in the majority of cases a precipitating event can be identified. These include:

- reduction in the dose of the immunosuppressive drugs as part of the process of defining the minimal immunosuppressive requirements in individual patients or in response to adverse effects of immunosuppression;
- poor patient compliance;
- inability to take medication e.g. vomiting; and
- changes in concomitant medication leading to accelerated metabolism of the calcineurin inhibitors (tacrolimus, cyclosporin) e.g. phenytoin or changes in the components of highly active retroviral treatment (HAART) therapy.

Some causes of graft dysfunction developing after 1 year post-transplant	
Immune mediated	Acute cellular rejection Ductopenic rejection De novo autoimmune hepatitis
Recurrent disease	Viral <ul style="list-style-type: none"> <li>• Hepatitis B viral infection</li> <li>• Hepatitis C virus infection</li> </ul> Autoimmune diseases <ul style="list-style-type: none"> <li>• Primary biliary cirrhosis</li> <li>• Primary sclerosing cholangitis</li> <li>• Autoimmune hepatitis</li> </ul> Cancer Alcohol associated liver disease Non-alcoholic fatty liver disease
Structural	Hepatic artery thrombosis Biliary strictures <ul style="list-style-type: none"> <li>• Anastomotic</li> <li>• Non-anastomotic</li> </ul> Cholangitis Portal venous thrombosis Hepatic venous thrombosis
Infections	
Drug toxicity	

**Table 41.2** Some causes of graft dysfunction developing after 1 year post-transplant.

It has also been suggested that intercurrent viral infections may trigger rejection episodes in patients on stable and previously effective immunosuppression regimens. The biochemical and histologic features of rejection are very similar to that seen in the early post-transplant period (Ch. 40). The treatment of the rejection is also similar to that for early rejection although the response to therapy may be slower and less satisfactory and some cases progress inexorably to chronic rejection. In those patients with histologically mild rejection, merely increasing the dose of calcineurin inhibitor may be sufficient; for histologically moderate or severe rejection, the usual treatment is with a short course of high-dose corticosteroids. More than one episode of rejection or multiple episodes of rejection should prompt reassessment of the immunosuppressive regimen.

### Chronic ductopenic rejection

Chronic ductopenic rejection usually develops during the first postoperative year but may occur at any time. In contrast to acute, cellular rejection, the time course of ductopenic rejection is slower. Risk factors for the development of chronic rejection include:

- prior retransplantation for chronic rejection;
  - primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) as original liver disease;
  - recurrent episodes of acute cellular rejection;
  - late (>3 months) acute rejection; and
  - inadequate immunosuppression.
- The clinical features include:
- progressive jaundice;

- symptoms associated with cholestasis (such as pruritus) in the later stages;
- cholestatic liver tests, initially the serum alkaline phosphatase and  $\gamma$ -glutamyltransferase levels rise followed by the serum bilirubin; and
- impaired synthetic function in advanced disease with reduction in the serum albumin level.

Abnormal coagulation tests may be due to vitamin K deficiency or impaired synthetic function.

Liver histology typically shows features of a 'vanishing bile duct syndrome', with progressive reduction in the intensity of the associated inflammatory infiltrate. Occasionally, a vascular pattern of damage is captured in the biopsy with occlusion of the arterial lumen by foamy macrophages. Chronic rejection may be reversible and is more likely to respond to treatment when more than 50% of portal tracts retain bile ducts.

Treatment is not always effective at reversing graft dysfunction. Increasing immunosuppression, by switching from cyclosporin to tacrolimus (where appropriate) or the institution of either mycophenolate or rapamycin/sirolimus may be of benefit if the damage is not too great. For those with end-stage disease, as shown by serum bilirubin in excess of 150  $\mu$ mol/L or 20 mg/dL, liver replacement is the only effective therapeutic option.

## HEPATIC ARTERY THROMBOSIS

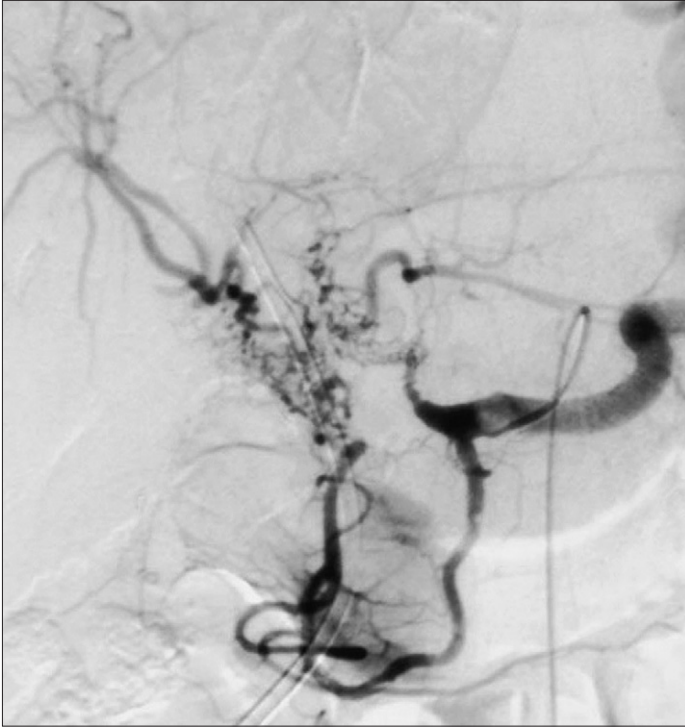
The late or delayed presentation of hepatic artery thrombosis (HAT) differs from the early presentation in that ischemic hepatitis and graft infarction do not typically occur. Some cases are clinically silent and are detected on routine scanning. The usual presentations are with ischemic cholangiopathy, liver abscess or sepsis. The initial investigation should include ultrasound and Doppler examination of the artery. Occasionally, pulsation of the artery proximal to the thrombosis may give rise to the mistaken belief that the artery is patent. The diagnosis should be confirmed either by conventional angiography or computed tomography (CT)/magnetic resonance (MR) angiography (Fig. 41.1).

Treatment of the blockage by stenting, dilatation or use of thrombolysis is usually ineffective. Most cases of late HAT progress with the development of multiple intra- and extra-hepatic abscesses and biliary strictures. The only effective treatment is by liver transplantation. It is important that regrafting is not postponed while attempts are made to treat with systemic antibiotics, local drainage or biliary stenting since these approaches are rarely effective and procrastination results in the patient being in a worse clinical state when transplantation is eventually undertaken.

## PORTAL VEIN THROMBOSIS

Portal vein thrombosis or stenosis is rare and is usually detected on routine examination at ultrasound. Occasionally it presents with complications of portal hypertension such as variceal hemorrhage or hypersplenism. The stenosis or thrombosis should be confirmed by vascular imaging (direct or with CT or MR). A stenosis can sometimes be dilated through an angiographic approach. Definitive treatment (with the construction of a shunt) is not always necessary, and varices treated either pharmacologically with  $\beta$  blockade (with propranolol or carvedilol), or endoscopic band ligation or sclerotherapy.





**Figure 41.1** Hepatic artery thrombosis.



**Figure 41.2** Anastomotic stricture.

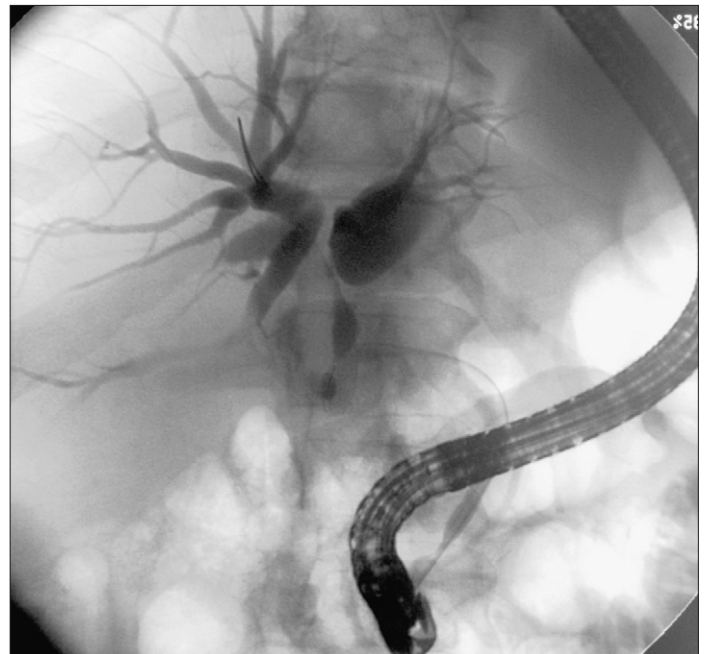
## BILIARY PROBLEMS

The biliary tract remains the Achilles heel of liver transplantation and is the source of most technical complications. The biliary tree is susceptible to ischemic damage because most of its blood supply is derived from a plexus of small collateral arteries that originate in the duodenum, with the remainder being supplied from the right hepatic artery. The use of cut-down and reduced sized livers, and livers derived from non-heart beating donors is more likely to be associated with late biliary strictures.

### Anastomotic strictures

Anastomotic strictures (Fig. 41.2) usually present in the first few months after transplantation but may present later. The clinical history is of obstructive jaundice with pruritus, pale stools and dark urine. Sometimes there is associated cholangitis. Liver function tests typically show an obstructive pattern but it is important to appreciate that this may be subtle or even absent. Ultrasound may demonstrate dilated intrahepatic bile ducts, but the absence of duct dilatation does not reliably exclude a significant biliary obstruction in the liver transplant recipient. The diagnosis is confirmed by imaging the biliary tree, using magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) (usually in patients with a Roux loop and a choledochojejunostomy).

The treatment of anastomotic strictures involves either endoscopic dilatation and stenting (which may be repeated) or formal biliary reconstruction with the fashioning of a Roux choledochojejunostomy. When the stricture arises at the Roux anastomosis, the optimal treatment is with percutaneous intrahepatic dilatation. Sometimes, it is necessary to place a stent



**Figure 41.3** Non-anastomotic strictures.

across this anastomosis but this is best avoided as the long-term consequences are unknown.

### Non-anastomotic strictures

The main causes of non-anastomotic strictures (Fig. 41.3) are:

- ischemic cholangitis;
- biliary sepsis;
- recurrent PSC.

Non-anastomotic strictures are usually multiple but solitary strictures can develop at the hilum. The treatment of single non-anastomotic strictures is similar to that of anastomotic strictures. However, mechanical intervention is usually futile in cases with multiple strictures and the initial management is usually directed at symptoms. Ursodeoxycholic acid (UDCA), at a dose of 10–15 mg/kg/day is usually given to help mitigate the effects of the retention of potentially toxic hydrophobic bile acids, although there is no strong evidence for a mitigating effect on the progression of liver damage. Most cases do progress leading to retransplantation because of uncontrolled symptoms or the consequences of secondary biliary cirrhosis.

RECURRENT DISEASES

Liver transplantation is curative in many indications (Table 41.3), but some diseases will recur in the graft. While this may not necessarily affect graft function and survival, it is important to recognize and, where appropriate, consider altering immunosuppression and offer specific treatment.

Metabolic diseases treated by liver transplantation	
Cured	$\alpha_1$ -Antitrypsin deficiency Antithrombin III deficiency Protein S deficiency Protein C deficiency Wilson disease Hemophilia type A and B Tyrosinosis Byler disease Galactosemia Crigler-Najjar syndrome
Recurrent/persistent	Amyloid Congenital protoporphyria Cystic fibrosis Gaucher disease* Hemochromatosis Niemann-Pick disease* Sea blue histiocyte disease*
*Commonly contraindications for liver transplantation.	

Table 41.3 Metabolic diseases treated by liver transplantation.

Viral infections

Recurrent hepatitis viral infection is covered in greater detail in Chapter 42, but de novo infection may also occur (Table 41.4). Many programs routinely offer immunization with hepatitis A and B virus prior to transplantation.

Hepatitis A virus

Hepatitis A viral infection may rarely affect the graft but the clinical picture is one of a mild, transient hepatitis occurring shortly after transplantation and is of little significance. De novo hepatitis A infection in the graft runs a similar pattern to that seen in the person with a native liver.

Hepatitis B virus

The management of hepatitis B virus (HBV) infected patients before and after transplantation is covered elsewhere. Infection of the graft with HBV may occur:

- when viral replication has not been suppressed prior to transplantation;
- when a non-immune, not infected recipient is transplanted with a graft from a donor who is anti-HBc-positive (hepatitis B core) in the absence of prophylaxis; and
- when HBV prophylaxis is inadequate or ineffective e.g. insufficient duration of treatment, non-compliance, viral mutation resulting in drug resistance.

In general, those who are at risk of developing HBV infection of the graft should be offered treatment with hyperimmune B immunoglobulin (HBIG) and lamivudine. The optimal dose and duration of HBIG treatment is unknown. Lamivudine should be given at 100 mg/day. Occasionally, viral mutations will occur so that the virus is not longer controlled by lamivudine. Therefore, those grafted for HBV-associated liver disease should be monitored with routine liver tests every 3 months together with assessment of serum HBV markers, including HBV DNA. Increases in serum HBV DNA levels suggest the development of viral mutations (such as the YMDD mutation). In this situation, adefovir 10 mg/day can be added to the regimen. Occasionally, HBV is resistant to adefovir but is sensitive to lamivudine.

Those who are given a graft from a donor who is anti-HBc positive should also receive long-term lamivudine and be screened for the development of HBV infection of the graft.

Hepatitis C virus

Recurrent infection of the graft by hepatitis C virus (HCV) is almost invariable. The patterns of damage associated with HCV

Recurrent viral hepatitis disease			
	Hepatitis A virus	Hepatitis B virus (HBV)	Hepatitis C virus
Frequency	Uncertain	Dependent on level of HBV DNA pre-orthotopic liver transplantation	Almost invariable
Outcome	Little significance	Little, if replication treated	Graft disease
Treatment	None needed	Lamivudine Adefovir	Interferon and ribavirin
Effect of immunosuppression	None	None	Worse with high dose steroids

Table 41.4 Recurrent viral hepatitis disease.

Recurrent autoimmune liver disease			
	<i>Primary biliary cirrhosis</i>	<i>Primary sclerosing cholangitis</i>	<i>Autoimmune hepatitis</i>
Incidence at 10 years	50%	40%	Uncertain
Effect on graft survival*	+/-	++	++
Treatment	UDCA	UDCA	Steroids
Diagnosis	Liver histology	MRCP/PTC	Serology and liver histology
Risk factors	Unknown	?Intact colon	Unknown
*+/- little effect, ++ significant effect; MRCP, magnetic resonance cholangiography and pancreatography; PTC, percutaneous transhepatic cholangiography; UDCA, ursodeoxycholic acid.			

**Table 41.5** Recurrent autoimmune liver disease.

recurrence varies from a mild inflammation to severe fibrosis and cirrhosis. As with HBV, a form of cholestatic hepatitis may develop. The role of treatment with peginterferon and ribavirin is effective in a small minority as the treatment is usually poorly tolerated. The risk factors associated with HCV recurrence and its effects are discussed in detail in Chapter 42.

### Autoimmune diseases

Autoimmune diseases may recur after liver transplantation (Table 41.5). Recognition is important because this may have an impact on the graft survival so recipients need to be advised. Furthermore, recognition is important with respect to understanding the pathophysiology of disease, managing the patient with allograft dysfunction and possible modification of the treatment.

#### Primary biliary cirrhosis

Following liver transplantation for PBC, the antimitochondrial and antinuclear antibodies show a transient fall but within 1 year reach or exceed titers measured before transplantation. Conditions associated with PBC, such as thyroid disease or sclerodactyly, may also develop for the first time after liver transplantation.

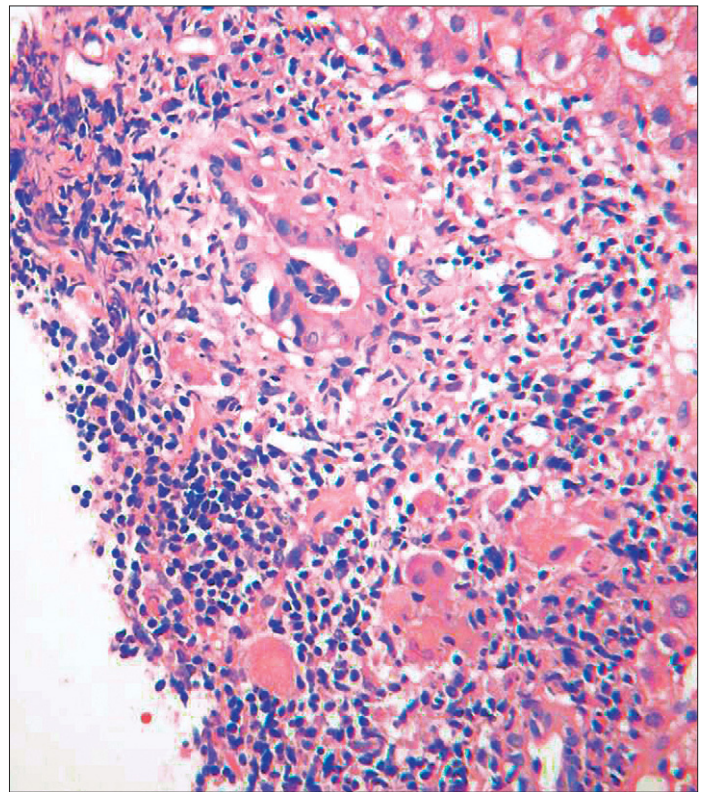
The diagnosis of recurrent PBC is made on the basis of

- cholestatic liver tests in the absence of biliary obstruction,
- granulomatous destruction of the middle-sized intrahepatic bile ducts on liver histology (Fig. 41.4).

It is now established that PBC may develop in the graft, affecting up to 60% by 10 years. The diagnosis is made on histologic examination of the liver. Risk factors have not been established but recent evidence suggests that recurrence is more rapid and more severe in those maintained on tacrolimus compared to those on cyclosporin-based immunosuppression. There is no effective treatment but most centers use UDCA (10–15 mg/kg/day). The effect of disease recurrence in the graft appears to be of little clinical significance within the first 10 years, although cirrhosis requiring retransplantation may develop in less than 3% at this time.

#### Autoimmune hepatitis and de novo autoimmune hepatitis

The reported incidence and risk factors for recurrence of autoimmune hepatitis (AIH) is conflicting. This is due partly to

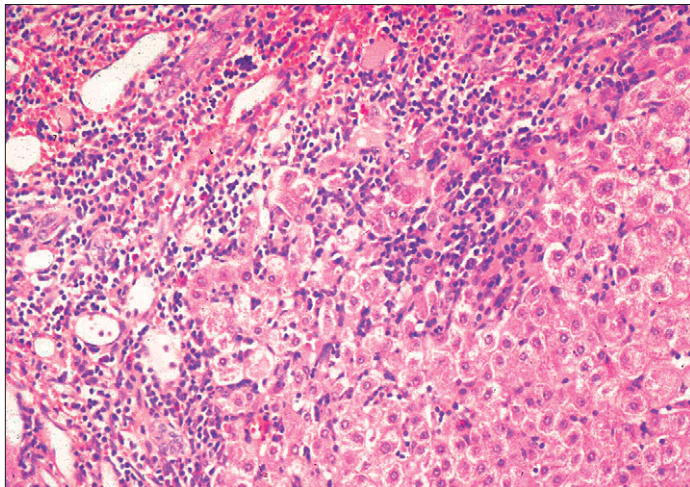
**Figure 41.4** Liver biopsy showing recurrent primary biliary cirrhosis.

varying criteria for defining recurrent disease. Criteria for recurrent AIH include:

- hepatic liver tests;
- elevated serum immunoglobulins;
- autoantibodies in significant titers;
- liver histology showing interface hepatitis (Fig. 41.5); and
- exclusion of other causes of graft dysfunction.

The likelihood of recurrent AIH is probably less than 30%. There is no consensus about risk factors or the response to corticosteroids. Many studies suggest that the majority of cases respond satisfactorily to increased doses of corticosteroids. However, there are other reports of progression to graft failure





**Figure 41.5** Liver biopsy showing recurrent autoimmune hepatitis.

despite additional immunosuppression. Because of the risk of recurrent disease, most centers advocate the continued use of a small dose of corticosteroids (such as prednisolone 5–10 mg/day) for at least 1 year and often longer term.

#### **De novo autoimmune hepatitis**

There have been several reports of a clinical and histologic appearance of AIH developing in patients transplanted for conditions other than AIH. This is more likely to occur in children but is seen in adults. Whether this represents a true 'autoimmune disease' or is a response to graft antigens (and therefore should be considered as a form of rejection) is unclear. The term 'graft dysfunction mimicking autoimmune hepatitis', while more cumbersome, is more accurate. Treatment is with increased immunosuppression and, where appropriate, introduction of corticosteroids, but this is not always effective in preventing progression of liver damage. Some patients develop graft cirrhosis and require regrafting.

#### **Primary sclerosing cholangitis**

The diagnosis of recurrent PSC in the graft may be difficult to substantiate as there are many causes for non-anastomotic strictures in the graft but the following criteria should be used:

- multiple non-anastomotic strictures;
- no severe reperfusion injury;
- no evidence of biliary tree ischemia;
- ABO identical graft; and
- no biliary obstruction.

Recurrent PSC presents with signs and symptoms of cholestasis. Liver ultrasound is usually normal but multiple intrahepatic strictures may be shown by PTC or MRCP (**Fig. 41.6**). ERCP is usually not technically possible since most, if not all, liver allograft recipients will have a Roux loop. Sometimes, the histologic features of PSC may be seen in the allograft (ductopenia, pericentral fibrosis).

Treatment is symptomatic. UDCA is usually offered but there is no evidence yet that this will affect the rate or severity of progression. Recurrent PSC is seen in about 40% by 10 years. Risk factors are unknown although it has been suggested that



**Figure 41.6** Recurrent primary sclerosing cholangitis.

those with a colectomy done before or during transplantation may be protected from recurrent disease.

#### **Metabolic diseases**

Transplantation will cure those metabolic diseases where the defect arises within the liver (such as protein C deficiency or Wilson disease) (Table 41.3). Where the defect arises mainly, or solely, outside the liver, transplantation will not be curative and the recipient must be screened, and where appropriate, treated.

#### **Hemochromatosis**

It is believed that the main defect in hemochromatosis lies in the gastrointestinal tract so that the liver graft may be at risk of accumulation of iron. It is recommended that the recipient be screened with serial estimation of iron, iron bonding capacity and ferritin.

#### **Non-alcohol fatty liver disease**

Non-alcohol fatty liver disease (NAFLD) may recur, or develop de novo in the allograft. The recipient should be advised to maintain ideal body weight and control diabetes (see below). Risk factors are as for fatty liver in the native liver, such as alcohol, obesity, diabetes and small bowel bypass surgery.

#### **Malignancy**

Refining the indications for transplantation for those with hepatic malignancy has meant that persisting disease (usually termed recurrent) is less likely. Rarely, malignancy in the donor may be transmitted to the recipient.

**Hepatocellular carcinoma**

Recurrent hepatocellular carcinoma (HCC) is usually apparent within the first 2 years but can present up to 10 years after transplantation. The tumor may arise in the graft, abdomen, chest or bones. The diagnosis may also be made on the basis of reappearance of raised  $\alpha$ -fetoprotein levels in serum. Few centers routinely screen recipients for evidence of recurrence as there is currently no effective therapy available, except for the occasional case with isolated resectable lesions e.g. in the adrenal bed. The choice of immunosuppression may affect the rate of recurrence. While there is limited data in humans apart from retrospective analysis, it has been suggested that cyclosporin may be associated with more rapid tumor cell division, while, conversely, rapamycin may retard progression by inhibiting tumor angiogenesis.

**Cholangiocarcinoma**

Few patients with cholangiocarcinoma are candidates for transplantation because of the high rates of recurrence as the tumor spreads early through the lymphatic system and nerves. The use of chemotherapy and brachytherapy may allow a selected group of patients to undergo transplantation but the risk of recurrence persists for at least 3–5 years after transplantation. There is no current effective treatment for recurrent cholangiocarcinoma.

**Budd-Chiari syndrome**

There are many factors that may underlie thrombosis of the hepatic veins. About 30–50% are associated with a myeloproliferative disorder and in these patients the risk of further thrombosis remains after transplantation. In other cases, the cause of the prothrombotic tendency is known and is considered to be corrected by liver transplantation (such as protein C deficiency), but nevertheless recipients are currently also routinely considered for long-term anticoagulation. Anticoagulation is commenced immediately after liver transplantation unless contraindicated by catastrophic bleeding. The diagnosis of recurrent hepatic venous outflow obstruction will present with right upper abdominal pain and the onset of ascites. The diagnosis is confirmed by demonstrating thrombosis of the hepatic veins. Stenting or local thrombolysis can be effective but regrafting may be required.

A Budd-Chiari-like syndrome can develop in patients transplanted for other indications in whom the cava is preserved and the hepatic veins are anastomosed using the 'piggy-back' technique. This presents with ascites, usually in the weeks immediately after liver transplantation. This phenomenon may be triggered by a separate cause of graft dysfunction if it causes swelling of the liver and distortion of the angles of drainage of the hepatic veins.

**Sarcoidosis**

Sarcoidosis may recur with florid granulomatous infiltration in the graft but it usually responds rapidly to increased corticosteroid therapy.

**Alcoholic liver disease**

The proportion of patients grafted for alcoholic liver disease who return to alcohol consumption varies greatly in reported series, in part because of the definition of recidivism. Less than 20% return to a pattern of drinking that is potentially harmful

(more than 200 g/week) and fewer than 5% develop problems with graft damage or non-compliance, as a consequence of alcohol consumption.

All persons grafted for alcoholic liver disease should be counseled to avoid alcohol altogether since it is not possible to identify those who are able to return to a safe pattern of drinking. When relapse does occur, the patient should be counseled and offered help to abstain. Screening with markers such as carbohydrate-deficient transferrin or  $\gamma$ -glutamyl-transferase levels, is not routinely done.

**CONSEQUENCES OF IMMUNOSUPPRESSION**

Lifelong immunosuppression is required for most liver allograft recipients. A small proportion of patients can stop immunosuppression with continued good graft function. A small number of transplant programs are currently attempting withdrawal of immunosuppression but this should not be attempted outside these experimental protocols and only after full and informed consent has been obtained from the patient. There are no accurate markers for the prospective identification of clinical tolerance. The advent of newer immunosuppressive regimens (such as those using Campath-1H) may be more effective in inducing tolerance and so permit either less intense immunosuppression or total withdrawal.

Consideration for withdrawal of immunosuppression should be confined to patients who:

- are 3 or more years post transplant;
- have stable and normal liver function; and
- have normal liver biopsies.

Those grafted for autoimmune diseases, such as PBC, PSC or AIH, are less likely to be withdrawn successfully. Immunosuppression should be withdrawn slowly and, any change in liver tests must indicate an increase in immunosuppression.

Immunosuppression is associated with toxicity and, as with any medication, the benefits must be balanced against the side-effects of treatment. Side-effects are either class related (such as increased risk of infection and malignancy) or drug-specific (for example, corticosteroid-associated osteoporosis). The introduction into clinical practice of newer immunosuppressive drugs allows the clinician to tailor the drugs used to the patient. Increasingly, immunosuppressive regimens are being changed over time. Immunosuppression, including choice of drug regimens and specific side-effects, are discussed in detail in Chapter 39.

**GENERAL HEALTH ISSUES**

Liver allograft recipients should be encouraged to return to a normal life but will need to be aware of the possible increased risks associated with a transplant and the consequences.

**Medical problems****Cardiovascular risks**

Most, but not all, studies have suggested that liver allograft recipients have a two- to threefold increased risk of cardiovascular morbidity and mortality. The reasons for this increased risk are multifactorial and include:

- weight gain and obesity post-transplantation;
- hypertension;

- hyperlipidemia;
- diabetes mellitus; and
- renal impairment.

Some of these risk factors may be affected by the immunosuppression regimen. It is therefore important that regular assessment is made of these risk factors and recipients are counseled and treated appropriately.

### Hypertension

The majority of liver allograft recipients are not hypertensive prior to transplantation, partly because of the vasodilatation that is characteristic of advanced cirrhosis. Significant hypertension develops in up to 70% of patients post-transplant. The etiology is multifactorial and is likely to be a consequence, in part, of the effects of the drugs, especially the calcineurin inhibitors and corticosteroids, and renal impairment. Important features of hypertension occurring in the liver allograft recipient include the loss of the nocturnal fall in blood pressure seen in non-grafted persons, and increased systemic vascular resistance.

Blood pressure is usually assessed at each clinic visit but it is well established that these readings correlate poorly with the patients' mean blood pressure readings. A high reading at a clinic should be followed by a series of readings in the community to get a better evaluation of the problem. Twenty-four hour blood pressure profiles are the best way of evaluating patients. Confirmed hypertension should be actively treated. The ideal target blood pressure is not clear in this situation but it seems reasonable to aim for a maximum systolic blood pressure of 140 mmHg and diastolic of 80 mmHg, and with more stringent control in patients with diabetes mellitus and renal impairment.

Drug therapy is usually required and most agents can be used in association with the typical immunosuppressive regimens. However, diuretics tend not to be very effective in this situation. First line therapy is often with a calcium channel blocker, despite a high incidence of dependent edema. Angiotensin-converting enzyme (ACE) inhibitors may be less effective in patients with a high systemic vascular resistance, but their action on the effect of the calcineurin inhibitors on renal blood flow can result in effective blood pressure control. For those who develop troublesome side-effects from ACE inhibitors, angiotensin II receptor antagonists can be used with good effect.

The threshold for treatment should be lowered in those with associated renal disease or proteinuria. In these patients, caution must be exercised with ACE inhibitors because of the potential for precipitating renal failure (Table 41.6). ACE inhibitors are the treatment of choice in patients with type I diabetes. Outside the context of liver transplantation, thiazides,  $\beta$  blockers, ACE inhibitors and the dihydropyridine class of calcium channel antagonists have been shown to be of benefit in slowing the progression of renal damage in patients with type 2 diabetes. However, this benefit has not been confirmed in a similar population of liver graft recipients. Caution must be observed with the use of antihypertensive drugs because of potential side-effects and drug interactions (see Table 41.6).

### Hyperlipidemia

Hyperlipidemia is common after liver transplantation, as with other organ transplantations. The major risk factors include immunosuppressive drugs (especially sirolimus and corticosteroids), coexisting renal damage, diabetes and obesity.

Treatment should be as for those without liver transplantation:

- general measures like avoidance of smoking and excessive alcohol consumption, increase in physical activity;
- pharmacologic treatments, e.g. the use of statins, should be considered in those with significant hyperlipidemia.

'Statins' should be used with caution in the liver allograft recipient because of possible drug interactions. There is an increased risk of rhabdomyolysis when combining a 'statin'

Drug treatment for hypertension in the liver allograft recipient	
<b><math>\beta</math> blockers</b>	
Mode of action:	Reduce cardiac output Block peripheral adrenoceptors Some depress plasma renin
Contraindications:	Asthma Uncontrolled heart failure
Side-effects:	Bronchospasm Bradycardia Peripheral vasoconstriction Fatigue Sleep disturbance
Interactions with immunosuppression:	None major Carvedilol may increase calcineurin inhibitor level
<b>Angiotensin-converting enzyme inhibitors</b>	
Mode of action:	Inhibits conversion of angiotensin I to angiotensin II
Contraindications:	Angioedema Reno-vascular disease
Side-effects:	Profound hypotension Renal impairment Persistent cough Gastrointestinal upset Blood dyscrasia Rarely hepatotoxicity
Interactions with immunosuppression:	Increased risk of hyperkalemia
<b>Calcium channel blockers</b>	
Mode of action:	Interfere with inward displacement of calcium ions through the slow channels of cell membranes
Contra-indications:	Pregnancy Unstable angina Severe left heart failure
Side-effects:	Flushing Headaches Gum hypertrophy
Interactions with immunosuppression:	Some may increase calcineurin inhibitor levels

**Table 41.6 Drug treatment for hypertension in the liver allograft recipient.**

Note: the list of drugs, side-effects and contraindications is not comprehensive



[3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors) with cyclosporin, nicotinic acid and a fibrate (especially gemfibrozil) so these combinations should be used only after full counseling and the introduction of suitable patient monitoring. Fenofibrate in combination with cyclosporin may increase the risk of renal toxicity.

### Gout

Hyperuricemia is seen commonly after transplantation but actual gout is rare. The causes of hyperuricemia include renal impairment and the action of the calcineurin inhibitors on renal urate handling. Treatment of the acute attack of gout is a challenge since the non-steroidal anti-inflammatory drugs (NSAIDs) may precipitate renal failure in those taking calcineurin inhibitors, so colchicine is preferable. Colchicine may cause diarrhea, so drug doses need to be closely monitored. Preventative treatment is required when there are frequent, recurrent attacks, the presence of gouty tophi or signs of gouty arthritis. Treatment with allopurinol is contraindicated in those also taking azathioprine because of the high risk of neutropenia. Probenecid (note interaction with captopril) and sulfinpyrazone may be used.

### Diabetes mellitus

New onset diabetes mellitus develops in about 15% of liver allograft recipients. The risk factors include the use of calcineurin inhibitors (especially tacrolimus), corticosteroids and concomitant HCV infection. Treatment is as for the diabetic with a native liver. Oral hypoglycemic agents of all classes are safe and effective although many patients will require insulin treatment.

### Renal disease

There are many causes for renal impairment in the allograft recipient, and these include:

- calcineurin inhibitor toxicity;
- diabetic renal disease;
- hypertension;
- immunoglobulin A (IgA) nephropathy;
- HCV-associated glomerulonephritis;
- other pre-existing renal disease; and
- drug toxicity.

Calcineurin inhibitor toxicity remains the single greatest cause of renal failure in the liver allograft recipient. A retrospective analysis from the USA analyzed the probability of renal failure in just under 70 000 non-renal allograft recipients and found that during a median follow-up of 36 months, 16% developed end-stage renal disease (defined as a glomerular filtration rate of less than 29 mL/min/1.73 m<sup>2</sup> of body surface area). The risk factors for renal failure are shown in **Table 41.7**. Just under one-third of these patients required renal support or renal transplantation. The onset of renal failure was associated with a 4.5-fold increased risk of death. Several studies have shown that the risk of late renal failure is determined within the first postoperative year and levels and doses of calcineurin inhibitor are strong predictors of later renal failure. There does not appear to be a significant difference between cyclosporin and tacrolimus with respect to the incidence or severity of calcineurin inhibitor associated nephrotoxicity.

The probability of renal impairment and renal failure increases with time, so renal function should be regularly monitored. Simple measurement of serum creatinine and urea may be

misleading and formal measurement of creatinine clearance is often impractical. The use of calculated measures of creatinine clearance (such as the Cockcroft-Gault formula) is less accurate but easier to perform. The mechanism of calcineurin inhibitor nephrotoxicity is not well understood but probably relates, at least in part, to renal ischemia. Kidney histology is not specific but shows interstitial fibrosis, tubular atrophy and vascular sclerosis.

The strategy for the prevention of renal failure is based on a combination of factors, including:

- avoidance of precipitating factors – avoid high levels of calcineurin inhibitor, especially during the first year, avoid concomitant use of drugs such as NSAIDs;
- treatment of exacerbating risk factors – aggressive treatment of hypertension and diabetes;
- early cessation of calcineurin inhibitors; and
- reduction of calcineurin inhibitor in combination with other agents, such as mycophenolate or rapamycin/sirolimus.

The level of creatinine clearance at which calcineurin inhibitor should be reduced or discontinued is not clear. Many programs are considering modification of calcineurin inhibitor regimens with creatinine clearance thresholds of 60–70 mL/min. Depending on the clinical situation, calcineurin inhibitor is either reduced or withdrawn. Many patients retain excellent graft function despite 'suboptimal' immunosuppression, probably reflecting tolerance or near-tolerance of the graft. These patients may get a satisfactory improvement in renal function with a reduction in cyclosporin levels <50 ng/mL or tacrolimus <3 ng/mL. In other patients, a similar outcome is achieved with additional immunosuppression from mycophenolate or sirolimus. When complete withdrawal of calcineurin inhibitors is contemplated, recipients are started on a combination of prednisolone (7.5–10 mg/day) and mycophenolate or sirolimus. Once the patients are stabilized on the new medications, the calcineurin inhibitor is gradually withdrawn completely. Renal function improves or stabilizes in about 70% of patients with these alterations to the immunosuppressive regimens.

Although some recipients can be maintained on monotherapy with mycophenolate, there is a risk of developing acute and chronic rejection and this approach is not recommended. The addition of a low dose of corticosteroids appears to reduce the risk of graft-threatening rejection.

Risk factors for the development of renal failure in non-renal allograft recipients	
Post-operative renal failure	2.13
Diabetes mellitus	1.42
Increasing age (per 10-year increment)	1.36
Hepatitis C virus infection	1.15
Hypertension	1.18
Female gender	0.744
Reproduced from Ojo et al (03) by permission of New Engl J Med.	

**Table 41.7 Risk factors for the development of renal failure in non-renal allograft recipients.**

**Bone disease**

Osteopenia is seen fairly commonly after liver transplantation and identified risk factors include pre-existing osteopenia, prolonged bed-rest, poor diet and use of corticosteroids. Management is aimed at prevention, early detection and intervention. Prevention should start during the management of chronic liver disease and continue after transplantation with early mobilization, curtailment of long-term use of corticosteroids, encouragement of active weight-bearing exercise, a diet adequate in calcium and vitamin D, avoidance of smoking and excess alcohol. There is no consensus as yet for the use of routine DEXA (dual energy X-ray absorptiometry) screening of the liver allograft recipient to screen for osteopenia. There should be a low threshold for the use of DEXA screening in those who are female and post-menopausal, with a family history of osteopenia or a history of non-traumatic fracture. Interventional studies are few but there is now some preliminary evidence that risendronate is effective in reversing the osteoporosis in liver transplant recipients. Spontaneous improvement in bone density has also been observed at 12–18 months after liver transplantation in patients with pre-existing chronic cholestatic disease.

**Bowel disease**

Patients with PSC and other cholestatic diseases have an increased risk of colitis. Following transplantation, the natural history of the colitis may change. In about one-third, the colitis will improve, in about one-third the colitis will remain unaltered and in the remainder, the colitis will deteriorate, requiring either increased treatment or even colectomy. In the liver allograft recipient with good liver function, colectomy is a safe procedure and is indicated in those with severe, recurrent flare-ups, severe dysplasia or malignancy. As indicated above, there is an increased risk of colon cancer in these patients. These cancers are often in the ascending colon.

Although there is no common consensus, it has been suggested that all patients with active colitis receive long-term treatment with UDCA as this has been shown in the non-transplant setting to be associated with a reduction in the rate of formation of colonic polyps and of colonic cancers. We also recommend annual colonoscopy, with multiple biopsies of the entire colon.

**Neurologic problems**

Most neurologic problems present in the first few weeks after transplantation but may be problematic in the long-term survivor. Neurologic problems may be seen as:

- cognitive problems;
- mood disorders e.g. anxiety or depression;
- tremor;
- headaches and migraine;
- dementia; and
- seizures.

In the liver allograft recipient, headaches are not uncommon and may be associated with calcineurin inhibitor use. There is also a risk of migraine, especially in those with a history prior to transplantation. Treatment is as for migraine in the non-transplant recipient e.g. with analgesia, propranolol, or proprietary drug combinations marketed for the treatment of migraine. In severe cases, it may be necessary to switch from calcineurin inhibitors to alternative immunosuppressive agents.

Some neurologic problems may be associated with co-morbid conditions, such as a peripheral neuropathy associated with alcohol excess or diabetes, cerebrovascular disease which may be related to age and exacerbated by drug treatment, and rarely progressive multifocal leukoencephalopathy. When neurologic changes develop, drug effects should be considered and treatment amended. Investigation, including magnetic resonance imaging (MRI) examination, is not often helpful except for when it demonstrates structural problems such as abscess, large or small infarcts or metastatic deposits.

**GENERAL HEALTH AND LIFESTYLE ISSUES****Quality of life post-transplant**

Although the quality of life following liver transplantation is usually very good, it cannot be considered normal. The physical and psychologic sequelae are discussed below.

**Weight gain and obesity**

Weight gain occurs in up to 70%. There are many factors responsible for the weight gain, including the use of corticosteroids, the loss of anorexia associated with liver failure, liberation from dietary restrictions and better absorption and utilization of foods. There is also evidence of a reduction in the basal metabolic rate after liver transplantation. Weight gain is greatest in the first post-transplant year and in those who were overweight prior to the onset of liver disease. The obesity may cause fatty liver disease in the allograft and may even lead to cirrhosis. Liver allograft recipients should be counseled about sensible eating habits and lifestyle activity. Weight should be monitored and dietary advice given early.

**Skin and dental problems**

Many liver allograft recipients have poor dentition as a consequence of their previous liver disease. Dental work may carry a risk of bacteremia. There is no consensus about the need for antibiotic prophylaxis in the liver allograft recipient. If antibiotic therapy is indicated, then care must be taken to ensure there is no interaction with the immunosuppression.

Gum hypertrophy is sometimes seen and may be associated with the use of cyclosporin and with nifedipine. Treatment is of the hypertrophy itself and consideration should be given to switching from cyclosporin to another regimen.

Hirsutism is seen in some of those on cyclosporin treatment and may be distressing to women. Sometimes the hirsutism will resolve after 6 months. If not, treatment is either with topical treatments or by switching to an alternative treatment regimen. Hair loss may occur either as a result of the trauma of the surgery or from the use of immunosuppressive treatment, especially azathioprine.

As discussed above, skin cancers are seen in those on immunosuppression. Any suspicious skin lesion should be biopsied and treatment given accordingly. Basal cell carcinoma is the commonest skin malignancy but squamous cell carcinoma, melanoma and Kaposi sarcoma all occur with higher frequency than in the general population.

**Sexual health and reproduction**

The number of liver allograft recipients who become pregnant is still relatively low but clear trends are emerging. There is a return to normal menstrual pattern with the return of good liver

function. Fertility appears to be similar to that of the general population but there is a decrease in sexual function in women. There are concerns about the potential effects of the immunosuppressive drugs. It should be noted that animal studies suggest that both mycophenolate and rapamycin are teratogenic and these drugs are not indicated in those who wish to become pregnant and alternative immunosuppressive regimens should be chosen. The teratogenic effects of corticosteroids, calcineurin inhibitors and azathioprine are relatively weak.

Women who wish to become pregnant after liver transplantation are advised to postpone pregnancy until at least 1 year and preferably 2 years and when liver tests and liver function is normal. Blood pressure and other complications should be actively treated. The woman should be counseled about the potential complications and risks of teratogenicity associated with drugs. Vitamin (especially folate) and nutritional deficiencies should be corrected. The woman needs also to consider the likely long-term graft survival. Reports from registries of pregnancy in liver allograft recipients suggest that there may be an increase in pre-term delivery and low birth weight babies but no major increase in fetal abnormalities. There may be transient elevations of liver tests either before or after delivery, but these do not appear to be associated with major problems. Risks of hypertension and pre-eclampsia are also increased.

Potential parents need to be aware that some conditions may affect the baby e.g. genetic conditions such as Wilson disease or genetic hemochromatosis, and viral hepatitis will require additional interventions. Breast feeding is contraindicated in those on the major antirejection drugs as these drugs are secreted in the breast milk.

There are little data on the additional risk of contraception in the liver allograft recipient but general experience suggests that it is probably small. The use of hormonal contraception, while effective, is associated with added problems related to the vascular consequences, and this is of particular relevance to patients transplanted for Budd-Chiari syndrome. Intrauterine devices may be associated with an increased risk of infection. The recipient should be counseled and made aware of any additional risks and these risks need to be balanced against the risk/benefit/acceptability ratio of other methods of contraception.

Sexual dysfunction is common in men with chronic liver disease and may have been present for many years prior to transplantation. There is often a sudden and dramatic return of potency as early as the end of the first week after transplantation. Others continue to have some sexual dysfunction. Sildenafil should be used with caution and it is recommended to start at a low dose and titrate upwards.

### Alcohol consumption

The issues surrounding alcohol consumption post-transplantation are controversial. Those transplanted for alcohol-associated liver disease are usually directed to abstain from alcohol completely. While it can be argued that those who are not alcohol dependent could be allowed to return to a safe pattern of alcohol consumption, the dilemma is that it is very difficult to determine in advance who is likely to return to a damaging pattern of alcohol consumption. Because of possible patient exploitation of differences or inconsistencies in advice from different clinicians, it is suggested that the advice given is clear and documented in writing.

Cancers in the liver allograft recipient		
Organ	Tumor	Virus association
Skin	Kaposi sarcoma Squamous cell carcinoma Basal cell carcinoma	Human herpes virus 8
Blood	Lymphoma	Epstein-Barr virus
Uterine cervix	Squamous carcinoma	Papilloma virus
Gastrointestinal tract	Oropharyngeal* Colon† Anal canal	Papilloma virus
*Especially in those grafted for alcoholic liver disease; †Especially in those with inflammatory bowel disease.		

**Table 41.8 Cancers in the liver allograft recipient.**

For those who were grafted for conditions other than alcoholic liver disease, it would seem reasonable to allow the recipient to return to a safe pattern of alcohol consumption, provided liver function is normal and that there are no other features (such as fatty liver or hepatitis C infection) that suggest the recipient is susceptible to graft dysfunction with modest alcohol consumption.

### Psychologic and psychiatric health

A liver transplant is a major procedure not just for the recipient but also the entire family. It is not surprising therefore that there are associated problems, despite the overall major improvement in health-related quality of life. Studies have shown that there is an increase in psychologic distress and relatively poor psychosocial adjustment. There is also an increase in depression and symptoms of post-traumatic stress disorders that can impair the quality of life. Clinicians caring for liver recipients need to be constantly aware of these concurrent conditions and, where they develop, offer appropriate treatment. Antidepressants are well tolerated.

### Cancer

There is an increased risk of de novo cancer in liver allograft recipients (Table 41.8). Cancers that are associated with viral infection are likely to be increased by immunosuppression (such as lymphoma). Others are associated with the indication for transplantation (such as the increased risk of bowel cancer in those with ulcerative colitis associated with PSC). Those transplanted for alcohol-associated cirrhosis have an increased incidence of cancer of the throat, and esophagus, possibly related to smoking rather than the alcohol per se.

#### Lymphoma (post-transplant lymphoproliferative disease)

The reported incidence of post-transplant lymphoproliferative disease (PTLD) is between 2 and 4%. About half the cases develop in the first postoperative year but PTLD may develop at any stage. Most, but not all, are associated with Epstein-Barr virus (EBV) infection or reactivation although early PTLD is more likely to be associated with primary infection and with heavy induction regimens including OKT-3. There is a spectrum of disease from a polyclonal non-specific reactive hyperplasia to an aggressive malignant lymphoma.



The clinical presentation is variable and it is often difficult to make the diagnosis. In contrast to lymphoma in the non-immunosuppressed patient, there is less lymph node involvement. Presenting symptoms include non-specific symptoms such as fever, weight loss, night sweats, lymphadenopathy, abdominal symptoms (such as pain, perforation or obstruction), renal impairment, pulmonary infiltrates or those consequent upon intracranial space occupying lesions. Liver involvement may be diffuse and masquerade as rejection.

When PTLTD is suspected, standard tests are rarely helpful or diagnostic. There is often evidence of EBV infection with high levels of EBV infected cells [usually > 60 000/million peripheral blood mononuclear cells (PBMCs)]. Imaging, with CT or MR, may show masses in the abdomen, chest or skull and biopsy of lesions will usually confirm the diagnosis. Fine needle aspiration is rarely adequate to make the diagnosis. The diagnosis is made on histologic examination. Treatment will include reduction in immunosuppression, and although this approach may be effective in helping resolution of the lymphoma, the price may be graft loss from ductopenic rejection. Antiviral therapy with aciclovir or ganciclovir has been used with limited effect. Others require a combination of radiotherapy or chemotherapy, using conventional regimens such as CHOP (cyclophosphamide, doxorubicin, prednisolone and vincristine). The advent of rituximab, a monoclonal antibody to CD20, is associated with improved responses although full remission is obtained in only about half the cases. Imiquimod has been suggested to be effective, by stimulating the Toll-like receptors and releasing interferon- $\alpha$ .

### Cancer screening

The increase in cancers (particularly those associated with virus infection such as the human polyoma virus with skin, anal canal and uterine cervix, and human herpes virus 8 and Kaposi sarcoma) in the transplant population has led to calls for the use of screening protocols. There is no good evidence that additional screening will detect early cancers and improve outcomes although it makes good sense that usual screening regimens be closely followed, e.g. as for breast and uterine cancer. The recipients should regularly look for new skin lesions and any new lesion should be assessed and treated as needed. For those with a history of inflammatory bowel disease, there is an increased risk of bowel cancer especially in the right side of the colon.

### Infection

Infections in the long-term liver allograft recipient sometimes present as a major problem. Both the recipient and the clinician need to be aware of the increased susceptibility to common infections and the greater potential to become infected with unusual organisms. Bacterial, viral and fungal infections should be considered in any allograft recipient who becomes unwell. Infective episodes require prompt diagnosis and treatment.

Cytomegalovirus (CMV) infection occurs predominantly during the first few months after transplantation and is covered in greater detail in Chapter 17. Herpes virus infections, herpes simplex and varicella-zoster, are relatively common and may occur at any stage. Herpes simplex virus type 1 presents with oral ulceration and herpes simplex type 2 with genital ulcerations.

Reduction of immunosuppression, where possible, may be adequate to allow resolution of infection but topical or systemic therapy may be required. Varicella-zoster infection may reactivate in allograft recipients. Shingles should be treated actively with oral ganciclovir or valganciclovir. Primary infections may be severe with disseminated disease including hemorrhagic pneumonia, encephalitis, hepatitis, pancreatitis and disseminated intravascular coagulation.

Human herpes virus 8 is associated with the development of Kaposi sarcoma. Reactivation of EBV occurs in up to 50% of liver allograft recipients but clinically significant EBV infection is rare in the adult liver allograft recipient. In children, it is particularly associated with the development of lymphoma. West Nile virus is rarely seen but carries a worse outlook. Polyoma JC virus is a cause of progressive multifocal leukoencephalopathy. This presents as a progressive demyelinating disease with characteristic findings on MRI of the brain. The condition is progressive although immunosuppression reduction may slow progression. Human herpes virus 6 can also result in encephalitis. Papilloma virus infection can induce warty skin tumors that may undergo malignant transformation, especially where there is exposure to the sun. Cervical papilloma virus infection is associated with increased risk of squamous cell carcinoma of the uterine cervix. Reduction of immunosuppression may modify the progression of the infection.

### Drug prescribing

Liver allograft recipients will, like normal subjects, require medication for intercurrent problems. It is important that clinicians and recipients are aware of potential interactions with immunosuppressive agents. These are described in detail in Chapter 39. It must be stressed to the patient that it is not just those drugs prescribed by clinicians that might interfere with immunosuppressive agents, but also 'over the counter' medicines, such as non-steroidal anti-inflammatory agents, and healthcare remedies as well as 'natural' remedies such as St John's wort that might affect drug levels and lead to either toxicity or, with low levels, to allograft rejection.

### Vaccination

Vaccination with live or attenuated vaccines is contraindicated in any person on immunosuppression. Other vaccines can be safely administered (unless there is any other contraindication) (Table 41.9).

### Non-compliance

Non-compliance (sometimes termed non-concordance) with medication occurs and may lead to graft damage and even graft loss. There are many causes ranging from forgetfulness to experimentation to deliberate non-compliance. Sometimes it is a manifestation of associated depression. It is also a regular event in adolescents. Non-compliance is easy to detect when there is a sudden variation in blood levels of drugs, but some crafty exponents of the practice will comply with therapy immediately before a scheduled clinic visit. A history from both the patient and the care-giver may be unreliable. Clinicians need to be aware of the possibility of non-compliance and, where this occurs, there needs to be full and frank discussions between the clinician, the recipient and the care-givers and suitable help, support and counseling given.

<b>Vaccines for the liver allograft recipient. Live/attenuated vaccines should be avoided</b>	
Live/attenuated vaccines	BCG vaccine Measles Oral polio* Rubella Typhoid Ty21A Varicella-zoster Yellow fever
Denatured/polysaccharide vaccines	Anthrax Cholera Diphtheria Hemophilus Hepatitis A Hepatitis B Influenza Meningococcal Pneumococcal Polio (inactivated) Rabies Typhoid (not all typhoid vaccines)
*Note that household contacts should also avoid oral polio vaccines as the attenuated virus is excreted in the feces.	

**Table 41.9 Vaccines for the liver allograft recipient.** Live/attenuated vaccines should be avoided.

### **Routine liver biopsies post-transplant**

There is no overall consensus about the use of routine, protocol liver biopsy of the allograft. Arguments in favor include the fact that liver tests do not necessarily reflect the liver histology and some conditions, such as azathioprine-associated nodular regenerative hyperplasia will only be detected after liver biopsy.

Rejection and disease recurrence may develop in the absence of significant disturbance of liver function. Others argue that there is a small but real risk with the liver biopsy and that the procedure should be performed only when there is a specific indication.

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## SECTION V: LIVER TRANSPLANTATION

### CHAPTER

# 42

## Recurrent Viral Hepatitis in Liver Transplant Recipients

Scott W. Biggins and Norah A. Terrault

### INTRODUCTION

The majority of liver transplants performed worldwide are for complications of chronic viral hepatitis. Medical and surgical advances over the last two decades have made long-term survival after liver transplant a reality. However, recurrent disease due to viruses accounts for a significant proportion of the cost, morbidity and mortality post-transplantation, and the prevention and treatment of recurrent viral hepatitis is one of the most critical areas for improvement in liver transplant outcomes.

Hepatotropic viruses resulting in recurrent infection following liver transplantation are listed in **Table 42.1**. In terms of magnitude, hepatitis B virus (HBV), hepatitis C virus (HCV) and cytomegalovirus (CMV) account for the vast majority of infections and are the main contributors to post-transplant morbidity and graft loss. Less frequently, herpes simplex virus (HSV), herpes

zoster virus (HZV), Epstein-Barr virus (EBV), can recur. In this latter group, EBV infection is most serious, resulting in post-transplant lymphoproliferative disease (PTLD). Uncommon causes of recurrent viral infection manifesting as hepatitis include adenovirus, human herpesvirus-6 (HHV-6) and HHV-7.

In the USA, chronic HBV infection accounts for 8% of liver transplants performed. Initial results were poor, with 5-year survival rates of 50% in the early 1990s. The advent of effective prophylactic therapies to prevent recurrent HBV infection led to significant improvements in outcome and current survival rates at 5 years are 80% or greater. The importance of preventing recurrent infection to ensure prolonged graft survival is highlighted by the HBV experience. HCV is the most common indication for liver transplantation in the USA and Europe accounting for up to 50% of transplants in some programs. Unlike HBV, there are presently no effective prophylactic

**Recurrent hepatotropic viruses in liver transplant recipients**

<i>Virus</i>	<i>Prevalence %<sup>a</sup> US LT 2003</i>	<i>% Recurrent disease</i>	<i>References</i>
HBV	2.6 5–10	2.7–10 (with combination HBIg/antiviral prophylaxis) <sup>c</sup>	UNOS <sup>b</sup> Roche <sup>c</sup>
HCV	26.4	100	UNOS <sup>b</sup>
HDV	NA, <0.1 <sup>b</sup>	80 (if HBV recurrence) <sup>d</sup>	Samuel <sup>d</sup>
CMV	7.9 <sup>b</sup> (IgG+) 27 <sup>a</sup> 60 <sup>a</sup>	4.8 <sup>d</sup> (all) with Rx 18 <sup>d</sup> (all) without Rx 14.8 <sup>d</sup> (D+R-) with Rx 44 <sup>d</sup> (D+R-) without Rx 0.7 <sup>e</sup> (D+R+) with Rx 5.2 <sup>e</sup> (D+R-) with Rx	Burak <sup>a</sup> Gane <sup>f</sup> Seehofer <sup>e</sup>
EBV	54 <sup>b</sup> (IgG+), 4.2 <sup>b</sup> (DNA+) 58 (DNA+)	NA (adults) 69 (peds)	Barkholt <sup>g</sup> Krieger <sup>h</sup>
HSV	35 <sup>i</sup>	1 (liver), 53 (oral) <sup>i</sup>	Singh <sup>i</sup>
VZV	7 <sup>i</sup>	3 (1 year) <sup>j</sup> , 14 (5 years) <sup>j</sup>	Singh <sup>i</sup> Herrero <sup>j</sup>
Adenovirus	5.8 <sup>k</sup>	3.6 <sup>k</sup>	McGrath <sup>k</sup>
HHV-6, -7	14–82 <sup>l</sup>	1–6.7 <sup>l</sup>	Razonable <sup>l</sup>

HBIg, hepatitis B immunoglobulin; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; VZV, varicella-zoster virus; HHV-6, -7, human herpes virus-6 and -7; LT, liver transplantation; Rx, prophylactic treatment; D/R, donor CMV antibody and recipient CMV antibody status

<sup>a</sup>Burak et al (02); <sup>b</sup>Based on OPTN data as of January 1, 2004; <sup>c</sup>Roche & Samuel (04); <sup>d</sup>Samuel et al (97); <sup>e</sup>Seehofer et al (02); <sup>f</sup>Gane et al (97);

<sup>g</sup>Barkholt et al (96); <sup>h</sup>Krieger et al (00); <sup>i</sup>Singh et al (88); <sup>j</sup>Herrero et al (04); <sup>k</sup>McGrath et al (98); <sup>l</sup>Razonable & Paya (02).

**Table 42.1 Recurrent hepatotropic viruses in liver transplant recipients.**

Therapeutic strategies used in the management of recurrent viral infections		
Timing/Type	Definition	Examples
Pretransplant	Initiated before transplantation and continued for variable periods post-transplantation with the goal to prevent recurrent infection	ADV or LAM for HBV decompensated cirrhosis LADR protocol of interferon $\alpha 2b$ plus ribavirin for HCV cirrhosis
Prophylactic	Initiated at the time of transplantation and continued for variable periods post-transplantation with the goal of preventing recurrent infection	HBIG begun in anhepatic phase of transplant for HBV; Ganciclovir for CMV negative recipient of CMV positive donor liver
Pre-emptive	Initiated early in the post transplant period, typically within the first 4 weeks following transplantation, and before biochemical and histological disease is manifest	PEG-IFN and RBV after transplant for HCV with normal ALT without biopsy
Recurrent disease	Initiated after biochemical and histological evidence of recurrent disease	PEG-IFN and RBV for HCV with elevated ALT and stage $\geq 2$ fibrosis on biopsy ADV for HBV recurrence with elevated ALT and progressive histology Ganciclovir for CMV hepatitis after transplant
ADV, adefovir; CMV, cytomegalovirus; HBIG, hepatitis B immunoglobulin; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, interferon; LADR, low accelerating dose regimen; LAM, lamivudine; PEG-IFN, pegylated IFN; RBV, ribavirin.		

**Table 42.2 Therapeutic strategies used in the management of recurrent viral infections.**

therapies to prevent graft reinfection. Recurrent infection is universal in those with viremia pretransplantation, and progressive histologic disease leading to cirrhosis occurs in up to 30% within 5–10 years of transplantation.

Therapeutic approaches for the management of viral hepatitis in the setting of transplantation generally fall in one of several categories (Table 42.2). Pretransplant therapies include those focused on achieving viral eradication prior to transplantation and are most applicable to HBV and HCV. Prophylactic therapies are given from the time of transplantation for defined risk periods or indefinitely. Prophylactic approaches have been used successfully to prevent recurrent HBV and CMV infections. Pre-emptive therapies are used when recurrent viral infection has occurred but before liver disease is manifest. This strategy has been used in the management of HCV-infected patients. Finally, for those with established recurrent infection and evidence of graft or other organ injury, antiviral therapies have been used to eradicate infection and control disease progression.

## HEPATITIS B VIRUS

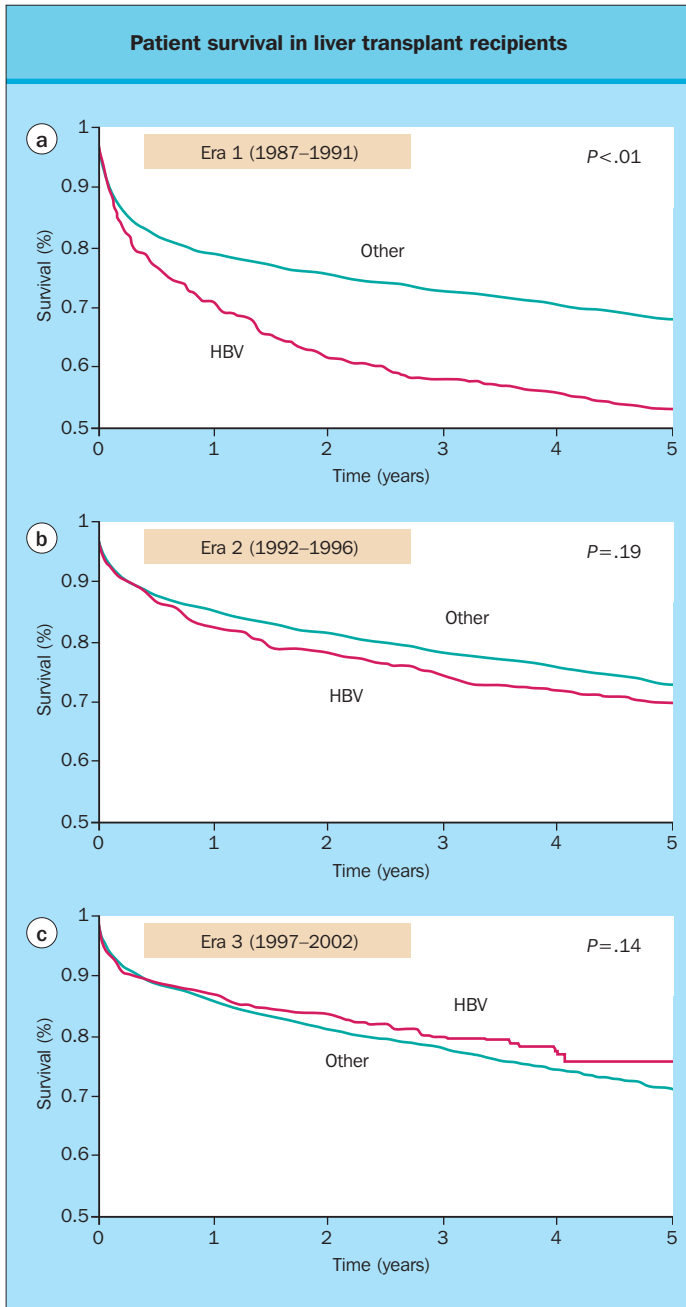
Successful treatment advances in the last decade have significantly improved outcomes of liver transplantation for HBV. The early experience of liver transplantation for HBV was vexed by recurrent HBV infection in the majority of patients with often aggressive disease leading to high rates of graft loss. Hepatitis B immunoglobulin (HBIG) given perioperatively and for prolonged periods post-transplantation, markedly reduced HBV recurrence with 5-year survival rates increasing from 50 to 75%. Nucleoside/nucleotide agents including lamivudine, tenofovir and adefovir dipivoxil, given pre- or post-transplantation have further contributed to improvements in graft and patient survival (Fig. 42.1). Current graft survival rates for patients with HBV are comparable or superior to other indications for transplantation.

## Diagnosis and pathology of recurrent HBV

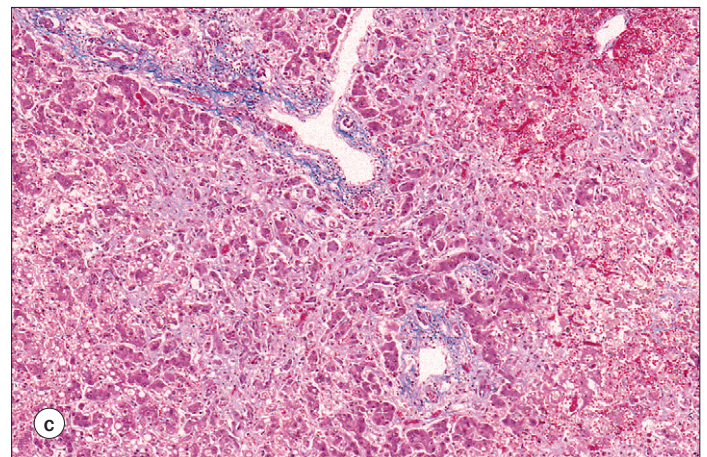
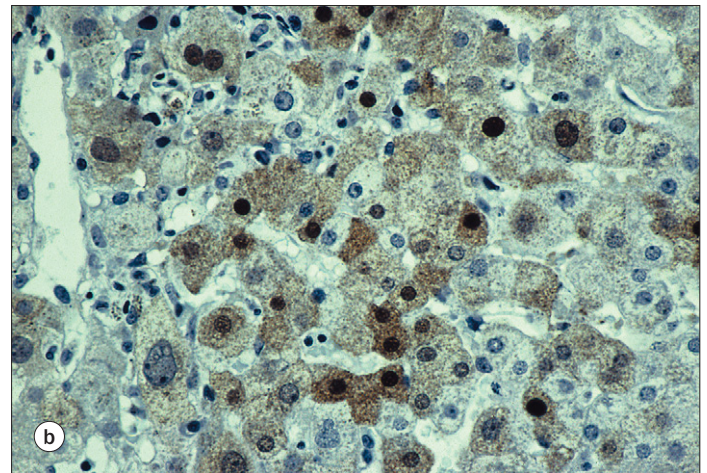
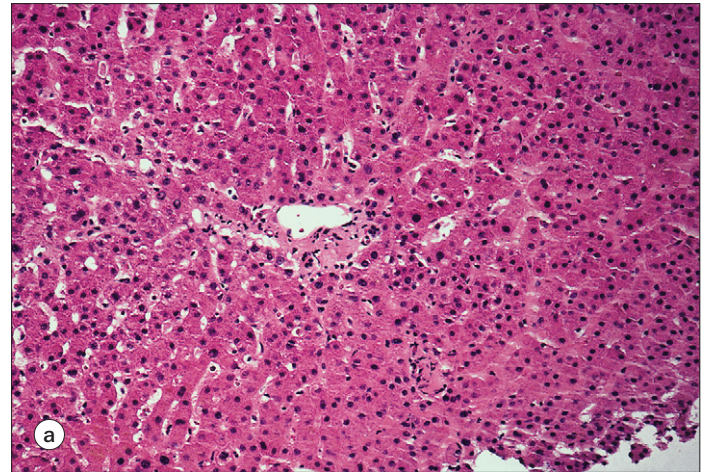
Recurrence of HBV is defined typically by the reappearance of HBsAg in the serum and is associated with variable levels of HBV DNA in serum and liver. The reappearance of these serologic and virologic markers is accompanied by clinical evidence of hepatitis with elevation of serum aminotransferase levels and acute or chronic hepatitis on liver biopsy (Fig. 42.2a). Immunohistochemical staining for hepatitis B surface (HBs) antigen (HBsAg) and hepatitis B core (HBc) antigen (HBcAg) are positive in those with histologic evidence of recurrent HBV and active replication (Fig. 42.2b). In most patients the histologic features are the same as those found in non-transplant patients. Early changes are those of acute hepatitis with a predominance of lobular necroinflammation evolving to portal-based disease with variable degrees of hepatocyte necrosis, mononuclear inflammation and fibrosis. The remarkable features of post-transplant HBV infection are the more rapid rate of fibrosis progression, with evolution to cirrhosis occurring in as short a period as 2 years. In approximately 5% of transplanted patients with recurrent HBV, a variant form of hepatitis, called fibrosing cholestatic hepatitis or FCH, develops. FCH is a hyperaggressive form of HBV infection unique to the transplant setting. The variant is associated with very high levels of HBV DNA in serum and high expression of HBsAg and HBcAg in hepatocytes, together with cholestasis, ballooned hepatocytes and a paucity of inflammatory cells (Fig. 42.2c). These findings suggest a direct cytopathic injury to hepatocytes by HBV.

## Natural history of recurrent HBV after liver transplantation

Without prophylactic therapy, HBV recurrence occurs in 80–100% which, without treatment, leads to cirrhosis and graft loss within 5 years in 50% of patients. Predictors of a more favorable outcome after transplant are hepatitis D virus co-infection, fulminant hepatic failure, absence of HBeAg and low levels of HBV DNA



**Figure 42.1 Patient survival in liver transplant recipients.** Survival in transplant recipients with HBV versus other diagnoses. A progressive improvement in survival is seen in patients transplanted for chronic HBV in the past 15+ years. While patient survival was significantly lower in HBV patients versus other diagnoses in era 1, these differences are not apparent in subsequent cohorts. The improvement in survival coincides with improvements in therapies to prevent and treat HBV infection. (a) Era 1 (1987–1991), (b) era 2 (1992–1996), and (c) era 3 (1997–2002). Reproduced from Kim et al (04) by permission of Wiley-Liss, Inc.

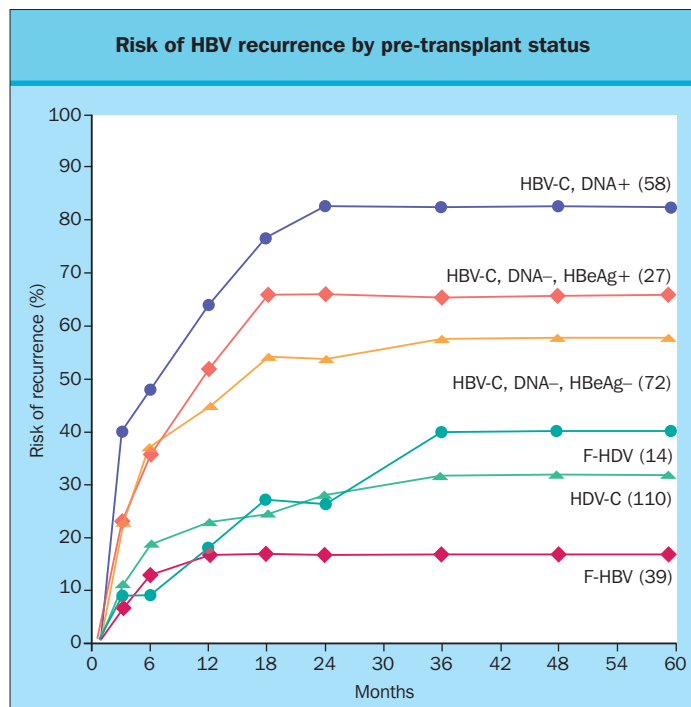


**Figure 42.2 Histologic features of recurrent HBV infection after liver transplantation.** (a) Recurrent HBV, chronic hepatitis; (b) Immunohistochemical stains for HBcAg and HBsAg in acute recurrent HBV; (c) recurrent HBV, fibrosing cholestatic variant. (Courtesy of Dr Linda Ferrell, University of California San Francisco.)

before transplantation (Fig. 42.3). For those who develop FCH, the mortality rate is essentially 100% in the absence of antiviral 'rescue therapy'. With current therapies to prevent and treat recurrent HBV infection, survival rates are currently 85% overall. The treatment approaches utilized prior to and following liver transplantation to prevent and treat HBV infection are detailed in Figure 42.4 and discussed below.

The natural history of HBV after transplantation is accelerated, presumably in large part, due to the effects of immunosuppressive therapy. In the absence of prophylaxis therapies to prevent HBV recurrence, HBV DNA levels increase after transplantation. There is a corticosteroid responsive element on the hepatitis B core protein which upregulates HBV replication. Lymphocyte-





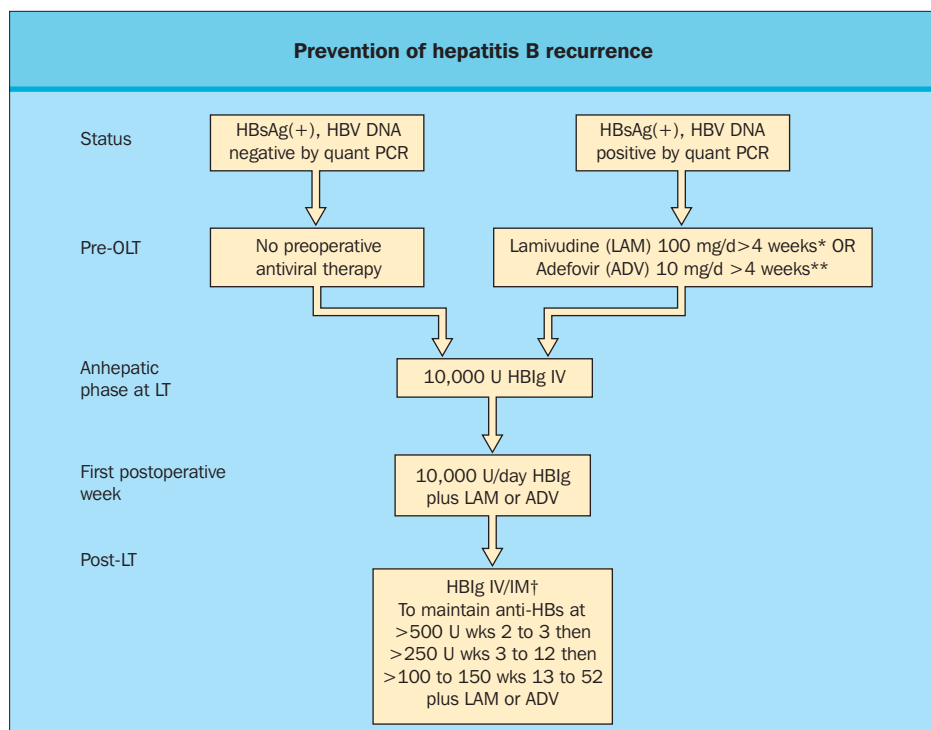
**Figure 42.3 Risk of HBV recurrence by pretransplant status.** The risk of HBV recurrence, defined by presence of HBsAg in serum, is highest among those with indices of active viral replication pretransplantation (HBeAg-positive and HBV DNA-positive by hybridization assay) and lowest in patients with low levels of HBV DNA pretransplantation, including those with fulminant hepatitis and hepatitis D co-infection. Reproduced from Samuel et al (93) by permission of the Massachusetts Medical Society. F-HDV, fulminant HDV; HDV-C, HDV cirrhosis; F-HBV, fulminant HBV; HBV-C, HBV cirrhosis.

depleting therapy (OKT-3) was associated with a rapid increase in HBV DNA levels in one study. Azathioprine also increases HBV replication in vitro, but cyclosporin does not. The effects of other calcineurin inhibitors and inosine monophosphate dehydrogenase (IMPDH) inhibitors on HBV replication are less well characterized.

Acute rejection is less common in liver transplant recipients with chronic HBV compared to those transplanted for other causes of cirrhosis. The immunosuppressive effects of chronic HBV infection are cited as the probable reason for this lower rate of rejection. With the widespread use of prophylactic therapies to prevent HBV recurrent post-transplantation, recurrent HBV infection is now an uncommon event, and the effects of chronic HBV infection on risk of acute rejection are less relevant. However, for those patients with recurrent HBV infection, suppression of HBV replication using antiviral agents such as lamivudine or adefovir may theoretically increase the risk for acute rejection. Recovery of viral-specific immune responses has been documented in patients treated with lamivudine therapy. While a consideration, an enhanced risk of rejection in liver transplant recipients with recurrent HBV disease on treatment with antivirals has not been reported.

### Pretransplant management of HBV

The antiviral drugs lamivudine and adefovir are well tolerated in patients with decompensated liver disease allowing patients with advanced disease to be treated safely and effectively while awaiting transplantation (Table 42.3). Use of interferon in cirrhotic patients, especially those with evidence of reduced liver synthetic function or symptoms of decompensation, is limited by cytopenias, a heightened risk of bacterial compli-



**Figure 42.4 Prevention of hepatitis B recurrence.** Most transplant programs use a combination of HBIG and nucleoside/nucleotide analogs as prophylaxis. The duration of antiviral therapy pretransplantation is dictated by the replication status of the patient (presence of HBV DNA by quantitative PCR).

†Post-transplant HBIG dosing is variable in terms of dose and duration. The doses presented here represent a more conservative approach of indefinite combination therapy. However, lower doses of HBIG and discontinuation of HBIG with or without vaccination, plus continued nucleoside/nucleotide analog therapy have been used with some success.

\*Patients with lamivudine resistance require treatment with adefovir.

\*\*Patients with adefovir resistance require treatment with lamivudine.

<b>Treatment options in patients with decompensated cirrhosis awaiting liver transplantation</b>			
	<i>Lamivudine</i>	<i>Adefovir</i>	<i>Entecavir</i>
Current status	FDA-approved for HBV	FDA-approved for HBV	FDA-approved for HBV
Dose (q.d.)	100 mg	10 mg	0.5 mg 1.0 mg lamivudine-resistant
Route of administration	oral	oral	oral
Duration of treatment	12 months + (HBeAg+) 12 months ++ (HBeAg-)	12 months + (HBeAg+) 12 months ++ (HBeAg-)	12 months + (HBeAg+) 12 months ++ (HBeAg-)
HBeAg seroconversion	16% at 1 year 27% at 2 years 40% at 3 years	12% at 1 year	21% at 1 year
HBsAg loss	0% at 1 year	0% at 1 year	0% at 1 year
Histological improvement	55% at 1 year	53–64% at 1 year	55–72% at 1 year
Side-effects	Elevated amylase, CK but pancreatitis rare	Renal toxicity with higher doses	Rare
Frequency of contraindications	± Dose adjustment for renal insufficiency	± Dose adjustment for renal insufficiency	± Dose adjustment for renal insufficiency
Risk of drug resistance	20% at 1 year 70% at 5 years	~2–3% at 2 years	0% at 1 year if wild-type HBV 7% at 1 year lamivudine-resistant
Cost <sup>†</sup>	Moderate (\$7 per day)	High (~\$18 per day)	High (~\$20 per day)
CK, creatinine; FDA, federal drug administration; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus. <sup>†</sup> Average wholesale price.			

**Table 42.3 Treatment options in patients with decompensated cirrhosis awaiting liver transplantation.**

cations, and the potential to precipitate worsening of liver function. Tenofovir fumarate has been used in patients with HIV and HBV infection, and appears to be a safe and effective anti-HBV agent. Lamivudine, adefovir and tenofovir require dose-adjustment in patients with renal dysfunction. Adefovir has been associated with elevated creatinine levels in 3% of treated patients and these changes are usually reversible. Renal dysfunction, manifested by Fanconi syndrome and elevation in creatinine, has also been described with tenofovir.

By achieving suppression of HBV replication pretransplantation, the risk of recurrent HBV infection post-transplantation is reduced. In addition, important and significant biochemical and clinical improvements can be achieved, leading to a delay in the need for liver transplantation in some patients. Since clinical and biochemical improvements tend to lag behind virologic response, patients with severely decompensated liver disease may die before the clinical benefits of antiviral treatment can be realized. In a multicenter study of 154 North American patients with decompensated chronic hepatitis B treated with lamivudine for a median of 16 months, 78% of deaths occurred within the first 6 months of treatment and were due to complications of liver failure. In contrast, those treated patients surviving at least 6 months had an actuarial 3-year survival of 88%.

Lamivudine was the first nucleoside analog to be used in the treatment of decompensated cirrhotics and there is abundant evidence attesting to its benefits in this population. In a study of 23 patients with severely decompensated HBV cirrhosis, defined

as a Child-Pugh-Turcotte (CPT) score of 10, treated with lamivudine, the median change in CPT scores was –3.0 versus +1.0 in an untreated historical control group ( $P = 0.016$ ). Liver transplantation was performed in 34.8% of treated patients versus 73.9% of controls ( $P = 0.04$ ) and the time to death or transplantation was significantly longer in treated patients than in controls ( $P < 0.001$ ). Adefovir offers similar clinical benefits. In a large multicenter trial, 128 patients with decompensated cirrhosis and lamivudine resistant HBV treated with adefovir, 81% became aviremic by quantitative polymerase chain reaction (PCR) and 90% had improved CPT scores. Tenofovir is another nucleoside analog like adefovir, with activity against both wild-type and lamivudine-resistant HBV. Since tenofovir has not been approved for treatment of chronic HBV, information on efficacy and long-term safety in liver transplant recipients is largely lacking. Entecavir has activity against wild-type, lamivudine-resistant and adefovir-resistant HBV. Since this drug was only recently approved, data on efficacy in liver transplant patients is not yet available.

Hepatitis ‘flares’ during lamivudine and adefovir treatment occur in about 10% of treated patients after 1 year of therapy and are also frequent with treatment discontinuation. Consequently in cirrhotic patients, especially those with evidence of reduced hepatic reserve or frank decompensation, prolonged treatment is used. Prolonged therapy, however, is associated with an increased frequency of resistance and the consequences of drug resistance in cirrhotic patients can be significant. The risk

of drug resistance is around 20% after 1 year with lamivudine and 2% after 2 years with adefovir. The emergence of drug resistant HBV has been linked with an increased frequency of hepatitis flares, worsening of histology, and clinical deterioration in patients with cirrhosis, including liver decompensation and liver-related death. The emergence of drug-resistant HBV pretransplantation also increases the risk of recurrent HBV after transplantation. Thus, careful monitoring of HBV DNA levels is warranted in cirrhotic patients and changes in antiviral therapy should be considered when HBV DNA levels increase by 1.0 log<sub>10</sub> or more and before clinical decompensation occurs. Considering the risks associated with the development of drug resistance with prolonged therapy, combination nucleoside/nucleotide analogs would appear to be a good strategy. Studies to date, however, have not determined the optimal drug combinations nor have the benefits of combination therapy been established.

## Post-transplant treatment strategies

### Prophylactic HBIg therapy

While HBIg monotherapy is largely of historical interest since the current practice is to use HBIg in combination with one or more antivirals, the factors influencing treatment efficacy and failure with HBIg monotherapy are relevant to today's prophylactic strategies. Before the use of HBIg, many transplant programs considered 'active' HBV infection a relative or absolute contraindication to liver transplantation. HBIg was the first therapy shown to prevent HBV recurrence in the transplant setting. While short-term HBIg given for 6 months or less was not effective prophylaxis, subsequent studies using longer duration, higher dose HBIg demonstrated a significant decrease in HBV recurrence and a parallel improvement in survival. In the largest study of 334 transplant recipients with HBV, patients treated with HBIg at least 6 months post-transplant had a recurrence rate of 36% compared to 75% in patients not receiving HBIg.

Pretransplant replication status was and remains an important determinant of the success of prophylactic therapy. Active HBV replication, defined by presence of HBeAg and/or serum HBV DNA levels >10<sup>5</sup> copies/mL, predicts a higher risk of treatment failure with HBIg than 'inactive' chronic HBV disease. Most protocols administer high dose HBIg (typically 10 000 IU) intraoperatively during the anhepatic phase and for the first postoperative week. Dosing after this immediate postoperative period is more variable; some protocols titrating HBIg to maintain specific anti-HBs titers while others providing a fixed monthly dose of HBIg. Pharmacokinetic studies demonstrate that 'effective prophylaxis' requires HBIg doses titrated to achieve anti-HBs titers of 500 IU/L during the first week, of 250 IU/L during weeks 2–12, and of 100 IU/L thereafter. Thus both prolonged treatment periods and high anti-HBs titers, especially in the first 12 weeks post-transplantation are important in achieving high treatment efficacy. A recent pharmacokinetic study of patients receiving pretransplant lamivudine followed by combination lamivudine and HBIg therapy post-transplantation reaffirms that importance of pretransplant replication status in determining HBIg requirements. Recipients with low levels of HBV DNA (<10<sup>5</sup> copies/mL) pretransplantation had lower HBIg requirements than those with higher levels of HBV DNA. Interestingly, the HBIg requirements of native non-replicators (i.e. low levels of HBV DNA prior to receipt of antiviral therapy) were lower than patients with low HBV DNA levels due to

lamivudine suppression, who in turn, had lower HBIg requirements than patients with high levels of HBV DNA (despite lamivudine therapy) at the time of liver transplantation. These data suggest that the pretreatment replication status and pretransplant success of antiviral therapy are both factors influencing HBIg requirements and the risk of HBV recurrence post-transplantation.

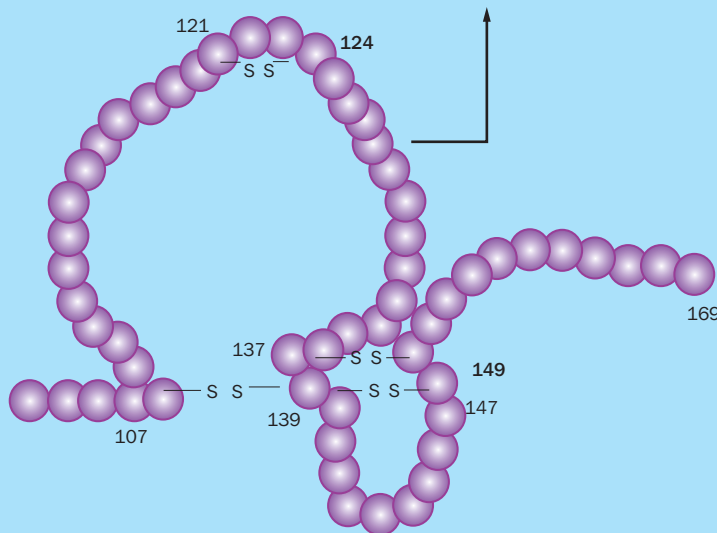
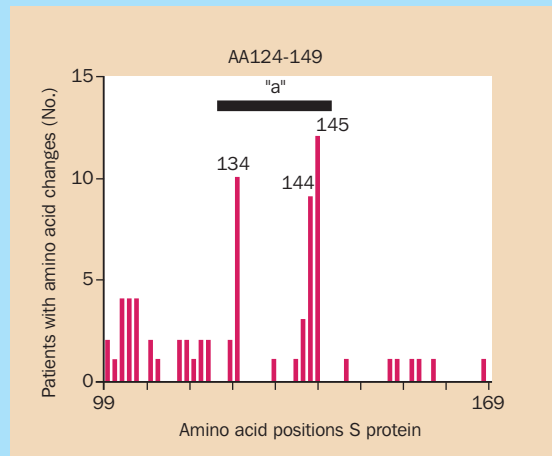
Prophylaxis using HBIg monotherapy failed to prevent recurrent HBV in 20% of patients on average, with very low rates (5% or less) in those with low level HBV replication pretransplantation but up to 50% in those with active replication pretransplantation. Early failures were due to inadequate suppression of HBV replication due to high pretransplant HBV DNA levels, inadequate dosing of HBIg, or both. Treatment failures occurring later (after at least 6 months of HBIg) were due to the development of HBV surface escape mutants. Escape mutations were located in the immunodominant epitope of the surface protein, the 'a' determinant, most commonly at amino acid positions 134, 144 and 145 (Fig. 42.5) and associated with reduced binding of anti-HBs in vitro. Given the reduced binding affinity, continuing HBIg after the emergence of escape mutants is ineffective and alternative therapies are needed to prevent progressive HBV disease. HBIg escape mutants are sensitive to lamivudine and adefovir.

Studies have evaluated whether transplant recipients on HBIg monotherapy who are HBsAg-negative and HBV DNA-negative can be transitioned from HBIg to lamivudine monotherapy. In a study of 16 HBsAg-negative, HBV DNA-negative transplant recipients who switched from HBIg to lamivudine 2 years after transplantation, none developed recurrent HBV with average follow-up of 51 months. Another study reported less success with this strategy with 4 of 12 patients developing recurrent HBV viremia after 34 months of follow-up. The failure or success of this strategy may be related to the pretransplant replication status of the patient. Patients who have active replication pretransplantation are at higher risk for treatment failure with either HBIg monotherapy and with lamivudine monotherapy. In those patients on long-term HBIg prophylactic therapy who are HBsAg-negative and without clinical evidence of HBV recurrence, HBV DNA has been identified in serum, lymphocytes or liver in nearly half of the patients 10 years post-transplantation, but at low levels only detectable by PCR methods. This suggests the risk of recurrent HBV persists for years, even in the presence of effective prophylactic therapy. Such data suggest long-term and possibly indefinite treatment may be required in these transplant recipients. Whether those patients lacking HBV DNA in all compartments can be safely discontinued from anti-HBV therapy is unknown.

### Lamivudine monotherapy prophylaxis

In the search for prophylactic therapies which were safe and well-tolerated in transplant recipients, easy to administer and of lower cost, lamivudine monotherapy was initially examined as an alternative to HBIg monotherapy. The largest study was of 77 HBV-infected patients awaiting transplantation, given lamivudine 100 mg daily pretransplantation and continued up to 2 years after transplantation. HBV recurrence rates were similar to historical controls treated with HBIg monotherapy after 2 years (Fig. 42.6). As seen with HBIg monotherapy, pretransplant replication status strongly influenced the success of



**Immunodominant epitope and common mutations in patients failing HBIG**

**Figure 42.5 Immunodominant epitope and common mutations in patients failing HBIG.** The region of the surface protein involved in binding of HBs antibody is described as a cysteine-rich web and single amino acid changes in the immunodominant “a” determinant (amino acids 124–149) result in reduced binding of anti-HBs in vitro. The most common escape mutations reported in patients failing HBIG monotherapy are at amino acid positions 134, 144 and 145 of the surface protein.

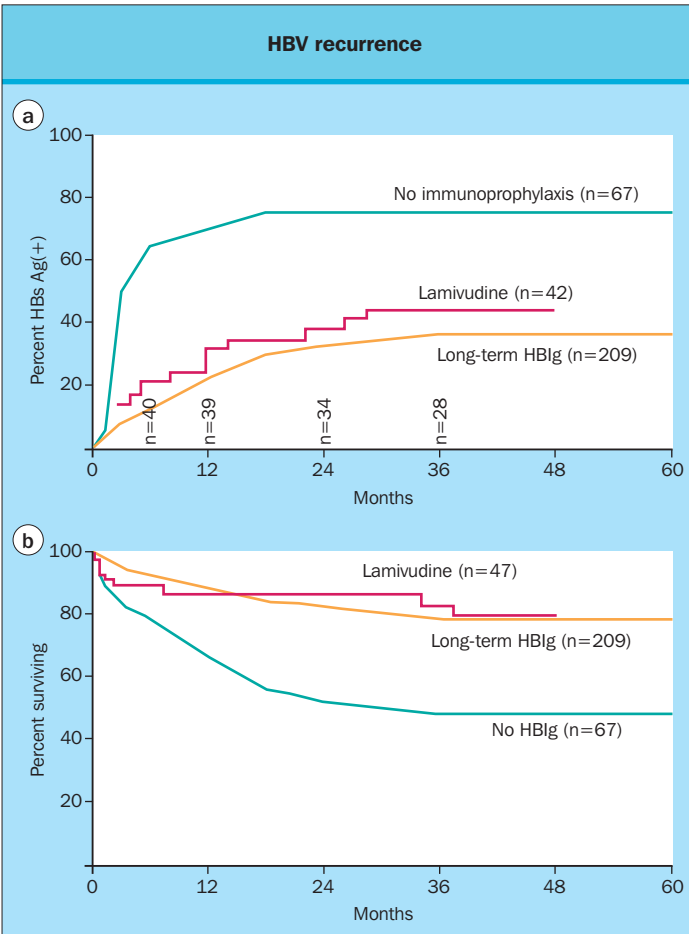
lamivudine monotherapy. At 2 years post-transplantation, none of the patients who were HBeAg and HBV DNA-negative pre-lamivudine therapy had recurrent HBV infection compared to 61% of patients who were HBeAg and HBV DNA-positive before starting treatment. A limitation of lamivudine monotherapy is the development of drug resistance during prolonged therapy. In transplant recipients receiving prophylactic lamivudine monotherapy, resistance developed in 20–40% after 1 year.

#### **Combination prophylaxis with HBIG and lamivudine or other antivirals**

In the majority of transplant programs, combination immunoprophylaxis, with HBIG and an antiviral agent such as lamivudine or adefovir, is the current standard of care for patients with HBV undergoing liver transplantation. The combination of HBIG and lamivudine is more efficacious than either agent alone and high efficacy is reported for both HBeAg-positive and HBeAg-negative (presumed precore mutation) chronic HBV. With follow-up periods of 13–30 months post-transplantation, HBV recurrence

rates in patients receiving prophylactic treatment with lamivudine plus HBIG range from 0–10%.

High dose HBIG protocols can cost up to \$100 000 in the first year post-transplantation and \$10 000–\$50 000 in subsequent years depending upon the doses given. Several different HBIG preparations are available (Table 42.4). The significant cost of long-term HBIG therapy has spurred investigation of alternative protocols using lower doses of HBIG in combination with antiviral agents, short-term HBIG with long-term antivirals and alternative immunoprophylaxis agents (see below). In general, protocols that give HBIG to maintain anti-HBs trough titers at a specified level (usually 100 IU/mL) are more cost-effective than regimes giving a fixed monthly dose of HBIG, and intramuscular administration of HBIG is less costly than intravenous administration. Intramuscular administration of HBIG has a 40–45% reduction in the delivery of anti-HBs to the intravascular space, but titer levels >100 IU/L can be achieved and maintained. Protocols using lower doses of HBIG in combination with lamivudine have similar efficacy rates as protocols using higher



**Figure 42.6 HBV recurrence.** Patients treated with lamivudine monotherapy pre and post-transplantation were compared to historical data from patients treated with HBIg monotherapy post-transplantation (for <6 months and >6 months). (a) Rate of HBV recurrence post-transplantation, (b) Actuarial rate of patient survival. Reproduced from Perrillo et al (2001) by permission of Wiley-Liss, Inc.

doses of HBIg, suggesting that the use of HBIg in conjunction with a nucleoside/nucleotide analog may allow lower doses of HBIg to be administered. Alternative forms of HBs antibody are also being evaluated in clinical trials including hepatitis B immune plasma and monoclonal anti-HBs.

Combination therapy using HBIg plus a nucleoside/nucleotide analog for an indefinite duration may not be required in all liver transplant recipients with pretransplant HBV infection. In patients with naturally occurring low level HBV replication (i.e. inactive chronic HBV infection) awaiting transplantation, treatment with HBIg monotherapy, lamivudine monotherapy or combination therapy are similarly successful. Whether the small risk of treatment failure with monotherapy warrants long-term combination therapy in this population is unknown. In contrast, patients with active HBV replication pretransplantation have high rates of treatment failure with HBIg and lamivudine monotherapies and combination therapy provides superior benefits. Recent studies has evaluated whether HBIg can be stopped after a defined period of combination therapy and lamivudine monotherapy be used to provide ongoing prophylaxis. In a study comparing long-term HBIg and lamivudine versus short-term (1 month) HBIg and long-term lamivudine, all patients were HBsAg-negative at 18 months post-transplant and there was no difference in recurrence of HBV DNA by group (3/14 on long-term combination therapy versus 1/15 in short-term HBIg group). HBV vaccination as an alternative to HBIg has been also studied (Table 42.5). In HBsAg-negative, HBV DNA-negative transplant recipients on combination therapy with HBIg and lamivudine, the success of inducing and maintaining anti-HBs levels >10 IU/L with vaccination was variable (18–64%), possibly related to the vaccination preparation or dosing schedule. However, among the vaccine responders, HBIg was discontinued with no evidence of recurrent HBV with follow-up periods up to 40 months. Lamivudine was continued as prophylaxis in all vaccine responders. Longer-term studies are needed to define the subsets of patients who may be suitable for HBIg discontinuation.

Licensed hepatitis B immunoglobulin (HBIg) preparations				
Product	Protein IgG%	Anti-HBs (IU/L)	Manufacturer/Reference	Country
HBIg (before 1999)	16.5	200	NABI	USA
NABI-HB	5	300	NABI-Rockville	USA
Hyperhep/Bayhep	16.5	200	Cutter/Miles/Bayer	USA
Hep-B-Gammagee	16.5	>100	Merck, Sharp and Dome	USA
Hepatect	5	50	Biotest Pharma	Europe
Hepatitis B IG	5	50	BioProduct Lab	UK
Uman-B IG	–	200/50	Farma Biagini	Italy/Europe
Hebagam IM	–	100	Natal Bioproducts Institute	South Africa
Hepuman	–	200	Berna	Europe
anti-HBs, antibody to hepatitis B surface antigen; IgG, immunoglobulin G. Modified from Terrault & Vyas (03) by permission of Elsevier.				

**Table 42.4 Licensed hepatitis B immunoglobulin (HBIg) preparations.**

HBV vaccination as alternative to HBIg							
Reference	N	Vaccine Strategy	% Response at Specific Anti-HBs Cut-off			Recurrence N	Duration Follow-up after HBIg Stopped
			>10 IU	>100 IU	>500 IU		
Barcena, 1999	5	3 doses, 0, 1, and 2 mos + 3 add'n doses if needed	80	23	NA	0/4	5–8 mos
Angelico, 2002	17	Double doses, 3 courses	18	12	6	NA	40 mos
Sanchez-Fueyo, 2002	22	3 doses, routine schedule	63	23.5	9.1	0/14 (2 had fall in anti-HB titer to <10 IU/L)	14 mos
Bienzie, 2003	20	Adjuvant + vaccine $\times 3$ and prn*	80	NA	NA	0/16	13.5 mos
Albeniz-Arbizu, 2003	12	3 doses, 0, 1 and 2 mos	75	17	NA	1/12	19 mos
*Additional doses given if initial anti-HBs titer <10 IU/L Abbreviations: anti-HBs = anti-hepatitis B surface antibody titer, HBIg = hepatitis B immunoglobulin Roche B, Samuel D. Liver Transplantation 2004.							

**Table 42.5 HBV vaccination as alternative to HBIg.**

The development of lamivudine-resistant HBV prior to transplantation presents additional challenges in the post-transplant period. Variable success has been reported using high-dose HBIg monotherapy to prevent reinfection in these patients. With the availability of antiviral agents with efficacy against lamivudine-resistant HBV, the preferred strategy is adefovir, entecavir or tenofovir in combination with HBIg. An additional concern is the potential for cross-resistance in patients receiving HBIg and nucleoside/nucleotide analogs in combination or sequentially. Due to the overlapping reading frames of HBV, mutations in the polymerase potentially can cause changes in the surface gene and vice versa. Thus, the selection of resistance mutations in the polymerase with pretransplant treatment with lamivudine or adefovir may be associated with mutations in the surface gene, which may limit the efficacy of subsequent HBIg treatment, at least theoretically.

The ideal time to begin antiviral therapy pretransplantation is determined primarily by liver disease severity and the replication status of the patient. For those with inactive HBV disease (normal serum aminotransferase levels and low levels of HBV DNA, typically  $\leq 10^4$  copies/mL), antiviral therapy can be started at or near the time of transplantation. For those with active HBV disease (HBeAg-positive or HBV DNA  $>10^4$  copies/mL or abnormal liver enzymes), the duration of nucleoside/nucleotide therapy should be sufficient to achieve viral suppression, achieve the desired clinical benefits and yet minimize emergence of drug resistance. To achieve reductions in HBV DNA levels to of  $\leq 10^4$  copies/mL usually requires a period of 3–6 months of lamivudine or adefovir treatment depending upon baseline HBV DNA levels, and clinical improvements in patients with decompensated disease requires at least 6 months of treatment. Since the timing of transplantation is usually not known, prolonged treatment with antiviral agents is common in patients awaiting transplantation and drug resistance may develop in this population. Antiviral agents that have a low rate of viral resistance may be better suited for patients with decompensated liver disease requiring prolonged periods of pretransplant therapy.

### Management of recipients of anti-HBc-positive allografts

Due to the discrepancy between the static number of donor organs and increasing number of patients awaiting liver transplantation, use of anti-HBc-positive allografts to expand the donor pool is a common practice. Most anti-HBc-positive, HBsAg-negative allografts have normal or near normal liver enzymes and histology, but there is a risk of de novo HBV infection in recipients. HBV-naïve and anti-HBs-negative recipients are at highest risk of de novo HBV with receipt of an anti-HBc-positive organ with a reported incidence of 33–78%. The lowest risk is seen in anti-HBs-positive recipients. Prophylactic therapies reduce the risk of de novo infection in patients receiving anti-HBc-positive grafts. Prophylaxis must take into account the pretransplant serologic status of the recipient (Table 42.6). For recipients with pre-existing chronic HBV infection, a combination of HBIg and a nucleoside/nucleotide analog is used as prophylaxis. Lamivudine alone with or without HBIg has been used to prevent de novo HBV in HBsAg-negative recipients of anti-HBc-positive allografts. Pretransplant vaccination, even when anti-HBs titers are successfully induced and maintained, does not prevent de novo HBV post-transplant in all patients. Therefore, prophylaxis is generally undertaken even if pretransplant HBV vaccination was successful. For patients with pre-existing immunity against HBV infection (anti-HBc-positive with or without anti-HBs), the risk of de novo infection is so low some experts suggest that an alternative to long-term prophylaxis may be close serologic and virologic monitoring and initiation of nucleoside analog therapy at the first signs of infection. Larger-scale studies to determine the optimal treatment algorithms in these patients with different levels of risk are needed.

### Treatment of recurrent HBV in transplant recipients

Graft survival is less in those who develop recurrent HBV than those who remain HBsAg-negative post-transplantation. In patients with recurrent HBV infection, the goals of therapy are to suppress viral replication, stabilize graft function and prevent histologic progression. Challenges associated with the treatment



of recurrent HBV infection in transplant patients include the presence of immunosuppression, which may affect treatment efficacy, the frequent presence of drug-resistant HBV, and the rapid development of recurrent cirrhosis in the absence of effective antiviral therapy. Emergence of resistance with single drug therapy occurs with prolonged treatment, albeit at a different rate with each drug (Fig. 42.7). Sequential use of anti-HBV therapies can lead to an accumulation of polymerase and surface gene mutations and an increasingly drug-resistant form of HBV infection. Knowledge of the preceding drug exposure history is critical in making treatment decisions. In general, the treatment options for drug-experienced patients with recurrent HBV are more limited than those with wild-type HBV infection (recurrent or de novo). Despite the theoretical advantages of combination nucleoside/nucleotide analog therapy, the optimal

combination of available antivirals that will achieve the desired benefits of high efficacy and low toxicity has not been established.

Lamivudine and adefovir are the mainstays of therapy for those with recurrent disease (Table 42.7). Experience with entecavir is limited, but the safety and efficacy data in non-transplant patients suggest this drug will be useful in treatment of recurrent HBV disease. Famciclovir, ganciclovir (intravenous) and interferon were used for the management of patients with recurrent disease prior to the availability of lamivudine, but the antiviral effects of these agents were modest and less consistently achievable than with lamivudine and adefovir. Thus famciclovir, ganciclovir and interferon should be considered second-line agents for use in patients failing first-line therapies with lamivudine, entecavir and/or adefovir. Tenofovir was used to treat lamivudine-resistant HBV infection prior to the availability of adefovir and entecavir; although experience is limited, tenofovir appears to be another treatment option for patients with lamivudine-resistant recurrent HBV infection. Given the limited data on long-term safety and efficacy of tenofovir in transplanted patients, tenofovir should be considered second-line therapy. Future agents may include the new nucleoside/nucleotide analogs currently under study such as clevudine, emtricitabine and telbivudine.

Lamivudine is effective in liver transplant recipients with recurrent HBV due to wild-type HBV, those who have failed HBIg therapy and those with adefovir-resistant HBV. Additionally, lamivudine has been shown to have efficacy in FCH. In the largest clinical study in North America of 52 liver transplant recipients with recurrent HBV (including 50% who had failed HBIg prophylaxis), lamivudine 100 mg daily for a median of 49 weeks resulted in normalization of liver enzymes in 72%, decline in HBV DNA levels in 60%, HBeAg loss in 50% and HBsAg loss in 6%. However, 27% had a virologic breakthrough and 36% of these patients experienced clinical deterioration with the development of drug resistance. Other studies show similar results; while the majority of patients respond initially, emergence of lamivudine resistance limits long-term efficacy and monitoring for virologic breakthrough is essential. There is cross-resistance between lamivudine and

Management of recipients of anti-HBc positive allografts		
Recipient status	Risk of de novo HBV in absence of prophylaxis	Prophylaxis regimes reported to be effective
HBsAg+	Unknown	HBIg plus antiviral (same as HBsAg+ patient not receiving anti-HBc + organ)
HBsAg–, anti-HBc–, anti-HBs–	33–78%	Lamivudine (or other antiviral) monotherapy HBIg (short or long term) plus antivirals
HBsAg–, anti-HBc+, anti-HBs+	0–13%	Lamivudine (or other antiviral) monotherapy No prophylaxis – treat if de novo HBV
anti-HBc, anti-hepatitis B core antibody; anti-HBs, anti-hepatitis B surface antibody; HBeAg, hepatitis B e antigen; HBIg, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus DNA.		

Table 42.6 Management of recipients of anti-HBc positive allografts.

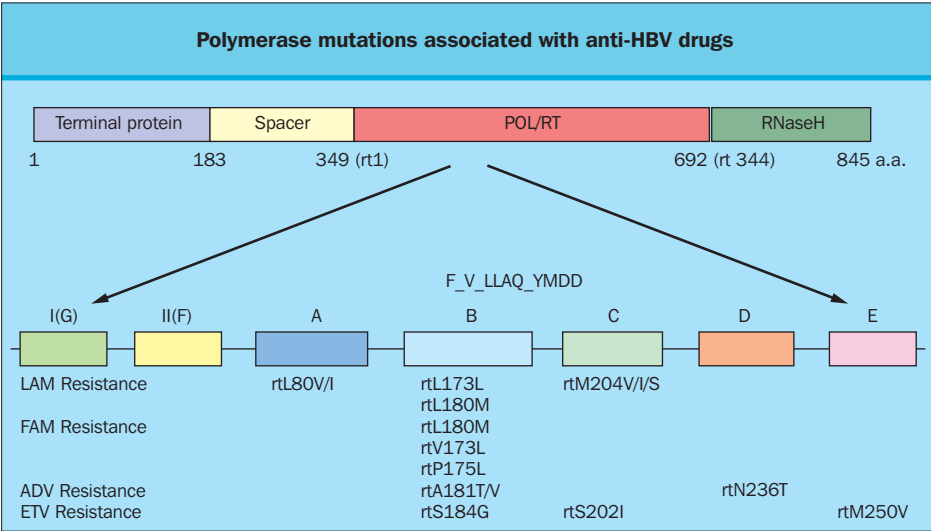


Figure 42.7 Mutations associated with anti-HBV drugs. Genotype-independent numbering scheme for the HBV polymerase/RT domain is shown. The primary location for mutations associated with LAM-resistance is in domains B and C; similar to those for FAM. The signature mutation associated with ADV-resistance is in domain D. Entecavir resistance mutations have only been identified in patients with preexisting lamivudine-resistance mutations. LAM: lamivudine, ADV: adefovir, FAM: famciclovir. (Courtesy of S. Locarnini, Victoria Infectious Diseases Reference Laboratory, Melbourne, Australia.)

<b>Treatment options for patients with recurrent HBV</b>			
<i>Patient population</i>	<i>First-line agents</i>	<i>Second-line agents</i>	<i>Comments</i>
No prior therapy	Lamivudine, adefovir, entecavir	Tenofovir i.v., ganciclovir, interferon	Risk of resistance with prolonged therapy higher with lamivudine than adefovir or entecavir. Adefovir and entecavir are more costly than lamivudine.
HBIG treatment failure	Lamivudine, adefovir, entecavir	Tenofovir i.v., ganciclovir, interferon	Lamivudine and adefovir are effective against surface gene mutants.
Lamivudine resistance	Adefovir, entecavir	Tenofovir i.v., ganciclovir, interferon	Case report of patients treated with sequential HBIG and lamivudine resistance who had increased viral replication in the presence of lamivudine.
Famciclovir resistance	Adefovir, entecavir	Lamivudine, tenofovir i.v., ganciclovir, interferon	Lamivudine is effective in short-term, but lamivudine resistance emerges more rapidly if prior famciclovir exposure.
Adefovir resistance	Lamivudine, entecavir	Tenofovir, interferon	Only lamivudine has been demonstrated to be effective. IFN likely to be option; efficacy of other antiviral unknown.

**Table 42.7 Treatment options for patients with recurrent HBV.**

famciclovir and prior treatment with famciclovir increases the risk of lamivudine resistance.

Adefovir is effective against wild-type and lamivudine-resistant HBV. In 196 liver transplant recipients with recurrent and lamivudine-resistant HBV disease, treatment with adefovir 10 mg daily for 52 weeks led to undetectable HBV DNA (<200 copies/mL) levels in 35%, normalization of liver enzymes in 46%, and improvement in CPT score in over 90%. Adefovir was well tolerated but renal toxicity was a concern. A total of 13% of treated patients had a 0.5 mg/dL increase in serum creatinine during treatment but all patients had other potential risk factors for abnormal creatinine levels including concurrent use of calcineurin inhibitors. Dose adjustments in patients with reduced creatinine clearance and close monitoring of renal function are recommended during adefovir therapy. Adefovir resistance is seen in 2 and 4% of patients after 2 and 3 years of treatment. Adefovir-resistant HBV is sensitive to lamivudine and entecavir.

Entecavir is now available and is active against both HBeAg-positive and HBeAg-negative hepatitis B, including lamivudine-refractory disease. Little is known about its effectiveness and safety in the transplant setting.

### Retransplantation for recurrent HBV

Retransplantation for graft failure due to recurrent HBV was associated with dismal outcomes in the pre-HBIG and pre-nucleoside era, with mortality rates of 68–95%. The contemporary experience is significantly better. Using prophylactic regimens of HBIG with and without antiviral agents, low rates of recurrent HBV disease in the second graft and prolonged survival are achievable. For patients who are HBV DNA-positive and requiring retransplantation, high dose HBIG with anti-HBs target titers >500 IU/L appear more successful than lower dose HBIG using target titers of 100 IU/L. The addition of pretransplant nucleoside/nucleotide analogs to reduce HBV DNA levels pre-transplantation enhances the efficacy of prophylaxis further and prevents recurrent HBV in the majority of these patients. The

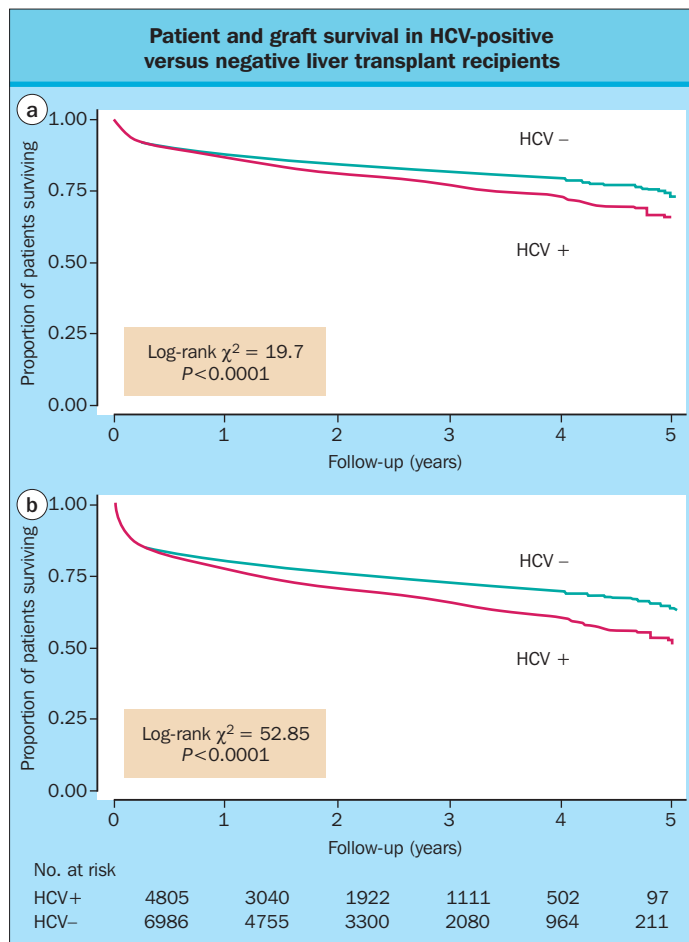
optimal dose of HBIG therapy and choice of antiviral agent(s) must be individualized and based upon the patient's prior drug exposures and likelihood of harboring specific surface and polymerase mutations.

## HEPATITIS C VIRUS

More than 20 000 patients worldwide have undergone liver transplantation for HCV cirrhosis, and in North America, HCV accounts for 25–50% of the liver transplants performed. Unfortunately, recurrent HCV is essentially universal in patients who are viremic pretransplantation and is associated with a more rapid progression to cirrhosis in the transplanted liver. The risk of death and allograft failure are increased in HCV-positive transplant recipients [hazard ratio (HR) 1.23 and HR 1.30] compared HCV-negative recipients (**Fig. 42.8**). Further limiting graft outcomes in transplant patients are a poor response to and limited tolerability of interferon and ribavirin therapy. High baseline viral load and a predominance of genotype 1 infection reduce even further the likelihood of achieving viral clearance. However, in selected patients, viral eradication or slowing of disease progression appears to be achievable. For the remaining patients with progressive histologic disease and non-response to treatment, retransplantation is the only current option. Unfortunately, retransplantation for recurrent HCV is associated with 5-year survival rate of 50%, making this a controversial indication for retransplantation.

### Natural history of recurrent HCV after transplantation

Liver transplant recipients with HCV have increased viral replication, increased risk of fibrosis and an accelerated time to cirrhosis compared to patients not on immunosuppressive therapy (**Table 42.8**). Viral kinetic studies of the early events following transplantation have shown that HCV RNA levels drop abruptly during the anhepatic phase and again during the 8–24 h after reperfusion. By 24–72 h there is an increase of



**Figure 42.8 Patient and graft survival in HCV-positive and HCV-negative liver transplant recipients.** (a) Kaplan-Meier estimates of patient survival according to HCV infection status. (b) Kaplan-Meier estimates of allograft survival according to HCV infection status. Reproduced from Forman et al (02) by permission of Elsevier.

serum HCV RNA to detectable levels, with levels increasing over postoperative weeks 2–4 and peaking between weeks 4 and 16 before stabilizing. At 1-year post-transplantation, viral levels are typically 1.0 log<sub>10</sub> higher than pretransplant levels. While the correlation between post-transplant HCV viral titer and histologic severity of recurrent disease is poor, some studies have found an association between pretransplant viral load and risk of cirrhosis and/or graft loss post-transplantation.

Recurrent infection defined by the presence of detectable HCV RNA in serum and liver occurs universally and early in patients who are viremic pretransplantation. Clinical and histologic presentations among infected transplant recipients are more variable. Acute recurrent HCV disease, when manifest, usually occurs within 3 to 6 months after transplantation and is characterized by elevated serum aminotransferase levels and histologic findings of lobular hepatitis, focal hepatocellular necrosis, and acidophilic bodies (Fig. 42.9a). Chronic recurrent HCV disease occurs with or without a preceding acute hepatitis phase. The histologic changes of chronic HCV disease include mixed portal and periportal inflammation with or without periportal fibrosis, similar to the histologic picture of non-transplant

#### Natural history of hepatitis C post-transplantation

	Post-transplantation	Non-immunosuppressed persons with chronic HCV
Fibrosis progression (units fibrosis/year)	0.3/year (0.004–2.19/year)	0.2/year (0.09–0.8/year)
Median time to cirrhosis	8–12 years	20–30 years
Decompensation rate after cirrhosis	50% at 1 year	4% per year
Survival after decompensation	41% at 1 year	50% at 5 years
The natural history of HCV disease post-transplantation is truncated compared to that of non-immunosuppressed persons with chronic HCV (i.e. disease progression prior to transplantation). Modified from Berenguer (03).		

**Table 42.8 Natural history of hepatitis C post-transplantation.**

patients with chronic HCV infection (Fig. 42.9b). Approximately 20% of patients develop chronic hepatitis without acute hepatitis; this presentation appears to have a more favorable outcome in terms of disease progression. The majority, 70%, develops acute hepatitis and evolves to chronic hepatitis. In 10% or less of transplant recipients, a severe and rapidly progressive form of hepatitis, termed cholestatic hepatitis, develops.

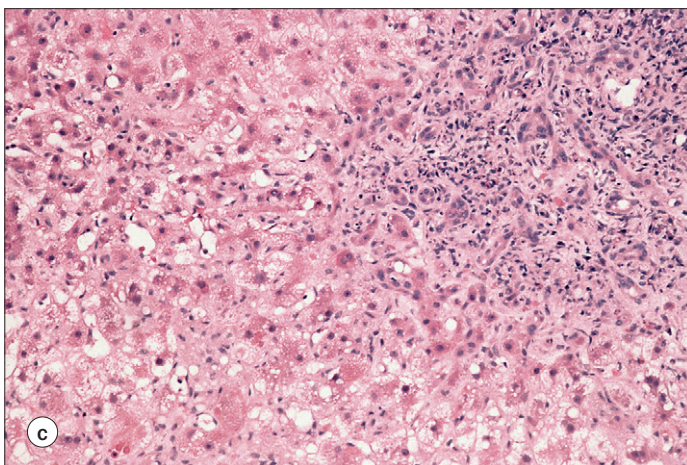
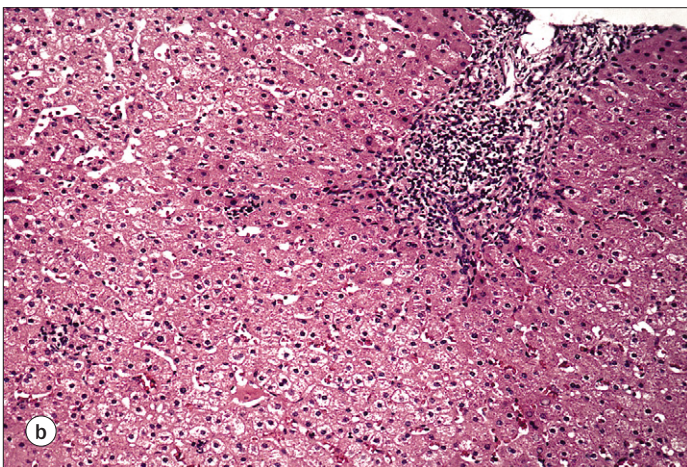
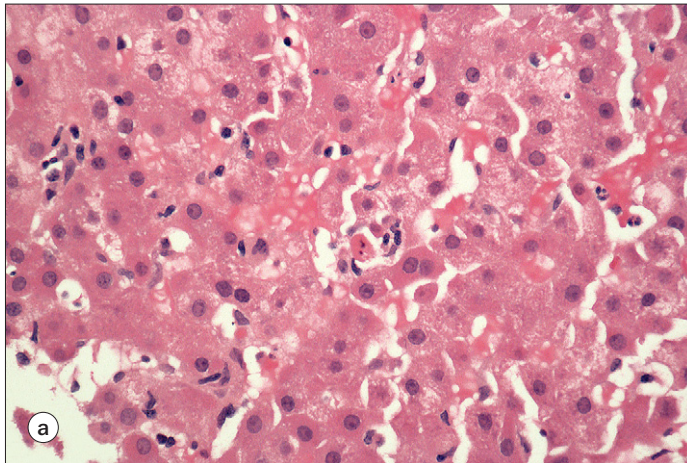
Cholestatic hepatitis is characterized by serum bilirubin >6 mg/dL, high serum HCV RNA levels (>10<sup>6</sup> copies/mL), and a characteristic histologic pattern of perivenular ballooning hepatocytes, confluent necrosis, bile duct proliferation and cholestasis, but with minimal inflammation (Fig. 42.9c). This form of recurrent disease has been historically associated with a poor prognosis, with mortality rates as high as 50% at 1 year and 18% at 3 years. However, stabilization of liver disease and prolonged survival has been achieved in some patients with use of antiviral therapy.

#### Immunosuppression, acute rejection and HCV

Excessive immunosuppression and rapid changes in immunosuppression are thought to be key factors influencing the severity of recurrent HCV disease. Steroid-free immunosuppressive regimens have shown some benefits in HCV infected recipients. When steroids are used, the rate of withdrawal rather than the absolute dose of steroids appears to be of importance. In contrast to HCV-negative recipients, who have reduced mortality if they experience an episode of acute cellular rejection, HCV infected recipients have a threefold increase in mortality after an episode of rejection. Rapid changes in immunosuppression, such as occurs with boluses of corticosteroids and lymphocyte-depleting agents like OKT-3 in treatment of acute rejection, are associated with increased risk of severe HCV disease and decreased time to HCV recurrence.

In terms of specific immunosuppressive agents, none has been identified as being harmful to HCV disease progression per se. Retrospective analyses of patients receiving tacrolimus versus





**Figure 42.9 Histologic features of HCV infection in liver transplant recipients.** (a) Acute hepatitis, (b) chronic hepatitis, (c) cholestatic variant. (Courtesy of Dr Linda Ferrell, University of California San Francisco.)

cyclosporin-based immunosuppression show no difference in disease severity between groups. Mycophenolate mofetil use was implicated in early multivariate analyses predicting disease severity, but two large prospective studies found no difference in HCV recurrence or outcomes in those transplant recipients who did and did not receive mycophenolate mofetil. The

### Factors associated with increased severity of HCV recurrence

<i>Consistently linked with disease severity</i>	<i>Conflicting studies on relationship to disease severity</i>
High pre-LT HCV viral load Necroinflammation at initial biopsy Acute rejection Later year of transplantation CMV infection	HCV Genotype I Older donor age Peak AST level HLA incompatibility
Non-white race	Live donor liver transplant
	Prolonged warm ischemia time
OKT-3 use	–

AST, aspartate aminotransferase; CMV, cytomegalovirus; HCV, hepatitis C virus; HLA, human lymphocyte antigen; LT, liver transplantation; MMF, mycophenolate mofetil; OKT-3; anti-CD3 monoclonal antibody.

**Table 42.9 Factors associated with increased severity of HCV recurrence.**

limited data on induction therapy using lymphocyte-depleting therapy indicate no adverse effects on disease progression. Sirolimus has not been studied sufficiently to draw conclusions.

The histologic features of early recurrent HCV and acute cellular rejection overlap and make the diagnosis of acute cellular rejection in the setting of recurrent HCV disease difficult. In a retrospective study of 285 liver transplant recipients, HCV was associated with an increased risk of early (<6 months after transplant) acute cellular rejection compared to other transplant indications (HR 1.7). In this same study, 49% of transplant recipients with HCV had at least one episode of early acute cellular rejection. In light of this difficulty and due to the reported association between treatment of acute rejection and increased risk of HCV disease progression, some experts have recommended that only acute rejection of moderate or severe degree should be treated. Corticosteroid boluses and leukocyte depleting agents should be used judiciously. Additionally, expert panels recommend against rapid withdrawal of immunosuppression. An exception to these guidelines may be cholestatic hepatitis in which rapid reduction in the level of immunosuppression has been considered the cornerstone to therapy.

### Factors associated with recurrent HCV

Several recipient, donor and viral factors have been associated with increased severity of HCV recurrence and reduced graft survival and patient survival (Table 42.9). The factors most firmly associated with high risk of progression to cirrhosis and graft loss due to HCV include: liver biopsy histology at 1 year, acute cellular rejection, use of OKT-3 for rejection, presence of CMV viremia and non-white race. Whether recipients of live donor allografts are at risk of earlier and more severe HCV recurrence than deceased donor recipients is controversial. A concerning trend is the decreasing graft and patients survival

rates over time for patients with HCV; this contrasts sharply with the increasing survival rates seen for non-HCV indications during the same time period. Two changes in clinical practice have been implicated as the cause for this trend in patients with HCV: the use of newer and more potent immunosuppressive agents, and the increasing use of older donors, particularly those over 50 years of age.

#### Anti-HCV-positive donors

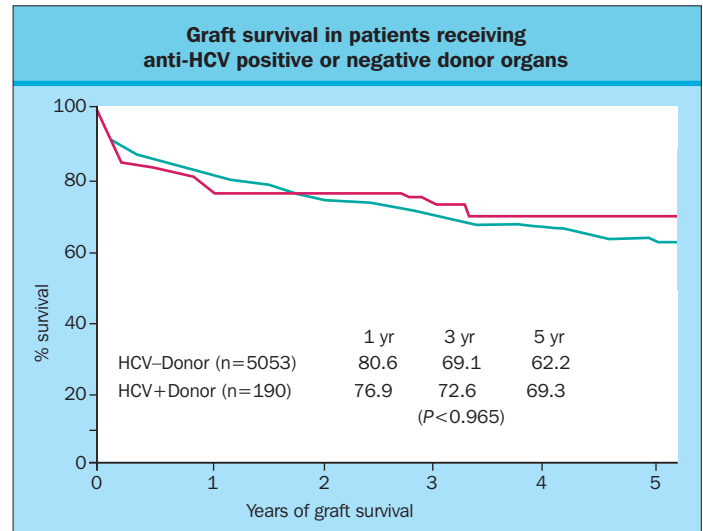
HCV antibody-positive donors are increasingly being used for patients whose urgency for an allograft precludes waiting for an antibody-negative donor allograft. Since spontaneous clearance of HCV virus can occur in up to 45% of those exposed to HCV, a sizeable portion of otherwise healthy potential donors who are HCV antibody-positive may not have an active HCV infection. Additionally, 80% of persons with chronic HCV infection and persistently normal liver enzymes have mild or minimal histologic disease. Since only antibody results are available at the time of transplantation, all anti-HCV-positive organs must be considered a source of HCV RNA and a risk for HCV disease post-transplantation. In general, anti-HCV organs should be restricted to HCV RNA-positive patients with a high need awaiting liver transplantation. Use of anti-HCV-positive organs in HCV uninfected persons is not recommended currently, as the organ presents a risk for de novo HCV infection and no effective prophylactic therapy is available. Similarly, since spontaneous or treatment-induced clearance for HCV RNA does not protect against future HCV infection, anti-HCV-positive, HCV RNA-negative patients awaiting transplantation should not receive organs from anti-HCV-positive donors.

Virologic analyses of the organs of anti-HCV-positive recipients of anti-HCV-positive organs indicate that the majority are infectious and that the recipient viral strain typically predominates post-transplantation. However, the possible negative consequences of giving a recipient infected with a favorable HCV genotype for treatment (e.g. HCV genotype 2 or 3) a donor organ which may be infected with a less variable genotype, must be fully discussed with potential recipients. To date, the outcome data available indicate that patient and graft survival are at least as good and perhaps better for anti-HCV-positive recipients receiving an allograft from an anti-HCV-positive donor compared to those receiving an anti-HCV-negative donor (Fig. 42.10). Thus, the use of allografts that are anti-HCV-positive represents a reasonable expansion of the donor pool in selected anti-HCV-positive patients who are well informed and HCV RNA-positive.

#### Pretransplant antiviral therapy

Recurrent HCV after liver transplantation requires the presence of HCV RNA pretransplantation and limited studies suggest the severity of HCV disease after transplantation is related to pretransplant levels of HCV RNA. The impact of a treatment-induced reduction in viral load pretransplantation on the risk of HCV recurrence is unknown, but if a sustained virologic response is achieved pretransplantation, HCV recurrence is rare post-transplantation.

In advanced liver disease, treatment with interferon and ribavirin is associated with reduced tolerability, frequent dose reductions and possible precipitation of clinical decompensation. However, with the selection of appropriate candidates and using a low accelerating dosage regimen (LADR) of interferon plus



**Figure 42.10 Graft survival.** Graft survival in patients receiving anti-HCV-positive and anti-HCV-negative donor organs. Number of grafts analyzed each year is indicated. There was no difference in survival between groups. Reproduced from Velidedeoglu et al (02) by permission of Lippincott, Williams and Wilkins.

ribavirin, sustained responses can be achieved. In a study of 102 patients awaiting liver transplant with mean CPT score of 7.1  $\pm$  2.0, 40 patients achieved an end-of-treatment response, 21 achieved a sustained viral response, and only 16 of 102 could not tolerate treatment. The sustained virologic response (SVR) rates were genotype-dependent, 11% and 50% for genotypes 1 and non-1, respectively. None of the 10 patients who were HCV RNA-negative pretransplantation had recurrent HCV after transplantation but all 22 patients who were HCV RNA-positive had recurrence. In this study, interferon  $\alpha$ 2b was started at half dose with ribavirin 200 mg daily, and if tolerated, the doses were increased incrementally every 2 weeks (interferon first, then ribavirin) until target doses were achieved. Adverse events in this study were common with thrombocytopenia ( $<45,000/\text{mL}$ ) and leukopenia (neutrophil count  $<1000/\text{mL}$ ) the most frequent. In contrast to these results, another study of patients with more severe liver disease (mean CPT score 11.9  $\pm$  1.2) using interferon  $\alpha$ 2b 1 MU thrice weekly with ribavirin, 1 MU daily alone or 3 MU thrice weekly alone reported greater intolerance to treatment regimens and a high rate of serious adverse events. Whether growth factors such as granulocyte-macrophage colony stimulating factor (GM-CSF) and erythropoietin can increase the tolerability of interferon and ribavirin in this population is unknown. While additional studies to define the optimal treatment strategy are needed, the available data suggest patients with mildly decompensated liver disease may be appropriate candidates for pretransplant antiviral therapy, whereas the risk of complications may outweigh benefits in those with more advanced stages of decompensation.

#### Post-transplant management of HCV infection

Treatment approaches can be divided into three general categories (prophylactic, pre-emptive and treatment of established disease) based upon the timing of the intervention (Table 42.2). Prophylactic therapy is started at the time of transplantation and continued for variable periods post-transplantation. As with any



form of 'post-exposure' prophylaxis, the goal of prophylaxis is to prevent infection. Pre-emptive treatment of recurrent HCV refers to initiating antiviral therapy early in the post-transplant period, typically within the first 4 weeks following transplantation, and before biochemical and histologic disease is manifest. Treatment of recurrent HCV disease refers to initiating antiviral therapy for biochemical and histologically evidence of recurrent disease.

Dose reductions are more common in transplant patients receiving antiviral therapy for recurrent HCV than in non-transplant populations. Dose reductions, in turn, likely contribute to the lower response rates seen in this population. Cytopenias are frequent due to concurrent use of immunosuppressive agents and alterations in renal function related to calcineurin inhibitor therapy or other post-transplant factors affecting ribavirin clearance. Indeed, the optimal dose of ribavirin in transplant patients has not been determined. Growth factors may be an important method of managing cytopenias and improving quality of life during antiviral treatment.

As interferon has immunomodulatory effects, specifically upregulation of human leukocyte antigen (HLA) expression on bile duct epithelia, there has been persistent concern regarding the risk of rejection related to use of interferon in liver transplant recipients. Additionally, this risk of acute rejection may be greater if interferon is used pre-emptively rather than later post-transplantation, since the risk of acute rejection is highest in the first 3 months after transplantation. In uncontrolled retrospective studies, rates of acute cellular rejection reported in patients on antiviral therapy range from 11 to 34%. In controlled studies, the frequency of acute rejection has not been significantly different in interferon-treated patients compared to untreated controls.

### **Prophylactic therapy**

There are no established prophylactic therapies for HCV. In the transplant setting, HCV antibody therapy is being evaluated as a potential means of preventing HCV infection post-transplantation. Preclinical and retrospective cohort data support this approach. In a retrospective observational study of transplant recipients with HBV and HCV receiving HBIg, the prevalence of recurrent HCV disease after transplantation was lower in those who received HBIg prior to screening for HCV compared to those who received HBIg after screening for HCV began. These results suggested that the HCV antibodies in HBIg prior to HCV screening reduced the risk of recurrent disease. In a study of two chimpanzees treated with high dose hepatitis C immune globulin (HCIG) immediately before and continued after HCV inoculation, both were initially viremic but subsequently had undetectable HCV RNA and had no evidence of biochemical hepatitis.

At least three HCV antibody preparations are being studied in prospective clinical trials. A preliminary report of the Canadian HCV immune globulin study (Cangene Corp, Canada) found no benefit with HCV RNA detectable in all treated patients post-transplantation and no appreciable difference in level of viremia or histologic disease in patients receiving HCIG prophylaxis versus controls. Other studies using different antibody products and alternative dosing schedules are underway, but at the present time, prophylactic therapy has no established role in the management of patients with HCV undergoing liver transplantation.

### **Pre-emptive antiviral therapy**

The lower HCV viral loads present in the early post-transplant period and the absence of significant histologic disease may favorably affect the success of antiviral therapy in the transplant setting. This rationale underlies the pre-emptive therapy strategy. Studies have shown that pre-emptive antiviral therapy can lead to eradication of HCV infection, but tolerability of therapy can be limiting. Unlike non-transplant and acutely infected patients, liver transplant recipients are typically on multiple immunosuppressants, prophylactic antimicrobial agents, have variable renal function, and are recovering from a major operative procedure in the setting of advanced liver disease. In a study of 110 patients transplanted for HCV, only 60% were found to be candidates for interferon and ribavirin therapy at 8 weeks post-transplantation by traditional criteria. Patients with a high pre-transplant model of end-stage liver disease (MELD) and CPT scores were less suitable for this therapeutic approach.

Clinical trials using pre-emptive interferon monotherapy, though generally positive, demonstrated a modest benefit, primarily in delaying time to histologic recurrence. Sustained virologic responses were infrequent, occurring in 0–22%. More recent studies using interferon and ribavirin suggest combination therapy is more efficacious. An uncontrolled study from Italy of 36 patients (83% genotype 1b) treated with combination standard interferon  $\alpha 2b$  3 MU thrice weekly and ribavirin 10 mg/kg/day initiated within 3 weeks after transplant and continued for 52 weeks, reported sustained virologic responses in 33%, all of whom had normal serum aminotransferase levels and histology after completion of therapy. Viral clearance was more frequent in patients with non-1 HCV genotypes (100%) compared to those with genotype 1b (20%).

In contrast to the Italian experience, a US study of pre-emptive standard or pegylated (PEG)-interferon  $\alpha 2b$ , 3 MU thrice weekly or 1.5  $\mu\text{g/kg/week}$ , or interferon/peginterferon  $\alpha 2b$  plus ribavirin, 600 mg daily increasing to 1.0–1.2 g daily, for a total of 48 weeks, reported sustained virologic responses in only 9.1%, with most responders in the combination therapy group. Dose reductions and discontinuations were required in 85% and 37% of patients, respectively. The inability to achieve full-dose therapy may explain the low rate of sustained virologic response. The differences in applicability and tolerability of pre-emptive therapy in the Italian and US studies may reflect the clinical status of the patients in the early post-transplant period. The subgroups of patients for which pre-emptive therapy is best suited is unknown, and whether pre-emptive treatment is better than delaying treatment until recurrent disease is established also requires study.

### **Treatment of established HCV disease**

Study protocols for the treatment of established recurrent HCV disease have generally initiated treatment between 6 and 24 months after transplantation. Theoretical advantages of initiating treatment when recurrent disease is clinically or histologically apparent rather than as pre-emptive treatment include lower doses of immunosuppression (may enhance response to antiviral therapy), improved clinical status to better tolerate treatment, lower risk of acute rejection, and cost savings since only those with progressive disease are treated. Potential disadvantages of the 'wait and treat recurrent disease' approach include higher



viral load and more fibrosis which may reduce likelihood of response.

Treatment of established disease with interferon or ribavirin monotherapy has been disappointing. The combination interferon and ribavirin resulted in sustained virologic responses in approximately 20% of treated patients. Histologic improvements were reported in both responders and non-responders.

Most of the studies evaluating combination therapy for established recurrent HCV disease were uncontrolled and of small sample size, and with variable interferon and ribavirin doses and treatment duration. Doses of interferon  $\alpha$  were typically 3 MU trice weekly (range 1.5–5 MU) with ribavirin typically 600–1000 mg daily (range 400–1200 mg daily). Duration of therapy typically was 1 year. End of therapy biochemical and virologic responses ranged from 25% to 100% and 9% to 64%, respectively. There was a wide range of reported sustained virologic responses (assessed at 6 months after completion of therapy), 5–56%. Generally, adverse events during combination treatment were common with withdrawal of treatment required in up to 50% of patients; anemia, leukopenia and thrombocytopenia were the most common causes.

Peginterferon has not been compared directly to standard interferon in combination therapy for established recurrent HCV disease and published data using peginterferons are limited. The reported rates of sustained viral response with peginterferon and ribavirin were at least as good as standard interferon and ribavirin, ranging from 26% to 37.5%. Despite titrated regimens using low initial doses of peginterferon and ribavirin, dose reductions and discontinuations were frequent.

The optimal duration of treatment for recurrent HCV disease is unknown. In an immunosuppressed population of liver transplant recipients, longer treatment periods may be necessary to enhance sustained virologic responses. Prolonged low dose 'maintenance' interferon is being evaluated for histologic and clinical benefits in patients who fail to clear HCV after combination interferon and ribavirin therapy in several clinical trials (HALT-C, EPIC and CO-PILOT). There are no data regarding whether this strategy is effective in the transplant setting.

The cost-effectiveness of antiviral therapy for recurrent HCV disease was examined in one study. Using a Markov-based decision analytic model to simulate costs and health outcomes, treatment of recurrent HCV with antiviral therapy (interferon and ribavirin) was cost effective. Treatment of 100 men, aged 55 years, with recurrent HCV would prevent 29 cases of cirrhosis, seven deaths, and had an incremental cost effectiveness ratio of \$29 100 per year of life saved.

### Retransplantation for recurrent HCV

In the USA, 8–9% of all transplants performed are retransplants, with recurrent HCV accounting for approximately 40%. Compared to primary transplantation, retransplantation for all indications is associated with longer hospital stays, 40% greater costs and a 10–20% reduction in survival. Outcomes of retransplantation for recurrent HCV disease are at best similar to other indications yet most studies report inferior results. In a large study of over 10 000 patients in the UNOS database, anti-HCV-positive retransplant recipients had reduced survival compared to anti-HCV-negative retransplant recipients, with 5-year survival rates of 61% and 54%, respectively. Studies specifically evaluating retransplantation for recurrent HCV disease (not just

HCV-positive status) are smaller and from single centers but show similar or worse outcomes. Predictors of poor outcome following retransplantation included recipient age over 50 years, serum creatinine greater than 2.0 mg/dL, and serum bilirubin greater than 10 mg/dL. In general, outcomes are predicted to be best if retransplantation occurs before the onset of liver decompensation or significant renal dysfunction. Additionally, those with a longer period of time from first to second transplant appear to have a better outcome. Overall, however, retransplantation for recurrent HCV disease remains controversial. Currently, most transplant programs use a selective approach, choosing HCV infected patients with favorable clinical characteristics for retransplantation.

## CYTOMEGALOVIRUS

CMV is an important pathogen in liver transplant recipients, contributing directly to morbidity and mortality. Infection usually develops within the first 3 months after transplantation. Without antiviral prophylaxis, the overall incidence of CMV infection is 50–60%, and of CMV disease is 20–30%. The clinical impact of CMV in transplant recipients relates to the direct effects of the infectious disease syndromes, and the indirect effects of viral-associated immune modulation leading to an increased risk of superinfection with other opportunistic pathogens, chronic allograft rejection, and possibly EBV-associated lymphoproliferative disease.

### Diagnosis of CMV in liver transplant recipients

The diagnosis of CMV infection can be established by serologic and virologic techniques. Serology is most useful in assessing past exposure to CMV in both the donor and recipient. Serial testing with documentation of either seroconversion or rising immunoglobulin G (IgG) titers is an indirect measure of active viral infection, but these are not routinely tested after transplantation. The cornerstone for CMV diagnosis is the demonstration of the virus in the blood, respiratory specimens, urine or tissue. The gold standard for CMV diagnosis has been the growth of the virus in cultures with demonstration of cytopathic effect. Typical histologic features in tissue biopsies include the presence of 'cytomegalic' cells with intranuclear inclusions and associated acute, focal inflammation. However, more rapid methods are available (Table 42.10). The sensitive CMV antigen test, which detects a structural protein of CMV, pp65, is usually the diagnostic test of choice. Alternatively, detection of virus in blood or tissues using PCR can identify presence of infection but has lower specificity in identifying patients with CMV disease. However, since viremia precedes the clinical symptoms by 2–5 days, the PCR detection of viremia may be useful in situations in which pre-emptive therapy is desirable. Additionally, the presence of CMV antigens in tissues using monoclonal antibody staining or in situ hybridization supports the diagnosis of CMV organ involvement.

### Natural history and clinical presentation

Three patterns of viral transmission are observed. Primary CMV infection develops in a CMV-seronegative individual who receives blood products and/or an organ from a seropositive donor or acquires de novo infection from a community source. Secondary CMV infection or reactivation occurs when latent CMV re-

<b>Diagnostic methods for CMV</b>				
<i>Test</i>	<i>Methodology</i>	<i>Interpretation of positive result</i>	<i>Advantages</i>	<i>Disadvantages</i>
Conventional culture	Culture sample with human fibroblasts, evaluate for cytopathic changes	Presence of virus in sample, not necessarily active disease	None	Low sensitivity Low reproducibility 1–6 weeks for results
Shell vial culture	Culture sample with human fibroblasts, evaluate for CMV antigen in culture	Presence of virus in sample, not necessarily active disease	Faster than conventional culture Lower cost	Low sensitivity Low reproducibility 1–3 days for results
Serology IgG 2 weeks apart and IgM	ELISA and other assays to detect seroconversion and/or increasing titers	Seroconversion implies exposure and increased risk of disease	Lower cost	Requires samples 2 weeks apart for ideal use Antibodies can be undetectable up to 4 weeks after primary infection; rheumatoid factor can cause false positive
CMV antigenemia assay (pp65)	Test peripheral blood leukocytes for CMV antigen	Increasing proportion of + cells correlates well with active viremia, suggestive of active disease	High sensitivity Quantitative Can monitor response to treatment	Higher cost
CMV DNA	PCR hybridization	Active viremia (with blood sample)	High sensitivity Quantitative Can monitor response to treatment	Various techniques, not standardized Higher cost
Histology	Tissue biopsy, evaluate for histopathologic changes	Active disease	Gold standard	Low sensitivity High specificity
ELISA, enzyme linked immunosorbent assays; IgG, anti-CMV immunoglobulin G; IgM, anti- CMV immunoglobulin M.				

**Table 42.10 Diagnostic methods for CMV.**

activates after transplantation in a CMV-seropositive recipient. Superinfection or reinfection with CMV occurs in a CMV-seropositive recipient who receives cells and/or organ from a seropositive donor, with reactivation of latent virus of donor origin.

The risk factors for CMV disease in transplant recipients include donor–recipient CMV mismatch (seronegative recipient receives organ from a CMV-seropositive donor), use of anti-lymphocyte antibody preparations, transplantation for fulminant hepatic failure, and impaired renal function (creatinine clearance below 40 mL/min). Mismatch of donor and recipient CMV status is the single most important risk factor for CMV infection, followed by the type and intensity of immunosuppression. The use of antilymphocyte antibody therapy in addition to conventional immunosuppression increases the incidence of CMV disease 2.9 fold. These recognized risk factors for CMV disease post-transplantation provide the basis for currently used prophylactic protocols.

CMV infection exhibits a wide range of clinical manifestations. In over half of infected patients, CMV is isolated from body samples without evidence of disease. In one-quarter of patients, the isolation of the virus is accompanied by a mild and self-

limited febrile syndrome that includes anorexia, malaise, fever, myalgias and arthralgias. Hematologic abnormalities are common with leukopenia with or without thrombocytopenia present in up to a third of patients. Atypical lymphocytes may be detected on peripheral blood smears. Organ involvement with CMV develops in 20–25% of patients with CMV infection and most commonly occurs in the liver especially in the CMV donor-seropositive–recipient-seronegative situation. Another frequent site of CMV infection is the gastrointestinal tract and any segment of the gastrointestinal tract may be affected. Endoscopic findings are non-specific and biopsy is essential for diagnostic confirmation. Less frequently, CMV infection involves the pancreas, biliary tract, lungs, retina, epididymis, endometrium and central nervous system.

CMV infection has immunomodulatory and cellular effects which may have detrimental consequences in liver transplant recipients. CMV can directly impair cytokine secretion, suppress cytotoxic T cells and alter T-cell subpopulations leading to increased susceptibility to other opportunistic infections. CMV infection has been associated with reduced patient and graft survival. In a study of 146 liver transplant recipients, CMV disease was associated with a 3.9-fold increased risk of mortality

CMV prophylaxis in liver transplant recipients*			
CMV Ab recipient status	CMV Ab donor status	Post-transplant risk of CMV disease	CMV prophylaxis, stopped 3 months after transplant (adjust for renal function)
Negative	Positive	High	Ganciclovir 1000 mg PO t.i.d.
Positive	Positive	Intermediate	Ganciclovir 1000 mg PO t.i.d.
Positive	Negative	Low–intermediate	Acyclovir 800 mg PO q.i.d. <sup>†</sup>
Negative	Negative	Low	Acyclovir 800 mg PO q.i.d. <sup>†</sup>
CMV Ab, cytomegalovirus antibody; OKT-3, anti-CD3 monoclonal antibody.			
*Suggested algorithm – prophylaxis strategies are based on predicted risk of post-transplant disease.			
<sup>†</sup> Ganciclovir is substituted for acyclovir when OKT-3 or thymoglobulin is given.			

Table 42.11 CMV prophylaxis in liver transplant recipients\*.

and was dependent upon recipient and donor CMV serologic status; 1-year mortality rates were 11% if seronegative donor and recipient, 22% if seronegative donor and seropositive recipient, 30% if seropositive donor and recipient and 44% if seropositive donor and seronegative recipient. Finally, CMV infection has been linked with an increased frequency of chronic rejection although this association may be confounded by changes in immunosuppression regimens. The indirect association of CMV with malignancies in transplant patients has been difficult to establish and remains controversial.

### Prophylaxis of CMV in liver transplant recipients

Prophylactic regimens are highly effective in preventing CMV infection. A variety of treatments have shown efficacy in the transplant setting including intravenous immunoglobulin, CMV hyperimmune globulin, acyclovir, ganciclovir and most recently valganciclovir. In a meta-analysis of 11 studies with different donor–recipient CMV serostatuses, different organ types, and acyclovir or ganciclovir as prophylactic agents, the relative risk of CMV infection was 0.5 for all patients on either prophylactic agent compared to no prophylaxis. CMV prophylactic agents can be administered in a universal prophylaxis approach, or alternatively, pre-emptively to patients at increased risk of CMV disease, identified by markers of early viral replication or epidemiologic tools. Using the former approach, at least three randomized studies have demonstrated that it is possible to reduce CMV disease in recipients of CMV donor-positive organs. Universal prophylaxis is achieved through the prolonged administration of intravenous globulin or oral ganciclovir, or sequential use of ganciclovir followed by oral acyclovir. Pre-emptive prophylaxis with ganciclovir based on CMV detection by surveillance cultures or at the time of OKT-3 or thymoglobulin use is also effective. Controversy remains regarding the specific prophylactic regimen for patients in different risk groups. In general, those at high or intermediate risk of post-transplant CMV disease are given ganciclovir prophylaxis and those at low or low to intermediate risk receive acyclovir prophylaxis (Table 42.11). The duration of CMV prophylaxis following transplantation is generally for 3 months though longer treatment protocols are being studied.

Treatment of CMV infection		
CMV Ag status	Invasive CMV disease	Treatment (adjust for renal function)
Positive	No	Valganciclovir 900 mg PO b.i.d. × 14 days then q.d. × 3 months
Positive	Yes	Ganciclovir 5 mg/kg i.v. b.i.d. × 14 days (or less) Check CMV Ag status every 2 weeks When CMV Ag– change to valganciclovir 900 mg PO q.d. × 8 weeks
CMV Ag, cytomegalovirus antigen.		

Table 42.12 Treatment of CMV infection.

### Treatment of CMV disease in transplant recipients

Ganciclovir, valganciclovir, foscarnet and cidofovir, all have excellent activity against all herpesviruses. Common side-effects of ganciclovir include bone marrow suppression, which is observed infrequently in liver transplant recipients. Valganciclovir, a prodrug of ganciclovir, is as effective as ganciclovir and has a longer half-life allowing for one daily dosing, yet is more costly and may be associated with higher rates of neutropenia. Patients responding to ganciclovir may be considered for transition to oral valganciclovir to complete the treatment course. The use of foscarnet and cidofovir in the liver transplant population is restricted to patients who fail to respond to ganciclovir therapy due to acquired viral resistance. The major adverse effect of both these agents is nephrotoxicity. The appropriate duration of therapy for both asymptomatic infection and CMV disease is unclear. One treatment approach is presented in Table 42.12. Since CMV reactivation is a marker of excessive therapeutic immunosuppression, concurrent reduction in immunosuppressive therapy is frequently undertaken.



## OTHER RECURRENT VIRAL INFECTIONS POST-TRANSPLANTATION

### Epstein-Barr virus

Primary EBV infection in transplant patients may be acquired from the allograft, leukocyte-containing blood products or from the community. In adults, the great majority of EBV-related infections represent reactivation in individuals who are EBV-seropositive. Clinical presentations of EBV infection after liver transplantation include asymptomatic infection, mononucleosis syndromes, hepatitis and PTLT.

The clinical presentation of EBV-associated PTLT is variable. The presence of unexplained fevers with adenopathy, tonsillitis and pharyngitis consistent with a mononucleosis-like syndrome may be present. In addition, more severe presentations including bowel perforation or obstruction, hepatocellular dysfunction, impairment of renal function, weight loss, pulmonary infiltrates and central nervous system involvement are encountered clinically. Histologically, the spectrum of PTLT represents a continuum from a polyclonal non-specific reactive hyperplasia that follows a benign course to a malignant monoclonal polymorphic B-cell lymphoma. The diagnosis of PTLT relies on the demonstration of abnormal lymphoid proliferation in biopsy material and/or the identification of cellular clonal abnormality by cytologic analysis. EBV serologic conversion and high EBV DNA titer may be supportive of but are not diagnostic of PTLT.

The reported incidence of PTLT following liver transplantation varies from 2% to 4%. The main risk factors for PTLT are EBV seronegativity before transplantation, age less than 18 years, use of OKT-3 therapy for rejection treatment, CMV donor-positive-recipient-negative serostatus, and the development of CMV disease following liver transplantation.

The best therapy remains to be defined. Drastic reduction or cessation of immunosuppression appears to be the most effective measure. The addition of high-dose acyclovir or ganciclovir inhibits viral replication in the oropharynx but has not been shown to improve disease prognosis, especially in those cases in which cell transformation by EBV has occurred. Patients with extranodal, multifocal and brain involvement may require further therapy; including surgical extirpation of tumor masses, cytotoxic chemotherapy and/or radiation therapy. Other therapies that have been used with variable success are anti-B-cell monoclonal antibody and  $\alpha$  interferon.

Clearly, prevention is preferred. Reduced immunosuppression, especially avoiding the use of antilymphocyte therapy, should be considered in patients at risk. Moreover, given the association between CMV disease and risk of PTLT, strategies to prevent CMV disease may be useful. Anecdotally, in non-randomized trials, the incidence of PTLT was reported to be reduced with the use of CMV prophylactic therapy in high-risk patients. No single effective strategy to prevent the evolution of EBV infection to PTLT has been established.

### Herpes simplex virus

Virtually all of the infections caused by HSV result from reactivation of latent virus. Primary infection can be transmitted by person to person contact or via the allograft. HSV can cause hepatitis in the liver allograft recipient, which may result in liver failure but this complication is extremely rare. Oral mucocutaneous lesions are most common and typically occur during

the first month after transplantation. Most orolabial lesions are mild, but can be severe and complicated by bacterial superinfection and esophageal involvement. Anogenital infection may occur; this usually presents as large, coalescing and ulcerating lesions. For mucocutaneous disease, the clinical examination is usually highly suggestive of the diagnosis, and a Tzanck smear showing multinucleated giant cells is a rapid, morphologic method to establish the diagnosis. The gold standard for diagnosis is the detection of antigen or isolation of virus from tissue. More rapid methods including PCR tests have been shown to be sensitive and specific but a positive test does not distinguish latent from lytic infections.

Acyclovir, ganciclovir and famciclovir are all effective anti-HSV drugs. Mucocutaneous infections may be treated by oral medications, while disseminated or deep HSV disease requires the use of the intravenous therapy. The recent availability of oral valacyclovir, an ester formulation of acyclovir that produces high serum drug levels, may be a valid alternative to the intravenous medications. While usually treatment-responsive, antiviral prophylaxis has reduced the occurrence of HSV infections in the transplant setting.

### Varicella-zoster virus

Primary infection occurs in a minority of liver transplant recipients; the vast majority of disease represents reactivation infection in varicella-zoster virus (VZV)-seropositive individuals. Clinical disease with VZV is uncommon earlier than 2 months after transplantation. The incidence of VZV reactivation in liver transplant recipients was 3, 10 and 14% at 1, 3 and 5 years post-transplantation in one large series.

Localized dermatomal reactivation results in herpes zoster. Typically, two or three adjoining dermatomes are involved with a few distant sites of cutaneous dissemination. A syndrome of unilateral pain without cutaneous lesions has been described. A clinical diagnosis is made if the characteristic vesicular lesions distributed in a dermatomal pattern are present. Rarely, the disease may be characterized by life-threatening hemorrhagic pneumonia, skin lesions, encephalitis, hepatitis, pancreatitis and disseminated intravascular coagulation. A microbiologic diagnosis is made by direct immunofluorescence of a swab taken from a lesion or culture, or demonstration of multinucleated giant cells on a Tzanck smear.

High-dose intravenous acyclovir is usually effective, especially if the disease is recognized early and treatment is initiated within 24 h of the skin eruption. Both valacyclovir and famciclovir may be valid therapeutic agents. Because of the high mortality associated with severe primary VZV infection in organ transplant recipients, varicella-zoster immune globulin (VZIG) and antiviral agents should be administered promptly after a documented or suspected exposure.

### Other rare viral causes of recurrent hepatitis

An emerging role for human herpesvirus (HHV)-6 and -7 as pathogens in liver transplant has been recently recognized. Most infections are due to reactivation of the latent virus in the recipient, although donor transmission also occurs. Both viruses can present as febrile illnesses; HHV-6 has been associated with profound thrombocytopenia, a skin rash and a propensity for neural invasion, presenting clinically as encephalitis/encephalopathy. Hepatitis may be a clinical manifestation of these

infections in liver transplant recipients. HHV-6 and HHV-7 have been linked with an increased risk of CMV infection and other opportunistic infections and possibly with allograft rejection. The diagnostic methods used include viral isolation by culturing peripheral blood mononuclear cells, immunohistochemical staining of tissue specimens, PCR assays and serologic studies. The virus is susceptible to ganciclovir and foscarnet, although the role of antiviral therapy and prophylaxis in this setting remains to be determined.

Adenovirus infection predominantly causes a benign illness in the general population, but has a propensity for causing life-threatening lung and gastrointestinal tract disease in pediatric

liver recipients. An aggressive hepatitis leading to graft loss and requiring retransplantation has been reported. There is no effective treatment.

Hepatitis G virus (also known as GBV-C) is frequent in liver transplant recipients with reported prevalence rates of ~50%. Extensive studies have failed to show an association between HGV and acute, fulminant or chronic liver disease. However, HGV may modify the course of other viral infections as has been shown in persons with HIV infection. At the present time, complications of HGV reactivation or exacerbation in transplant recipients have not been identified, thus there is no clear need to test for this virus.

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