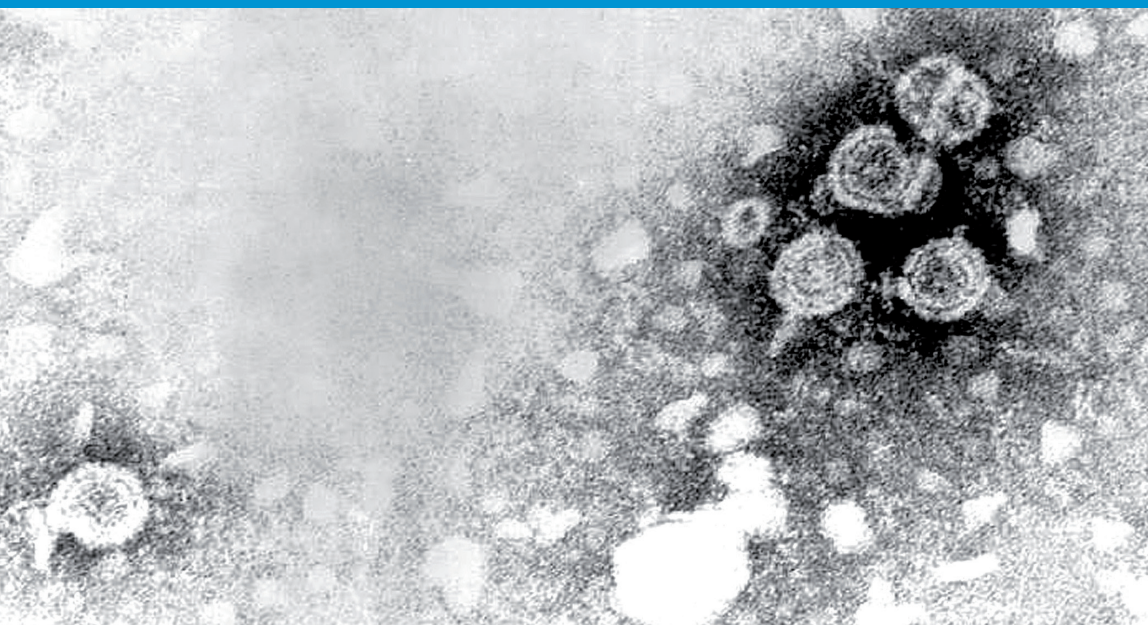
Mauss - Berg - Rockstroh - Sarrazin - Wedemeyer



HEPATOLOGY

A clinical textbook

SECOND EDITION

www.HepatologyTextbook.com

Flying Publisher

This textbook was made possible through unrestricted grants from
Roche Pharma, Germany
F Hoffman LaRoche Ltd, Switzerland
Gilead Sciences, Germany

Hepatology - A Clinical Textbook

Second Edition

www.HepatologyTextbook.com

Editors

Stefan Mauss
Thomas Berg
Jürgen Rockstroh
Christoph Sarrazin
Heiner Wedemeyer

Copy Editor: Rob Camp
Layout and design: Ismael Sala Salas

Flying Publisher

Disclaimer

Hepatology is an ever-changing field. The editors and authors of **Hepatology - A Clinical Textbook** have made every effort to provide information that is accurate and complete as of the date of publication. However, in view of the rapid changes occurring in medical science, as well as the possibility of human error, this site may contain technical inaccuracies, typographical or other errors. Readers are advised to check the product information currently provided by the manufacturer of each drug to be administered to verify the recommended dose, the method and duration of administration, and contraindications. It is the responsibility of the treating physician who relies on experience and knowledge about the patient to determine dosages and the best treatment for the patient. The information contained herein is provided “as is” and without warranty of any kind. The editors and Flying Publisher disclaim responsibility for any errors or omissions or for results obtained from the use of information contained herein.

D-40237 Düsseldorf
Printed in Germany
ISBN: 978-3-924774-70-7

Preface

Hepatology is a rapidly evolving medical field and will continue to be very exciting over the next few decades. The situation in viral hepatitis is similar to the HIV field 10-15 years ago when it started to become better understood and treatable. Today, hepatitis B viral replication can be suppressed by potent antiviral drugs but there are risks for the emergence of resistance. Strategies to enhance the eradication rates of HBV infection still need to be developed. On the other hand, hepatitis C virus infection can be eradicated by treatment with pegylated interferon plus ribavirin. However, particularly in those infected by HCV genotype 1 the sustained virologic response rates are still suboptimal. Many new antiviral drugs, especially protease and polymerase inhibitors, are currently in preclinical and clinical development, and the first data from larger clinical trials provide some optimism that the cure rates for patients with chronic hepatitis C will be enhanced with these new agents. In other areas of hepatology, e.g., for hereditary and metabol ic liver diseases, our knowledge is rapidly increasing and new therapeutic options are on the horizon.

Are books in rapidly evolving areas such as hepatology the right medium to gather and summarize the current knowledge? Are these books not likely to be outdated the very day they are published? This is indeed a challenge that can be convincingly overcome only by rapid internet-based publishing with regular updates. Another unmatched advantage of a web-based book is the free and unrestricted access everywhere. Viral hepatitis and other liver diseases are a global burden and timely information is important for physicians, scientists, patients and health care officials all around the world.

The editors of this web-based book – Thomas Berg, Stefan Mauss, Jürgen Rockstroh, Christoph Sarrazin and Heiner Wedemeyer – are young, bright, and already internationally renowned hepatologists who have created an excellent state-of-the-art textbook on clinical hepatology. The book is well-structured and written and provides in-depth information without being lengthy and redundant. I am convinced that all five will remain very active in the field and will update this book regularly as the science progresses. The book should rapidly become an international standard.

Frankfurt, 24 January 2009
Stefan Zeuzem

Preface

Therapeutic options and diagnostic procedures in hepatology have quickly advanced over the last decade. In particular, the management of viral hepatitis has completely changed since the early nineties. Before nucleoside and nucleotide analogues were licensed to treat hepatitis B and before interferon alpha / ribavirin combination therapy was approved for the treatment of chronic hepatitis C, very few patients infected with

HBV or HCV were treated successfully. The only option for most patients with end-stage liver disease or hepatocellular carcinoma was liver transplantation. However, even if the patients were lucky enough to be successfully transplanted, re-infection of the transplanted organs remained a major challenge. In the late eighties and early nineties discussions were ongoing about whether to reject patients with chronic hepatitis from the waiting lists because post-transplant outcome was poor. Today, just 15 years later, hepatitis B represents one of the best indications for liver transplantations as almost all re-infection can be prevented. In addition, the proportion of patients who need to be transplanted is declining because almost all HBV-infected patients can nowadays be treated successfully and reach complete suppression of HBV replication and some patients can even clear HBsAg, the ultimate endpoint of hepatitis B treatment. Hepatitis C has also become a curable disease with sustained responses of 50-80% using pegylated interferons in combination with ribavirin. The future of HCV treatment using direct HCV enzyme inhibitors is in Phase I-III trials but has not reached clinical practice yet.

Major achievements for the patients sometimes lead to significant challenges for the treating physician. Is the diagnostic work-up complete? Did I miss any recent developments in order to evaluate correctly the stage and grade of liver disease? What sensitivities are really necessary for assays to detect hepatitis viruses? When exactly do I need to determine HBV polymerase variants before and during treatment of hepatitis B? When can I safely stop treatment without risking a relapse? How to treat acute hepatitis B and C? When does a health-care worker need a booster vaccination for hepatitis A and B? These are just some of the many questions we have to ask ourselves frequently during our daily routine practice. With the increasing number of publications, guidelines and expert opinions it is getting more and more difficult to stay up-to-date and to make the best choices for the patients. In this respect, HEPATOLOGY 2009 - A Clinical Texbook is a very useful new tool which gives a state-of-the art update on various aspects of HAV, HBV, HCV, HDV and HEV infections. The editors are international experts in the field of viral hepatitis; all have made significant contributions to understanding the pathogenesis of virus-induced liver disease, diagnosis and treatment of hepatitis virus infections.

Hepatology 2009 - A Clinical Texbook gives a comprehensive overview on the epidemiology, virology, and natural history of all hepatitis viruses including hepatitis A, D and E. Subsequent chapters cover all major aspects of the management of hepatitis B and C including coinfections with HIV and liver transplantation. Importantly, complications of chronic liver disease such as HCC and recent developments in assessing the stage of liver disease are also covered. Finally, interesting chapters on autoimmune and metabolic non-viral liver diseases complete the book.

We are convinced that this new up-to-date book covering all clinically relevant aspects of viral hepatitis will be of use for every reader. The editors and authors have to be congratulated for their efforts.

Michael P Manns
Hannover, 24 January 2009

Foreword 2010

Because hepatology is such a dynamic and exciting area of medicine, regular updates are mandatory in keeping a clinical textbook useful. We are delighted to present this Second Edition of **Hepatology - A Clinical Textbook**. The First Edition was a major success, with more than 80,000 downloads worldwide. In addition, a Romanian translation was carried out by Camelia Sultana and Simona Ruta shortly after the appearance of the first edition. We invite qualified people everywhere to do the same, into any appropriate language! This web-based free-of-charge concept made possible by unrestricted grants from Roche and Gilead has allowed the material to reach countries usually not easily covered by print media, a special quality of this project. We hope this Second Edition of **Hepatology - A Clinical Textbook** will continue to be a valuable source of information for our readers.

The Editors
Thomas Berg, Stefan Mauss, Jürgen Rockstroh, Christoph Sarrazin, Heiner Wedemeyer

Contributing Authors

Fernando Agüero
Infectious Diseases Service
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Susanne Beckebaum
Interdisplinary Liver Transplant Unit
University Hospital Essen
Hufelandstr. 55
45122 Essen, Germany

Thomas Berg
Dept of Internal Medicine, Neurology and Dermatology
University Clinic Leipzig
Medical Clinic II
Liebigstrasse 20
04103 Leipzig, Germany
Thomas.berg@medizin.uni-leipzig.de

Florian van Bömmel
University Clinic Leipzig
Medical Clinic II
Liebigstrasse 20
04103 Leipzig, Germany

Christoph Boesecke
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany

Carlos Cervera
Infectious Diseases Service
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Vito R. Cicinnati
Interdisciplinary Liver Transplant Unit
University Hospital Essen
Hufelandstr. 55
45122 Essen, Germany

Markus Cornberg
Dept. of Gastroenterology, Hepatology and Endocrinology
Medical School of Hannover
Carl-Neuberg-Str. 1
30625 Hannover, Germany

Juan Carlos García Valdecasas
Liver Transplant Unit, Dept of Surgery
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Guido Gerken
Department of Gastroenterology
University Hospital Essen
Hufelandstr. 55
45122 Essen, Germany

Frank Grünhage
Medical Department II
Saarland University Hospital
Kirrbergerstr. 1
66421 Homburg, Germany

Bernd Kupfer
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany

Montserrat Laguno
Infectious Diseases Service
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Frank Lammert
Saarland University Hospital
Medical Department II
Kirbergerstr. 1
66421 Homburg, Germany

Christian Lange
J. W. Goethe-University Hospital
Medical Department 1
Theodor-Stern-Kai 7
60590 Frankfurt am Main, Germany

Johannes Lenz
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany

Jessica Lüsebrink
Institute of Pathology
Kliniken der Stadt Cologne gGmbH
Ostmerheimer Strasse 200
51109 Cologne, Germany

Michael P. Manns
Dept. of Gastroenterology, Hepatology
and Endocrinology
Medical School of Hannover
Carl-Neuberg-Strasse 1
30625 Hannover, Germany

Stefan Mauss
Center for HIV and
Hepatogastroenterology
Grafenberger Allee 128a
40237 Duesseldorf, Germany
Stefan.mauss@center-duesseldorf.de

José M. Miró
Infectious Diseases Service
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Asunción Moreno
Infectious Diseases Service
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Claus Niederau
Katholische Kliniken Oberhausen
gGmbH
Academic Teaching Hospital of
University of Duisburg-Essen
Mülheimer Str. 83
46045 Oberhausen, Germany

Karl-Philipp Puchner
University Clinic Leipzig
Medical Clinic II
Liebigstrasse 20
04103 Leipzig, Germany

Antonio Rimola
Liver Transplant Unit - CIBEREHD
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

J. K. Rockstroh
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany
juergen.rockstroh@ukb.uni-bonn.de

Christoph Sarrazin
J. W. Goethe-University Hospital
Department of Medicine 1
Theodor-Stern-Kai 7
60590 Frankfurt am Main, Germany
sarrazin@em.uni-frankfurt.de

Martin Schäfer
Dept of Psychiatry and Psychotherapy
Kliniken Essen-Mitte
Ev. Huyssenstift
Henricistraße 92
45136 Essen, Germany

Carolynne Schwarze-Zander
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany

Oliver Schildgen
Cologne City Clinic
Pathology Institute
Ostmerheimer Strasse 200
51109 Cologne, Germany

Verena Schildgen
Kliniken der Stadt Koln
Institut fur Pathologie
Ostmerheimer Strasse 200
51109 Cologne, Germany

Ulrich Spengler
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany

Christian P. Strassburg
Gastroenterology, Hepatology and
Endocrinology Clinic
Medical Teaching Scool Hannover
Carl- Neuberg-Strasse 1
30625 Hannover

Montserrat Tuset
Pharmacy Department
Hospital Clínic - IDIBAPS
University of Barcelona
Villarroel, 170
08036 Barcelona, Spain

Jan-Christian Wasmuth
Bonn University Hospital
Department of Medicine I
Sigmund-Freud-Strasse 25
53105 Bonn, Germany

Heiner Wedemeyer
Dept. of Gastroenterology, Hepatology
and Endocrinology
Medical School of Hannover
Carl-Neuberg-Strasse 1
30625 Hannover, Germany
heiner.wedemeyer@gmail.com

Johannes Wiegand
University Clinic Leipzig
Medical Clinic II
Liebigstrasse 20
04103 Leipzig, Germany

Stefan Zeuzem
J. W. Goethe-University Hospital
Medical Department 1
Theodor-Stern-Kai 7
60590 Frankfurt am Main, Germany

Part 1 **The Basics**

Chapter 1 **Hepatitis A** 3
Epidemiology, transmission and natural history
Johannes Lenz

Chapter 2 **Hepatitis B** 7
Epidemiology, transmission and natural history
Jan-Christian Wasmuth

Chapter 3 **Hepatitis C** 19
Epidemiology, transmission and natural history
Jan-Christian Wasmuth

Chapter 4 **Hepatitis E** 31
Epidemiology, transmission and natural history
Johannes Lenz

Chapter 5 **HBV** 37
Classification, biology, life cycle
Jessica Lüsebrink, Verena Schildgen, Oliver Schildgen

Chapter 6 **HCV** 61
Virology
Bernd Kupfer

Chapter 7 **Viral hepatitis** 83
Prophylaxis & vaccination
Heiner Wedemeyer

Part 2 **Hepatitis B and D**

Chapter 8 **Acute and chronic hepatitis B** 95
Diagnostic tests
Oliver Schildgen

Chapter 9 **HBV Treatment** 101
Standard of care
Florian van Bömmel, Johannes Wiegand, Thomas Berg

Chapter 10 **HBV** 131
Resistance and implications for therapeutic strategies
Stefan Mauss and Heiner Wedemeyer

Chapter 11 **Hepatitis D** 145
Diagnostic procedures and therapy
Heiner Wedemeyer

Part 3 **Hepatitis C**

Chapter 12 **Acute and chronic hepatitis C Diagnostic tests** 159
Christian Lange and Christoph Sarrazin

Chapter 13 **Standard of care** 171
Markus Cornberg, Michael P Manns, Heiner Wedemeyer

Chapter 14 **New agents for treatment** 197
Christian Lange and Christoph Sarrazin

Chapter 15 **Management of adverse drug reactions** 231
Martin Schäfer and Stefan Mauss

Chapter 16 **Extrahepatic manifestations** 239
Karl-Philipp Puchner and Thomas Berg

Part 4 **Coinfections**

Chapter 17 **HBV/HIV Coinfection Management** 259
Stefan Mauss and Jürgen Rockstroh

Chapter 18 **HCV/HIV Coinfection Management** 269
Christoph Boesecke, Stefan Mauss, Jürgen Rockstroh

Chapter 19 **HBV/HCV Coinfection Management** 283
Carolynne Schwarze-Zander and Jürgen Rockstroh

Part 5 **Liver fibrosis**

Chapter 20 **Assessment of hepatic fibrosis in chronic viral hepatitis** 291
Frank Grünhage and Frank Lammert

Part 6 **Hepatocellular Carcinoma**

Chapter 21 **Diagnosis, Prognosis & Therapy** 303
Ulrich Spengler

Part 7 Liver transplantation

Chapter 22 **Liver transplantation in hepatitis B and C monoinfection** 315
Susanne Beckebaum, Guido Gerker, Vito R Cicinatti

Chapter 23 **Liver transplantation in hepatitis B and C and HIV coinfection** 353
José Maria Miro, Fernando Agüero, Montserrat Laguno, Christian Manzardo, Montserrat Tuset, Carlos Cervera, Asunción Moreno, Juan Carlos García Valdecasas, Antonio Rimola, Hospital Clinic OLT in HIV Working Group

Part 8 Autoimmune and metabolic liver disease

Chapter 24 **Metabolic Liver Diseases Haemochromatosis** 373
Claus Niederau

Chapter 25 **Metabolic Liver Diseases NASH** 395
Claus Niederau

Chapter 26 **Metabolic Liver Diseases Wilson's Disease** 405
Claus Niederau

Chapter 27 **Autoimmune liver diseases: AIH, PBC and PSC** 421
Christian P Strassburg

Chapter 28 **Alcoholic Hepatitis** 467
Claus Niederau

Abbreviations

ADV: adefovir dipivoxil
AHA: autoimmune-haemolytic anaemia
Alb-IFN: albumin interferon
ALT: alanine aminotransferase
ASH: alcoholic steato-hepatitis
AST: aspartate aminotransferase
BID: twice a day
cccDNA: covalently closed circular DNA
CIFN: consensus interferon
CNI: calcineurin inhibitors
CP: Child-Pugh
CPT: Child-Pugh-Turcotte
EASL: European Association for the Study of the Liver
EBV: Epstein-Barr virus
EHM: extrahepatic manifestation
ERC: endoscopic retrograde cholangiography
ER: endoplasmic reticulum
ETV: entecavir
EVL: everolimus
EVR: early virologic response
GFR: glomerular filtration rate
GH: growth hormone
GM-CSF: granulocyte macrophage colony stimulating factor
GN: glomerulonephritis
HBcAg: hepatitis B core antigen
HBeAg: hepatitis B early antigen
HBsAg: hepatitis B surface antigen
HBV: hepatitis B virus
HCV: hepatitis C virus
HCV RNA: riboneucleic acid of hepatitis C virus
HCC: hepatocellular carcinoma
HRS: hepatorenal syndrome
IFN α: interferon α
IGF-1: insulin growth factor-1
INR: international normalised ratio
IPF: idiopathic pulmonary fibrosis
ITP: immune thrombocytopenic purpura
LAM: lamivudine
LDL: low density lipoproteins
LDLT: living donor liver transplantation
LdT: telbivudine
LT(X): liver transplantation
LPS: lipopolysaccharide
MELD: Model for End-Stage Liver Disease
MPGN: membranoproliferative glomerulonephritis
NASBA: nucleic acid sequence based amplification
NASH: non-alcoholic steato-hepatitis
NHL: non-Hodgkin lymphoma
NTR: non-translated regions
PCR: polymerase chain reaction
PCT: porphyria cutanea tarda
PDGF: platelet-derived growth factor
PEG-IFN: pegylated interferon
PT: prothrombin time
QD: once a day
QW: once a week
RF: rheumatoid factor
RVR: rapid virologic response
SSRI: serotonin uptake inhibitor
SVR: sustained virologic response
TGF-β: transforming growth factor β
RBV: ribavirin
SRL: sirolimus
TID: three times a day
TDF: tenofovir disoproxil fumarate
VLDL: very low density lipoproteins

Part 1

The Basics

Chapter 1: Hepatitis A - Epidemiology, transmission and natural history

Johannes Lenz

Genomic Organisation

The hepatitis A virus was identified in 1973 (Feinstone 1973). It is a 27 nm, positive-stranded RNA, non-enveloped, icosahedral virus of the heparnavirus genus of the Picornaviridiae. Its viral genome contains 7474 nucleotides that are grouped into three regions: a 5' and a 3' non-coding region and a 6681 nucleotide open reading frame. The polypeptide encoded by the open reading frame is processed by a viral protease, resulting in eleven proteins of which four are structural and seven are non-structural. Four distinct HAV genotypes in humans have been identified, although significant biological differences have not been found (Lemon 1992).

Epidemiology

Hepatitis A infection occurs worldwide sporadically or in epidemic outbreaks. There is an estimated caseload of 1.4 million cases per year (Viral Hepatitis Prevention Board 1997). As it is transmitted and spread via the faecal-oral route (Hollinger 1996), it shows higher prevalence in areas with low socio-economic status where adequate sanitation or adequate hygienic practices are lacking. The incidence of 1.5 per 100,000 in industrialised countries, e.g., the United States or Germany (Wasley 2007; RKI 2006), is low compared to developing countries (parts of Africa, Asia, Central and South America) where it may reach up to 150 per 100,000 per year (WHO).

Transmission

HAV is generally acquired via the faecal-oral route either by person-to-person contact or ingestion of contaminated food or water, as well as sexually via analingus. Hepatitis A is an enteric infection spread by contaminated excreta. High concentrations of virus are shed in the stools of patients 3 to 10 days prior to the onset of illness and until one to two weeks after the onset of jaundice. Faecal excretion of HAV persists longer in children and in immunocompromised persons (up to 4 to 5 months after infection) (Hollinger 1996).

Persons in psychiatric institutions or day-care centres, health care providers, military personnel and men who have sex with men (especially when practicing anal intercourse) are at higher risk of infection. Parenteral transmission via IV drug use or transfusion of blood products is rare because of the short period of HAV viraemia during acute infection. Mother-to-foetus transmission has not been reported.

Clinical course

Hepatitis A infection can take a wide spectrum of clinical courses ranging from asymptomatic or subclinical infection to cholestatic presentation or even to fulminant liver failure. In children most infections are asymptomatic, while in adults 70% show clinical illness. Anicteric symptomatic HAV is more frequent than icteric disease, as only 30% of patients develop jaundice.

The incubation time averages 30 days (15 to 49 days). The illness begins with the abrupt onset of unspecific prodromal symptoms including fatigue, malaise, nausea, vomiting, anorexia, fever, abdominal discomfort, and right upper quadrant pain (Lednar 1985). Within one week, patients with an icteric course note darkened urine, light-coloured acholic stool, jaundice, and often pruritus. The prodromal symptoms usually diminish when jaundice appears. The jaundice is most intense typically within the first two weeks. Decrease and subsequent normalisation of serum aminotransferases occurs rapidly and before a decrease or normalisation of serum bilirubin.

A biphasic or relapsing form of viral hepatitis A occurs in 6-10% of cases. The initial episode lasts 3-5 weeks and is followed by a period of remission characterised by normal liver chemistries lasting 4-5 weeks. Relapse may mimic the initial episode of the acute hepatitis. The full duration of the illness ranges from 16-40 weeks from the onset, and HAV-IgM antibodies persist throughout the clinical course (Schiff 1992).

A severe fulminant course of HAV with hepatic failure is found more often in patients with underlying liver disease. Patients with chronic hepatitis C have a greatly increased risk of hepatic failure, while HBV coinfection is less perilous (Vento 1989). Other risk factors are age, malnutrition and immunosuppression.

The available data on HAV in pregnant women is not conclusive. Some data show a risk of gestational complications and premature birth (Elinav 2006; Zhang 1990) while others have not observed such complications (Tong 1981).

Hepatitis A infection has been reported as a trigger for autoimmune chronic active hepatitis (CAH) in genetically susceptible individuals (Vento 1991). In 58 monitored relatives of patients with CAH, three cases of subclinical HAV occurred. Two of these developed CAH within 5 months of HAV infection. Both showed a defective T cell control of immune responses to the asialoglycoprotein receptor with ongoing T helper cell activation after the clearance of HAV.

Overall, a lethal course of HAV occurs in 0.1% of children, in 0.4% of persons aged 15-39 years, and in 1.1% in persons older than 40 years (Lemon 1985). Although a relapsing form of HAV (mentioned above) is known, the infection does not progress to a chronic state.

Clinical presentation

Jaundice and hepatomegaly are the two main findings in a physical examination. They are seen in 70 and 80% of symptomatic patients, respectively (Tong 1995). Other findings are splenomegaly, evanescent rash, cervical and other lymphadenopathies.

Extrahepatic manifestations

Although less frequent than in HBV infection, extrahepatic manifestations have been associated with acute HAV infection (Schiff 1992). Cutaneous vasculitis is typically located on the legs and buttocks. Skin biopsies reveal the presence of anti-HAV IgM and components of the complement system in the blood vessel walls. Also, arthritis appears to have a predilection for the lower extremities. Both arthritis and vasculitis have been associated with cryoglobulinaemia. Manifestations in the nervous system such as transverse myelitis, optic neuritis, and polyneuritis may also be immunocomplex-related. Haematological complications include thrombocytopenia, aplastic anaemia, and red cell aplasia. These conditions appear to be more likely in patients with prolonged symptoms.

Laboratory findings

In symptomatic patients typical laboratory findings are marked elevations of serum aminotransferases, alkaline phosphatase, and serum bilirubin (Tong 1995). Serum alanine aminotransferase (ALT) usually shows higher values than serum aspartate aminotransferase (AST) and concentrations exceeding 1000 IU/L are common.

The increase of serum aminotransferase precedes the elevation of serum bilirubin and the peak of bilirubin concentration occurs after the peak of aminotransferase concentration. Serum bilirubin often exceeds a concentration of 10 mg/dl. Other laboratory abnormalities include elevations of acute phase reactants, an elevated erythrocyte sedimentation rate, and increased immunoglobulins.

Diagnosis

The specific diagnosis of acute HAV infection is made by the detection of serum anti-HAV IgM antibodies in those with symptoms of acute hepatitis. This antibody is present in 99% of patients by the time of appearance of clinical symptoms. Therefore, it is the gold standard for detection of acute HAV. Anti-HAV IgM concentration peaks in the second month of infection and then gradually decreases until it becomes undetectable, usually after 6 to 12 months. Sometimes anti-HAV IgM persists longer and therefore, detection in asymptomatic individuals does not necessarily indicate acute infection, as it could be an effect of previous asymptomatic HAV contact (CDC 2005).

Detection of HAV in stool, body fluids, serum and liver tissue by either electron microscopy or polymerase chain reaction (PCR) is more complicated and expensive. Anti-HAV IgG antibodies are formed in the early convalescent phase, remain positive for decades, and provide long-lasting, if not lifetime immunity to re-infection.

Treatment

Because acute hepatitis A is a self-limiting disease and in most cases resolves spontaneously without residual damage or sequelae and no specific therapy is available, the treatment is based on monitoring. In 85% of cases, clinical symptoms and laboratory abnormalities resolve within 3 months. After 6 months almost all patients have complete recovery (Koff 1992). More severe courses require hospitalisation. In an outbreak in Pennsylvania, USA, 20% of patients had to be admitted to hospital (Wheeler 2005). The rare cases that progress to fulminant hepatic failure (impaired synthetic function, hepatic encephalopathy) require aggressive monitoring therapy. These patients should be transferred to a centre that is capable of performing liver transplantation.

Prevention

HAV is predominantly transmitted faecal-orally by ingestion of contaminated foods and water. Therefore proper preparation of foods especially in areas where HAV is endemic is crucial to avoid infection (“cook it, peel it, or leave it”). A second option is vaccination. There are several effective and highly immunogenic vaccines commercially available (Hammitt 2008). The issue is discussed in more detail in Chapter 7, ‘Prophylaxis and vaccination of viral hepatitis’.

References

CDC (Centers for Disease Control). Positive test results for acute hepatitis A virus infection among persons with no recent history of acute hepatitis. MMWR 2005; 54: 453-6.

Elinav E, Ben-Dov IZ, Shapira Y, et al. Acute hepatitis A infection in pregnancy is associated with high rates of gestational complications and preterm labor. Gastroenterology. 2006; 130: 1129-34.

Feinstone SM, Kapikian AZ, Purceli RH. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. Science 1973; 182:1026.

Hammitt LL, Bulkow L, Hennessy TW et al. Persistence of antibody to hepatitis A virus 10 years after vaccination among children and adults. J. Infect. Dis. 2008: 198, 1776-1782.

Hollinger FB and Ticehurst JR. Hepatitis A virus. In: Fields BN, Knipe DM, and Howley PM, editors. Fields Virology, 3rd ed. Philadelphia, Lippincott - Raven, 1996: 735-782.

Koff RS. Clinical manifestations and diagnosis of hepatitis A virus infection. Vaccine 1992;10 Suppl 1:S15-7.

Lemon SM, Jansen RW, Brown EA. Genetic, antigenic and biological differences between strains of hepatitis A virus. Vaccine 1992; 10: S40-4.

Lemon SM. Type A viral hepatitis. New developments in an old disease. N Engl J Med. 1985; 313: 1059-67.

Lednar WM, Lemon SM, Kirkpatrick JW, Redfield RR, Fields ML, Kelley PW. Frequency of illness associated with epidemic hepatitis A virus infections in adults. Am J Epidemiol 1985; 122: 226-33.

RKI (Robert Koch Institut, Germany): Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2006. 2006.

Schiff ER. Atypical clinical manifestations of hepatitis A. Vaccine 1992; 10: S18-20.

Tong MJ, Thursby M, Rakela J, McPeak C, Edwards VM, Mosley JW. Studies on the maternal-infant transmission of the viruses which cause acute hepatitis. Gastroenterology 1981; 80: 999-1004.

Tong MJ, el-Farra NS, Grew MI. Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. J Infect Dis 1995; 171: S15-8.

Vento S, Garofano T, Di Perri G, Dolci L, Concia E, Bassetti D. Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. Lancet 1991; 337: 1183-7.

Vento S, Garofano T, Renzini C et al. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. N Engl J Med 1998; 338: 286-90.

Viral Hepatitis Prevention Board. News from the VHPB meeting in St. Julians, Malta. Viral Hepatitis 1997; 6: 6.

Wasley A, Miller JT, Finelli L. Surveillance for acute viral hepatitis--United States, 2005.

MMWR Surveill Summ. 2007; 56: 1-24.

Wheeler C, Vogt TM, Armstrong GL et al. An outbreak of hepatitis A associated with green onions. N Engl J Med 2005; 353: 890-7.

WHO (World Health Organization): Hepatitis A. World Health Organization. Department of Communicable Disease Surveillance and Response.

Zhang RL, Zeng JS, Zhang HZ. Survey of 34 pregnant women with hepatitis A and their neonates. Chin Med J (Engl) 1990; 103: 552-5.

Chapter 2: Hepatitis B - Epidemiology, transmission and natural history

Jan-Christian Wasmuth

Introduction

It is estimated that 40% of the world's population has had contact with or are carriers of the hepatitis B virus (HBV). This corresponds to an estimated 350 million HBV carriers (Goldstein 2005). Thus, HBV infection is one of the most important infectious diseases worldwide. Around one million persons die of HBV-related causes annually. There is a wide range of HBV prevalence rates in different parts of the world. HBV prevalence varies from 0.1% up to 20%. Low prevalence areas (0.1-2%) are Western Europe (with wide variation within Europe), United States and Canada, Australia and New Zealand; intermediate prevalence (3-5%) are the Mediterranean countries, Japan, Central Asia, the Middle East, and Latin and South America; and high prevalence areas (10-20%) southeast Asia, China, and sub-Saharan Africa. This diversity is probably related to differences in the age at infection, which correlates with the risk of chronicity. The progression rate from acute to chronic HBV infection decreases with age. It is approximately 90% for an infection acquired perinatally, and is as low as 5% (or even lower) for adults (Stevens 1975; Wasley 2008).

The incidence of new infections has decreased in most developed countries, most likely due to the implementation of vaccination strategies (Rantala 2008). However, exact data are difficult to generate as many cases will remain undetected due to the asymptomatic nature of many acute and chronic infections (RKI 2007). Nevertheless, in Germany 2524 cases of acute hepatitis B were documented in the year 2006, corresponding to an incidence rate of 1.4 per 100,000 inhabitants. In 1997 there were 6135 documented cases of acute hepatitis B. Likewise, the incidence of acute hepatitis B in the United States has decreased by 78% from 1990 to 2005 (Wasley 2008). It is expected that this number will further decrease in countries with implementation of vaccination programs. In Germany 87% of all children starting school were completely vaccinated in 2006 with a trend toward increasing coverage (Poethko-Muller 2007).

Although the incidence of acute HBV infection has decreased in most countries due to the implementation of vaccination programs, HBV-related complications such as cancers and deaths have been on the increase (Gomaa 2008). Reasons might be the delay of vaccination effects, improved diagnosis, and better documentation of HBV cases. Although a drop in prevalence has been observed in many countries, estimates are difficult due to a continuously growing migration from high or medium prevalence areas to low prevalence areas (Belongia 2008).

Transmission

The routes of HBV transmission:

- Sexual
- Percutaneous (Intravenous Drug Use)
- Perinatal

- Horizontal
- Transfusion
- Nosocomial infection (including needle-stick injury)
- Organ transplantation

There is considerable variation in the predominance of transmission modes in different geographic areas. For example, in low prevalence areas such as Western Europe, the routes are mainly unprotected sexual intercourse and intravenous drug use. In high prevalence areas like Sub-Saharan Africa perinatal infection is the predominant mode of transmission. Horizontal transmission, particularly in early childhood, is regarded as the major route of transmission in intermediate prevalence areas.

Sexual transmission

In low prevalence areas sexual transmission is the major route of transmission. Approximately 40% of new HBV infections in the United States is considered to be transmitted via heterosexual intercourse, and 25% occur in men who have sex with men (MSM) (Wasley 2008). Measures to prevent HBV transmission are vaccination and safer sex, i.e., use of condoms. However, there is ongoing debate regarding what to advise low-viremic patients.

Percutaneous innoculation

Percutaneous transmission seems to be an effective mode of HBV transmission. The most important route is sharing of syringes and needles in intravenous drug users. In low prevalence areas such as Europe and the United States about 15% of newly diagnosed HBV infections is in IVDU (Wassley 2008). The risk of HBV transmission increases with the number of years of drug use, frequency of injection, and sharing of drug preparation equipment.

Other situations with possible percutaneous inoculation of HBV are sharing shaving razors or toothbrushes, although the exact number remains unkown. In addition, certain practices like acupuncture, tattooing, and body piercing have been associated with transmission of hepatitis B. Public health education and the use of disposable needles or equipment are important in preventing this mode of transmission.

Perinatal transmission

Transmission from an HBeAg-positive mother to her infant may occur in utero, at the time of birth, or after birth. The rate of infection can be as high as 90%. However, neonatal vaccination is highly efficacious (95%). Its efficacy indicates that most infections occur at or shortly before birth. On the other hand, caesarean section seems not be protective as it is in other vertically transmitted diseases like HIV.

The risk of transmission from mother to infant is related to the HBV replicative rate in the mother. There seems to be a direct correlation between maternal HBV DNA levels and the likelihood of transmission. In mothers with highly replicative HBV the risk of transmission may be up to 85 to 90%, and it continusously lowers with lower HBV DNA levels (Burk 1994; Wang 2003). In some studies there has been almost no perinatal transmission if the mother has no significant replication ($<10^5$ log copies/ml) (Li 2004).

It is possible to reduce the risk of perinatal transmission in several ways. The first step is identification of persons at risk. Testing for HBsAg should be performed in all women at the first prenatal visit and repeated later in pregnancy if appropriate. Newborns born to HBV-positive mothers can be effectively protected by passive-active immunization (>90% protection rate) (del Canho 1997). Hepatitis B immunoglobulin for passive immunization should be given as early as possible (within 12 hours), but can be given up to seven days after birth, if seropositivity of the mother is detected later. Active immunization follows standard schemes and is given at three time points (10 µg at day 0, month 1, and month 6). Anti-HBV treatment of the mother with nucleoside analogues may be discussed especially in mothers with high HBV DNA levels, although it is not known whether antiviral treatment has a protective effect in addition to immunization. At the moment there are no substantiated guidelines. If appropriate, lamivudine seems to be the treatment of choice. Telbivudine may be an alternative, whereas adefovir, entecavir and tenofovir are not recommended in pregnancy, unless clearly indicated (Cornberg 2007).

As mentioned earlier, caesarean section should not be performed routinely, whereas it is recommended in the setting of other infectious diseases like HIV (according to the viral replication rate). If vaccination was performed in the child, the child may be breastfed (Hill 2002).

Horizontal transmission

Children may acquire HBV infection through horizontal transmission via minor breaks in the skin or mucous membranes or close bodily contact with other children. In addition, HBV can survive outside the human body for a prolonged period; as a result, 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 hepatitis B carriers, there is no firm evidence of HBV transmission via body fluids other than blood.

Transfusion

Blood donors are routinely screened for hepatitis B surface antigen (HBsAg). Therefore incidence of transfusion-related hepatitis B has significantly decreased. The risk of acquiring posttransfusion hepatitis B depends on several factors like prevalence and donor testing strategies. In low prevalence areas it is estimated to be one to four per million blood components transfused (Dodd 2000; Polizzotto 2008). In high prevalence areas it is considerably higher (around 1 in 20,000) (Shang 2007).

There are different strategies for donor screening. Most countries use HBsAg screening of donors. Others, like the United States, use both HBsAg and anti-HBc. Routine screening of anti-HBc remains controversial, as the specificity is low and patients with cleared hepatitis have to be excluded. Screening of pooled blood samples or even individual samples may be further improved by nucleic acid amplification techniques. However, this is an issue of continuous debate due to relatively low risk reduction and associated costs.

Nosocomial infection

Nosocomial infection can occur from patient to patient, from patient to health care worker and vice versa. HBV is considered the most commonly transmitted blood-borne virus in the healthcare setting.

In general, nosocomial infection of hepatitis B can and should be prevented. Despite prevention strategies nosocomial infections occur, and there are documented cases (Williams 2004). However, the exact risk of nosocomial infection is unknown. The number of infected patients reported in the literature is likely to be an underestimate of true figures as many infected patients may be asymptomatic and only a fraction of the exposed patients are recalled for testing.

Strategies to prevent nosocomial transmission of hepatitis B are use of disposable needles and equipment, sterilization of surgical instruments, infection control measures and vaccination of healthcare workers.

Due to the implementation of routine vaccination of health care workers the incidence of HBV infection among them is lower than in the general population (Duseja 2002; Mahoney 1997). Therefore, transmission from healthcare workers to patients is a rare event, while the risk of transmission from an HBV-positive patient to a health care worker seems to be higher.

Healthcare workers positive for hepatitis B are not generally prohibited from working. However, the individual situation has to be evaluated in order to decide on the necessary measures. Traditionally HBeAg-negative healthcare workers are considered not be infective, whereas HBeAg-positive healthcare workers should perform measures such as wearing double gloves and not performing certain activities, to be defined on an individual basis. However, there have been cases of transmission of hepatitis B from HBsAg-positive, HBeAg-negative surgeons to patients (Teams 1997). Hepatitis B virus was identified that had a precore stop codon mutation resulting in non-expression of HBeAg despite active HBV replication. Therefore, HBV DNA testing has been implemented in some settings, although this may not be reliable in all situations due to fluctuating levels of HBV DNA. In most developed countries guidelines for hepatitis B positive healthcare workers have been established and should be consulted.

Organ transplantation

Transmission of HBV infection has been reported after transplantation of extrahepatic organs from HBsAg-positive donors (e.g., kidney, cornea) (Dickson 1997). Therefore, organ donors are routinely screened for HBsAg. The role of anti-HBc is controversial, as it is in screening of blood donors. Reasons are the possibility of false positive results, the potential loss of up to 5% of donors even in low endemic areas, and the uncertainty about the infectivity of organs, especially extrahepatic organs, from donors who have isolated anti-HBc (De Feo 2005). There is an increased risk of HBV infection for the recipient if organs of such donors are transplanted as compared to anti-HBc negative donors.

Postexposure prophylaxis

In case of exposure to HBV in any of the circumstances mentioned above, postexposure prophylaxis is recommended for all nonvaccinated persons. A passive-active immunization is recommended. The first dose of active immunization should be given as early as

possible. 12 hours after the exposure usually is considered the latest time point for effective postexposure prophylaxis. One dose of hepatitis B-immunoglobulin (HBIG) should be administered at the same time, if the source is known to be HBsAg-positive. The other two doses of vaccine should be administered according to the usual schedule.

Vaccinated individuals with a documented response do not need postexposure prophylaxis. Individuals who have had no postvaccination testing should be tested for anti-HBs titer as soon as possible. If this is not possible, or the anti-HBs titer is insufficient (<100 IE/l), they will require a second course of vaccination.

Individuals who are documented non-responders will require two doses of HBIG given one month apart.

Natural history and clinical manifestations

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Extrahepatic manifestations can occur in both acute and chronic infection.

Acute hepatitis

After HBV transmission, the incubation period lasts from one to four months. A prodromal phase may appear before acute hepatitis develops. During this period a serum sickness-like syndrome may develop. This syndrome manifests with fever, skin rash, arthralgia and arthritis. It will usually cease with the onset of hepatitis. At least 70% of patients will then have subclinical or anicteric hepatitis, while less then 30% will develop icteric hepatitis. The most prominent clinical symptoms of hepatitis are right upper quadrant discomfort, nausea, jaundice and other unspecific constitutional symptoms. In case of coinfection with other hepatitis viruses or other underlying liver disease the clinical course may be more severe. The symptoms including jaundice generally disappear after one to three months, but some patients have prolonged fatigue even after normalisation of serum aminotransferase concentrations.

Concentrations of alanine and aspartate aminotransferase levels (ALT and AST) may rise to 1000-2000 IU/L in the acute phase. ALT is typically higher than AST. Bilirubin concentration may be normal in a substantial portion of patients. In patients who recover, normalisation of serum aminotransferases usually occurs within one to four months. Persistent elevation of serum ALT for more than six months indicates progression to chronic hepatitis.

The rate of progression from acute to chronic hepatitis B is primarily determined by the age at infection (Ganem 2004; McMahon 1985). In adult-acquired infection the chronicity rate is 5% or less, whereas it is higher if acquired at younger ages. It is estimated to be approximately 90% for perinatally-acquired infection, and 20-50% for infections between the ages of one and five years.

Until recent years it has been assumed that patients who recover from acute hepatitis B actually clear the virus from the body. However, there is a lot of evidence now that even in patients positive for anti-HBs and anti-HBc HBV DNA may persist for long

periods of time and this latent infection maintains the T cell response that keeps the virus under control (Yotsuyanagi 1998). Complete eradication rarely occurs. This is an important finding, as immunosuppression can lead to reactivation of the virus, e.g., after organ transplant or during chemotherapy.

Fulminant hepatic failure is unusual, occurring in approximately 0.1-0.5% of patients. Reasons and risk factors for fulminant hepatitis B are not well understood (Garfein 2004). There may be correlation with substance abuse or coinfections with other viruses. Fulminant hepatitis B is believed to be due to massive immune-mediated lysis of infected hepatocytes. This is why many patients with fulminant hepatitis B have no evidence of HBV replication at presentation.

Antiviral treatment of patients with acute hepatitis B usually is not recommended (Cornberg 2007). The likelihood of fulminant hepatitis B is less than 1%, and the likelihood of progression to chronic hepatitis B is less than 5% in adults. Therefore, treatment of acute hepatitis B is mainly supportive in the majority of patients. Treatment can be considered in certain subsets of patients, e.g., patients with a severe or prolonged course of hepatitis B, patients coinfected with other hepatitis viruses or underlying liver diseases, patients with immunosuppression, or patients with fulminant liver failure undergoing liver-transplantation (Kondili 2004; Tillmann 2006). It should be checked whether any contacts could be exposed to hepatitis B.

Chronic hepatitis

The HBV chronicity rate is around 5% or less in adult-acquired infection, as mentioned earlier. In perinatally acquired infection it is estimated to be approximately 90%, and 20-50% for infections between the age of one and five years (Ganem 2004; McMahon 1985). However, most patients will not have a history of acute hepatitis.

Most patients with chronic hepatitis B are clinically asymptomatic. Some may have nonspecific symptoms such as fatigue. In most instances, significant clinical symptoms will develop only if liver disease progresses to decompensated cirrhosis. In addition, extrahepatic manifestations may cause symptoms.

Accordingly, physical examination will be normal in most instances. In advanced liver disease there may be stigmata of chronic liver disease such as splenomegaly, spider angiomata, Caput medusae, palmar erythema, testicular atrophy, gynecomastia, etc. In patients with decompensated cirrhosis jaundice, ascites, peripheral edema, and encephalopathy may be present.

Laboratory testing shows mild to moderate elevation in serum AST and ALT in most patients, whereas normal transaminases occur rarely. During exacerbation, serum ALT concentration may be as high as 50 times the upper limit of normal. Alfa-fetoprotein (AFP) concentrations correlate with disease activity. In exacerbations of hepatitis B concentrations as high as 1000 ng/mL may be seen.

The natural course of chronic HBV infection is determined by the interplay between viral replication and the host immune response. Other factors that may play a role in the progression of HBV-related liver disease include gender, alcohol consumption, and concomitant infection with other hepatitis virus(es). The outcome of chronic HBV infection depends upon the severity of liver disease at the time HBV replication is arrested. Liver fibrosis is potentially reversible once HBV replication is controlled.

There are two different states that are distinguished in chronic HBV infection: firstly, a high-replicative state with active liver disease and elevated serum ALT. HBV DNA and HBeAg are present. Secondly, a low or non-replicative phase, where serum ALT may normalize, HBeAg disappears, and anti-HBe antibodies appear. In some patients, virus replication stops completely, as demonstrated by sensitive HBV DNA assays, although they remain HBsAg-positive. These patients have undetectable HBV DNA in serum and normal ALT concentrations. No sign of ongoing liver damage or inflammation is found on liver biopsy. This state is called inactive carrier state.

A small percentage of patients continue to have moderate levels of HBV replication and active liver disease (elevated serum ALT and chronic inflammation on liver biopsies) but remain HBeAg-negative. These patients with HBeAg-negative chronic hepatitis may have residual wild type virus or HBV variants that cannot produce HBeAg due to precore or core promoter variants.

The first high-replicative phase may switch into the nonreplicative phase spontaneously or upon antiviral treatment. Conversely, the non-replicative phase may reactivate to the high-replicative phase either spontaneously or with immunosuppression (e.g., in HIV infection or with chemotherapy).

In perinatally acquired chronic HBV infection there are three different states: An immune tolerance phase, an immune clearance phase, and a late non-replicative phase.

The immune tolerance phase, which usually lasts 10-30 years, is characterized by high levels of HBV replication, as manifested by the presence of HBeAg and high levels of HBV DNA in serum. However, there is no evidence of active liver disease as seen by normal serum ALT concentrations and minimal changes in liver biopsy. It is thought that this lack of liver disease despite high levels of HBV replication is due to immune tolerance to HBV (Dienstag 2008), although the exact mechanisms are unknown. This phenomenon of immune tolerance is believed to be the most important reason for the poor response to interferon therapy in HBeAg-positive patients with normal ALT levels. During this phase there is a very low rate of spontaneous HBeAg clearance. It is estimated that the rate of spontaneous HBeAg clearance is only 15% after 20 years of infection.

During the second to third decade the phase of immune tolerance may convert to a phase of immune clearance. The spontaneous HBeAg clearance rate increases. It is estimated to be 10 to 20% annually. If HBeAg seroconversion occurs, very often exacerbations of hepatitis with abrupt increases in serum ALT are observed. These exacerbations follow an increase in HBV DNA and might be due to a sudden increase in immune-mediated lysis of infected hepatocytes. Most often there are no clinical symptoms during exacerbation, and rise of ALT is only detected by routine examinations. Some patients may develop symptoms mimicking acute hepatitis. Titers of anti-HBc IgM may rise as well as alfa-fetoprotein. If such patients are not known to be HBV infected, misdiagnosis of acute hepatitis B can be made. HBeAg-seroconversion and clearance of HBV DNA from the serum is not always achieved after exacerbations. In these patients recurrent exacerbations with intermittent disappearance of serum HBV DNA with or without HbeAg loss may occur. The non-replicative phase is usually characterized by the absence of HBV DNA and normalisation of serum ALT as in adult chronic HBV.

Very few patients with chronic HBV infection become HBsAg negative in the natural course of infection. The annual rate of HBsAg clearance has been estimated to be less than 2% in Western patients and even lower (0.1-0.8%) in patients of Asian origin (Liaw 1991). If loss of HBsAg occurs, prognosis is considered favourable. However, clearance of HBsAg does not exclude development of cirrhosis or hepatocellular carcinoma in some patients, although the exact rate of these complications is not known. This phenomenon is thought to be linked to the fact that HBV DNA may still be present in hepatocytes despite HBsAg loss.

Prognosis

As clinical course varies among patients, there is a wide variation in clinical outcome and prognosis of chronic HBV infection. The lifetime risk of a liver-related death has been estimated to be 40-50% for men and 15% for women. The risk of progression appears to be higher, if immune activation occurs.

The estimated five-year rates of progression (Fattovich 2008; Lok 2008):

- Chronic hepatitis to cirrhosis – 10-20%
- Compensated cirrhosis to hepatic decompensation – 20-30%
- Compensated cirrhosis to hepatocellular carcinoma – 5-15%

Accordingly, the survival rates are:

- Compensated cirrhosis — 85% at five years
- Decompensated cirrhosis — 55-70% at one year and 15-35% at five years

There are several factors known to influence survival.

- ***Viral replication:*** In patients with signs of viral replication (i.e., HBeAg-positive) there is consistently worse survival than in patients who are HBeAg-negative. However, in recent decades, infections with HBeAg-negative precore mutants prevail by far in newly-acquired infections, resulting in a different pattern of HBeAg-negative and HBV DNA positive hepatitis with fibrosis progression and HCC in a substantial proportion of patients. In recent years, the amount of HBV DNA has also been linked to disease progression and has replaced HBeAg postivity as a marker for disease activity (Chen 2006). This is true both for progression to cirrhosis as well as for the risk of HCC. Therefore, most treatment guidelines today are based on the level of HBV viremia. A reasonable cut-off to distinguish patients with a low risk of progression from patients with a high risk of progression and indication for antiviral treatment is 10^4 copies/ml (corresponding to approximately 2 x 10^3 IU/ml), although other cut-offs may be used. The duration of viral replication is obviously linked with the risk of development of cirrhosis and HCC. As necroinflammation may persist longer in patients with a prolonged replicative phase, the risk of disease progression is elevated. Conversely, even in patients with decompensated cirrhosis, suppression of HBV replication and delayed HBsAg clearance can result in improvement in liver disease (Fung 2008).

- ***Alcoholism:*** HBV infection in alcoholics is associated with faster progression to liver injury and an elevated risk of developing cirrhosis and HCC (Bedogni 2008; Marcellin 2008). Survival is reduced compared to HBV-negative alcoholics. However, there is no clear evidence that alcoholics have an enhanced risk of chronic HBV infection, although prevalence of HBV is estimated to be fourfold higher than in controls (Laskus 1992) with variation among regions and cohorts (Rosman 1996).
- ***Hepatitis C coinfection:*** If coinfection of HCV and HBV occurs, HCV usually predominates. This may lead to lower levels of transaminases and HBV DNA (Jardi 2001). The rate of HBsAg-seroconversion even appears to be increased, although this finding may be due to the fact that around one third of patients coinfected with HBV and HCV lack markers of HBV infection (i.e., HBsAg) although HBV DNA is detectable. Despite lower aminotransferases and HBV DNA levels, liver damage is worse in most instances. The risk of severe hepatitis and fulminant hepatic failure seems to be elevated if both infections occur simultaneously regardless of whether it is an acute coinfection of HBV and HCV or acute hepatitis C in chronic hepatitis B (Liaw 2004).
- ***Hepatitis D coinfection:*** Acute HBV and HDV coinfection tends to be more severe than acute HBV infection alone. It is more likely to result in fulminant hepatitis. If HDV superinfection in patients with chronic HBV infection occurs, HDV usually predominates, and HBV replication is suppressed (Jardi 2001). Severity of liver disease is worse and progression to cirrhosis is accelerated in such patients (Fattovich 2000).

It is very difficult to predict the individual course of hepatitis B due to the many factors influencing disease progression. Several predictive models of disease progression that include clinical parameters (e.g., hepatic decompensation) and laboratory parameters (e.g., bilirubin, INR) have been evaluated, but none of these models is used routinely in the clinic at present. In patients with cirrhosis, the MELD score (Model for End-Stage Liver Disease) and the Child-Pugh score are used (see Chapter 3).

Extrahepatic manifestations

The two major extrahepatic complications of chronic HBV are polyarteritis nodosa and glomerular disease. They occur in 10-20% of patients with chronic hepatitis B and are thought to be mediated by circulating immune complexes (Han 2004).

- ***Polyarteritis nodosa:*** The clinical manifestations are similar to those in patients with polyarteritis who are HBV-negative. There may be some clinical benefit to antiviral therapy.
- ***Nephropathy/Glomerulonephritis:*** HBV can induce both membranous nephropathy and, less often, membranoproliferative glomerulonephritis. Most cases occur in children. The clinical hallmark is proteinuria. In contrast to polyarteritis nodosa, there is no significant benefit of antiviral treatment.

For further details, please refer to extrahepatic manifestations in Chapter 16.

References

Bedogni G, Miglioli L, Masutti F, et al. Natural course of chronic HCV and HBV infection and role of alcohol in the general population: the Dionysos Study. Am J Gastroenterol 2008;103(9):2248.

Belongia EA, Costa J, Gareen IF, et al. NIH Consensus Development Statement on Management of Hepatitis B: Draft. NIH Consens State Sci Statements 2008;25(2).

Burk RD, Hwang LY, Ho GY, Shafritz DA, Beasley RP. Outcome of perinatal hepatitis B virus exposure is dependent on maternal virus load. J Infect Dis 1994;170(6):1418.

Cornberg M, Protzer U, Dollinger MM, et al. Prophylaxis, diagnosis and therapy of hepatitis B virus (HBV) infection: the German guidelines for the management of HBV infection. Z Gastroenterol 2007;45(12):1281.

Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. Jama 2006;295(1):65.

Dickson RC, Everhart JE, Lake JR, et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. Gastroenterology 1997;113(5):1668.

Dienstag JL. Hepatitis B virus infection. N Engl J Med 2008;359(14):1486.

Dodd RY. Current viral risks of blood and blood products. Ann Med 2000;32(7):469.

Duseja A, Arora L, Masih B, et al. Hepatitis B and C virus--prevalence and prevention in health care workers. Trop Gastroenterol 2002;23(3):125.

Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48(2):335.

Fung J, Lai CL, Yuen MF. New paradigms for the treatment of chronic hepatitis B. J Gastroenterol Hepatol 2008;23(8 Pt 1):1182.

Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 2004;350(11):1118.

Garfein RS, Bower WA, Loney CM, et al. Factors associated with fulminant liver failure during an outbreak among injection drug users with acute hepatitis B. Hepatology 2004;40(4):865.

Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol 2005;34(6):1329.

Gomaa AI, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. World J Gastroenterol 2008;14(27):4300.

Han SH. Extrahepatic manifestations of chronic hepatitis B. Clin Liver Dis 2004;8(2):403.

Hill JB, Sheffield JS, Kim MJ, Alexander JM, Sercely B, Wendel GD. Risk of hepatitis B transmission in breast-fed infants of chronic hepatitis B carriers. Obstet Gynecol 2002;99(6):1049.

Jardi R, Rodriguez F, Buti M, et al. Role of hepatitis B, C, and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. Hepatology 2001;34(2):404.

Kondili LA, Osman H, Mutimer D. The use of lamivudine for patients with acute hepatitis B (a series of cases). J Viral Hepat 2004;11(5):427.

Laskus T, Radkowski M, Lupa E, Horban A, Cianciara J, Slusarczyk J. Prevalence of markers of hepatitis viruses in out-patient alcoholics. J Hepatol 1992;15(1-2):174.

Lok ASF. Clinical manifestations and natural history of hepatitis B virus infection. UpToDate, 2008. (Accessed October 23, 2008, at http://www.uptodateonline.com.)

Li XM, Shi MF, Yang YB, et al. Effect of hepatitis B immunoglobulin on interruption of HBV intrauterine infection. World J Gastroenterol 2004;10(21):3215.

Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. Hepatology 1991;13(4):627.

Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM. Impact of acute hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. Gastroenterology 2004;126(4):1024.

Mahoney FJ, Stewart K, Hu H, Coleman P, Alter MJ. Progress toward the elimination of hepatitis B virus transmission among health care workers in the United States. Arch Intern Med 1997;157(22):2601.

Marcellin P, Pequignot F, Delarocque-Astagneau E, et al. Mortality related to chronic hepatitis B and chronic hepatitis C in France: evidence for the role of HIV coinfection and alcohol consumption. J Hepatol 2008;48(2):200.

McMahon BJ, Alward WL, Hall DB, et al. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. J Infect Dis 1985;151(4):599.

Poethko-Muller C, Kuhnert R, Schlaud M. [Vaccination coverage and predictors for vaccination level. Results of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS)]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 2007;50(5-6):851.

Polizzotto MN, Wood EM, Ingham H, Keller AJ. Reducing the risk of transfusion-transmissible viral infection through blood donor selection: the Australian experience 2000 through 2006. Transfusion 2008;48(1):55.

Rantala M, van de Laar MJ. Surveillance and epidemiology of hepatitis B and C in Europe - a review. Euro Surveill 2008;13(21).

Anonymous. Hepatitis C: RKI-Ratgeber Infektionskrankheiten. Epidemiologisches Bulletin 2004;17:141.

Rosman AS, Waraich A, Galvin K, Casiano J, Paronetto F, Lieber CS. Alcoholism is associated with hepatitis C but not hepatitis B in an urban population. Am J Gastroenterol 1996;91(3):498.

Shang G, Seed CR, Wang F, Nie D, Farrugia A. Residual risk of transfusion-transmitted viral infections in Shenzhen, China, 2001 through 2004. Transfusion 2007;47(3):529.

Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292(15):771.

Teams TII. Transmission of hepatitis B to patients from four infected surgeons without hepatitis B e antigen. The Incident Investigation Teams and others. N Engl J Med 1997;336(3):178.

Tillmann HL, Hadem J, Leifeld L, et al. Safety and efficacy of lamivudine in patients with severe acute or fulminant hepatitis B, a multicenter experience. J Viral Hepat 2006;13(4):256.

Wasley A, Grytdal S, Gallagher K. Surveillance for acute viral hepatitis--United States, 2006. MMWR Surveill Summ 2008;57(2):1.

Williams IT, Perz JF, Bell BP. Viral hepatitis transmission in ambulatory health care settings. Clin Infect Dis 2004;38(11):1592.

Yotsuyanagi H, Yasuda K, Iino S, et al. Persistent viremia after recovery from self-limited acute hepatitis B. Hepatology 1998;27(5):1377.

Chapter 3: Hepatitis C - Epidemiology, transmission and natural history

Jan-Christian Wasmuth

Epidemiology

Hepatitis C is a disease with a significant global impact. According to the World Health Organization there are 170 million people infected with the hepatitis C virus (HCV), corresponding to 3% of the world's total population. There are considerable regional differences. In some countries, e.g., Egypt, the prevalence is as high as 20%. In Africa and the Western Pacific the prevalence is significantly higher than in North America and Europe (Anonymous 2004).

It is estimated that there are 2-5 million HCV-positive persons in Europe. The prevalence of HCV-antibodies in otherwise healthy blood donors is approximately 1.6% in the United States, 1.15% in Italy, 0.4% in Germany, and 0.23% in Scandinavia (Anonymous 2004). The number of patients actually HCV RNA positive is estimated to be around 80 to 90% of all HCV-antibody positive persons. Certain groups are preferentially affected: The highest risk factor in most instances is injection drug use. But patients undergoing hemodialysis and persons who received blood transfusions before 1991 are at risk also. In Europe and the United States chronic hepatitis C is the most common chronic liver disease. The majority of liver transplants performed in these regions are for chronic HCV.

It is difficult to determine the number of new HCV infections, as most acute cases will not be noticed clinically. Fewer than 25% of acute cases of hepatitis C are clinically apparent. In addition, the age of infection upon diagnosis is not possible to determine in most cases. Nevertheless, it has to be assumed that the number of new infections has considerably decreased over the past decades. For the United States it is estimated that the number of new cases of acute HCV infection has fallen from approximately 230,000 per year in the 1980s to about 20,000 cases per year currently (Wasley 2008). This decrease is primarily associated with reduced infections in injection drug users, a probable consequence of changes in injection practices motivated by education about human immunodeficiency virus (HIV) transmission. Transfusion-associated hepatitis C has had little impact on this decline, as the number of cases has been reduced almost to zero.

Transmission

Parenteral exposure to the hepatitis C virus is the most efficient means of transmission. Accordingly, the majority of patients infected with HCV in Europe and the United States acquired the disease through intravenous drug use or blood transfusion. The latter has become rare since routine testing of the blood supply for HCV began in the early 1990s. Other types of parenteral exposure are important in specific regions in the world.

The following possible routes of infection have been identified in anti-HCV-positive blood donors (in descending order of transmission risk):

- Injection drug use
- Blood transfusion
- Sex with an intravenous drug user
- Having been in jail more than three days
- Religious scarification
- Having been struck or cut with a bloody object
- Pierced ears or body parts
- Immunoglobulin injection

Very often in patients with newly diagnosed HCV infection no clear risk factor can be identified.

Injection drug use

Injection drug use has been the most commonly identified source of acute HCV infection. It is estimated that most newly acquired infections occur in individuals who have injected illegal drugs. The seroprevalence of anti-HCV antibodies in groups of intravenous drug users may be up to 70% with considerable variation depending on factors such as region, risk behaviour, socioeconomic status and others, underscoring the efficiency of transmission via direct blood contact (Sutton 2008). HCV infection also has been associated with a history of intranasal cocaine use, presumably due to blood on shared straws or other sniffing paraphernalia.

Blood transfusion

In the past, blood transfusion or use of other blood products was a major risk factor for transmission of HCV. In some historic cohorts 10% or more of patients who received blood transfusions were infected with hepatitis C (Alter 1989). However, blood donor screening for HCV since the early 1990s has nearly eliminated this transmission route. Blood donors are screened for anti-HCV antibodies and HCV RNA – at least in developed countries. The risk is now estimated to be between 1:500,000 and 1:1,000,000 units (Pomper 2003).

In cohorts of multiply transfused patients such as hemophiliacs, over 90% of patients were infected with hepatitis C in the past (Francois 1993). Since the use of routine inactivated virus (e.g., heat inactivation or pasteurization) or recombinant clotting factors, new cases of hepatitis C infection have become uncommon in these patients.

Organ transplantation

Transplant recipients who receive organs from HCV-positive donors have a high risk of acquiring HCV infection. Transmission rates in different cohorts vary from 30 to 80% (Pereira 1991; Roth 1994). Therefore, most transplant organisations have developed strategies for screening and selective utilization of organs from anti-HCV positive donors.

Sexual or household contact

Usual household contacts do not pose a risk of HCV transmission. The efficiency of HCV transmission by sexual contact is very low. However, there is no doubt that sexual transmission of hepatitis C is possible.

The exact risk of HCV transmission in monogamous heterosexual relationships has been difficult to determine. It appears that the risk in long-term partnerships is very low. In prospective cohorts of monogamous, heterosexual couples, there was a long-term transmission risk of 0.01% or lower (Vandelli 2004). Factors that may increase the risk of HCV infection include greater numbers of sex partners, history of sexually transmitted diseases, and failure to use a condom. Whether underlying HIV infection increases the risk of heterosexual HCV transmission to an uninfected partner is unclear. Very often it is difficult to rule out the possibility that transmission results from risk factors other than sexual exposure.

Outbreaks of cases of acute hepatitis C in several cities in Europe and the United States among men who have sex with men (MSM) have focused attention on sexual transmission of HCV. There is clear evidence that no other route than unprotected sex can account for the transmission of HCV. Unprotected anal sex, fisting, having many sex partners in a short time period, and a concomitant sexually transmitted disease were identified risk factors (Danta 2007). It appears that mucosal damage is a prerequisite for HCV transmission. According to these observations, the seroprevalence of HCV in MSM ranges from about 4 to 8%, which is higher than the HCV prevalence reported for general European populations.

Patients with acute or chronic HCV infection should be advised that transmission to sexual contacts is a possibility, although the risk is extremely low in heterosexual relationships. It is likely that the use of condoms will lower the risk of sexual transmission further. However, in most countries there are no firm recommendations to use barrier precautions in stable monogamous sexual partnerships. The transmission risk in MSM is considerably higher so that – in conjunction with the risk of other sexually transmitted diseases – safer sex practices should be advised in this group.

Perinatal transmission

The risk of perinatal transmission of HCV in HCV RNA positive mothers is estimated to be 5% or less (Ohto 1994). In mothers coinfected with HIV this risk correlates with immunosuppression and has been described to reach up to 20%. Today, there are no specific recommendations for prevention of perinatal transmission (Pembrey 2005). Caesarean section has not been shown to reduce the transmission risk. There is no evidence that breastfeeding is a risk for infection among infants born to HCV-infected women. Early diagnosis of infection in newborns requires HCV RNA testing since anti-HCV antibodies are passively transferred from the mother.

Hemodialysis

Patients who participate in chronic hemodialysis programs are at increased risk for hepatitis C. The prevalence of HCV antibodies in such patients reaches 15%, although it has declined in recent years (Fissell 2004). A number of risk factors have been identified for HCV infection among dialysis patients. These include blood transfusions, the duration of hemodialysis, the prevalence of HCV infection in the dialysis unit, and the type of dialysis. The risk is higher with in-hospital hemodialysis as opposed to peritoneal dialysis. The best strategy to prevent hemodialysis-associated HCV transmission is subject to debate.

Other rare transmission routes

Rare sources of percutaneous transmission of HCV are contaminated equipment used during medical procedures, procedures involved in traditional medicine (e.g., scarification, cupping), tattooing, and body piercing (Haley 2001). All these routes bear the potential of transmiting HCV. However, in most instances it is not clear if the risk is due to the procedure itself, or whether there are possible contacts with persons involved who are HCV-positive. In addition, transmission via these routes is so rare that persons with exposure are not at increased risk for acquiring hepatitis C.

Needle-stick injury

There is some risk of HCV transmission for health care workers after unintentional needle stick injury or exposure to other sharp objects. The incidence of seroconversion after exposure to an HCV-positive source is generally estimated to be less than 2% (Anonymous 2001). However, data are divergent and figures ranging from 0 to 10% can be found (Mitsui 1992). Exposure of HCV to intact skin has not been associated with HCV transmission.

Clinical manifestations and natural history of HCV infection

The spectrum of clinical manifestations of HCV infection varies in acute versus chronic disease. Acute infection with HCV is most often asymptomatic. It leads to chronic infection in about 80% of cases. The manifestations of chronic HCV range from an asymptomatic state to cirrhosis, and hepatocellular carcinoma. HCV infection usually is slowly progressive. Thus, it may not result in clinically apparent liver disease in many patients if the infection is acquired later in life. Approximately 20-30% of chronically infected individuals develop cirrhosis over a 20-30 year period of time.

Acute hepatitis C

After inoculation of HCV, there is a variable incubation period. HCV RNA in blood (or liver) can be detected by PCR within several days to eight weeks (Hoofnagle 1997). Aminotransferases become elevated approximately 6-12 weeks after exposure (range 1-26 weeks). The elevation of aminotransferases varies considerably among individuals, but tends to be more than 10-30 times the upper limit of normal (typically around 800 U/l). HCV antibodies can be found for the first time around 8 weeks after exposure although in some patients it may take several months before HCV antibodies are detected by ELISA testing.

However, the majority of newly-infected patients will be asymptomatic and have a clinically nonapparent or mild course. Jaundice as a clinical feature of acute hepatitis C will be present in less than 25% of infected patients. Therefore, acute hepatitis C will not be noticed in most patients. Periodic screening for infection may be warranted in certain groups of patients who are at high risk for infection, e.g., homosexually-active patients with HIV infection.

Other symptoms that may occur are similar to those in other forms of acute viral hepatitis, including malaise, nausea, and right upper quadrant pain. In patients who

experience such symptoms of acute hepatitis, the illness typically lasts for 2-12 weeks. Along with clinical resolution of symptoms, aminotransferases levels will normalize in about 40% of patients. Loss of HCV RNA, which indicates cure from hepatitis C, occurs in fewer than 20% of patients – regardless of normalisation of aminotransferases.

Fulminant hepatic failure due to acute HCV infection is very rare. It may be more common in patients with underlying chronic hepatitis B virus infection (Chu 1999).

Chronic hepatitis C

The risk of chronic HCV infection is high. 80-100% of patients remain HCV RNA positive after acute hepatitis C (Alter 1999). Most of these will have persistently elevated liver enzymes in further follow-up. By definition, hepatitis C is regarded to be chronic after persistence of more than six months. Once chronic infection is established, there is a very low rate of spontaneous clearance.

It is unclear why infection with HCV results in chronic infection in most cases. Genetic diversity of the virus and its tendency toward rapid mutation may allow HCV to constantly escape immune recognition. Host factors may also be involved in the ability to spontaneously clear the virus. Factors that have been associated with successful HCV clearance are HCV-specific CD4 T cell responses, high titers of neutralising antibodies against HCV structural proteins, and specific HLA-DRB1 and DQB1 alleles (Lauer 2001). Infection with HCV during childhood appears to be associated with a lower risk of chronic infection, approximately 50-60% (Vogt 1999). Finally, there seem to be ethnic differences, with lower risk of chronicity in certain populations.

Most patients with chronic infection are asymptomatic or have only mild nonspecific symptoms as long as cirrhosis is not present (Lauer 2001; Merican 1993). The most frequent complaint is fatigue. Less common manifestations are nausea, weakness, myalgia, arthralgia, and weight loss. HCV infection has also been associated with cognitive impairment. All these symptoms are non-specific and do not reflect disease activity or severity (Merican 1993). Very often symptoms may be caused by other underlying diseases (e.g., depression), and it can be difficult to distinguish between different diseases. Fatigue as the most common symptom may be present in many other situations (including healthy control groups within clinical studies). Hepatitis C is rarely incapacitating.

Aminotransferase levels can vary considerably over the natural history of chronic hepatitis C. Most patients have only slight elevations of transaminases. Up to one third of patients have a normal serum ALT (Martinot-Peignoux 2001; Puoti 2002). About 25% of patients have a serum ALT concentration of more than twice normal, but usually less than 5 times above the upper limit of normal. Elevations of 10 times the upper limit of normal are very seldomly seen.

There is a poor correlation between concentrations of aminotransferases and liver histology. Even patients with normal serum ALT show histologic evidence of chronic inflammation in the majority of cases (Mathurin 1998). The degree of injury is typically minimal or mild in these patients. Accordingly, normalisation of aminotransferases after interferon therapy does not necessarily reflect histologic improvement.

Natural history

The risk of developing cirrhosis within 20 years is estimated to be around 10 to 20%, with some studies showing estimates up to 50% (de Ledinghen 2007; Poynard 1997; Sangiovanni 2006; Wiese 2000). Due to the long course of hepatitis C the exact risk is very difficult to determine, and figures are divergent for different studies and populations. In fact, chronic hepatitis C is not necessarily progressive in all affected patients. In several cohorts it has been shown that a substantial number of patients will not develop cirrhosis over a given time. It is estimated that about 30% of patients will not develop cirrhosis for at least 50 years (Poynard 1997).

Therefore, studies with short observation periods sometimes fail to show an increase in mortality. In addition, survival is generally not impaired until cirrhosis has developed. On the other hand, there is no doubt that patients with chronic hepatitis C have a high risk of cirrhosis, decompensation, and hepatocellular carcinoma in long-term follow-up. For example, in a cohort of patients with post-transfusion hepatitis C evaluated more than 20 years after transfusion 23% had chronic active hepatitis, 51% cirrhosis, and 5% hepatocellular carcinoma (Tong 1995). It is not completely understood why there are such differences in disease progression. An influence of host and viral factors has to be assumed.

Cirrhosis and hepatic decompensation

Complications of hepatitis C occur almost exclusively in patients who have developed cirrhosis. Interestingly, non-liver related mortality is higher in cirrhotic patients as well. However, cirrhosis may be very difficult to diagnose clinically, as most cirrhotic patients will be asymptomatic as long as hepatic decompensation does not occur. Findings that can be associated with cirrhosis are hepatomegaly and/or splenomegaly on physical examination, elevated serum bilirubin concentration, hyperalbuminemia, or low platelets. Other clinical findings associated with chronic liver disease may be found such as spider angiomata, Caput medusae, palmar erythema, testicular atrophy, or gynaecomastia. Most of these findings are found in less than half of cirrhotic patients, and therefore none is sufficient to establish a diagnosis of cirrhosis.

Hepatic decompensation can occur in several forms. Most common is ascites, followed by variceal bleeding, encephalopathy and jaundice. As mentioned earlier, hepatic decompensation will develop only in cirrhotic patients. However, not all patients with cirrhosis actually show signs of decompensation over time. The risk for decompensation is estimated to be close to 5% per year in cirrhotics (Poynard 1997). Once decompensation has developed the 5-year survival rate is roughly 50% (Planas 2004). For this group of patients liver transplantation is the only effective therapy.

Similar to decompensation, hepatocellular carcinoma (HCC) develops solely in patients with cirrhosis (in contrast to chronic hepatitis B). The risk for HCC has been estimated to be less than 3% per year once cirrhosis has developed (Di Bisceglie 1997; Fattovich 1997). However, HCV-associated HCC has significant impact on survival (see Chapter 21).

Elevated concentrations of alpha-fetoprotein (AFP) do not necessarily indicate HCC. AFP may be mildly elevated in chronic HCV infection (i.e., 10 to 100 ng/mL). Levels above 400 ng/mL as well as a continuous rise in AFP over time are suggestive of HCC.

Disease progression

Chronic hepatitis C has different courses among individuals. It is not completely understood why there are differences in disease progression. Several factors have been identified that may be associated with such differences. However, other factors not yet identified may also be important.

- **Age and gender:** Acquisition of HCV infection after the age of 40 to 55 may be associated with a more rapid progression of liver injury, as well as male gender (Svirtlih 2007). On the contrary, children appear to have a relatively low risk of disease progression (Child 1964). In one cohort, for example, only 1 of 37 patients with HCV RNA in serum had elevated levels of serum aminotransferases, and only 3 of 17 (18%) who had liver biopsies approximately 20 years after exposure had histologic signs of progressive liver disease.

- **Ethnic background:** Disease progression appears to be slower and changes in liver histology less severe in African-Americans (Sterling 2004).

- **HCV-specific cellular immune response:** The severity of liver injury is influenced by the cellular immune response to HCV-specific targets. Inflammatory responses are regulated by complex mechanisms and probably depend on genetic determinants such as HLA expression (Hraber 2007). Whether this determines progression of liver disease is not clear.

- **Alcohol intake:** Alcohol increases HCV replication, enhances the progression of chronic HCV, and accelerates liver injury (Gitto 2008). Even moderate amounts of alcohol appear to increase the risk of fibrosis. Accordingly, in alcoholic patients with cirrhosis and liver failure a high prevalence of anti-HCV antibodies has been described. Alcohol intake should be avoided in all patients with chronic hepatitis C. There is no clear amount of safe alcohol intake.

- **Daily use of marijuana:** Daily use of marijuana has been associated with more rapid fibrosis progression, possibly through stimulation of endogenous hepatic cannabinoid receptors.

- **Other host factors:** Genetic polymorphisms of certain genes might influence the fibrosis progression rate (Jonsson 2008). For example, transforming growth factor B1 (TGF B1) phenotype and fibrosis stage are correlated. Patients with moderate to severe steatosis are at higher risk for developing hepatic fibrosis.

- **Viral coinfection:** Progression of hepatitis C clearly is accelerated in HIV-infected patients (see section on coinfection).Acute hepatitis B in a patient with chronic hepatitis C may be more severe. Chronic hepatitis B may be associated with decreased HCV replication as opposed to HCV monoinfected patients, al-

though HCV usually predominates. Nevertheless, liver damage is usually worse and progression faster in patients with dual HBV/HCV infections.Around one third of patients coinfected with HBV and HCV lack markers of HBV infection (i.e., HBsAg) although HBV DNA is detectable.

- **Geography and environmental factors:** There are some obvious geographic differences (Lim 2008). For example, hepatocellular carcinoma is observed more often in Japan than in the United States. The reason for this phenomenon is not clear.

- **Use of steroids:** It is well known that use of steroids increases the HCV viral load, while the effect on aminotransferases is variable. They tend to decrease in most patients, although increases in transaminases and bilirubin have also been described. Reducing doseage of corticosteroids returns HCV viral load to baseline. However, the clinical consequences of corticosteroid use are largely unknown. It seems to be reasonable to assume that short-term use of corticosteroids is not associated with significant changes in long-term prognosis.

- **Viral factors:** The influence of viral factors on disease progression is unclear. Overall, there seems to be no significant role of different genotypes and quasispecies on fibrosis progression or outcome. However, coinfection with several genotypes may have a worse outcome as compared to monoinfection.

It is very difficult to predict the individual course of hepatitis C due to the many factors influencing disease progression. Today, liver biopsy is the best predictor of disease progression (Gebo 2002). The grade of inflammation and stage of fibrosis are useful in predicting further clinical course. In patients with severe inflammation or bridging fibrosis virtually all patients will develop cirrhosis within ten years. In contrast, patients with mild inflammation and no fibrosis have an annual progression risk to cirrhosis of around 1%.

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin, mg/dL	<2	2-3	>3
Albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time			
Seconds over control	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

Table 1. Child-Pugh classification of severity of liver disease (Child 1964).
A total score of 5-6 is considered stage A (well-compensated disease); 7-9 is stage B (significant functional compromise); and 10-15 is stage C (decompensated disease). These grades correlate with one- and two-year patient survival: stage A - 100 and 85 percent; stage B - 80 and 60 percent; and stage C - 45 and 35 percent.

Several predictive models of disease progression that include clinical parameters (e.g., hepatic decompensation) and laboratory parameters (e.g., bilirubin, INR) have been evaluated, but none of these models is routinely used in the clinic at present. In patients with cirrhosis, the MELD score (Model for End-Stage Liver Disease) and the CHILD score (Table 1) are used to stage disease and to describe the prognosis in the near future (see Chapters 22 & 23). The MELD Score is used especially to estimate relative disease severity and likely survival of patients awaiting liver transplant. It is calculated as: MELD Score = 10 x ((0.957 x ln(creatinine)) + (0.378 x ln(bilirubin)) + (1.12 x ln(INR))) + 6.43. An online calculator and further information can be found at the website of The United Network for Organ Sharing (UNOS) (http://www.unos.org).

Extrahepatic manifestations

Around 30 to 40% of patients with chronic hepatitis C have an extrahepatic manifestation of HCV (Zignego 2008). There is a wide variety of extrahepatic manifestations described as being associated with HCV:

- Hematologic manifestations (essential mixed cryoglobulinemia, lymphoma)
- Autoimmune disorders (thyroiditis, presence of various autoantibodies)
- Renal disease (membranoproliferative glomerulonephritis)
- Dermatologic disease (porphyria cutanea tarda, lichen planus)
- Diabetes mellitus

For further details refer to Chapter 16.

References

Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989;321(22):1494.

Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 1999;341(8):556.

Anonymous. Hepatitis C: RKI-Ratgeber Infektionskrankheiten. Epidemiologisches Bulletin 2004;17:141.

Anonymous. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. MMWR Recomm Rep 2001;50(RR-11):1.

Child CGI, Turcotte JG. Surgery and Portal Hypertension. In: Child CGI, ed. The Liver and portal hypertension. Philadelphia: WB Saunders; 1964:5.

Chu CM, Yeh CT, Liaw YF. Fulminant hepatic failure in acute hepatitis C: increased risk in chronic carriers of hepatitis B virus. Gut 1999;45(4):613.

Danta M, Brown D, Bhagani S, et al. Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours. Aids 2007;21(8):983.

de Ledinghen V, Trimoulet P, Mannant PR, et al. Outbreak of hepatitis C virus infection during sclerotherapy of varicose veins: long-term follow-up of 196 patients (4535 patient-years). J Hepatol 2007;46(1):19.

Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology 1997;26(3 Suppl 1):34S.

Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997;112(2):463.

Fissell RB, Bragg-Gresham JL, Woods JD, et al. Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. Kidney Int 2004;65(6):2335.

Francois M, Dubois F, Brand D, et al. Prevalence and significance of hepatitis C virus (HCV) viremia in HCV antibody-positive subjects from various populations. J Clin Microbiol 1993;31(5):1189.

Gebo KA, Herlong HF, Torbenson MS, et al. Role of liver biopsy in management of chronic hepatitis C: a systematic review. Hepatology 2002;36(5 Suppl 1):S161.

Gitto S, Micco L, Conti F, Andreone P, Bernardi M. Alcohol and viral hepatitis: A mini-review. Dig Liver Dis 2008.

Haley RW, Fischer RP. Commercial tattooing as a potentially important source of hepatitis C infection. Clinical epidemiology of 626 consecutive patients unaware of their hepatitis C serologic status. Medicine (Baltimore) 2001;80(2):134.

Hraber P, Kuiken C, Yusim K. Evidence for human leukocyte antigen heterozygote advantage against hepatitis C virus infection. Hepatology 2007;46(6):1713.

Jonsson JR, Purdie DM, Clouston AD, Powell EE. Recognition of genetic factors influencing the progression of hepatitis C : potential for personalized therapy. Mol Diagn Ther 2008;12(4):209.

Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345(1):41.

Lim YS, Kim WR. The global impact of hepatic fibrosis and end-stage liver disease. Clin Liver Dis 2008;12(4):733.

Martinot-Peignoux M, Boyer N, Cazals-Hatem D, et al. Prospective study on anti-hepatitis C virus-positive patients with persistently normal serum alanine transaminase with or without detectable serum hepatitis C virus RNA. Hepatology 2001;34(5):1000.

Mathurin P, Moussalli J, Cadranel JF, et al. Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. Hepatology 1998;27(3):868.

Merican I, Sherlock S, McIntyre N, Dusheiko GM. Clinical, biochemical and histological features in 102 patients with chronic hepatitis C virus infection. Q J Med 1993;86(2):119.

Mitsui T, Iwano K, Masuko K, et al. Hepatitis C virus infection in medical personnel after needlestick accident. Hepatology 1992;16(5):1109.

Ohto H, Terazawa S, Sasaki N, et al. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. N Engl J Med 1994;330(11):744.

Pembrey L, Newell ML, Tovo PA. The management of HCV infected pregnant women and their children European paediatric HCV network. J Hepatol 2005;43(3):515.

Pereira BJ, Milford EL, Kirkman RL, Levey AS. Transmission of hepatitis C virus by organ transplantation. N Engl J Med 1991;325(7):454.

Planas R, Balleste B, Alvarez MA, et al. Natural history of decompensated hepatitis C virus-related cirrhosis. A study of 200 patients. J Hepatol 2004;40(5):823.

Pomper GJ, Wu Y, Snyder EL. Risks of transfusion-transmitted infections: 2003. Curr Opin Hematol 2003;10(6):412.

Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349(9055):825.

Puoti C, Castellacci R, Montagnese F, et al. Histological and virological features and follow-up of hepatitis C virus carriers with normal aminotransferase levels: the Italian prospective study of the asymptomatic C carriers (ISACC). J Hepatol 2002;37(1):117.

Roth D, Zucker K, Cirocco R, et al. The impact of hepatitis C virus infection on renal allograft recipients. Kidney Int 1994;45(1):238.

Sangiovanni A, Prati GM, Fasani P, et al. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. Hepatology 2006;43(6):1303.

Sterling RK, Stravitz RT, Luketic VA, et al. A comparison of the spectrum of chronic hepatitis C virus between Caucasians and African Americans. Clin Gastroenterol Hepatol 2004;2(6):469.

Sutton AJ, Hope VD, Mathei C, et al. A comparison between the force of infection estimates for blood-borne viruses in injecting drug user populations across the European Union: a modelling study. J Viral Hepat 2008;15(11):809.

Svirtlih N, Jevtovic D, Simonovic J, et al. Older age at the time of liver biopsy is the important risk factor for advanced fibrosis in patients with chronic hepatitis C. Hepatogastroenterology 2007;54(80):2324.

Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995;332(22):1463.

Vandelli C, Renzo F, Romano L, et al. Lack of evidence of sexual transmission of hepatitis C among monogamous couples: results of a 10-year prospective follow-up study. Am J Gastroenterol 2004;99(5):855.

Vogt M, Lang T, Frosner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341(12):866.

Wasley A, Grytdal S, Gallagher K. Surveillance for acute viral hepatitis--United States, 2006. MMWR Surveill Summ 2008;57(2):1.

Wiese M, Berr F, Lafrenz M, Porst H, Oesen U. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in germany: a 20-year multicenter study. Hepatology 2000;32(1):91.

Zignego AL, Craxi A. Extrahepatic manifestations of hepatitis C virus infection. Clin Liver Dis 2008;12(3):611.

Chapter 4: Hepatitis E - Epidemiology, transmission and natural history

Johannes Lenz

Introduction

Like hepatitis A, the hepatitis E virus is a non-enveloped single stranded RNA virus of an icosahedral shape, measuring 27-34 nm in diameter. It is the sole member of the genus Hepevirus in the family of Hepeviridiea (Emerson 2004). Its existence was hypothesised when a retrospective analysis of clinical samples collected during hepatitis outbreaks in India in 1955 with newly developed essays for hepatitis A and B showed a high prevalence of close to 100% for anti-HAV IgG but no sign of acute hepatitis A or B. Thus the conclusion was that there must be another infectious agent for enterically transmitted non-A non-B hepatitis (ET-NANB) (Khuroo 1980; Wong 1980). HEV was first visualised in 1983. It was transmitted to a human volunteer in Russia and to cynomologus monkeys, causing acute hepatitis in both, and thus establishing its etiologic role in ET-NANB hepatitis (Balayan 1983).

Three large open reading frames (ORFs) of the positive-sense RNA of HEV have been described. While the largest ORF consisting of 1693 codons encodes for non-structural proteins responsible for the processing and replication of the virus, the other two ORFs (660 and 123 codons, respectively) encode for structural polypeptides (Koonin 1992). Four genotypes and multiple subtypes of HEV have been identified by phylogenetic analysis of stored HEV sequences.

Genotype 1 HEV is the main cause of hepatitis E in developing regions of Asia, Africa, and South America. In patients in Mexico, Chad, and Nigeria genotype 2 has been identified (Buisson 2000; Cuyck-Gandre 1997; Tam 1991). Genotype 3 has been found in cases of autochthonous hepatitis E in many developed regions (Banks 2004; Garkavenko 2001; Wibawa 2004) while genotype 4 has been found in industrialized regions of Asia (Lu 2006; Wang 2002). Genotypes 1 and 2 HEV appear to be confined to humans only, genotype 3 and 4 have been found in swine and wild animals (Lu 2006). Only one serotype of HEV is known.

Epidemiology and transmission

The characteristics of hepatitis E epidemiology are similar to those of the hepatitis A virus. Areas with endemic infections and high incidence are in Asia, Africa, Central America and the Middle East (Belabbes 1985; Gupta 1957; Arankalle 1988; Tsega 1991; Velazquez 1990). Here the predominant mode of infection is faecal-oral via contaminated water (Belabbes 1985; Naik 1992). Large outbreaks of HEV have been described. The largest documented incident was in China between 1986 and 1988 involving over 100,000 individuals (Zhuang 1992). Parenteral transmission by blood transfusion seems to occur especially in areas where HEV occurs endemically (Matsubayashi 2004; Khuroo 2004).

In industrialised countries the disease occurs sporadically. Most infections are diagnosed in individuals who travel to countries where HEV is endemic. It has however

been questioned if all cases are imported, for example when high rates of hepatitis E antibodies were found in drug users in Denmark and Sweden (Sylvan 1998; Christensen 2002). This may indicate parenteral transmission by needle sharing within the group. Furthermore, HEV was found in sewage samples collected in France, Spain and the United States (Buti 2003). There is evidence of autochthonous HEV in these areas. In a retrospective analysis of 28 patients in the United Kingdom who were previously diagnosed with drug-induced liver injury 21% actually were actually infected with HEV (Dalton 2007). One could conclude from these findings that the incidence of HEV in industrialised countries may currently be underestimated and that HEV infection may well be underdiagnosed.

Zoonotic transmission needs also to be mentioned. People with occupational contact with swine in the United States (veterinarians and farmers) show a high seroprevalence of anti-HEV antibodies (Meng 2002; Karetnyi 1999). Rodents may also function as a reservoir in some regions (He 2006). Two case studies from Japan demonstrated transmission by undercooked wild boar and deer meat to humans (Tei 2003; Li 2005). To this day the extent of endemic or zoonotic transmission is not fully understood.

Vertical transmission of HEV infection from mother to child has been identified. In one study of eight pregnant women with acute hepatitis E, five blood specimens collected from their babies at birth tested positive for HEV RNA (Khuroo 1995).

Clinical features

The disease may range in severity from sub-clinical to fulminant liver failure. Pregnant women are at especially high risk with a death rate approaching 20%. Overall fulminant fatal hepatitis E occurs in 0.5-3% (Herrera 1993).

After an incubation period of 15 to 60 days (Khuroo 1980; Bayalan 1983) the infected patient develops symptoms and clinical signs that resemble those seen with other forms of acute viral hepatitis. The most prominent feature is jaundice accompanied by general symptoms such as malaise, anorexia and fever, as well as abdominal pain, nausea, vomiting and hepatomegaly. Other clinical symptoms are diarrhoea, pruritus, arthralgia and rash. In biochemical analyses elevated serum concentrations of bilirubin, alanine aminotransferase and aspartate aminotransferase can be seen. Laboratory and clinical symptoms usually resolve within a few weeks to two months. Compared to hepatitis A the disease appears to be more severe with protracted coagulopathy and cholestasis in more than half of patients (Chau 2006).

A study from Japan compared the clinical features of patients infected with genotypes 3 and 4 and saw that genotype 4 tends to have more severe clinical manifestations than genotype 3 (Ohnishi 2006). It was observed that genotype 4 infected individuals had significantly higher alanine aminotransferase peak levels (median 3430 IU/L vs. 1052 IU/L), a lower trough prothrombin time (61 vs. 84%) and that the median time in hospital was longer (26.5 vs. 18 days).

Liver histology in a study of eleven patients with sporadic acute hepatitis E showed acute hepatic lesions in all cases. Nine samples displayed marked necro-inflammatory activity and in five, confluent necrosis was present. Siderosis and cholestasis were diagnosed in eleven and nine patients, respectively (Peron 2007).

The sero-epidemiology of hepatitis E suggests that individuals previously infected with HEV are protected during epidemics of the disease, indicating that immunity to HEV is induced and prevents reinfection (Bryan 1994).

Hepatitis E is widely accepted to be self-limiting and not to progress to chronic disease. However, recent reports describe patients who underwent organ transplant and subsequent immunosuppressive therapy and who may develop chronic HEV infection. In a group of 14 organ recipients (liver, kidney, pancreas) that were diagnosed with acute HEV, chronic hepatitis developed in 8 patients as confirmed by persistently elevated aminotransferase levels, serum HEV RNA, and histologic features of chronic hepatitis (Kamar 2008). Two cases of chronic HEV infection in liver transplant recipients leading to cirrhosis and graft-failure have been reported (Haagsma 2008). The same group found a prevalence of HEV infection acquired after liver transplantation in 274 patients of only 1% (Haagsma 2009). It remains to be determined if there is a substantial risk for immunosuppressed patients of developing chronic HEV infection.

Diagnosis

Diagnosis of acute hepatitis E is based upon the detection of antibodies to HEV or detection of HEV RNA in serum or faeces. HEV RNA may be found very early in faeces and serum. It usually becomes undetectable within one to six weeks after the onset of symptoms (Takahashi 2005). Anti-HEV IgM antibodies are also present early in infection and remain positive for months. Formation of anti-HEV IgG can be detectable as early as in the second week of clinical symptoms.

Combined testing for anti-HEV IgG and either anti-HEV IgA or HEV RNA may be helpful in areas of higher HEV prevalence to distinguish ongoing from remote infection (Takahashi 2005), as anti-HEV IgM (or anti-HEV IgA) alone may be present in individuals with previous HEV contact. Also IgM rheumatoid factor may cause false positive results.

Pregnancy

Fulminant hepatic failure occurs more frequently in pregnant women, resulting in a remarkably high mortality rate of 15 to 25%, primarily in women in the third trimester (Khuroo 1981). The foetal and obstetric outcomes of pregnant women with jaundice and acute viral hepatitis E appear to be worse compared to hepatitis due to other causes (Patra 2007). In 220 consecutive pregnant women with icteric acute hepatitis in a hospital in New Delhi fulminant hepatic failure was more common and maternal mortality was higher (relative risk 2.7 and 6.0, respectively) in HEV-infected women than in those with other aetiologies. The relative risks for obstetric complications were: 4.1 for antepartum haemorrhage, 1.9 for intrauterine foetal death, 1.2 for preterm delivery, and 1.8 for stillbirth.

Treatment

Specific treatment is not available for hepatitis E infection and only monitoring is possible. As in most cases the infection is self-limiting and is followed by complete recovery without chronic sequelae, and no specific interventions are required. Patients with hepatic failure should be transferred to a centre capable of performing liver transplants.

Prevention

In areas with endemic HEV infection food and water sanitation is warranted especially for individuals that are immunosuppressed. It has to be kept in mind that at least some of the HEV infections in industrialized countries are autochthonous infections. These are not associated with travelling to high incidence countries but may be due to zoonotic transmission (Wichmann 2008). A vaccine based on ORF2 has been developed and successfully tested in a phase II trial in Nepal (Shrestha 2007). Another HEV vaccine based on the 50 kD recombinant capsid protein went through Phase III clinical trials at the Xiamen University in China (Feng-Cai 2009). To our knowledge the data of this trial has not been published yet. This topic is discussed in more detail in Chapter 7.

References

Arankalle VA, Chada MS, Mehendale SM, Banerjee K. Outbreak of enterically transmitted non-A, non-B hepatitis among school children. Lancet 1988; 2:1199.

Balayan MS, Andjaparidze AG, Savinskaya SS et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 1983; 20: 23-31.

Banks M, Heath GS, Grierson SS, et al. Evidence for the presence of hepatitis E virus in pigs in the United Kingdom. Vet Rec 2004; 154: 223–7.

Belabbes EH, Bouguermouh A, Benatallah A, Illoul G. Epidemic non-A, non-B viral hepatitis in Algeria: strong evidence for its spreading by water. J Med Virol 1985; 16: 257-63.

Bryan JP, Tsarev SA, Iqbal M et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. J Infect Dis. 1994; 170: 517-21.

Buisson Y, Grandadam M, Nicand E, et al. Identifi cation of a novel hepatitis E virus in Nigeria. J Gen Virol 2000; 81: 903–9.

Buti M, Jardi R, Martin M et al. Hepatitis E virus epidemiology in industrialized countries. Emerg Infect Dis. 2003; 9: 448-54.

Chau TN, Lai ST, Tse C et al. Epidemiology and clinical features of sporadic hepatitis e as compared with hepatitis a. Am J Gastroenterol 2006; 101: 292-6.

Christensen PB, Engle RE, Jacobsen SE, Krarup HB, Georgsen J, Purcell RH. High prevalence of hepatitis E antibodies among Danish prisoners and drug users. J Med Virol. 2002; 66: 49-55.

Cuyck-Gandre H, Zhang HY, Tsarev SA, et al. Characterization of hepatitis E virus (HEV) from Algeria and Chad by partial genome sequence. J Med Virol 1997; 53: 340–7.

Dalton HR, Fellows HJ, Stableforth W, et al. The role of hepatitis E virus testing in drug-induced liver injury. Aliment Pharmacol Ther 2007; 26: 1429–35.

De Feo TM, Poli F, Mozzi F, Moretti MP, Scalamogna M. Risk of transmission of hepatitis B virus from anti-HBC positive cadaveric organ donors: a collaborative study. Transplant Proc 2005;37(2):1238.

Emerson SU, Anderson D, Arankalle A et al. Virus taxonomy VIIIth report of the ICTV. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Hepevirus. 2004 London: Elsevier/Academic Press. Pp 851-3.

Feng-Cai Z. A Phase 3, Randomized, Double-blind, Placebo (Hepatitis B Vaccine) Controlled Clinical Trial of Recombinant (E. Coli) Hepatitis E Vaccine. ClinicalTrials.gov Identifier: NCT01014845. November 13, 2009

Garkavenko O, Obriadina A, Meng J, et al. Detection and characterisation of swine hepatitis E virus in New Zealand. J Med Virol 2001; 65: 525–29.

Gupta, DN, Smetana, HF. The histopathology of viral hepatitis as seen in the Delhi epidemic (1955-56). Indian J Med Res 1957; 45: 101.

Haagsma EB, van den Berg AP, Porte RJ et al. Chronic hepatitis E virus infection in liver transplant recipients. Liver Transpl. 2008; 14 : 547-53.

Haagsma E, Niesters H, van den Berg A, et al. Prevalence of Hepatitis E Virus Infection in Liver Transplant Recipients. Liver Transpl. 2009; 15: 1225-8.

He J, Innis BL, Shrestha MP et al. Evidence that rodents are a reservoir of hepatitis E virus for humans in Nepal. J Clin Microbiol 2006; 44: 1208.

Herrera JL. Hepatitis E as a cause of acute non-A, non-B hepatitis. Arch Intern Med 1993; 153: 773-5

Kamar N, Selves J, Mansuy JM et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med. 2008; 358: 811-7.

Karetnyi YV, Gilchrist MJ, Naides SJ. Hepatitis E virus infection prevalence among selected populations in Iowa. J Clin Virol 1999; 14: 51-5.

Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am J Med 1980; 68: 818-24.

Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. Am J Med 1981; 70: 252-5.

Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. Lancet. 1995; 345: 1025-6.

Khuroo MS, Kamili S, Yattoo GN. Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. J Gastroenterol Hepatol 2004; 19: 778-84.

Koonin EV, Gorbalenya AE, Purdy MA, Rozanov MN, Reyes GR, Bradley DW. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. Proc Natl Acad Sci U S A 1992; 89: 8259-63.

Li TC, Chijiwa K, Sera N, et al. Hepatitis E virus transmission from wild boar meat. Emerg Infect Dis 2005; 11: 1958-60.

Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Rev Med Virol. 2006; 16: 5-36.

Matsubayashi K, Nagaoka Y, Sakata H et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004; 44: 934-40.

Meng XJ, Wiseman B, Elvinger F et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol 2002; 40: 117-22.

Naik SR, Aggarwal R, Salunke PN, Mehrotra NN. A large waterborne viral hepatitis E epidemic in Kanpur, India. Bull World Health Organ 1992; 70: 597-604.

Ohnishi S, Kang JH, Maekubo H et al. Comparison of clinical features of acute hepatitis caused by hepatitis E virus (HEV) genotypes 3 and 4 in Sapporo, Japan. Hepatol Res 2006; 36: 301-307.

Patra S, Kumar A, Trivedi SS, Puri M, Sarin SK. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. Ann Intern Med. 2007; 147: 28-33.

Peron JM, Danjoux M, Kamar N et al. Liver histology in patients with sporadic acute hepatitis E: A study of 11 patients from southwest France. Virchows Arch 2007; 450: 405-410.

Shrestha MP, Scott RM, Joshi DM, et al. Safety and efficacy of a recombinant hepatitis E vaccine. N. Engl. J. Med. 2007: 356, 895-903.

Sylvan, SP. The high rate of antibodies to hepatitis E virus in young, intravenous drug-abusers with acute hepatitis B-virus infection in a Swedish community: a study of hepatitis markers in individuals with intravenously or sexually acquired hepatitis B-virus infection. Scand J Infect Dis 1998; 30: 429.

Takahashi M, Kosakai S, Mizuo H et al. Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitisE virus (HEV) is highly specific for diagnosis of acute HEV infection. J Clin Microbiol 2005; 43: 49-56.

Tam AW, Smith MM, Guerra ME, et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology 1991; 185: 120–31.

Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362: 371-3.

Tsega E, Krawczynski K, Hansson BG et al. Outbreak of acute hepatitis E virus infection among military personnel in northern Ethiopia. J Med Virol 1991; 34: 232-6.

Velazquez O, Stetler HC, Avila C, et al. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986-1987. JAMA 1990; 263: 3281-5.

Wang YC, Zhang HY, Xia NS, et al. Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. J Med Virol 2002; 67: 516–21.

Wibawa ID, Muljono DH, Mulyanto, et al. Prevalence of antibodies to hepatitis E virus among apparently healthy humans and pigs in Bali, Indonesia: identifi cation of a pig infected with a genotype 4 hepatitis E virus. J Med Virol 2004; 73: 38–44.

Wichmann O, Schimanski S, Koch J, et al. Phylogenetic and case-control study on hepatitis E virus infection in Germany. J. Infect. Dis. 2008: 198, 1732-1741.

Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM. Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. Lancet 1980; 2: 876-9.

Zhuang, H. Hepatitis E and strategies for its control. Viral Hepatitis in China: Problems and Control Strategies. Monogr Virol 1992; 19: 126.

Chapter 5:The human hepatitis B virus –Classification, biology, life cycle, *in vitro* and *in vivo* models

Jessika Lüsebrink, Verena Schildgen, Oliver Schildgen

Introduction

In 1965, while searching for tools to identify and track genetic differences in different human populations, a novel antigen was found in the sera of Australian aborigines (Blumberg 1965). This antigen was first called Australia antigen and was associated with clinical courses of hepatitis in the following years (Blumberg 1967; Blumberg 1968) and, immediately thereafter, to a form of what was then called serum hepatitis (Okochi 1970; Okochi 1968; Okochi 1993). Electron microscopy studies revealed that patients testing positive for the Australia antigen had two different types of particles in their serum containing the Australia antigen, namely small particles of spherical and rod-like shape with a diameter of approximately 22 nm, and the so-called Dane particles, of 42 nm (Dane 1970), which are the intrinsic infectious viral particles containing the viral genome and are now called human hepatitis B virus (HBV) (Heerman 1984; Kaplan 1973; Robinson 1975; Robinson 1974; Robinson 1974b; Robinson 1976; Robinson 1976b). Subsequently it was seen that a number of HBV-like viruses exist, most of them displaying a very narrow host range; all together these viruses form the viral family Hepadnaviridae.

Taxonomic classification of the *Hepadnaviridae*

The family name Hepadnaviridae is based on the clinical picture of infection and the target organ (**hepa**titis; liver, classical Greek: το επαρ, phonetic: to **hepa**r) and its nucleic acid, the **DNA**. The family of Hepadnaviridae contains two genera, the *orthohepadnaviruses* that infect only mammals, and the *avihepadnaviruses* that infect birds.

Taxonomically, the *Hepadnaviridae* form their own group because of biological characteristics not observed in any other viral family. The *Hepadnaviridae* contain one of the smallest pathogen genomes known, measuring only 3-3.3 kbp. The reading frames on the genome are organized in a unique and highly condensed way and overlap, contributing to a unique replication strategy. This includes a reverse transcription step that is also observed during retrovirus replication, although in contrast to retroviruses the nucleic acid packaged into hepadnaviral infectious particles is DNA rather than RNA.

The sub-classification into the two genera is based on differences in the host range as well as on phylogenetic differences between mammalian and avian hepadnaviruses (Figure 1). Until now, two major species have been assigned to the avihepadnaviruses, the duck hepatitis B virus (DHBV) and the heron hepatitis B virus (HHBV). Additionally, a number of other avihepadnaviruses have been described that have yet to be integrated into the viral taxonomy system (Guo 2005). The orthohepadnavirus genus includes the four best-known species HBV, WHV, GSHV and WHMV. The prototype species is the human hepatitis B virus (HBV) that can also be used experimentally to infect chimpanzees. WHV, the woodchuck hepatitis virus, is a well-studied orthohepadnavirus that occurs naturally

in marmots and cannot be transferred to other rodents like its relative GSHV, the ground squirrel hepatitis virus. Interestingly, GSHV can infect woodchucks, its host range not being as narrow as that for WHV. The last virus mentioned above, the woolly monkey hepatitis B virus (WMHV), despite having a non-human primate as its natural host, in contrast to HBV, is not infectious in chimpanzees (Lanford 2003; Lanford 1998; Seeger 1991; Seeger 1987). A further member of the genus, the arctic ground squirrel hepatitis virus (AGSHV) is most closely related to GSHV, but further delineation on its host range has not been published (Robbins 1995). Hepadnavirus isolates from chimpanzees, gorillas, orangutans, and gibbons were initially believed to be distinct species but are considered HBV subtypes versus distinct species (Hu 2000; Starkman 2003; Testut 1996; Thornton 2001; Verschoor 2001; Warren 1999; Zuckerman 1975; Zuckerman 1978). In humans, HBV is divided into eight genotypes, A-H, However, it cannot be excluded that more genotpyes may be discovered or evolve in the future. The different genotypes display pairwise differences between 8 and 17% (Arauz-Ruiz 2002; Arauz-Ruiz 1997; Arauz-Ruiz 1997b; Arauz-Ruiz 2001; Fung 2004; Norder 2003; Norder 1994).

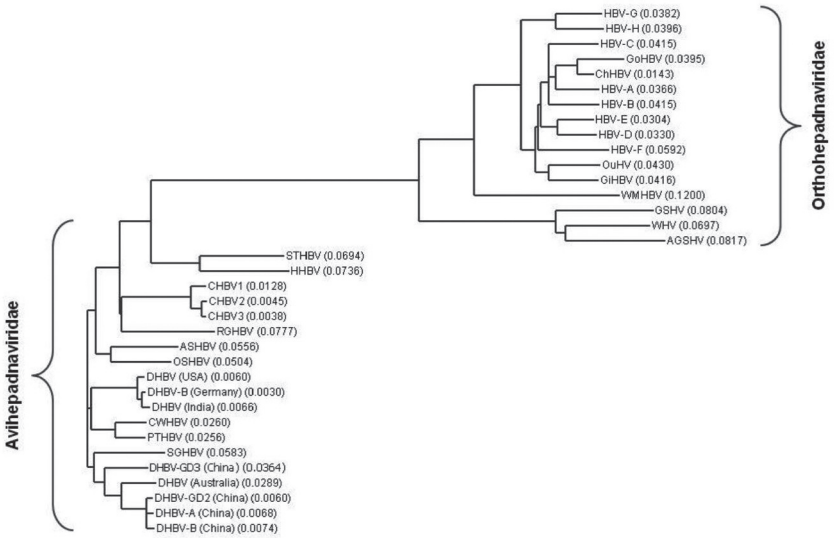


Figure 1. The phylogenetic tree of reference strains of orthohepadnaviruses and avihepadnaviruses. The following prototype sequences were used: AB064316, AB113876, AF193864, AF493986, AJ006350, AJ131567, AJ251937, AJ441111, AJ441112, AJ441113, AY226578, AY433937, AY494849, AY494850, AY494851, AY494852, AY521226, AY521227, AY536371, AY781186, CS388973, CS388974, CS388977, CS388980, CS409746, CS409749, D00220, M11082, NC_001484, NC_001486, NC_005890, NC_005950, U29144, X12798, X74623.

Abbreviations: AGSHV=arctic ground squirrel hepatitis virus, ASHBV=ashy headed sheldgoose HBV, CHBV=crane HBV, ChHBV=chimpanzee HBV, GiHBV=gibbon HBV, GoHBV=gorilla HBV, GSHV=ground squirrel hepatitis virus, CWHBV=chileo wigeon HBV, HHBV=heron HBV, OSHBV=orinoco sheldgoose HBV, OuHV=orangutan hepadnavirus, PTHBV=puna teal HBV, RGHBV=ross' goose HBV, SGHBV=snow goose HBV, STHBV=stork HBV, WHV=woodchuck hepatitis virus, WMHBV=woolly monkey HBV.

Structure of the viral particles and organization of the viral genome

The *Hepadnaviridae* are enveloped DNA viruses with a circular partially double-stranded DNA that in concert with the core protein forms the nucleocapsid. The infectious virus, i.e., the Dane particle, is of a spherical shape with a diameter of 42-47 nm. The viral membrane acquired by the virus during budding or while the viral particles are being transported through secretory pathways via the endoplasmatic reticulum (ER) and Golgi pathways forms the surface containing three viral surface proteins. These proteins, known by their sizes as small (HBsAg), middle (HBmAg), or large (HBlAg), are acquired during budding into the ER.

The nucleocapsid, which forms the inner part of the Dane particle, is around 28 nm in size and besides a single copy of the viral genome contains the viral polymerase, covalently bound to the viral genome, which in turn leads to problems in the molecular diagnostics of HBV infections (see Chapter 11). As with nearly all enveloped viruses there is also evidence that the viral particle contains proteins assumed to be of host origin for HBV (Albin 1980).

The average size of the viral genome is around 3.3 kbp, varying slightly from genotype to genotype and from isolate to isolate. Figure 2 shows the open reading frame organization of the HBV genome. All open reading frames have an identical orientation and overlap at least partially. Within the Dane particle the minus strand of the viral genome is present in full length, carrying the whole genome. In contrast, the plus strand spans only ~ 2/3 of the genome in length and its 3' end is variable in size (Lutwick 1977; Summers 1975). The viral polymerase is covalently bound to the minus strand by a phosphor-tyrosine bond. At the 5' end of the plus strand a short RNA oligomer from the pre-genomic (pg) RNA remains residually bound covalently after viral DNA synthesis. The minus strand, in contrast to the plus strand, contains a small redundancy of 8-9 nucleotides in length on both the 5' end and the 3' end named the r region. These redundant structures are essential for the viral replication mechanism (Lien 1986; Lien 1987; Seeger 1986; Will 1987).

The viral genome covers four open reading frames, all of them encoded by the minus strand, with 6 start codons, four promoters, two transcription enhancing elements, a poly-adenylation signal motif, and a number of signals for DNA replication (Figure 2). The major RNA transcripts are polyadenylated, capped, 3.5 kb, 2.4 kb, and 2.1 kb in length and named pre-C/C, preS, and S mRNAs (Cattaneo 1984; Ender 1985). A 0.7 kb long mRNA termed X mRNA occurs only occasionally. The 3' end of all HBV transcripts is common for all of them and created by the polyadenylation signal in the core (C) gene.

The viral genome encodes for the core protein, the pre-core protein also known as e-antigen, the polymerase, the three surface proteins, and the X-protein. While the core protein, also recognized by the immune system, is essential for the formation of nulceocapsids, the HBeAg which in its gene also contains the full core gene, is post-translationally processed and as a non-essential gene is important in the virus host-immunity interaction. HBeAg is also a marker for active virus replication and plays an important role in molecular diagnostics (Chen 2004).

The viral polymerase is the single enzyme encoded by the HBV genome and is an RNA-dependent DNA polymerase with RNaseH activity. The HBV polymerase consists of three functional domains and a so-called spacer region; at its N-terminal domain is located the terminal protein (TP) acting as a primer in minus-strand DNA synthesis. The C terminal region is separated by the spacer and functions as the RT polymerase and the RNaseH.

The three surface proteins L, M, and S share the C terminal s domain and are coded on one open reading frame that encodes three start codons (one for L: preS1, M: preS2, and S, respectively) and overlaps with the polymerase open reading frame (Seeger 2007). So far, the role of the X-protein has not been fully elucidated, although it has been associated with the nucleus and the cytoskeleton (Doria 1995; Henkler 2001; Lara-Pezzi 2001). However, HBX is required for efficient infection *in vivo* (Zhang 2001; Zoulim 1994).

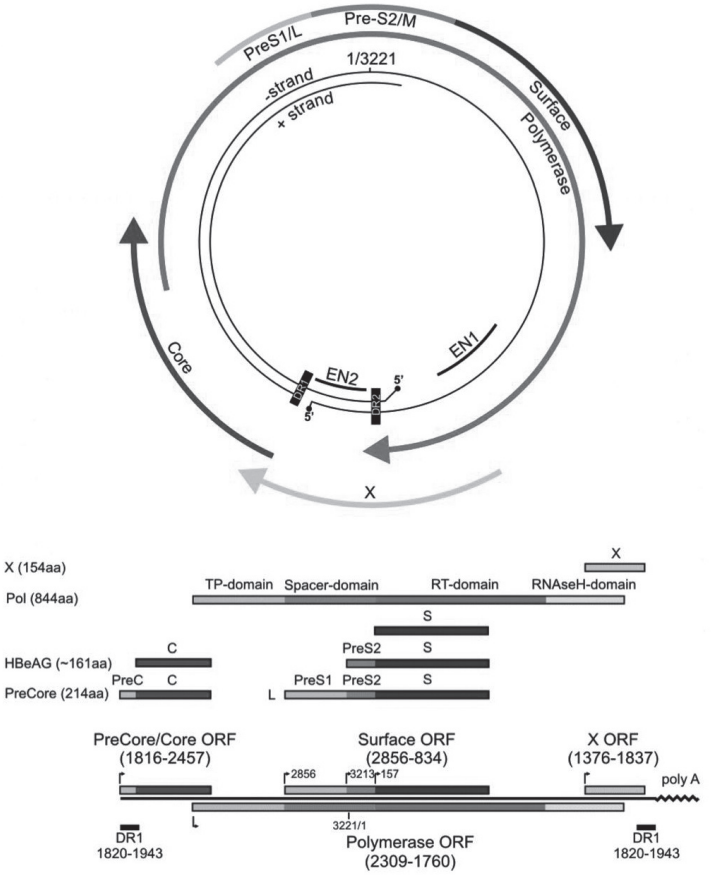


Figure 2. Genome organization and transcripts of the human hepatitis B virus.

The HBV replication cycle

Despite 40 years of HBV research no widely available cell lines permissive for HBV or any other member of the *Hepadnaviridae* family has been described. Studies on the replication cycle of *Hepadnaviridae*, i.e., attachment, entry, genome replication, transcription and expression of viral genes, assembly, and budding cannot be fully studied or are limited to small series of experiments with primary permissive hepatocytes (Aldrich 1989; Gripon 1993; Gripon 1988; Ochiya 1989; Tuttleman 1986). Unfortunately, these primary hepatocytes remain permissive for only a short time after being obtained from the intact liver.

It is assumed that virus entry and the host range of Hepadnaviridae is dependent on the N terminus of the large surface antigen (Chouteau 2001; Gripon 2005; Ishikawa 1995; Lambert 1990; Verschoor 2001). So far, the intrinsic HBV receptor has not been discovered, but from studies on DHBV in primary duck hepatocytes it is assumed that around 104 receptor molecules per cell mediate the rapid binding, followed by a slow uptake of the virus to the cell which can take up to 16 hours (Hagelstein 1997; Klingmuller 1993; Kock 1996; Pugh 1995; Pugh 1989; Rigg 1992). Following entry into the hepatocyte and uncoating, which may proceed in parallel, the nucleocapsid is transported into the cell's nucleus where the viral nucleic acid is released. Release of the viral DNA and disintegration of the nucleocapsid is assumed to take place at the nuclear pore complex (Kann 1997; Rabe 2003).

In the infected hepatocyte the viral DNA is immediately transformed into the covalently closed circular (ccc) DNA by cellular enzymes. The cccDNA in turn is the template for transcription of viral genes, and acts chemically and structurally like an episomal/extrachromosomal DNA and has a plasmid-like structure (Bock 1994; Bock 2001; Newbold 1995). Congruent with the fact that HBV infects hepatocytes, nearly all elements regulating viral transcription have binding sties for liver-specific transcription factors (Courtois 1987; Guo 1993; Lopez-Cabrera 1990; Lopez-Cabrera 1991; Raney 1995; Schaller 1991). Nevertheless, although a number of factors and interactions regulating viral transcription are known, the exact mechanisms of HBV transcription remains unclear. However, viral transcription occurs in the nucleus, and both messenger and pregenomic RNAs are transported into the cytoplasma where they are translated or used as the template for the production of progeny genome, respectively.

In the cytoplasma, the core protein which itself can be phosphorylated by several kinases forms the basis for the nucleocapid and plays an active role in binding and packaging of the pregenomic RNA, recruitment of the viral polymerase, and thus enables the RT polymerase/RNA complex to initiate reverse transcription within the newly forming nucleocapsids (Daub 2002; Gerlich 1982; Kann 1993; Kau 1998; Lau 1999; Liao 1995; Watts 2002).

The three surface proteins of HBV have two major properties: First, as transmembrane proteins they are anchored in the viral envelope and thus are located on the surface of the virus, being responsible for binding to the so-far-unknown viral receptor. Second, the three surface proteins are secreted as subviral particles that do not contain a functional nucleocapsid. The proteins differ in their N-terminal sequences that are longer in case of the L and M protein. All proteins have in common the S

domain, M additionally has the preS2 domain, L has both the preS2 and in addition the preS1 domain (Figure 2). The surface proteins of mammalian Hepdnaviridae have been shown to be N- and O-glycosylated. These glycosylations have been shown to be responsible for proper secretion of progeny viral particles and in turn may represent novel targets for therapies with glycosylation inhibitors (Block 1998; Block 1994; Lu 2003; Schildgen 2004; Schmitt 1999; Schmitt 2004). Moreover, the surface proteins have been demonstrated to be activators of transcription by acting in trans (Caselman 1990; Kekule 1990).

The viral polymerase, singlemost enzyme encoded by the hepadnaviral genome, consists of three functional domains – the terminal protein, the reverse transcriptase, and the RNaseH domain – and a spacer domain that separates the terminal protein domain from the polymerase domains. The terminal protein also serves as a primer for the reverse transcription (Lanford 1997; Wang 1992; Weber 1994). Before or during formation of the cccDNA the terminal protein but also one of the redundant terminal repeats present on the relaxed circular viral genomic DNA that is released from the nucleocapsid are removed and the cccDNA forms by not fully understood mechanism, most probably dependent on cellular ligases and maybe further enzymes. So far it is assumed that cellular DNA repair mechanisms become active and convey the relaxed circular form into the cccDNA (Seeger 2007).

As mentioned previously, the cccDNA is the template also for the pre-genomic RNA (pgRNA). This RNA is both the template for core and polymerase protein translation but also the matrix for the progeny genomes. The pgRNA bears a secondary structure - named ε-structure - that is present at both the 5'- and the 3'-ends. The ε-hairpin loops at the 5' end are first recognized by the viral polymerase and act as the initial packaging signal (Bartenschlager 1992; Hirsch 1990; Huang 1991). The synthesis of the DNA minus strand, i.e. the intrinsic reverse transcription, is then initiated by the formation of a covalent bond between the tyrosine Y65 residue of the terminal protein domain and a desoxy-guanosine-monophosphate (dGMP) (Lanford 1999; Wang 1992; Weber 1994; Zoulim 1994). The next few nucleotides following this initial dGMP are complement to a small part of the ε-structure. The small terminal protein bound primer is subsequently translocated to the 3' end via an unknown mechanism but remains covalently bound during the whole time. Perhaps this process is a prerequisite for the correct folding of the progeny genome within the newly forming nucleocapsid. Finally, the minus strand is fully synthesized by the reverse transcription reaction while the RNA is degraded by the RNaseH activity of the enzyme. The following plus strand synthesis is initiated by an 18mer capped RNA oligo that remains from the 5' end of the pgRNA (Lien 1986; Loeb 1991). Nevertheless, it is assumed that, while not actively replicating and even with conflicting data on its stability, there is evidence that cccDNA may be stable in infected hepatocytes, thus contributing to chronic HBV infection, leading to a need for long-term therapies to help eliminate the cccDNA positive cells.

The final replication step, i.e., assembly and release of Dane particles, is not fully understood, although from one study on usage of glycosylation inhibitors that at non-toxic doses suppress viremia in WHV-infected woodchucks there is indirect evidence that assembly and release occur via secretory pathways (Block 1998).

Pathogenesis of hepadnavirus infections

The transmission of HBV and other members of the *Hepadnaviridae* family occur both vertically and horizontally via body fluids. A maximum of 1010 to 1012 genome copies per ml serum or body fluid can be found. In chronic infections, the viremia is subject to natural fluctuations of one $\log_{10}$ (Schildgen 2006). The rate for chronicity, depending on the study, is >90% in neonates and approximately 10-15% in adults. The risk for transfusion-acquired and nosocomial infections in the past two decades decreased due to optimized molecular diagnostics and more strict hygiene and legal regulations, although there is still a remarkable number of such transmission caused by incautious behavior of healthcare personal.

Once having entered the host, the Dane particle reaches its major target cell, the hepatocyte, the main site for replication and persistence, as virtually all hepadnaviruses display a pronounced and distinct liver tropism. Furthermore, some other cell types have been shown to serve as non-hepatic reservoirs for mammalian hepadnaviruses. Within the infected liver in immunocompetent hosts there is a continued damage of infected hepatocytes by cytotoxic T lymphocytes (CTLs) that leads to uninterrupted expression of collagen fibres, that in the worst and untreated cases lead to liver cirrhosis (Liaw 2004; Mathew 1996; Maynard 2005; Papatheodoridis 2005; Pinzarri 1995; Rizzetto 2005; Rockey 2005; Yoshida 2004).

It is worth note that there is still no evidence that HBV is cytotoxic for the infected hepatocyte. In contrast to other viruses that can infect the liver like the herpes simplex virus (HSV), HBV is unable to induce cytopathic effects under normal infection conditions (Jilbert 1992; Kajino 1994; Thimme 2003; Wieland 2004). Liver damage (fibrosis, cirrhosis, and probably hepatocellular carcinoma) is believed to be induced by the ongoing immune reaction and the steady state inflammation in the liver. Consequently, confirmed by experimental data (Ando 1994; Guidotti 1994; Guidotti 1999; Guidotti 1994b; Guidotti 1996; Guidotti 2000; Guidotti 1999b; Kakimi 2001; Tsui 1995), it is generally assumed that massive CTL and NK T cell action resulting in the killing of infected hepatocytes is essential for elimination of the infection. It is further assumed that in those cases in which chronic infection evolves, the initial cellular immune response is too weak and thus not sufficient to control the infection (Ganem 2004). It remains unclear what mechanisms are responsible for the passage from acute to chronic infection, thus this part of the viral life cycle remains a matter of speculation. As a matter of fact it has been shown that a sufficient Th1 response involving CD8 positive CTLs, natural killer T cells (NK T), cytokines (TNF-alpha, interferon gamma), and cytokines like IL-12 and IL-15 and many others are involved in the sufficient suppression of transient infections (Seeger 2007).

Despite the fact that only antibodies against the s protein are neutralizing and are the only markers of immunity it was hypothesized that transient infection is kept in check by gamma interferon and other cytokines released by immune cells, leading in turn to a shutdown of viral replication (Pasquetto 2002; Schulz 1999; Schulz 1999b). However, this does not explain why only in those patients who clear the virus HBsAg antibodies are present. This is assumed to be a continuous control of the infection as cccDNA can be found in these patients decades later (Maynard 2005; Werle-Lapostolle 2004), while this is not the case if the infection passes to a chronic stage.

Animal models for HBV infections

As mentioned above it is crucial to make use of suitable model systems to study the biology and clinical features of any viral infection. Unfortunately, due to its narrow host range this option is limited for HBV because HBV refuses to replicate in anything other than primary hepatocytes. Consequently, researchers all over the world have tried to establish animal models and cell culture systems that can at least partially reproduce some stages of the infection and can be used, e.g., for the preclinical testing of novel antiviral drugs.

Chimpanzees

Both from epidemiological studies in captive animals and on the natural reservoirs of Hepadnaviridae as well as from experimental infection experiments it is known that chimps and other higher human primates can be infected with HBV. Chimpanzees have been used for preclinical testing of preventive and therapeutic vaccines (Acs 1987; Kim 2008; Komiya 2008; Lubeck 1989; Murray 2005; Ogata 1993; Pride 1998; Sallberg 1998; Sureau 1988; Wahl 1989; Will 1983). Fortunately, for ethical, economic and scientific reasons, experiments with chimpanzees have nearly been stopped.

Woodchucks and squirrels

The woodchuck turned out to be a model for HBV infections through a lucky chance at the end of the 70s. In the Philadelphia Zoo, where the Penrose Research Laboratory was located, it was observed that woodchucks captured in the US mid-Atlantic states housed in the Philadelphia Zoo frequently suffered from hepatocellular carcinoma (Summers 1978). In contrast, in a woodchuck population caught in New York and countryside no hepatomas were observed. Subsequently, the hepatocellular carcinoma was associated with an HBV-like virus, termed woodchuck hepatitis virus (WHV). WHV surprisingly cannot infect European marmots. Meanwhile the woodchuck together with the woodchuck hepatitis virus is an accepted model in preclinical testing for novel antiviral drugs as they are highly representative for HBV.

In spite of a number of advantages the woodchuck model is not very widespread. Despite some successful attempts to breed woodchucks under laboratory conditions like at Cornell University, where a woodchuck colony is housed, most other laboratories failed to breed or managed to breed a rather limited number of woodchucks only by chance. Consequently, most labs have to rely on wild captured animals with or without chronic infections, which entails complications. Wild captured animals bear the risk of being infected with other pathogens such as parasites or the rabies virus, may carry ectoparasites or other unknown comorbidities. Furthermore, research with wild captured animals requires special permissions, at least in Europe, independent of the fact that those animals may be captured as agrarian varmints, thus resulting in an overwhelming bureaucracy with customs and local legal authorities. Moreover, hibernation may influence the experimental setting. Finally, as wild captured woodchuck have a combat weigh-in of up to 7 kg body weight and are not willing to assist the researcher in the planned experiments, they have to be anesthetized before any manipulation can be performed. In any case, the number of secondary reagents needed for woodchuck research, though not commercially available, is increasing and will be useful tools for future research.

The ground squirrel hepatitis virus was detected shortly after WHV (Marion 1980) and like WHV but unlike DHBV can induce hepatocellular carcinoma. In their natural host GSHV seems to be less severe than WHV in woodchucks (Cullen 1996; Marion 1983; Marion 1983b; Marion 1986).

Ducks

Within the genus of avihepadnaviruses, the duck hepatitis virus, which infects the domestic duck, was the first described (Mason 1980). Surprisingly, in birds the hepadnavirus infection is totally apathogenic, likely because DHBV spread occurs vertically in most cases. The viral replication of DHBV in its host takes place in the yolk sac, liver, spleen, kidney, and pancreas. Although some aspects of orthohepadnavirus infections can be studied with this model, the model has some limitations. In contrast to what is observed in mammals, avihepadnaviruses have not yet been associated with liver damage as a consequence of infections, i.e., fibrosis, cirrhosis and subsequent carcinoma do not develop during the chronic infection.

Moreover, up to 50% of ducks develop a liver disease unrelated to DHBV that may overlay DHBV-induced side effects. Furthermore, if not transmitted vertically, DHBV infection is cleared within a few days post-infection in contrast to mammalian hepadnaviruses. Finally, despite the fact that the duck model was widely used in preclinical trials (Chen 2007; Chu 1998; Delmas 2002; Deres 2003; Foster 2005; Hafkemeyer 1996; Heijtink 1993; Kumar 2002; Kumar 2001; Kumar 2001b; Kumar 2001c; Le Guerhier 2003; Lofgren 1996; Offensperger 1996; Peck 2001; Seifer 1998; Seigneres 2001; Xin 1998) it has to be kept in mind that birds do have a biology distinct from mammalian biology in many aspects, a problem that was somewhat lost in the past.

Mouse models

Although some important aspects of HBV infections have been investigated in transgenic mice and have led to convincing results to the majority of HBV researchers, these results need to be handled with care. In transgenic mice expression of viral antigens is possible but does not necessarily reflect a natural infection, thus some observed aspects may be artificial. However, approaches using adenovirus vectors carrying the HBV genome may remain a beneficial tool as at least some aspects of the infection can be studied in a well-characterised *in vivo* model (Seeger 2007). Another approach makes use of mouse chimera that consist of immunosuppressed mice transplanted with human hepatocytes. These mice have been shown to be susceptible for hepatitis B and C viruses (Dandri 2001; Dandri 2001b; Mercer 2001). The major advantage of this model is that primary human hepatocytes remain susceptible to HBV for a long time; unfortunately, these models require extreme breeding conditions that limit their use.

Tupaia

Tupaias, tree shrews, belong to the zoological order of Scadentia with the two families *Tupalidae* and *Ptilocercidae*. So far 20 species organized in 5 genera have been described. One species, Tupaia belangeri chinesis, has been found to be susceptible to HBV (Su 1999; Yan 1996; Yan 1996b). The tupaia is a relatively new model, but as it is directly permissive for HBV it may be the model of choice in future studies.

Cell culture models for *in vitro* phenotyping

Mutations within the polymerase gene can be detected by various methods such as direct sequencing, line probe assay or cloning analysis. While the sequencing of PCR products directly from patient serum or from cloned vectors gives information about amino acid exchanges within the major population of a patient of a clone, a line probe assay can simultaneously detect several co-existing HBV populations, although only mutations which were included in the test probe can be found. However, the quantification of minor populations needs to be refined, and their clinical impact determined. As yet no cell line fully permissive for HBV has been identified and so a simple drug phenotyping system has not yet been established. Consequently, it remains difficult to perform phenotypic tests for each individual clinical resistance. For these reasons, in daily practice genotypic resistance testing is the method of choice. Besides classical sequence analysis methods (commercial or in-house) line probe assays rapidly deliver information on mutations in the viral genome known to be associated with resistance. Cell culture assays for the study of HBV drug resistance are only used for confirmation of newly observed mutations that may mediate antiviral resistance. These methods in general include site-directed mutagenesis of replication-competent HBV genomes, exchange of HBV genome fragments, PCR amplification of complete HBV genomes or cloning of amplified HBV genomes, followed by subsequent transfection of these genomes. HBV replication capacity and drug susceptibility are usually measured by quantification of the different species of viral nucleic acids that form in the cell culture system. In order to minimize variations in transient transfection assays, and to allow a more reproducible measure of drug susceptibility, mutant HBV genomes were integrated into permanent cell lines (Yang 2005) or into baculovirus for transfer into mammalian cells (Delaney 2001). These latter methods are more laborious, but take whole genome variability into account and allow cross-resistance testing for various drugs (Delaney 2001; Zoulim 2006). Although transfection or transduction of mutant HBV genomes allows replication capacity and drug susceptibility to be studied, viral fitness can be assessed only incompletely as the early steps of infection – viral uptake and entry into the hepatocyte – cannot be investigated in these systems. Furthermore, it is important to note that there is no standardization of methods used worldwide, thus it is difficult to compare the level of resistance caused by the individually tested mutations in any quantitative manner.

Future options for antiviral therapy and molecular mechanisms of therapeutic failure

Despite safe and efficient vaccines the human hepatitis B virus (HBV) remains a major medical problem worldwide. According to cautious appraisals by the World Health Organisation an estimated number of 2 billion people worldwide - i.e., one third of mankind - have been infected with HBV, of which 350-450 million people are chronically infected lifelong (www.who.int).

In Germany, 0.8% of all residents, some 640,000 people, are chronically infected with HBV (Robert Koch Institute, www.rki.de). In the European Community, with an estimated 459 million residents in 2005 (http://europa.eu), this same

percentage means more than 3.67 million chronically HBV-infected patients in Europe. Besides the medical problem, socio-economic costs per patient and year are calculated at 25,000€ which is a severe economic concern for the European Community, resulting in an economical worst case scenario of total costs of up to 94,000,000,000€ per year.

The high costs caused by a chronically HBV infected patient are a consequence of the biology of the HBV infection. Once it has become chronic, HBV is a major cause of severe subsequent liver disease that initiates with fibrosis, leads to cirrhosis and finally causes hepatocellular carcinoma that often can only be treated by liver transplantation or leads to a fatal outcome if untreated. Recent studies have shown that the progression of chronic HBV infection and thus progression of liver disease can be efficiently slowed by vigorous antiviral therapy (Liaw 2006; Liu 2006).

These antiviral therapies are based on interferons (with a broad range of mild to severe side effects) and an increasing number of nucleoside and nucleotide analogues (Hadzayannis 2006). The latter groups of compounds were initially used for HIV therapy in which they inhibit the viral RNA-dependent DNA polymerase (reverse transcriptase), an enzyme that is also encoded by the HBV genome. The mechanism of action of nucleos(t)ide analogues against HBV is similar as they also inhibit the viral reverse transcriptase which results in chain termination in viral replication and consequently to the reduction of viremia. Despite the increasing number of anti-HBV drugs in clinical studies or in development, only 5 compounds are approved for HBV therapy, namely lamivudine, adefovir, entecavir, tenofovir, and clebivudine. As seen with HIV therapy, the usage of antiviral agents frequently arrives to the development of agent resistance that in most cases is caused by mutations in the viral target enzymes, i.e., in HBV and HIV, the viral reverse transcriptase(s).

Taking those facts into account it was rather surprising that with the introduction of adefovir several cases of nonresponse to the drug were observed that were associated not only with mutations or newly detected variants of the virus (Potthot 2006; Schildgen 2006b; Tillman 2007). In fact, despite proven compliance, in some cases the drug was unable to reduce viremia, although so far no resistance mutations have been described (Schildgen 2007; Schildgen 2006; Schildgen 2006b; Schildgen 2004; Schildgen 2006c; Sirma 2007). Surprisingly, in 2005 the Gerlich group at Giessen University Germany in parallel to myself detected a very small cohort of patients with a putative primary resistance to adefovir that was subsequently associated with a novel mutation in the viral polymerase (Schildgen 2006c). However, further studies have revealed that there remains a remarkable proportion of patients that do not respond to adefovir and other drugs (Carroue-Durantel 2008; Curtis 2007; Durantel 2005; Tillman 2007; Villet 2008).

Those observations lead to the conclusion that in some patients (in case of adefovir and tenofovir) (a) either the applied prodrugs are not efficiently processed into the active metabolites, (b) the drugs are not efficiently delivered to the infected cell due to a defective or altered transport mechanism, or (c) that the drugs are not efficiently phosphorylated (Tillman 2007). These assumptions are supported by a number of previous observations on the metabolism of the nucleos(t)ide analogues:

• First, especially for adefovir and tenofovir which are administered as prodrugs, defective intracellular esterolytic cleavage may lead to treatment failures (Ray 2004).

• Second, it is known that for the ATP binding cassette transporters, there are significant inter-individual differences in the form of polymorphisms in the respective genes/enzymes resulting in altered drug response, which in turn may induce treatment failure or severe side effects (Cascorbi 2006). Thereby, one of the most important human ABC transporters, MDR-1, depending on its allelic structure, was shown to lead to multiple drug resistance (MDR) against anti-cancer drugs, of which some belong to the nucleos(t)ide analogue family. MDR-1 mediated resistance is believed to be associated also with an amplification of the gene within the drug's target cells (Gillet 2007).

• Third, studies on the metabolism of adefovir (9-(-2)-phosphonylmethoxyethyl) adenine), an adenosine derivative probably absorbed by the ATP binding cassette, revealed that the compound is actively transported into the cytoplasm by a 50 kDa protein against a concentration gradient (Cihlar 1995) before it is phosphorylated to its diphosphorylated derivate PMEApp by the 5-phosphoribosyl-1-pyrophosphate (PRPP) synthase and/or adenylate kinase 2; the diphosphorylated form can be incorporated into the viral genome (Balzarini 1991; Krejcova 2000; Ray 2004; Robbins 1995; Robbins 1995b). Thereby, adefovir can reach intracellular levels of up to 10 pmol per one million cells. Polymorphisms in any of the enzymes involved in these uptake cascades may also lead to impaired antiviral activity.

Nevertheless, from those earlier studies and also from newer studies on the metabolism of adefovir and tenofovir (Delaney 2006; Ray 2004), it remains unclear to what extent other enzymes are involved in the processing of these drugs, as no related data from the respective clinical cases with non-response of assumed metabolic origin have been published. However, it can be assumed that the battery of enzymes involved in the phosphorylation of cellular nucleotides and nucleosides is also involved in the processing of the respective antiviral analogues; these enzymes require serious attention in future studies and their role in the non-response to HBV antiviral therapy should be investigated.

A remarkable number of host enzymes involved in nucleos(t)ide metabolism is encoded by or located in the mitochondria of the host cells as can be deduced by the major side effects induced by nucleos(t)ide analogues. This side effect is the displacement of mitochondrial DNA, mainly by inhibition of DNA polymerase γ, and the subsequent loss of mitochondrial function. This side effect indicates that nucleos(t)ide analogues enter the interior of mitochondria either in phosphorylated form or in non-phosphorylated form; in the latter case they are phosphorylated during or after import into the mitochondria. The mitochondrial nucleoside diphosphate kinase is functionally coupled to oxidative phosphorylation (Lipskaya 2005; Lipskaya 2008). Furthermore, recently, it has been shown that the nucleoside diphosphate kinase D (NM23-H4) binds to the inner mitochondrial membrane and thereby couples the nucleotide transfer with the respiratory chain (Tokarska-Schlattner 2008). In turn, these recent observations give raise to the hypothesis that efficient phosphorylation and subsequent processing of HBV antiviral drugs are strongly dependent on a functional respiratory chain.

A functional respiratory chain is dependent on a large number of factors, of which some are understood and others remain to be investigated. The formation of functional supramolecular "respirasomes" is critically dependent on the stochiometry of their individual components (McKenzie 2006; Stroh 2004). This indicates that besides a very balanced protein expression pattern of respiratory chain molecules and the proper function of those proteins, the physicochemical gradient and the mitochondrial membrane integrity influence mitochondrial function. Nucleoside reverse transcriptase inhibitors have recently been shown to alter the expression of mitochondria-related genes in the mouse liver, an effect that is likely to occur also in the liver of HBV-positive patients treated with similar compounds (Desai 2008). Genes that were affected by the change in the expression profile include enzymes of the ATP-synthetase (complex V of the respiratory chain), ABC proteins, thymidine kinase 2, twinkle, and the solute carrier family 25 members 19, 3, and 4 (deoxynucleotide carrier, phosphate carrier, and adenine nucleotide translocator, respectively). Furthermore, genes involved in the formation of the respiratory chain complexes, namely NADH ubiquinone dehydrogenase (complex I), succinate ubiquinone dehydrogenase (complex II), ubiquinol cytochrome c reductase (complex III), and cytochrome c oxidase (complex IV) were affected (Desai 2008). Taking all these observations together it becomes likely that antiviral drugs of the nucleos(t)ide type are capable of involuntarily regulating their own processing once they have entered the interior of the target cell.

Unfortunately, the problem of the host-mediated non-response to antivirals or treatment failures induced by host factors becomes more complicated by an as yet only partially understood virus-host interaction: The human hepatitis B virus encodes for a protein with a not fully understood function, namely the X protein. The name of the protein is derived from the X-files, unsolved criminal cases of the Federal Bureau of Investigation (FBI) in the US, and comes from the fact that no clear function was attributed to the X-protein. As late as 2001, almost 35 years after the identification of HBV, it was reported that HBx interacted with mitochondria and subsequently altered the intracellular calcium signalling, a process that turned out to be a prerequisite for efficient HBV replication (Bouchard 2001). It has recently been further demonstrated that HBx localizes to the outer mitochondrial membrane, depolarizes it by modulation of the mitochondrial permeability transition pore, upregulates Ca signaling and thus HBV replication, and also prevents the mitochondria from being transferred to an apoptotic stage (Clippinger 2008; Acs 1987). The authors concluded that HBx has a direct impact on the cell metabolism, and their data give rise to the hypothesis that nucleic acid metabolism is strongly influenced by HBx and, in turn, the processing of nucleos(t)ide analogues as well. This assumption is supported by recent data that shows that HBx induces oxidative stress by a translocalisation of mitochondrial Raf-1 kinase (Chen 2007). Oxidative stress is a condition that may have an impact on the proper function of the respiratory chain and thus may negatively influence the processing of antiviral drugs.

Finally, there remains a chance that virus variants with heretofore known but underestimated mutations, genotype specific polymorphisms, or not yet recognized patterns or combinations of mutations not yet associated with resistance but localised in the periphery are responsible for treatment failures. Thus, viral strains isolated from

patients with treatment failures will be tested for *in vitro* susceptibility to antiviral nucleos(t)ides by *in vitro* phenotyping in cell lines. In the absence of a permanent permissive HBV cell line, recombinant systems need to be used. These phenotyping systems are able to produce and secrete HBV particles and to react to antivirals by a decrease in production and secretion of viral particles (Angus 2003; Lucifora 2008; Schildgen 2006c).

In *in vitro* phenotyping systems some of these underestimated mutations in the viral reverse transcriptase have already been described and were extensively investigated *in vitro* (Zoulim 2006b). However, for some other mutations it remains so far unclear whether they in fact induce resistance, whereas the roles of other mutations (that have clear clinical correlates) are still not believed to play a role in antiviral resistance (Curtis 2007; Tillman 2007; Wong 2006). Unfortunately, in all studies, only a small domain of the viral polymerase responsible for reverse transcription was taken into account for analyses, and the role and structural influence of the remaining domains still remain to be investigated.

One possible explanation for this discrepancy is that a crystal structure of the HBV polymerase, in contrast to HIV and other retroviruses reverse transcriptases, is still not available - most binding studies investigating the interaction between the antiviral compound and the viral reverse transcriptase have been performed in silico (Das 2001; Langley 2007). Although those in silico models have demonstrated to be a good tool for prediction of new resistance mutations (Das 2001) and/or analysis of the binding capacity of new drugs by the mutated HBV reverse transcriptase, they lack flexibility and remain accessible to a rather limited community, as those models are not freely available (i.e., no pdb file posted). Furthermore, it appears that most studies have been driven by commercial interest rather than addressing public scientific interest.

Based on earlier published methodology (Das 2001; Langley 2007), a more flexible model for prediction of new resistance mutations, including for drugs not yet approved that can be continuously adjusted to the biological features of the viral polymerase, is highly desirable. Such an in silico model will be useful both for the scientific community that develops new antivirals against HBV and the medical community that has to treat patients, while at the same time avoiding (a) the emergence of resistant HBV variants and (b) reducing costs by avoiding treatment approaches that use drugs that the individual HBV-infected patients will not respond to due to existing mutations in the reverse transcriptase.

References

Acs G, Sells MA, Purcell RH, Price P, Engle R, Shapiro M et al. Hepatitis B virus produced by transfected Hep G2 cells causes hepatitis in chimpanzees. Proc Natl Acad Sci US 1987; 84(13):4641-4644.

Albin C, Robinson WS. Protein kinase activity in hepatitis B virus. J Virol 1980; 34(1):297-302.

Aldrich CE, Coates L, Wu TT, Newbold J, Tennant BC, Summers J et al. In vitro infection of woodchuck hepatocytes with woodchuck hepatitis virus and ground squirrel hepatitis virus. Virology 1989; 172(1):247-252.

Ando K, Guidotti LG, Wirth S, Ishikawa T, Missale G, Moriyama T et al. Class I-restricted cytotoxic T lymphocytes are directly cytopathic for their target cells in vivo. J Immunol 1994; 152(7):3245-3253.

Angus P, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C, et al. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 2003; 125:292-7.

Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J Gen Virol 2002; 83(Pt 8):2059-2073.

Arauz-Ruiz P, Norder H, Visona KA, Magnius LO. Genotype F prevails in HBV infected patients of hispanic origin in Central America and may carry the precore stop mutant. J Med Virol 1997; 51(4):305-312.

Arauz-Ruiz P, Norder H, Visona KA, Magnius LO. Molecular epidemiology of hepatitis B virus in Central America reflected in the genetic variability of the small S gene. J Infect Dis 1997; 176(4):851-858.

Arauz-Ruiz P, Sundqvist L, Garcia Z, Taylor L, Visona K, Norder H et al. Presumed common source outbreaks of hepatitis A in an endemic area confirmed by limited sequencing within the VP1 region. J Med Virol 2001; 65(3):449-456.

Balzarini J, Perno CF, Schols D, De Clercq E. Activity of acyclic nucleoside phosphonate analogues against human immunodeficiency virus in monocyte/macrophages and peripheral blood lymphocytes. Biochem Biophys Res Commun 1991; 178:329-35.

Bartenschlager R, Schaller H. Hepadnaviral assembly is initiated by polymerase binding to the encapsidation signal in the viral RNA genome. EMBO J 1992; 11(9):3413-3420.

Block TM, Lu X, Mehta AS, Blumberg BS, Tennant B, Ebling M et al. Treatment of chronic hepadnavirus infection in a woodchuck animal model with an inhibitor of protein folding and trafficking. Nat Med 1998; 4(5):610-614.

Block TM, Lu X, Platt FM, Foster GR, Gerlich WH, Blumberg BS et al. Secretion of human hepatitis B virus is inhibited by the imino sugar N-butyldeoxynojirimycin. Proc Natl Acad Sci US 1994; 91(6):2235-2239.

Blumberg BS, Alter HJ, Visnich S. A "NEW" ANTIGEN IN LEUKEMIA SERA. JAMA 1965; 191:541-546.

Blumberg BS, Gerstley BJ, Hungerford DA, London WT, Sutnick AI. A serum antigen (Australia antigen) in Down's syndrome, leukemia, and hepatitis. Ann Intern Med 1967; 66(5):924-931.

Blumberg BS, Sutnick AI, London WT. Hepatitis and leukemia: their relation to Australia antigen. Bull NY Acad Med 1968; 44(12):1566-1586.

Bouchard MJ, Wang LH, Schneider RJ. Calcium signaling by HBx protein in hepatitis B virus DNA replication. Science 2001; 294:2376-8.

Bock CT, Schranz P, Schroder CH, Zentgraf H. Hepatitis B virus genome is organized into nucleosomes in the nucleus of the infected cell. Virus Genes 1994; 8(3):215-229.

Bock CT, Schwinn S, Locarnini S, Fyfe J, Manns MP, Trautwein C et al. Structural organization of the hepatitis B virus minichromosome. J Mol Biol 2001; 307(1):183-196.

Carrouee-Durantel S, Durantel D, Werle-Lapostolle B, Pichoud C, Naesens L, Neyts J, Trepo C, Zoulim F. Suboptimal response to adefovir dipivoxil therapy for chronic hepatitis B in nucleoside-naive patients is not due to pre-existing drug-resistant mutants. Antivir Ther 2008; 13:381-8.

Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. Pharmacol Ther 2006; 112:457-73.

Caselmann WH, Meyer M, Kekule AS, Lauer U, Hofschneider PH, Koshy R. A trans-activator function is generated by integration of hepatitis B virus preS/S sequences in human hepatocellular carcinoma DNA. Proc Natl Acad Sci US 1990; 87(8):2970-2974.

Cattaneo R, Will H, Schaller H. Hepatitis B virus transcription in the infected liver. EMBO J 1984; 3(9):2191-2196.

Chen, J., A. Siddiqui. 2007. Hepatitis B virus X protein stimulates the mitochondrial translocation of Raf-1 via oxidative stress. J Virol 81:6757-60.

Chen MT, Billaud JN, Sallberg M, Guidotti LG, Chisari FV, Jones J et al. A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. Proc Natl Acad Sci US 2004; 101(41):14913-14918.

Chen ZY, Cheng AC, Wang MS, Xu DW, Zeng W, Li Z. Antiviral effects of PNA in duck hepatitis B virus infection model. Acta Pharmacol Sin 2007; 28(10):1652-1658.

Chouteau P, Le Seyec J, Cannie I, Nassal M, Guguen-Guillouzo C, Gripon P. A short N-proximal region in the large envelope protein harbors a determinant that contributes to the species specificity of human hepatitis B virus. J Virol 2001; 75(23):11565-11572.

Chu CK, Boudinot FD, Peek SF, Hong JH, Choi Y, Korba BE et al. Preclinical investigation of L-FMAU as an anti-hepatitis B virus agent. Antivir Ther 1998; 3(Suppl 3):113-121.

Cihlar T, Rosenberg I, Votruba I, Holy A. Transport of 9-(2-phosphonomethoxyethyl)adenine across plasma membrane of HeLa S3 cells is protein mediated. Antimicrob Agents Chemother 1995; 39:117-24.

Clippinger AJ, Bouchard MJ. Hepatitis B virus HBx protein localizes to mitochondria in primary rat hepatocytes and modulates mitochondrial membrane potential. J Virol 2008; 82:6798-811.

Courtois G, Morgan JG, Campbell LA, Fourel G, Crabtree GR. Interaction of a liver-specific nuclear factor with the fibrinogen and alpha 1-antitrypsin promoters. Science 1987; 238(4827):688-692.

Cullen JM, Marion PL. Non-neoplastic liver disease associated with chronic ground squirrel hepatitis virus infection. Hepatology 1996; 23(6):1324-1329.

Curtis M, Zhu Y, Borroto-Esoda K. Hepatitis B virus containing the I233V mutation in the polymerase reverse-transcriptase domain remains sensitive to inhibition by adefovir. J Infect Dis 2007; 196:1483-6.

Dandri M, Burda MR, Gocht A, Torok E, Pollok JM, Rogler CE et al. Woodchuck hepatocytes remain permissive for hepadnavirus infection and mouse liver repopulation after cryopreservation. Hepatology 2001; 34(4 Pt 1):824-833.

Dandri M, Burda MR, Torok E, Pollok JM, Iwanska A, Sommer G et al. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 2001; 33(4):981-988. b

Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet 1970; 1(7649):695-698.

Das K, Xiong X, Yang H, Westland CE, Gibbs CS, Sarafianos SG, Arnold E. Molecular modeling and biochemical characterization reveal the mechanism of hepatitis B virus polymerase resistance to lamivudine (3TC) and emtricitabine (FTC). J Virol 2001; 75:4771-9.

Daub H, Blencke S, Habenberger P, Kurtenbach A, Dennenmoser J, Wissing J et al. Identification of SRPK1 and SRPK2 as the major cellular protein kinases phosphorylating hepatitis B virus core protein. J Virol 2002; 76(16):8124-8137.

Delaney WE, Edwards R, Colledge D, Shaw T, Torresi J, Miller TG et al. Cross-resistance testing of antihepadnaviral compounds using novel recombinant baculoviruses which encode drug-resistant strains of hepatitis B virus. Antimicrob Agents Chemother 2001; 45(6):1705-1713.

Delaney WE, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, Miller MD. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob Agents Chemother 2006; 50:2471-7.

Delmas J, Schorr O, Jamard C, Gibbs C, Trepo C, Hantz O et al. Inhibitory effect of adefovir on viral DNA synthesis and covalently closed circular DNA formation in duck hepatitis B virus-infected hepatocytes in vivo and in vitro. Antimicrob Agents Chemother 2002; 46(2):425-433.

Deres K, Schroder CH, Paessens A, Goldmann S, Hacker HJ, Weber O et al. Inhibition of hepatitis B virus replication by drug-induced depletion of nucleocapsids. Science 2003; 299(5608):893-896.

Desai VG, Lee T, Delongchamp RR, Leakey JE, Lewis SM, Lee F, Moland CL, Branham WS, Fuscoe JC. Nucleoside reverse transcriptase inhibitors (NRTIs)-induced expression profile of mitochondria-related genes in the mouse liver. Mitochondrion 2008; 8:181-95.

Doria M, Klein N, Lucito R, Schneider RJ. The hepatitis B virus HBx protein is a dual specificity cytoplasmic activator of Ras and nuclear activator of transcription factors. EMBO J 1995; 14(19):4747-4757.

Durantel D, Brunelle MN, Gros E, Carrouee-Durantel S, Pichoud C, Villet S, Trepo C, Zoulim F. Resistance of human hepatitis B virus to reverse transcriptase inhibitors: from genotypic to phenotypic testing. J Clin Virol 2005; 34 Suppl 1:S34-43.

Enders GH, Ganem D, Varmus H. Mapping the major transcripts of ground squirrel hepatitis virus: the presumptive template for reverse transcriptase is terminally redundant. Cell 1985; 42(1):297-308.

Foster WK, Miller DS, Scougall CA, Kotlarski I, Colonno RJ, Jilbert AR. Effect of antiviral treatment with entecavir on age- and dose-related outcomes of duck hepatitis B virus infection. J Virol 2005; 79(9):5819-5832.

Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology 2004; 40(4):790-792.

Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 2004; 350(11):1118-1129.

Gerlich WH, Goldmann U, Muller R, Stibbe W, Wolff W. Specificity and localization of the hepatitis B virus-associated protein kinase. J Virol 1982; 42(3):761-766.

Gillet JP, Efferth T, Remacle J. 2007. Chemotherapy-induced resistance by ATP-binding cassette transporter genes. Biochim Biophys Acta 1775:237-62.

Gripon P, Cannie I, Urban S. Efficient inhibition of hepatitis B virus infection by acylated peptides derived from the large viral surface protein. J Virol 2005; 79(3):1613-1622.

Gripon P, Diot C, Guguen-Guillouzo C. Reproducible high level infection of cultured adult human hepatocytes by hepatitis B virus: effect of polyethylene glycol on adsorption and penetration. Virology 1993; 192(2):534-540.

Gripon P, Diot C, Theze N, Fourel I, Loreal O, Brechot C et al. Hepatitis B virus infection of adult human hepatocytes cultured in the presence of dimethyl sulfoxide. J Virol 1988; 62(11):4136-4143.

Guidotti LG, Ando K, Hobbs MV, Ishikawa T, Runkel L, Schreiber RD et al. Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. Proc Natl Acad Sci US 1994; 91(9):3764-3768.

Guidotti LG, Borrow P, Brown A, McClary H, Koch R, Chisari FV. Noncytopathic clearance of lymphocytic choriomeningitis virus from the hepatocyte. J Exp Med 1999; 189(10):1555-1564.

Guidotti LG, Guilhot S, Chisari FV. Interleukin-2 and alpha/beta interferon down-regulate hepatitis B virus gene expression in vivo by tumor necrosis factor-dependent and -independent pathways. J Virol 1994; 68(3):1265-1270.

Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity 1996; 4(1):25-36.

Guidotti LG, McClary H, Loudis JM, Chisari FV. Nitric oxide inhibits hepatitis B virus replication in the livers of transgenic mice. J Exp Med 2000; 191(7):1247-1252.

Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999; 284(5415):825-829. b

Guo H, Mason WS, Aldrich CE, Saputelli JR, Miller DS, Jilbert AR et al. Identification and characterization of avihepadnaviruses isolated from exotic anseriformes maintained in captivity. J Virol 2005; 79(5):2729-2742.

Guo W, Chen M, Yen TS, Ou JH. Hepatocyte-specific expression of the hepatitis B virus core promoter depends on both positive and negative regulation. Mol Cell Biol 1993; 13(1):443-448.

Hadziyannis SJ New developments in the treatment of chronic hepatitis B. Expert Opin Biol Ther 2006; 6:913-21.

Hafkemeyer P, Keppler-Hafkemeyer A, al Haya MA, Janta-Lipinski M, Matthes E, Lehmann C et al. Inhibition of duck hepatitis B virus replication by 2',3'-dideoxy-3'-fluoroguanosine in vitro and in vivo. Antimicrob Agents Chemother 1996; 40(3):792-794.

Hagelstein J, Fathinejad F, Stremmel W, Galle PR. pH-independent uptake of hepatitis B virus in primary human hepatocytes. Virology 1997; 229(1):292-294.

Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-s sequence. J Virol 1984; 52(2):396-402.

Heijtink RA, De Wilde GA, Kruining J, Berk L, Balzarini J, De Clercq E et al. Inhibitory effect of 9-(2-phosphonylmethoxyethyl)-adenine (PMEA) on human and duck hepatitis B virus infection. Antiviral Res 1993; 21(2):141-153.

Henkler F, Hoare J, Waseem N, Goldin RD, McGarvey MJ, Koshy R et al. Intracellular localization of the hepatitis B virus HBx protein. J Gen Virol 2001; 82(Pt 4):871-882.

Hirsch RC, Lavine JE, Chang LJ, Varmus HE, Ganem D. Polymerase gene products of hepatitis B viruses are required for genomic RNA packaging as wel as for reverse transcription. Nature 1990; 344(6266):552-555.

Hu X, Margolis HS, Purcell RH, Ebert J, Robertson BH. Identification of hepatitis B virus indigenous to chimpanzees. Proc Natl Acad Sci US 2000; 97(4):1661-1664.

Huang MJ, Summers J. Infection initiated by the RNA pregenome of a DNA virus. J Virol 1991; 65(10):5435-5439.

Ishikawa T, Ganem D. The pre-S domain of the large viral envelope protein determines host range in avian hepatitis B viruses. Proc Natl Acad Sci US 1995; 92(14):6259-6263.

Jilbert AR, Wu TT, England JM, Hall PM, Carp NZ, O'Connell AP et al. Rapid resolution of duck hepatitis B virus infections occurs after massive hepatocellular involvement. J Virol 1992; 66(3):1377-1388.

Kajino K, Jilbert AR, Saputelli J, Aldrich CE, Cullen J, Mason WS. Woodchuck hepatitis virus infections: very rapid recovery after a prolonged viremia and infection of virtually every hepatocyte. J Virol 1994; 68(9):5792-5803.

Kakimi K, Lane TE, Chisari FV, Guidotti LG. Cutting edge: Inhibition of hepatitis B virus replication by activated NK T cells does not require inflammatory cell recruitment to the liver. J Immunol 2001; 167(12):6701-6705.

Kann M, Bischof A, Gerlich WH. In vitro model for the nuclear transport of the hepadnavirus genome. J Virol 1997; 71(2):1310-1316.

Kann M, Thomssen R, Kochel HG, Gerlich WH. Characterization of the endogenous protein kinase activity of the hepatitis B virus. Arch Virol Suppl 1993; 8:53-62.

Kaplan PM, Greenman RL, Gerin JL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. J Virol 1973; 12(5):995-1005.

Kau JH, Ting LP. Phosphorylation of the core protein of hepatitis B virus by a 46-kilodalton serine kinase. J Virol 1998; 72(5):3796-3803.

Kekule AS, Lauer U, Meyer M, Caselmann WH, Hofschneider PH, Koshy R. The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. Nature 1990; 343(6257):457-461.

Kim SH, Kim SH, Oh HK, Ryu CJ, Park SY, Hong HJ. In vivo hepatitis B virus-neutralizing activity of an anti-HBsAg humanized antibody in chimpanzees. Exp Mol Med 2008; 40(1):145-149.

Klingmuller U, Schaller H. Hepadnavirus infection requires interaction between the viral pre-S domain and a specific hepatocellular receptor. J Virol 1993; 67(12):7414-7422.

Kock J, Borst EM, Schlicht HJ. Uptake of duck hepatitis B virus into hepatocytes occurs by endocytosis but does not require passage of the virus through an acidic intracellular compartment. J Virol 1996; 70(9):5827-5831.

Komiya Y, Katayama K, Yugi H, Mizui M, Matsukura H, Tomoguri T et al. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C. Transfusion 2008; 48(2):286-294.

Krejcova R, Horska K, Votruba I, Holy A. Interaction of guanine phosphonomethoxyalkyl derivatives with GMP kinase isoenzymes. Biochem Pharmacol 2000; 60:1907-13.

Kumar R, Nath M, Tyrrell DL. Design and synthesis of novel 5-substituted acyclic pyrimidine nucleosides as potent and selective inhibitors of hepatitis B virus. J Med Chem 2002; 45(10):2032-2040.

Kumar R, Rai D, Sharma SK, Saffran HA, Blush R, Tyrrell DL. Synthesis and antiviral activity of novel 5-(1-cyanamido-2-haloethyl) and 5-(1-hydroxy(or methoxy)-2-azidoethyl) analogues of uracil nucleosides. J Med Chem 2001; 44(21):3531-3538.

Kumar R, Sharma N, Nath M, Saffran HA, Tyrrell DL. Synthesis and antiviral activity of novel acyclic nucleoside analogues of 5-(1-azido-2-haloethyl)uracils. J Med Chem 2001; 44(24):4225-4229. b

Kumar R, Tyrrell DL. Novel 5-vinyl pyrimidine nucleosides with potent anti-hepatitis B virus activity. Bioorg Med Chem Lett 2001; 11(22):2917-2920. c

Lambert V, Fernholz D, Sprengel R, Fourel I, Deleage G, Wildner G et al. Virus-neutralizing monoclonal antibody to a conserved epitope on the duck hepatitis B virus pre-S protein. J Virol 1990; 64(3):1290-1297.

Lan YT, Li J, Liao W, Ou J. Roles of the three major phosphorylation sites of hepatitis B virus core protein in viral replication. Virology 1999; 259(2):342-348.

Lanford RE, Chavez D, Barrera A, Brasky KM. An infectious clone of woolly monkey hepatitis B virus. J Virol 2003; 77(14):7814-7819.

Lanford RE, Chavez D, Brasky KM, Burns RB, III, Rico-Hesse R. Isolation of a hepadnavirus from the woolly monkey, a New World primate. Proc Natl Acad Sci US 1998; 95(10):5757-5761.

Lanford RE, Kim YH, Lee H, Notvall L, Beames B. Mapping of the hepatitis B virus reverse transcriptase TP and RT domains by transcomplementation for nucleotide priming and by protein-protein interaction. J Virol 1999; 73(3):1885-1893.

Lanford RE, Notvall L, Lee H, Beames B. Transcomplementation of nucleotide priming and reverse transcription between independently expressed TP and RT domains of the hepatitis B virus reverse transcriptase. J Virol 1997; 71(4):2996-3004.

Langley DR, Walsh AW, Baldick CJ, Eggers BJ, Rose RE, Levine SM, Kapur AJ, Colonno RJ, Tenney DJ. Inhibition of hepatitis B virus polymerase by entecavir. J Virol 2007; 81:3992-4001.

Lara-Pezzi E, Serrador JM, Montoya MC, Zamora D, Yanez-Mo M, Carretero M et al. The hepatitis B virus X protein (HBx) induces a migratory phenotype in a CD44-dependent manner: possible role of HBx in invasion and metastasis. Hepatology 2001; 33(5):1270-1281.

Le Guerhier F, Thermet A, Guerret S, Chevallier M, Jamard C, Gibbs CS et al. Antiviral effect of adefovir in combination with a DNA vaccine in the duck hepatitis B virus infection model. J Hepatol 2003; 38(3):328-334.

Liao W, Ou JH. Phosphorylation and nuclear localization of the hepatitis B virus core protein: significance of serine in the three repeated SPRRR motifs. J Virol 1995; 69(2):1025-1029.

Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004; 351(15):1521-1531.

Liaw YF. Hepatitis B virus replication and liver disease progression: the impact of antiviral therapy. Antivir Ther 2006; 11:669-79.

Lien JM, Aldrich CE, Mason WS. Evidence that a capped oligoribonucleotide is the primer for duck hepatitis B virus plus-strand DNA synthesis. J Virol 1986; 57(1):229-236.

Lien JM, Aldrich CE, Mason WS. Evidence that a capped oligoribonucleotide is the primer for duck hepatitis B virus plus-strand DNA synthesis. J Virol 1986; 57(1):229-236. b

Lien JM, Petcu DJ, Aldrich CE, Mason WS. Initiation and termination of duck hepatitis B virus DNA synthesis during virus maturation. J Virol 1987; 61(12):3832-3840.

Lipskaya TY, Voinova VV. Functional coupling between nucleoside diphosphate kinase of the outer mitochondrial compartment and oxidative phosphorylation. Biochemistry (Mosc) 2005; 70:1354-62.

Lipskaya TY, Voinova VV. Mitochondrial nucleoside diphosphate kinase: mode of interaction with the outer mitochondrial membrane and proportion of catalytic activity functionally coupled to oxidative phosphorylation. Biochemistry (Mosc) 2008; 73:321-31.

Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, and Chen DS. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. J Infect Dis 2006; 193:1258-65.

Loeb DD, Hirsch RC, Ganem D. Sequence-independent RNA cleavages generate the primers for plus strand DNA synthesis in hepatitis B viruses: implications for other reverse transcribing elements. EMBO J 1991; 10(11):3533-3540.

Lofgren B, Vickery K, Zhang YY, Nordenfelt E. 2',3'-dideoxy-3'-fluoroguanosine inhibits duck hepatitis B virus in vivo. J Viral Hepat 1996; 3(2):61-65.

Lopez-Cabrera M, Letovsky J, Hu KQ, Siddiqui A. Multiple liver-specific factors bind to the hepatitis B virus core/pregenomic promoter: trans-activation and repression by CCAAT/enhancer binding protein. Proc Natl Acad Sci US 1990; 87(13):5069-5073.

Lopez-Cabrera M, Letovsky J, Hu KQ, Siddiqui A. Transcriptional factor C/EBP binds to and transactivates the enhancer element II of the hepatitis B virus. Virology 1991; 183(2):825-829.

Lu X, Tran T, Simsek E, Block TM. The alkylated imino sugar, n-(n-Nonyl)-deoxygalactonojirimycin, reduces the amount of hepatitis B virus nucleocapsid in tissue culture. J Virol 2003; 77(22):11933-11940.

Lubeck MD, Davis AR, Chengalvala M, Natuk RJ, Morin JE, Molnar-Kimber K, et al. Immunogenicity and efficacy testing in chimpanzees of an oral hepatitis B vaccine based on live recombinant adenovirus. Proc Natl Acad Sci US 1989; 86(17):6763-6767.

Lucifora J, Durantel D, Belloni L, Barraud L, Villet S, Vincent IE, et al. Initiation of hepatitis B virus genome replication and production of infectious virus following delivery in HepG2 cells by novel recombinant baculovirus vector. J Gen Virol 2008; 89:1819-28.

Lutwick LI, Robinson WS. DNA synthesized in the hepatitis B Dane particle DNA polymerase reaction. J Virol 1977; 21(1):96-104.

Marion PL, Knight SS, Salazar FH, Popper H, Robinson WS. Ground squirrel hepatitis virus infection. Hepatology 1983; 3(4):519-527.

Marion PL, Oshiro LS, Regnery DC, Scullard GH, Robinson WS. A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. Proc Natl Acad Sci US 1980; 77(5):2941-2945.

Marion PL, Robinson WS. Hepadna viruses: hepatitis B and related viruses. Curr Top Microbiol Immunol 1983; 105:99-121. b

Marion PL, Van Davelaar MJ, Knight SS, Salazar FH, Garcia G, Popper H et al. Hepatocellular carcinoma in ground squirrels persistently infected with ground squirrel hepatitis virus. Proc Natl Acad Sci U S A 1986; 83(12):4543-4546.

Mason WS, Seal G, Summers J. Virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. J Virol 1980; 36(3):829-836.

Mathew J, Geerts A, Burt AD. Pathobiology of hepatic stellate cells. Hepatogastroenterology 1996; 43(7):72-91.

Maynard M, Parvaz P, Durantel S, Chevallier M, Chevallier P, Lot M et al. Sustained HBs seroconversion during lamivudine and adefovir dipivoxil combination therapy for lamivudine failure. J Hepatol 2005; 42(2):279-281.

McKenzie M, Lazarou M, Thorburn DR, Ryan MT. Mitochondrial respiratory chain supercomplexes are destabilized in Barth Syndrome patients. J Mol Biol 2006; 361:462-9.

Mercer DF, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfret A et al. Hepatitis C virus replication in mice with chimeric human livers. Nat Med 2001; 7(8):927-933.

Murray JM, Wieland SF, Purcell RH, Chisari FV. Dynamics of hepatitis B virus clearance in chimpanzees. Proc Natl Acad Sci US 2005; 102(49):17780-17785.

Newbold JE, Xin H, Tencza M, Sherman G, Dean J, Bowden S et al. The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. J Virol 1995; 69(6):3350-3357.

Norder H, Arauz-Ruiz P, Blitz L, Pujol FH, Echevarria JM, Magnius LO. The T(1858) variant predisposing to the precore stop mutation correlates with one of two major genotype F hepatitis B virus clades. J Gen Virol 2003; 84(Pt 8):2083-2087.

Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 1994; 198(2):489-503.

Ochiya T, Tsurimoto T, Ueda K, Okubo K, Shiozawa M, Matsubara K. An in vitro system for infection with hepatitis B virus that uses primary human fetal hepatocytes. Proc Natl Acad Sci US 1989; 86(6):1875-1879.

Offensperger WB, Offensperger S, Keppler-Hafkemeyer A, Hafkemeyer P, Blum HE. Antiviral activities of penciclovir and famciclovir on duck hepatitis B virus in vitro and in vivo. Antivir Ther 1996; 1(3):141-146.

Ogata N, Miller RH, Ishak KG, Purcell RH. The complete nucleotide sequence of a pre-core mutant of hepatitis B virus implicated in fulminant hepatitis and its biological characterization in chimpanzees. Virology 1993; 194(1):263-276.

Okochi K, Mayumi M, Haguino Y, Saito N. Evaluation of frequency of Australia antigen in blood donors of Tokyo by means of immune adherence hemagglutination technique. Vox Sang 1970; 19(3):332-337.

Okochi K, Murakami S. Observations on Australia antigen in Japanese. Vox Sang 1968; 15(5):374-385.

Okochi K, Murakami S. Observations on Australia antigen in Japanese. Vox Sang 1968:15:374-85. Vox Sang 1993; 65(1):75. b

Papatheodoridis GV, Petraki K, Cholongitas E, Kanta E, Ketikoglou I, Manesis EK. Impact of interferon-alpha therapy on liver fibrosis progression in patients with HBeAg-negative chronic hepatitis B. J Viral Hepat 2005; 12(2):199-206.

Pasquetto V, Wieland SF, Uprichard SL, Tripodi M, Chisari FV. Cytokine-sensitive replication of hepatitis B virus in immortalized mouse hepatocyte cultures. J Virol 2002; 76(11):5646-5653.

Peek SF, Cote PJ, Jacob JR, Toshkov IA, Hornbuckle WE, Baldwin BH et al. Antiviral activity of clevudine [L-FMAU, (1-(2-fluoro-5-methyl-beta, L-arabinofuranosyl) uracil)] against woodchuck hepatitis virus replication and gene expression in chronically infected woodchucks (Marmota monax). Hepatology 2001; 33(1):254-266.

Pinzani M. Novel insights into the biology and physiology of the Ito cell. Pharmacol Ther 1995; 66(2):387-412.

Potthoff A, Tillmann HL, Bara C, Deterding K, Pethig K, Meyer S, et al. Improved outcome of chronic hepatitis B after heart transplantation by long-term antiviral therapy. J Viral Hepat 2006; 13:734-41.

Pride MW, Bailey CR, Muchmore E, Thanavala Y. Evaluation of B and T-cell responses in chimpanzees immunized with Hepagene, a hepatitis B vaccine containing pre-S1, pre-S2 gene products. Vaccine 1998; 16(6):543-550.

Pugh JC, Di Q, Mason WS, Simmons H. Susceptibility to duck hepatitis B virus infection is associated with the presence of cell surface receptor sites that efficiently bind viral particles. J Virol 1995; 69(8):4814-4822.

Pugh JC, Summers JW. Infection and uptake of duck hepatitis B virus by duck hepatocytes maintained in the presence of dimethyl sulfoxide. Virology 1989; 172(2):564-572.

Rabe B, Vlachou A, Pante N, Helenius A, Kann M. Nuclear import of hepatitis B virus capsids and release of the viral genome. Proc Natl Acad Sci US 2003; 100(17):9849-9854.

Raney AK, Zhang P, McLachlan A. Regulation of transcription from the hepatitis B virus large surface antigen promoter by hepatocyte nuclear factor 3. J Virol 1995; 69(6):3265-3272.

Ray AS, Vela JE, Olson L, Fridland A. Effective metabolism and long intracellular half life of the anti-hepatitis B agent adefovir in hepatic cells. Biochem Pharmacol 2004; 68:1825-31.

Rigg RJ, Schaller H. Duck hepatitis B virus infection of hepatocytes is not dependent on low pH. J Virol 1992; 66(5):2829-2836.

Rizzetto M, Tassopoulos NC, Goldin RD, Esteban R, Santantonio T, Heathcote EJ et al. Extended lamivudine treatment in patients with HBeAg-negative chronic hepatitis B. J Hepatol 2005; 42(2):173-179.

Robbins BL, Connelly MC, Marshall DR, Srinivas RV, Fridland A. A human T lymphoid cell variant resistant to the acyclic nucleoside phosphonate 9-(2-phosphonylmethoxyethyl)adenine shows a unique combination of a phosphorylation defect and increased efflux of the agent. Mol Pharmacol 1995; 47:391-7.

Robbins BL, Greenhaw J, Connelly MC, Fridland A. Metabolic pathways for activation of the antiviral agent 9-(2-phosphonylmethoxyethyl)adenine in human lymphoid cells. Antimicrob Agents Chemother 1995; 39:2304-8. b

Robinson WS. DNA and DNA polymerase in the core of the Dane particle of hepatitis B. Am J Med Sci 1975; 270(1):151-159.

Robinson WS. DNA and DNA polymerase of a virus-like particle in hepatitis B. Dev Biol Stand 1975; 30:23-37. b

Robinson WS, Clayton DA, Greenman RL. DNA of a human hepatitis B virus candidate. J Virol 1974; 14(2):384-391.

Robinson WS, Greenman RL. DNA polymerase in the core of the human hepatitis B virus candidate. J Virol 1974; 13(6):1231-1236. b

Robinson WS, Lutwick LI. The virus of hepatitis, type B (first of two parts). N Engl J Med 1976; 295(21):1168-1175. (155) b. Robinson WS, Lutwick LI. The virus of hepatitis, type B. (Second of two parts). N Engl J Med 1976; 295(22):1232-1236. (156) Rockey DC. Antifibrotic therapy in chronic liver disease. Clin Gastroenterol Hepatol 2005; 3(2):95-107.

Sallberg M, Hughes J, Javadian A, Ronlov G, Hultgren C, Townsend K et al. Genetic immunization of chimpanzees chronically infected with the hepatitis B virus, using a recombinant retroviral vector encoding the hepatitis B virus core antigen. Hum Gene Ther 1998; 9(12):1719-1729.

Schaller H, Fischer M. Transcriptional control of hepadnavirus gene expression. Curr Top Microbiol Immunol 1991; 168:21-39.

Schildgen O, Fiedler M, Dahmen U, Li J, Lohrengel B, Lu M et al. Fluctuation of the cytokine expression in the liver during the chronic woodchuck hepatitis virus (WHV) infection is not related to viral load. Immunol Lett 2006; 102(1):31-37.

Schildgen O, Roggendorf M, Lu M. Identification of a glycosylation site in the woodchuck hepatitis virus preS2 protein and its role in protein trafficking. J Gen Virol 2004; 85(Pt 4):787-793.

Schildgen O. Novel lamivudine resistance. Antimicrob Agents Chemother 2007; 51:4533, author reply 4533-4.

Schildgen O, Hartmann H, Gerlich WH. Replacement of tenofovir with adefovir may result in reactivation of hepatitis B virus replication. Scand J Gastroenterol 2006; 41:245-6.

Schildgen O, Helm M, Gerlich W. Nonresponse to adefovir: host or virus dependent? J Clin Virol 2006: 37:327-8. b

Schildgen O, Schewe CK, Vogel M, Daumer M, Kaiser R, Weitner L, Matz B, and Rockstroh JK. Successful therapy of hepatitis B with tenofovir in HIV-infected patients failing previous adefovir and lamivudine treatment. AIDS 2004; 18:2325-7.

Schildgen O, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, et al. Variant of hepatitis B virus with primary resistance to adefovir. N Engl J Med 2006; 354:1807-12. c

Schmitt S, Glebe D, Alving K, Tolle TK, Linder M, Geyer H et al. Analysis of the pre-. J Biol Chem 1999; 274(17):11945-11957.

Schmitt S, Glebe D, Tolle TK, Lochnit G, Linder D, Geyer R et al. Structure of pre-. J Gen Virol 2004; 85(Pt 7):2045-2053.

Schultz U, Chisari FV. Recombinant duck interferon gamma inhibits duck hepatitis B virus replication in primary hepatocytes. J Virol 1999; 73(4):3162-3168.

Schultz U, Summers J, Staeheli P, Chisari FV. Elimination of duck hepatitis B virus RNA-containing capsids in duck interferon-alpha-treated hepatocytes. J Virol 1999; 73(7):5459-5465. b

Seeger C, Baldwin B, Hornbuckle WE, Yeager AE, Tennant BC, Cote P et al. Woodchuck hepatitis virus is a more efficient oncogenic agent than ground squirrel hepatitis virus in a common host. J Virol 1991; 65(4):1673-1679.

Seeger C, Ganem D, Varmus HE. Biochemical and genetic evidence for the hepatitis B virus replication strategy. Science 1986; 232(4749):477-484.

Seeger C, Marion PL, Ganem D, Varmus HE. In vitro recombinants of ground squirrel and woodchuck hepatitis viral DNAs produce infectious virus in squirrels. J Virol 1987; 61(10):3241-3247.

Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Knipe DM, et al., editors. Fields Virology. Philadelphia: Lippincott Williams & Wilkins, 2007: 2977-3029.

Seifer M, Hamatake RK, Colonno RJ, Standring DN. In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobucavir. Antimicrob Agents Chemother 1998; 42(12):3200-3208.

Seigneres B, Aguesse-Germon S, Pichoud C, Vuillermoz I, Jamard C, Trepo C et al. Duck hepatitis B virus polymerase gene mutants associated with resistance to lamivudine have a decreased replication capacity in vitro and in vivo. J Hepatol 2001; 34(1):114-122.

Sirma H, Funk A, Gerlich W, Schildgen O. Does pre-treatment with lamivudine prime for adefovir resistance of hepatitis B virus infection? J Antimicrob Chemother 2007; 60:448-9.

Starkman SE, MacDonald DM, Lewis JC, Holmes EC, Simmonds P. Geographic and species association of hepatitis B virus genotypes in non-human primates. Virology 2003; 314(1):381-393.

Stroh A, Anderka O, Pfeiffer K, Yagi T, Finel M, Ludwig B, Schagger H. Assembly of respiratory complexes I, III, and IV into NADH oxidase supercomplex stabilizes complex I in Paracoccus denitrificans. J Biol Chem 2004; 279:5000-7.

Su JJ, Qin GZ, Yan RQ, Huang DR, Yang C, Lotlikar PD. The expression of insulin-like growth factor II, hepatitis B virus X antigen and p21 in experimental hepatocarcinogenesis in tree shrews. Ann Acad Med Singapore 1999; 28(1):62-66.

Summers J, O'Connell A, Millman I. Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. Proc Natl Acad Sci US 1975; 72(11):4597-4601.

Summers J, Smolec JM, Snyder R. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc Natl Acad Sci US 1978; 75(9):4533-4537.

Sureau C, Eichberg JW, Hubbard GB, Romet-Lemonne JL, Essex M. A molecularly cloned hepatitis B virus produced in vitro is infectious in a chimpanzee. J Virol 1988; 62(8):3064-3067.

Testut P, Renard CA, Terradillos O, Vitvitski-Trepo L, Tekaia F, Degott C et al. A new hepadnavirus endemic in arctic ground squirrels in Alaska. J Virol 1996; 70(7):4210-4219.

Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH et al. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 2003; 77(1):68-76.

Thornton SM, Walker S, Zuckerman JN. Management of hepatitis B virus infections in two gibbons and a western lowland gorilla in a zoological collection. Vet Rec 2001; 149(4):113-115.

Tillmann HL. Antiviral therapy and resistance with hepatitis B virus infection. World J Gastroenterol 2007; 13:125-40.

Tokarska-Schlattner M, Boissan M, Munier A, Borot C, Mailleau C, Speer O, Schlattner U, Lacombe ML. The nucleoside diphosphate kinase D (NM23-H4) binds the inner mitochondrial membrane with high affinity to cardiolipin and couples nucleotide transfer with respiration. J Biol Chem 2008; 283:26198-207.

Tsui LV, Guidotti LG, Ishikawa T, Chisari FV. Posttranscriptional clearance of hepatitis B virus RNA by cytotoxic T lymphocyte-activated hepatocytes. Proc Natl Acad Sci US 1995; 92(26):12398-12402.

Tuttleman JS, Pugh JC, Summers JW. In vitro experimental infection of primary duck hepatocyte cultures with duck hepatitis B virus. J Virol 1986; 58(1):17-25.

Urban S, Gripon P. Inhibition of duck hepatitis B virus infection by a myristoylated pre-S peptide of the large viral surface protein. J Virol 2002; 76(4):1986-1990.

Verschoor EJ, Warren KS, Langenhuijzen S, Heriyanto, Swan RA, Heeney JL. Analysis of two genomic variants of orang-utan hepadnavirus and their relationship to other primate hepatitis B-like viruses. J Gen Virol 2001; 82(Pt 4):893-897.

Villet S, Pichoud C, Billioud G, Barraud L, Durantel S, Trepo C, Zoulim F. Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. J Hepatol 2008; 48:747-55.

Wahl M, Iwarson S, Snoy P, Gerety RJ. Failure of hepatitis B immune globulin to protect against exp infection in chimpanzees. J Hepatol 1989; 9(2):198-203.

Wang GH, Seeger C. The reverse transcriptase of hepatitis B virus acts as a protein primer for viral DNA synthesis. Cell 1992; 71(4):663-670.

Warren KS, Heeney JL, Swan RA, Heriyanto, Verschoor EJ. A new group of hepadnaviruses naturally infecting orangutans (Pongo pygmaeus). J Virol 1999; 73(9):7860-7865.

Watts NR, Conway JF, Cheng N, Stahl SJ, Belnap DM, Steven AC et al. The morphogenic linker peptide of HBV capsid protein forms a mobile array on the interior surface. EMBO J 2002; 21(5):876-884.

Weber M, Bronsema V, Bartos H, Bosserhoff A, Bartenschlager R, Schaller H. Hepadnavirus P protein utilizes a tyrosine residue in the TP domain to prime reverse transcription. J Virol 1994; 68(5):2994-2999.

Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004; 126(7):1750-1758.

Wieland S, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. Proc Natl Acad Sci US 2004; 101(17):6669-6674.

Will H, Darai G, Deinhardt F, Schellekens H, Schaller H. Hepatitis B after infection of a chimpanzee with cloned HBV DNA. Dev Biol Stand 1983; 54:131-133.

Will H, Reiser W, Weimer T, Pfaff E, Buscher M, Sprengel R et al. Replication strategy of human hepatitis B virus. J Virol 1987; 61(3):904-911.

Wong SN, Lok AS. Treatment of hepatitis B: who, when, and how? Arch Intern Med 2006; 166:9-12.

Xin W, Wang JH. Treatment of duck hepatitis B virus by antisense poly-2'-O-(2,4-dinitrophenyl)-oligoribonucleotides. Antisense Nucleic Acid Drug Dev 1998; 8(6):459-468.

Yan RQ, Su JJ, Huang DR, Gan YC, Yang C, Huang GH. Human hepatitis B virus and hepatocellular carcinoma. I. Experimental infection of tree shrews with hepatitis B virus. J Cancer Res Clin Oncol 1996; 122(5):283-288.

Yan RQ, Su JJ, Huang DR, Gan YC, Yang C, Huang GH. Human hepatitis B virus and hepatocellular carcinoma. II. Experimental induction of hepatocellular carcinoma in tree shrews exposed to hepatitis B virus and aflatoxin B1. J Cancer Res Clin Oncol 1996; 122(5):289-295. b

Yang H, Qi X, Sabogal A, Miller M, Xiong S, Delaney WE. Cross-resistance testing of next-generation nucleoside and nucleotide analogues against lamivudine-resistant HBV. Antivir Ther 2005; 10(5):625-633.

Yoshida EM, Ramji A, Chatur N, Davis JE, Owen DA. Regression of cirrhosis associated with hepatitis B e (HBe) antigen-negative chronic hepatitis B infection with prolonged lamivudine therapy. Eur J Gastroenterol Hepatol 2004; 16(3):355-358.

Zhang Z, Torii N, Hu Z, Jacob J, Liang TJ. X-deficient woodchuck hepatitis virus mutants behave like attenuated viruses and induce protective immunity in vivo. J Clin Invest 2001; 108(10):1523-1531.

Zoulim F. In vitro models for studying hepatitis B virus drug resistance. Semin Liver Dis 2006; 26(2):171-180.

Zoulim F, Saputelli J, Seeger C. Woodchuck hepatitis virus X protein is required for viral infection in vivo. J Virol 1994; 68(3):2026-2030.

Zoulim F, Seeger C. Reverse transcription in hepatitis B viruses is primed by a tyrosine residue of the polymerase. J Virol 1994; 68(1):6-13.

Zoulim, F. In vitro models for studying hepatitis B virus drug resistance. Semin Liver Dis 2006; 26:171-80.

Zuckerman AJ, Scalise G, Mazaheri MR, Kremastinou J, Howard CR, Sorensen K. Transmission of hepatitis B to the rhesus monkey. Dev Biol Stand 1975; 30:236-239.

Zuckerman AJ, Thornton A, Howard CR, Tsiquaye KN, Jones DM, Brambell MR. Hepatitis B outbreak among chimpanzees at the London Zoo. Lancet 1978; 2(8091):652-654.

Chapter 6: HCV - Virology

Bernd Kupfer

History

Hepatitis C virus (HCV) is a major cause of progressive liver disease with approximately 170 million people infected worldwide. HCV induces chronic infection in up to 80% of infected individuals. The main complications of HCV infection are severe liver fibrosis and cirrhosis, and 30-50% of individuals with cirrhosis go on to develop hepatocellular carcinoma (Tong 1995; Poynard 1997).

Until 1975, only two hepatitis viruses had been identified, the "infectious hepatitis virus" (hepatitis A virus, HAV) and the "serum hepatitis virus" (hepatitis B virus, HBV). However, other viruses were excluded from being the cause of approximately 65% of post-transfusion hepatitis. Therefore, these hepatitis cases were termed "non-A, non-B hepatitis" (NANBH) (Feinstone 1975). Inoculation of chimpanzees (Pan troglodytes) with blood products derived from humans with NANB hepatitis led to persistent increases of serum alanine aminotransferase (ALT) indicating that an infectious agent was the cause of the disease (Alter 1978; Hollinger 1978). Subsequently, it was demonstrated that the NANBH agent could be inactivated by chloroform (Feinstone 1983). Moreover, it was reported that the infectious agent was able to pass through 80 nm membrane filters (Bradley 1985). Taken together these findings suggested that the NANBH causing agent would be a small virus with a lipid envelope. However, the lack of a suitable cell culture system for cultivation of the NANBH agent and the limited availability of chimpanzees prevented further characterization of the causative agent of NANBH for several years. In 1989, using a newly developed cloning strategy for nucleic acids derived from plasma of NANBH infected chimpanzees the genome of the major causative agent for NANBH was characterized (Choo 1989). cDNA clone 5-1-1 encoded immunological epitopes that interacted with sera from individuals with NANBH (Choo 1989; Kuo 1989). The corresponding infectious virus causing the majority of NANBH was subsequently termed hepatitis C virus (HCV).

Taxonomy and genotypes

HCV is a small-enveloped virus with one single-stranded positive-sense RNA molecule of approximately 9.6 kb. It is a member of the Flaviviridae family. This viral family contains three genera, flavivirus, pestivirus, and hepacivirus. To date, only two members of the hepacivirus genus have been identified, HCV and GB virus B (GBV-B), a virus that had been initially detected together with the then-unclassified virus GB virus A (GBV-A) in a surgeon with active hepatitis (Thiel 2005; Ohba 1996; Simons 1995). However, the natural hosts for GBV-B and GBV-C seem to be monkeys of the Saguinus species (tamarins). Analyses of viral sequences and phylogenetic comparisons support HCV's membership in a distinct genus from flavivirus or pestivirus (Choo 1991).

The error-prone RNA polymerase of HCV together with the high replication rate of the virus is responsible for the large interpatient genetic diversity of HCV strains. Moreover, the extent of viral diversification of HCV strains within a single

HCV-positive individual increases significantly over time resulting in the development of quasispecies (Bukh 1995).

Comparisons of HCV nucleotide sequences derived from individuals from different geographical regions revealed the presence of six major HCV genotypes with a large number of subtypes within each genotype (Simmonds 2004; Simmonds 2005). Sequence divergence of genotypes and subtypes is 20% and 30%, respectively. HCV strains belonging to the major genotypes 1, 2, 4, and 5 are found in sub-Saharan Africa whereas genotypes 3 and 6 are detected with extremely high diversity in South East Asia. This suggests that these geographical areas could be the origin of the different HCV genotypes. The emergence of different HCV genotypes in North America and Europe and other non-tropical countries appears to represent more recent epidemics introduced from the countries of the original HCV endemics (Simmonds 2001; Ndjomou 2003). Besides epidemiological aspects determination of the HCV genotype plays an important role for the initiation of anti-HCV treatment since the response of different genotypes varies significantly with regard to specific antiviral drug regimens, e.g., genotype 1 is most resistant to the current therapy of the combination of pegylated interferon alpha and ribavirin (Manns 2001; Fried 2002).

Viral structure

Structural analyses of HCV virions are very limited since the virus is difficult to cultivate in cell culture systems, a prerequisite for yielding sufficient virions for electron microscopy. Moreover, serum-derived virus particles are associated with serum low-density lipoproteins (Thomssen 1992), which makes it difficult to isolate virions from serum/plasma of infected subjects by centrifugation. Visualization of HCV virus-like particles via electron microscopy succeeded only rarely (Kaito 1994; Shimizu 1996a; Prince 1996) and it was a point of controversy if the detected structures really were HCV virions. Nevertheless, these studies suggest that HCV has a diameter of 55 to 65 nm confirming size prediction of the NANBH agent by ultra-filtration (Bradley 1985). Various forms of HCV virions appear to exist in the blood of infected individuals: virions bound to very low density lipoproteins (VLDL), virions bound to low density lipoproteins (LDL), virions complexed with immunoglobulins, and free circulating virions (Bradley 1991; Thomssen 1992; Thomssen 1993; Agnello 1999; Andre 2002). The reasons for the close association of a major portion of circulating virions with LDL and VLDL remain unexplained. One possible explanation is that HCV theoretically enters hepatocytes via the LDL receptor (Agnello 1999; Nahmias 2006). Moreover, it is speculated that the association with LDL and/or VLDL protects the virus against neutralization by HCV-specific antibodies.

The design and optimization of subgenomic and genomic HCV replicons in the human hepatoma cell line Huh7 offered for the first time the possibility to investigate HCV RNA replication in a standardized manner (Lohmann 1999; Ikeda 2002; Blight 2002). However, despite the high level of HCV gene expression no infectious viral particles are actually produced. Therefore, it cannot be used for structural analysis of free virions.

Recently infectious HCV particles have been achieved in cell culture by using recombinant systems (Heller 2005; Lindenbach 2005; Wakita 2005; Zhong 2005; Yu 2007). However, even in these *in vitro* systems the limited production of viral particles prevents

3D structural analysis (Yu 2007). Very recently, it was shown by cryo-electron microscopy (cryoEM) and negative-stain transmission electron microscopy that HCV virions isolated from cell culture have a spherical shape with a diameter of approximately 50 to 55 nm (Heller 2005; Wakita 2005; Yu 2007) confirming earlier results that measured the size of putative native HCV particles from the serum of infected individuals (Prince 1996). The outer surface of the viral envelope seems to be smooth. Size and morphology are therefore very similar to other members of the Flaviviridae family such as the dengue virus and the West Nile virus (Yu 2007). Modifying a baculovirus system (Jeong 2004; Qiao 2004) the same authors were able to produce large quantities of HCV-like particles (HCV-LP) in insect cells (Yu 2007). Analysing the HCV-LPs by cryoEM it was demonstrated that the HCV E1 protein is present in spikes located on the outer surface of the LPs.

Using 3D modeling of the HCV-LPs together with genomic comparison of HCV and well-characterized flaviviruses it is assumed that 90 copies of a block of two heterodimers of HCV proteins E1 and E2 form the outer layer of the virions with a diameter of approximately 50 nm (Yu 2007). This outer layer surrounds the lipid bilayer that contains the viral nucleocapsid consisting of several molecules of the HCV core (C) protein. An inner spherical structure with a diameter of approximately 30-35 nm has been observed (Wakita 2005) suggesting the nucleocapsid that harbours the viral genome (Takahashi 1992).

Genome organization

The genome of the hepatitis C virus consists of one 9.6 kb single-stranded RNA molecule with positive polarity. Similar to other positive-strand RNA viruses, the genomic RNA of hepatitis C virus serves as messenger RNA (mRNA) for the translation of viral proteins. The linear molecule contains a single open reading frame (ORF) coding for a precursor polyprotein of approximately 3000 amino acid residues (Figure 1). During viral replication the polyprotein is cleaved by viral as well as host enzymes into three structural proteins (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). An additional protein (termed F [frameshift] or ARF [alternate reading frame]) is predicted as a result of ribosomal frameshifting during translation within the core-region of the genomic RNA (Xu 2001; Walewski 2001; Varaklioti 2002; Branch 2005). Detection of anti-F protein antibodies in the serum of HCV-positive subjects indicates that the protein is expressed during infection *in vivo* (Walewski 2001; Komurian-Pradel 2004).

The structural genes encoding the viral core protein and the viral envelope proteins E1 and E2 are located at the 5' terminus of the open reading frame followed downstream by the coding regions for the non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (Figure 1). The structural proteins are essential components of the HCV virions, whereas the non-structural proteins are not associated with virions but are involved in RNA replication and virion morphogenesis.

The ORF is flanked by 5' and 3' nontranslated regions (NTR; also called untranslated regions, UTR or noncoding regions, NCR) containing nucleotide sequences relevant for the regulation of viral replication. Both NTRs harbour highly conserved regions compared to the protein encoding regions of the HCV genome. The high grade of conservation of the NTRs makes them candidates i) for improved molecular diagnostics, ii) as targets for antiviral therapeutics, and iii) as targets for anti-HCV vaccine

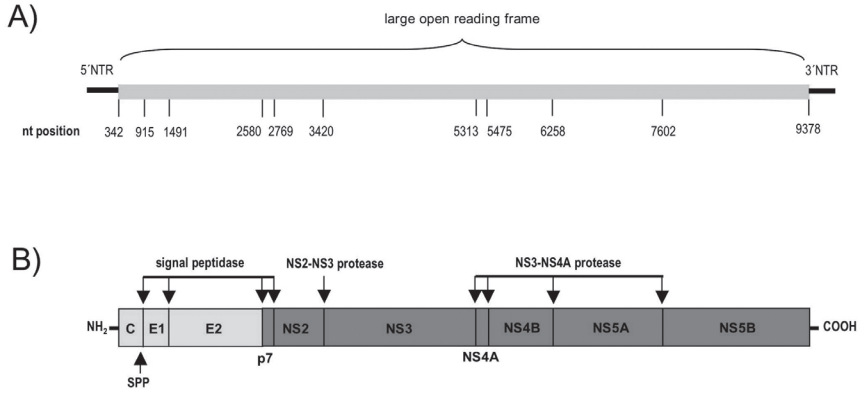


Figure 1. Genome organization and polyprotein processing.
A) Nucleotide positions correspond to the HCV strain H77 genotype 1a, accession number NC_004102. nt, nucleotide; NTR, nontranslated region. B) Cleavage sites within the HCV precursor polyprotein for the cellular signal peptidase the signal peptide peptidase (SPP) and the viral proteases NS2-NS3 and NS3-NS4A, respectively.

The 5'NTR is approximately 341 nucleotides long with a complex secondary structure of four distinct domains (I-IV) (Fukushi 1994; Honda 1999). The first 125 nucleotides of the 5'NTR spanning domains I and II have been shown to be essential for viral RNA replication (Friebe 2001; Kim 2002). Domains II-IV build an internal ribosome entry side (IRES) involved in ribosome-binding and subsequent cap-independent initiation of translation (Tsukiyama-Kohara 1992; Wang 1993).

The 3'NTR consists of three functionally distinct regions: a variable region, a poly U/UC tract of variable length, and the highly conserved X tail at the 3' terminus of the HCV genome (Tanaka 1995; Kolykhalov 1996; Blight 1997). The variable region of approximately 40 nucleotides is not essential for RNA replication. However, deletion of this sequence led to significantly decreased replication efficiency (Yanagi 1999; Friebe 2002). The length of the poly U/UC region varies in different HCV strains ranging from 30 to 80 nucleotides (Kolykhalov 1996). The minimal length of that region for active RNA replication has been reported to be 26 homouridine nucleotides in cell culture (Friebe 2002). The highly conserved 98 nucleotide X tail consists of three stem-loops (SL1-SL3) (Tanaka 1996; Ito 1997; Blight 1997) and deletions or nucleotide substitutions within that region are most often lethal (Yanagi 1999; Kolykhalov 2000; Friebe 2002; Yi 2003). Another so-called "kissing-loop" interaction of the 3'X tail SL2 and a complementary portion of the NS5B encoding region has been described (Friebe 2005). This interaction induces a tertiary RNA structure of the HCV genome that is essential for HCV replication in cell culture systems (Friebe 2005; You 2008). Finally, both NTRs appear to work together in a long-range RNA-RNA interaction possibly resulting in temporary genome circularization (Song 2006).

Genes and proteins

As described above, translation of the HCV polyprotein is initiated through involvement of some domains in NTRs of the genomic HCV RNA. The resulting polyprotein consists of ten proteins that are co-translationally or post-translationally cleaved from the polyprotein. The N-terminal proteins C, E1, E2, and p7 are processed by a cellular signal peptidase (SP) (Hijikata 1991). The resulting immature core protein still contains the E1 signal sequence at its C terminus. Subsequent cleavage of this sequence by a signal peptide peptidase (SPP) leads to the mature core protein (McLauchlan 2002).

The non-structural proteins NS2 to NS5B of the HCV polyprotein are processed by two virus-encoded proteases (NS2-NS3 and NS3) with the NS2-NS3 cysteine protease cleaving at the junction of NS2-NS3 (Santolini 1995) and the NS3 serine protease cleaving the remaining functional proteins (Bartenschlager 1993; Eckart 1993; Grakoui 1993a; Tomei 1993).

The positions of viral nucleotide and amino acid residues correspond to the HCV strain H77 genotype 1a, accession number NC_004102. Some parameters characterizing HCV proteins are summarised in Table 1.

Protein	# of aa	aa position in ref. seq.	MW of protein
Core immature	191	1-191	23 kd
Core mature	174	1-174	21 kd
F-protein or ARF-protein	126-161		~ 16-17 kd
E1	192	192-383	35 kd
E2	363	384-746	70 kd
p7	63	747-809	7 kd
NS2	217	810-1026	21 kd
NS3	631	1027-1657	70 kd
NS4A	54	1658-1711	4 kd
NS4B	261	1712-1972	27 kd
NS5A	448	1973-2420	56 kd
NS5B	591	2421-3011	66 kd

Table 1. Overview of the size of HCV proteins.
aa, amino acid; MW, molecular weight; kd, kilodalton; ref. seq., reference sequence (HCV strain H77; accession number NC_004102).

Core. The core-encoding sequence starts at codon AUG at nt position 342 of the H77 genome, the start codon for translation of the entire HCV polyprotein. During translation the polyprotein is transferred to the endoplasmic reticulum (ER) where the core protein (191 aa) is excised by a cellular signal peptidase (SP). The C terminus of the resulting core precursor still contains the signal sequence for ER membrane translocation of the E1 ectodomain (aa 174-191). This protein region is further processed by the cellular intramembrane signal peptide peptidase (SPP) leading to removal of the E1 signal peptide sequence (Hüssy 1996; McLauchlan 2002; Weihofen 2002).

The multifunctional core protein has a molecular weight of 21 kilodalton (kd). *In vivo*, the mature core molecules are believed to form homo-multimers located mainly at the ER membrane (Matsumoto 1996). They have a structural function since they form the viral capsid that contains the HCV genome. In addition, the core protein has regulatory functions including particle assembly, viral RNA binding, and regulation of RNA translation (Ait-Goughoulte 2006; Santolini 1994). Moreover, protein expression analyses indicate that the core protein may be involved in many other cellular reactions such as cell signalling, apoptosis, lipid metabolism, and carcinogenesis (Tellinghuisen 2002). However, these preliminary findings need to be analyzed further.

E1 and E2. Downstream of the core-coding region of HCV RNA genome two envelope glycoproteins are encoded, E1 (gp35; 192 aa) and E2 (gp70; 363 aa). During translation at the ER both proteins are cleaved from the precursor polyprotein by a cellular SP. Inside the lumen of the ER both polypeptides experience N-linked glycosylation post-translationally (Duvet 2002). Both glycoproteins E1 and E2 harbour 5 and 11 putative N-glycosylation sites, respectively.

E1 and E2 are type I transmembrane proteins with a large hydrophilic ectodomain of approximately 160 and 334 aa and a short transmembrane domain (TMD) of 30 aa. The TMD are responsible for the anchoring of the envelope proteins in the membrane of the ER and ER retention (Cocquerel 1998; Duvet 1998; Cocquerel 1999; Cocquerel 2001). Moreover, the same domains have been reported to contribute to the formation of E1-E2 heterodimers (Op de Beeck 2000). The E1-E2 complex is involved in adsorption of the virus to its putative receptors tetraspanin CD81 and low density lipoprotein receptor inducing fusion of the viral envelope with the host cell plasma membrane (Agnello 1999; Flint 1999; Wunschmann 2000). However, the precise mechanism of host cell entry is still not understood completely. Several other host factors have been identified to be involved in viral entry. These candidates include the scavenger receptor B type I (Scarselli 2002; Kapadia 2007), the tight junction proteins claudin-1 (Evans 2007) and occludin (Ploss 2009), the C-type lectins L-SIGN and DC-SIGN (Gardner 2003; Lozach 2003; Pöhlmann 2003) and heparan sulfate (Barth 2003).

Two hypervariable regions have been identified within the coding region of E2. These regions termed hypervariable region 1 (HVR1) and 2 (HVR2) differ by up to 80% in their amino acid sequence (Weiner 1991; Kato 2001). The first 27 aa of the E2 ectodomain represent HVR1, while the HVR2 is formed by a stretch of seven amino acids (position 91-97). The high variability of the HVRs reflects exposure of these domains to HCV-specific antibodies. In fact, E2-HVR1 has been shown to be the most important target for neutralizing antibodies (Farci 1996; Shimizu 1996b). However, the combination of the mutation of the viral genome with the selective pressure of the humoral immune response leads to viral escape via epitope alterations. This makes the development of vaccines that induce neutralizing antibodies challenging.

The p7 protein. The small p7 protein (63 aa) is located between the E2 and NS2 regions of the polyprotein precursor. During translation the cellular SP cleaves the E2-p7 as well as the p7-NS2 junction. The functional p7 is a membrane protein which is localized in the endoplasmic reticulum where it forms an ion channel (Haqshenas

2007; Pavlovic 2003; Griffin 2003). The p7 protein is not essential for RNA replication since replicons lacking the p7 gene replicate efficiently (Lohmann 1999; Blight 2000), however, it has been suggested that p7 plays an essential role for the formation of infectious virions (Sakai 2003; Haqshenas 2007).

NS2. The non-structural protein 2 (p21; 217 aa) together with the N-terminal portion of the NS3 protein form the NS2-3 cysteine protease which catalyses cleavage of the polyprotein precursor between NS2 and NS3 (Grakoui 1993b; Santolini 1995). The N-terminus of the functional NS2 arises from the cleavage of the p7-NS2 junction by the cellular SP. Moreover, after cleavage from the NS3 the protease domain of NS2 seems to play an essential role in the early stage of virion morphogenesis (Jones 2007).

NS3. The non-structural protein 3 (p70; 631 aa) is cleaved at its N terminus by the NS2-NS3 protease. The N terminus (189 aa) of the NS3 protein has a serine protease activity. However, in order to develop full activity of the protease the NS3 protease domain requires a portion of NS4A (Faila 1994; Bartenschlager 1995; Lin 1995; Tanji 1995; Tomei 1996). NS3 together with the NS4A cofactor are responsible for cleavage of the remaining downstream cleavages of the HCV polyprotein precursor. Since the NS3 protease function is essential for viral infectivity it is a promising target in the design of antiviral treatments.

The C-terminal portion of NS3 (442 aa) has ATPase/helicase activity, i.e., it catalyses the binding and unwinding of the viral RNA genome during viral replication (Jin 1995; Kim 1995). However, recent findings indicate that other non-structural HCV proteins such as the viral polymerase NS5B may interact functionally with the NS3 helicase (Jennings 2008). These interactions need to be investigated further in order to better understand the mechanisms of HCV replication.

NS4A. The HCV nonstructural protein 4A (p4) is a 54 amino acid polypeptide that acts as a cofactor of the NS3 serine protease (Faila 1994; Bartenschlager 1995; Lin 1995; Tanji 1995; Tomei 1996). Moreover, this small protein is involved in the targeting of NS3 to the endoplasmic reticulum resulting in a significant increase of NS3 stability (Wölk 2000).

NS4B. The NS4B (p27) consists of 217 amino acids. It is an integral membrane protein localized in the endoplasmic reticulum. The N-terminal domain of the NS4B has an amphipathic character that targets the protein to the ER. This domain is crucial in HCV replication (Elazar 2004; Gretton 2005) and therefore an interesting target for the development of anti-HCV therapeutics or vaccines. In addition, a nucleotide-binding motif (aa 129-134) has been identified (Einav 2004). Although the function of NS4B is still unknown, it has been demonstrated that the protein induces a membranous web that may serve as a platform for HCV RNA replication (Egger 2002).

NS5A. The NS5A protein (p56; 458 aa) is a membrane-associated phosphoprotein that appears to have multiple functions in viral replication. It is phosphorylated by different cellular protein kinases indicating an essential but still not understood role of

NS5A in the HCV replication cycle. In addition, NS5A has been found to be associated with several other cellular proteins (MacDonald 2004) making it difficult to determine the exact functions of the protein. One important property of NS5A is that it contains a domain of 40 amino acids, the so-called IFN-α sensitivity-determining region (ISDR), that plays a significant role in the response to IFN-α-based therapy (Enomoto 1995; Enomoto 1996). An increasing number of mutations within the ISDR showed positive correlation with sustained virological response to IFN-α-based treatment.

NS5B. The non-structural protein 5B (p66; 591 aa) represents the RNA-dependent RNA polymerase of HCV (Behrens 1996). The hydrophobic domain (21 aa) at the C terminus of NS5B inserts into the membrane of the endoplasmic reticulum, while the active sites of the polymerase are located in the cytoplasm (Schmidt-Mende 2001).

The cytosolic domains of the viral enzyme form the typical polymerase right-handed structure with "palm", "fingers", and "thumb" subdomains (Ago 1999; Bressanelli 1999; Lesburg 1999). In contrast to mammalian DNA and RNA polymerases the fingers and thumb subdomains are connected resulting in a fully enclosed active site for nucleotide triphosphate binding. This unique structure makes the HCV NS5B polymerase an attractive target for the development of antiviral drugs.

Using the genomic HCV RNA as a template, the NS5B promotes the synthesis of minus-stranded RNA that then serves as a template for the synthesis of genomic positive-stranded RNA by the polymerase.

Similar to other RNA-dependent polymerases, NS5B is an error-prone enzyme that incorporates wrong ribonucleotides at a rate of approximately 10-3 per nucleotide per generation. Unlike cellular polymerases, the viral NS5B lacks a proof-reading mechanism leading to the conservation of misincorporated ribonucleotides. These enzyme properties together with the high rate of viral replication promote a pronounced intra-patient as well as inter-patient HCV evolution.

F protein, ARFP. In addition to the ten proteins derived from the long HCV ORF, the F (frameshift) or ARF (alternate reading frame) or core+1 protein has been reported (Walewski 2001; Xu 2001; Varaklioti 2002). As the designations indicate the ARFP is the result of a -2/+1 ribosomal frameshift between codons 8 and 11 of the core protein-encoding region. The ARFP length varies from 126 to 161 amino acids depending on the corresponding genotype. *In vitro* studies have shown that ARFP is a short-lived protein located in the cytoplasm (Roussel 2003) primarily associated with the endoplasmic reticulum (Xu 2003). Detection of anti-F protein antibodies in the serum of HCV-positive subjects indicates that the protein is expressed during infection *in vivo* (Walewski 2001; Komurian-Pradel 2004). However, the functions of ARFP in the viral life cycle are still unknown and remain to be elucidated.

Viral lifecycle

Due to the absence of a small animal model system and efficient *in vitro* HCV replication systems it has been difficult to investigate the viral life cycle of HCV. The recent development of such systems has offered the opportunity to analyse in detail the different steps of viral replication.

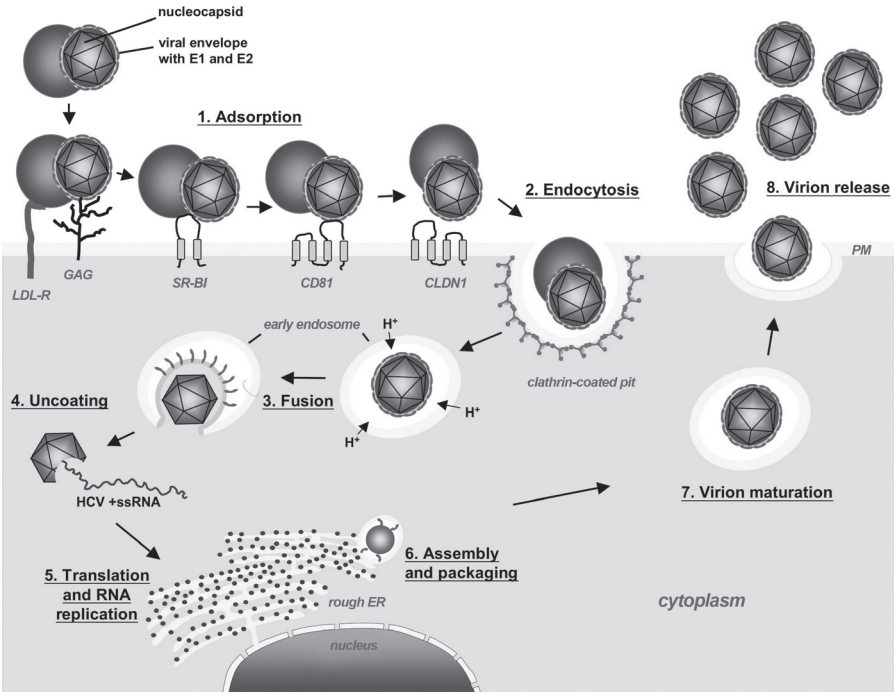


Figure 2. Current model of the HCV lifecycle.
Designations of cellular components are in itallics. For a detailed illustration of viral translation and RNA replication, see Pawlotsky 2007. HCV +ssRNA, single stranded genomic HCV RNA with positive polarity; rough ER, rough endoplasmic reticulum; PM, plasma membrane. For other abbreviations see text.

Adsorption and viral entry

The most likely candidate as receptor for HCV is the tetraspanin CD81 (Pileri 1998). CD81 is a ubiquitous 25 kd molecule expressed on the surface of a large variety of cells including hepatocytes and PBMCs. Experimental binding of anti-CD81 antibodies to CD81 were reported to inhibit HCV entry into Huh7 cells and primary human hepatocytes (Hsu 2003; Bartosch 2003a; Cormier 2004; McKeating 2004; Zhang 2004; Lindenbach 2005; Wakita 2005). Moreover, gene silencing of CD81 using specific siRNA molecules confirmed the relevance of CD81 in viral entry (Bartosch 2003b; Cormier 2004; Zhang 2004; Akazawa 2007). Finally, expression of CD81 in cell lines lacking CD81 made them permissive for HCV entry (Zhang 2004; Lavillette 2005; Akazawa 2007). However, more recent studies have shown that CD81 alone is not sufficient for HCV viral entry and that co-factors such as scavenger receptor B type I (SR-BI) are needed (Bartosch 2003b; Hsu 2003; Scarselli 2002, Kapadia 2007). Moreover, it appears that CD81 is involved in a post-HCV binding step (Cormier 2004; Koutsoudakis 2006; Bertaud 2006). These findings together with the identification of other host factors involved in HCV cell entry generate the current model for the early steps of HCV infection (Helle 2008).

Adsorption of HCV to its target cell is the first step of viral entry. Binding is possibly initiated by the interaction of the HCV E2 envelope glycoprotein and the glycosaminglycan heparan sulfate on the surface of host cells (Germi 2002; Barth 2003; Basu 2004; Heo 2004). Moreover, it is assumed that HCV initiates hepatocyte infection via LDL receptor binding (Agnello 1999; Monazahian 1999; Wünschmann 2000; Nahmias 2006; Molina 2007). This process may be mediated by VLDL or LDL, reported to be associated with HCV virions in human sera (Bradley 1991; Thomssen 1992; Thomssen 1993). After initial binding the HCV E2 glycoprotein interacts with the SR-BI in cell culture (Scarselli 2002). SR-BI is a protein expressed on the surface of the majority of mammalian cells. It acts as a receptor for LDL as well as HDL (Acton 1994; Acton 1996) emphasizing the role of these compounds for HCV infectivity. Alternative splicing of the SR-BI transcript leads to the expression of a second isoform of the receptor SR-BII (Webb 1998), which also may be involved in HCV entry into target cells (Grove 2007). As is the case for all steps of viral entry the exact mechanism of the HCVE2/SR-BI interaction remains unknown. In some studies it has been reported that HCV binding to SR-BI is a prerequisite for the concomitant or subsequent interaction of the virus with CD81 (Kapadia 2007; Zeisel 2007). The multi-step procedure of HCV cell entry was shown to be even more complex since a cellular factor termed claudin-1 (CLDN1) has been newly identified as involved in this process (Evans 2007). CLDN1 is an integral membrane protein that forms a backbone of tight junctions and is highly expressed in the liver (Furuse 1998). Inhibition assays reveal that CLDN1 involvement occurs downstream of the HCV-CD81 interaction (Evans 2007). Recent findings suggest that CLDN1 could also act as a compound enabling cell-to-cell transfer of hepatitis C virus independently of CD81 (Timpe 2007). Furthermore, it was reported that two other members of the claudin family claudin-6 and claudin-9 may play a role in HCV infection (Zheng 2007; Meertens 2008).

After the complex procedure of binding to the different host membrane factors HCV enters the cell in a pH-dependent manner indicating that the virus is internalized via clathrin-mediated endocytosis (Bartosch 2003b; Hsu 2003; Blanchard 2006; Codran 2006). The acidic environment within the endosomes is assumed to trigger HCV E1-E2 glycoprotein-mediated fusion of the viral envelope with the endosome membrane (Blanchard 2006; Meertens 2006, Lavillette 2007).

In summary, HCV adsorption and viral entry into the target cell is a very complex procedure that is not yet fully understood. Despite having identified several host factors that probably interact with the viral glycoproteins the precise mechanisms of interaction need to continue to be investigated. The fact that some human cell lines are not susceptible to HCV infection despite expressing SR-BI, CD81, and CLDN1 indicates that other cellular factors are involved in viral entry (Evans 2007). Very recently, a cellular four transmembrane domain protein named occludin (OCLN) was identified to represent an additional cellular factor essential for the susceptibility of cells to HCV infection (Ploss 2009). Similar to claudin-1 OCLN is a component of the tight junctions in hepatocytes. All tested cells expressing SR-BI, CD81, CLDN1, and OCLN were susceptible to HCV. Although the precise mechanism of HCV uptake in hepatocytes is still not clarified, these four proteins may represent the complete set of host cell factors necessary for cell-free HCV entry.

Besides the infection of cells through cell-free HCV it has been documented that HCV can also spread via cell-to-cell transmission (Valli 2006; Valli 2007). This transmission path may differ significantly with regard to the cellular factors needed for HCV entry into cells. CD81 is dispensable for cell-to-cell transmission in cultivated hepatoma cells (Witteveldt 2009). These findings require further investigation in order to analyze the process of cell-to-cell transmission of HCV both *in vitro* and *in vivo*. Antiviral treatment strategies must account for the cellular pathways of both cell-free virus and HCV transmitted via cell-to-cell contact.

Translation and posttranslational processes.

As a result of the fusion of the viral envelope and the endosomic membrane, the genomic HCV RNA is released into the cytoplasm of the cell. As described above, the viral genomic RNA possesses a nontranslated region (NTR) at each terminus. The 5'NTR consists of four distinct domains, I-IV. Domains II-IV form an internal ribosome entry side (IRES) involved in ribosome-binding and subsequent cap-independent initiation of translation (Fukushi 1994; Honda 1999; Tsukiyama-Kohara 1992; Wang 1993). The HCV-IRES binds to the 40S ribosomal subunit complexed with eukaryotic initiation factors 2 and 3 (eIF2 and eIF3), GTP, and the initiator tRNA resulting in the 48S preinitiation complex (Spahn 2001; Otto 2002; Sizova 1998; reviewed in Hellen 1999). Subsequently, the 60S ribosomal subunit associates with that complex leading to the formation of the translational active complex for HCV polyprotein synthesis at the endoplasmic reticulum. HCV RNA contains a large ORF encoding a polyprotein precursor. Posttranslational cleavages lead to 10 functional viral proteins Core, E1, E2, p7, NS2-NS5B. The viral F protein (or ARF protein) originates from a ribosomal frameshift within the first codons of the core-encoding genome region (Walewski 2001; Xu 2001; Varaklioti 2002). Besides several other cellular factors that have been reported to be involved in HCV RNA translation, various viral proteins and genome regions have been shown to enhance or inhibit viral protein synthesis (Zhang 2002; Kato 2002; Wang 2005; Kou 2006; Bradrick 2006; Song 2006).

The precursor polyprotein is processed by at least four distinct peptidases. The cellular signal peptidase (SP) cleaves the N-terminal viral proteins immature core protein, E1, E2, and p7 (Hijikata 1991), while the cellular signal peptide peptidase (SPP) is responsible for the cleavage of the E1 signal sequence from the C-terminus of the immature core protein, resulting in the mature form of the core (McLauchlan 2002). The E1 and E2 proteins remain within the lumen of the ER where they are subsequently N-glycosylated with E1 having 5 and E2 harbouring 11 putative N-glycosylation sites (Duvet 2002).

In addition to the two cellular peptidases HCV encodes two viral enzymes responsible for cleavage of the non-structural proteins NS2 to NS5B within the HCV polyprotein precursor. The zinc-dependent NS2-NS3 cysteine protease consisting of the NS2 protein and the N-terminal portion of NS3 autocatalytically cleaves the junction between NS2 and NS3 (Santolini 1995), whereas the NS3 serine protease cleaves the remaining functional proteins (Bartenschlager 1993; Eckart 1993; Grakoui 1993a; Tomei 1993). However, for its peptidase activity NS3 needs NS4A as a cofactor (Failla 1994; Tanji 1995; Bartenschlager 1995; Lin 1995; Tomei 1996).

HCV RNA replication

The complex process of HCV RNA replication is poorly understood. The key enzyme for viral RNA replication is NS5B, an RNA-dependent RNA polymerase (RdRp) of HCV (Behrens 1996). In addition, several cellular as well as viral factors have been reported to be part of the HCV RNA replication complex. One important viral factor for the formation of the replication complex appears to be NS4B which is able to induce an ER-derived membranous web containing most of the non-structural HCV proteins including NS5B (Egger 2002). This web could serve as the platform for the next steps of viral RNA replication. The RdRp uses the previously released genomic positive-stranded HCV RNA as a template for the synthesis of an intermediate minus-stranded RNA. Recently it has been reported that the cellular peptidyl-prolyl isomerases cyclophilin A, B and C (Cyp A, Cyp B, and Cyp C) could stimulate binding of the RdRp to the viral RNA resulting in increased HCV RNA synthesis (Watashi 2005; Nakagawa 2005; Yang 2008; Heck 2009). However, these reports are in part inconsistent and further studies are needed in order to investigate the involvement of cyclophilins in HCV RNA replication.

After the viral polymerase has bound to its template the NS3 helicase is assumed to unwind putative secondary structures of the template RNA in order to facilitate the synthesis of minus-strand RNA (Jin 1995; Kim 1995). In turn, again with the assistance of the NS3 helicase, the newly synthesized antisense RNA molecule serves as the template for the synthesis of numerous plus-stranded RNA. The resulting sense RNA may be used subsequently as genomic RNA for HCV progeny as well as for polyprotein translation.

Assembly and release

After the viral proteins, glycoproteins, and the genomic HCV RNA have been synthesized these single components have to be arranged in order to produce infectious virions. As is the case for all other steps in the HCV lifecycle viral assembly is a multi-step procedure involving most viral components along with many cellular factors. Investigation of viral assembly and particle release is still in its infancy since the development of *in vitro* models for the production and release of infectious HCV occurred only recently. Previously, it was reported that core protein molecules were able to self-assemble *in vitro*, yielding nucleocapsid-like particles. Very recent findings suggest that viral assembly takes place within the endoplasmic reticulum (Gastaminza 2008) and that lipid droplets (LD) are involved in particle formation (Moradpour 1996; Barba 1997; Miyanari 2007; Shavinskaya 2007; Appel 2008). It appears that LD-associated core protein targets viral non-structural proteins and the HCV RNA replication complex including positive and negative stranded RNA from the endoplasmic reticulum to the LD (Miyanari 2007). Beside the core protein, LD-associated NS5A seems to play a key role in the formation of infectious viral particles (Appel 2008). Moreover, E2 molecules are detected in close proximity to LD-associated membranes. Finally, spherical virus-like particles associated with membranes can be seen very close to the LD. Using specific antibodies the virus-like particles were shown to contain core protein as well as E2 glycoprotein molecules indicating that these structures may represent infectious HCV (Miyanari 2007). However, the precise mechanisms for the formation and release of infectious HCV particles are still unknown.

Model systems for HCV research

For a long time HCV research was limited due to a lack of small animal models and efficient cell culture systems. The development of the first HCV replicon system (HCV RNA molecule, or region of HCV RNA, that replicates autonomously from a single origin of replication) 10 years after the identification of the hepatitis C virus offered the opportunity to investigate the molecular biology of HCV infection in a standardized manner (Lohmann 1999).

HCV replicon systems. Using total RNA derived from the explanted liver of an individual chronically infected with HCV genotype 1b, the entire HCV ORF sequence was amplified and cloned in two overlapping fragments. The flanking NTRs were amplified and cloned separately and all fragments were assembled into a modified full-length sequence. Transfection experiments with *in vitro* transcripts derived from the full-length clones failed to yield viral replication. For this reason, two different subgenomic replicons consisting of the 5' IRES, the neomycin phosphotransferase gene causing resistance to the antibiotic neomycin, the IRES derived from the encephalomyocarditis virus (EMCV) and the NS2-3' NTR or NS3-3' NTR sequence, respectively, were generated (Figure 3).

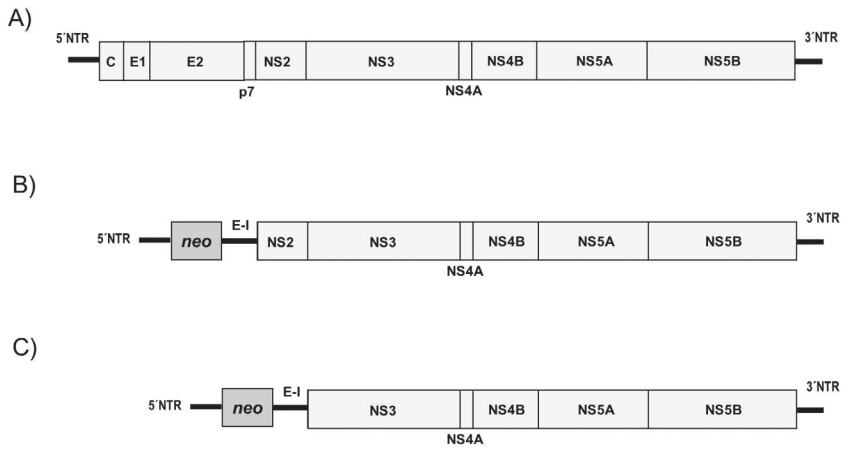


Figure 3. Structure of subgenomic HCV replicons (Lohmann 1999).
This figure illustrates the genetic information of *in vitro* transcripts used for Huh-7 transfection. A) Full-length transcript derived from the explanted liver of a chronically infected subject. B) Subgenomic replicon lacking the structural genes and the sequence encoding p7. C) Subgenomic replicon lacking C, E1, E2, p7, and NS2 genes. neo, neomycin phosphotransferase gene; E-I, IRES of the encephalomyocarditis virus (EMCV).

In vitro transcripts derived from these constructs lacking the genome region coding for the structural HCV proteins were used to transfect the hepatoma cell line Huh7 (Lohmann 1999). The transcripts are bicistronic, i.e., the first cistron containing the HCV IRES enables the translation of the neomycin phosphotransferase as a tool for

efficient selection of successfully transfected cells and the second cistron containing the EMCV IRES directs translation of the HCV-specific proteins. Only some Huh7 clones can replicate replicon-specific RNA in titres of approximately 10^8 positive-stranded RNA copies per microgram total RNA. Moreover, all encoded HCV proteins are detected predominantly in the cytoplasm of the transfected Huh7 cells. The development of this replicon is a milestone in HCV research with regard to the investigation of HCV RNA replication and HCV protein analyses.

More recently, the methodology has been improved in order to achieve significantly higher replication efficiency. Enhancement of HCV RNA replication was achieved by the use of replicons harbouring cell culture-adapted point mutations or deletions within the NS genes (Blight 2000; Lohmann 2001; Krieger 2001). Further development has led to the generation of selectable full-length HCV replicons, i.e., genomic replicons that also contain genetic information for the structural proteins Core, E1, and E2 (Pietschmann 2002; Blight 2002). This improvement offered the opportunity of investigating the influence of the structural proteins on HCV replication. Thus it has been possible to analyse the intracellular localisation of these proteins. However, using this methodology viral assembly and release has not been achieved.

Another important milestone was reached when a subgenomic replicon based on the HCV genotype 2a strain JFH-1 was generated (Kato 2003). This viral strain derived from a Japanese subject with fulminant hepatitis C (Kato 2001). The corresponding replicons showed higher RNA replication efficiency than previous replicons. Moreover, cell lines distinct from Huh7, such as HepG2 or HeLa were transfected efficiently with transcripts derived from the JFH-1 replicon (Date 2004; Kato 2005).

HCV pseudotype virus particles (HCVpp). The generation of retroviral pseudotypes bearing HCV E1 and E2 glycoproteins (HCVpp) offers the opportunity to investigate E1-E2-dependent HCVpp entry into Huh7 cells and primary human hepatocytes (Bartosch 2003a; Hsu 2003; Zhang 2004). In contrast to the HCV replicons where cells were transfected with HCV-specific synthetic RNA molecules, this method allows a detailed analysis of the early steps in the HCV life cycle, e.g., adsorption and viral entry.

Infectious HCV particles in cell culture (HCVcc). Transfection of Huh7 and "cured" Huh7.5 cells with full-length JFH-1 replicons led for the first time to the production of infectious HCV virions (Zhong 2005; Wakita 2005). The construction of a chimera with the core NS2 region derived from HCV strain J6 (genotype 2a) and the remaining sequence derived from JFH-1 improved infectivity. Importantly, the secreted viral particles are infectious in cell culture (HCVcc) (Wakita 2005; Zhong 2005; Lindenbach 2005) as well as in chimeric mice with human liver grafts as well as in chimpanzees (Lindenbach 2006).

In addition, an alternative strategy for the production of infectious HCV particles was developed (Heller 2005). A full-length HCV construct (genotype 1b) was placed between two ribozymes in a plasmid containing a tetracycline-responsive promoter. Huh7 cells were transfected with those plasmids, resulting in efficient viral replication with HCV RNA titres of up to 10^7 copies/ml cell culture supernatant.

The development of cell culture systems that allow the production of infectious HCV represents a breakthrough for HCV research and it is now possible to investigate the whole viral life cycle from viral adsorption to virion release. These studies will help to better understand the mechanisms of HCV pathogenesis and they should significantly accelerate the development of HCV-specific antiviral compounds.

References

Acton SL, Scherer PE, Lodish HF, et al. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. J Biol Chem 1994, 269:21003-21009.

Acton S, Rigotti A, Landschulz KT, et al. Identification of scavenger receptor BI as a high-density lipoprotein receptor. Science 1996, 271:518-520.

Agnello V, Abel G, Elfahal M, et al. Hepatitis C virus and other Flaviviridae viruses enter cells via low-density lipoprotein receptor. Proc Natl Acad Sci U S A 1999, 96:12766-12771.

Ago H, Adashi T, Yoshida A, et al. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. Structure 1999, 7:417-1426.

Ait-Goughoulte M, Hourioux C, Patient R, et al. Core protein cleavage by signal peptide peptidase is required for hepatitis C virus-like particle assembly. J Gen Virol 2006, 87:855–860.

Akazawa D, Date T, Morikawa K, et al. CD81 expression is important for the permissiveness of Huh7 cell clones for heterogeneous hepatitis C virus infection. J Virol 81:5036-5045.

Alter HJ, Holland PV, Purcell RH, et al. Transmissible agent in non-A, non-B hepatitis. Lancet 1978, 1:459-463.

Andre P, Komurian-Pradel F, Deforges S, et al. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol 2002, 76:69619-6928.

Appel N, Zayas M, Miller S, et al. Essential role of domain III of nonstructural protein 5A for hepatitis C virus infectious particle assembly. PLoS Pathog 4(3): e1000035.

Barba G, Harper F, Harada T, et al. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A 1997, 94:1200–1205.

Bartenschlager R, Ahlborn-Laake L, Mous J, et al. Nonstructural protein 3 of hepatitis C virus encodes a serine-type proteinase required for cleavage at the NS3/4 and NS4/5 junctions. J Virol 1993, 67:3835–3844.

Bartenschlager R, Lohmann V, Wilkinson T, et al. Complex formation between the NS3 serinetype proteinase of the hepatitis C virus and NS4A and its importance for polyprotein maturation. J Virol 1995, 69:7519–7528.

Barth H, Schäfer C, Adah MI, et al. Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulphate. J Biol Chem 2003, 278:41003-41012.

Bartosch B, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudoparticles containing functional E1-E2 envelope protein complexes. J Exp Med 2003a, 197:633-642.

Bartosch B, Vitelli A, Granier C, et al. Cell entry of hepatitis C virus requires a set of coreceptors that include CD81 and SR-B1 scavenger receptor. J Biol Chem 2003b, 278:41624-41630.

Behrens SE, Tomei L, DeFrancesco R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. EMBO J 1996, 15:12-22.

Bertaux C, Dragic T. Different domains of CD81 mediate distinct stages of hepatitis C virus pseudoparticle entry. J Virol 2006, 80:4940-4948.

Blanchard E, Belouzard S, Goueslain L, et al. Hepatitis C virus entry depends on clathrin-mediated endocytosis. J Virol 2006, 80:6964-6972.

Blight KJ and Rice CM. Secondary structure determination of the conserved 98-base sequence at the 3´terminus of hepatitis C virus genome RNA. J Virol 1997, 71:7345-7452.

Blight KJ, Kolykhalov AA, Rice CM. Efficient initiation of HCV RNA replication in cell culture. Science 2000, 290:1972-1974.

Blight KJ, McKeating JA, Rice CM. Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication. J Virol 2002, 76:13001-13014.

Bradley DW, McCaustland KA, Cook EH, et al. Post-transfusion non-A, non-B hepatitis in chimpanzees: physiochemical evidence that the tubule-forming agent is a small, enveloped virus. Gastro 1985, 88:773-779.

Bradley D, McCaustland K, Krawczynski K, et al. Hepatitis C virus: buoyant density of the factor VIII-derived isolate in sucrose. J Med Virol 1991, 34:206-208.

Bradrick SS, Walters RW, Gromeier M. The hepatitis C virus 3'-untranslated region or a poly(A) tract promote efficient translation subsequent to the initiation phase. Nucleic Acids Res 2006, 34:1293–1303.

Branch AD, Stumpp DD, Gutierrez JA et al. The hepatitis virus alternate reading frame (ARF) and its family of novel products: the alternate reading frame protein/F-protein, the double-frameshift protein, and others. Semin Liv Dis 2005, 25-105-117.

Bressanelli S, Tomei L, Roussel A, et al. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. Proc Natl Acad Sci USA 1999, 96:13034-13039.

Bukh J, Miller RH, Purcell RH. Biology and genetic heterogeneity of hepatitis C virus. Clin Exp Rheumatol 1995, Suppl 13:S3-7.

Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989, 244:359-362.

Choo QL, Richman KH, Han JH, et al. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci U S A 1991, 88:2451-2455.

Cocquerel L, Meunier JC, Pillez A, et al. A retention signal necessary and sufficient for endoplasmic reticulum localization maps to the transmembrane domain of hepatitis C virus glycoprotein E2. J Virol 1998, 72:2183-2191.

Cocquerel L, Duvet S, Meunier JC, et al. The transmembrane domain of hepatitis C virus glycoprotein E1 is a signal for static retention in the endoplasmic reticulum. J Virol 1999, 73:2641-2649.

Cocquerel L, Meunier JC, Op de Beeck A, et al. Coexpression of hepatitis C virus envelope proteins E1 and E2 in cix improves the stability of membrane insertion of E2. J Gen Virol 2001, 82:1629-1635.

Codran A, Royer C, Jaeck D, et al. Entry of hepatitis C virus pseudotypes into primary human hepatocytes by clathrin-dependent endocytosis. J Gen Virol 2006, 87:2583-2593.

Cormier EG, Tsamis F, Kajumo F, et al. CD81 is an entry coreceptor for hepatitis C virus. Proc Natl Acad Sci USA 2004, 101:7270-7274.

Date T, Kato T, Miyamoto M, et al. Genotype 2a hepatitis C virus subgenomic replicon can replicate in HepG2 and IMY-N9 cells. J Biol Chem 2004, 279:22371-22376.

Duvet S, Cocquerel L, Pillez A, et al. Hepatitis C virus glycoprotein complex localization in the endoplasmic reticulum involves a determinant for retention and not retrieval. J Biol Chem 1998, 273:32088-32095.

Duvet S, Op de Beeck A, Cocquerel L, et al. Glycosylation of the hepatitis C virus envelope protein E1 occurs posttranslationally in a mannosylphosphoryldolichol-deficient CHO mutant cell line. Glycobiology 2002, 12.95-101.

Eckart MR, Selby M, Maisiarz F, et al. The hepatitis C virus encodes a serine protease involved in processing of the putative non-structural proteins from the viral polyprotein precursor. Biochem Biophys Res Commun 1993, 192:399–406.

Egger D, Wölk , Gosert R, et al. Expression of hepatitis C virus proteins induce distinct membrane alterations including candidate viral replication complex. J Virol 2002, 76:5974-5984.

Einav S, Elazar M, Danieli T, et al. A nucleotide binding motif in hepatitis C virus (HCV) NS4B mediates HCV RNA replication. J Virol 2004, 78:11288-11295.

Elazar M, Liu P, Rice CM, et al. An N-terminal amphipathic helix in hepatitis C virus (HCV) NS4B mediates membrane association, correct localization of replication complex proteins, and HCV RNA replication. J Virol 2004, 78:11393-11400.

Enomoto N, Sakuma I, Asahina Y, et al. Comparison of full-length sequences of interferon sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Investig 1995, 96:224-230.

Enomoto N, Sakuma I, Asahina Y, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996, 334:77-81.

Evans MJ, von Hahn T, Tscherne DM, et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. Nature 2007, 446:801-805.

Failla C, Tomei L, DeFrancesco R. Both NS3 and NS4A are required for proteolytic processing of hepatitis C virus nonstructural proteins. J Virol 1994, 68:3753-3760.

Farci P, Shimoda A, Wong D, et al. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. Proc Nat. Acad Sci USA 1996, 93:15394-15399.

Feinstone SM, Kapikian AZ, Purcell RH, et al. Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Engl J Med 1975, 292:767-770.

Feinstone SM, Mihalik KB, Purcell AH, et al. Inactivation of hepatitis B virus and non-A, non-B hepatitis by chloroform. Infect Immun 1983, 41:767-770.

Flint M, Maidens C, Loomis-Price LD, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J Virol 1999, 73:6235-6244.

Friebe P, Lohmann V, Krieger N, et al. Sequences in the 5' nontranslated region of hepatitis C virus required for RNA replication. J Virol 2001, 75:12047-12057.

Friebe P and Bartenschlager R. Genetic analysis of sequences in the 3' nontranslated region of hepatitis C virus that are important for RNA replication. J Virol 2002, 76:5326-5338.

Friebe P, Boudet J, Simorre JP, et al. Kissing-loop interaction in the 3' end of the hepatitis C virus genome essential of RNA replication. J Virol 2005, 79:380-392.

Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon Alfa-2a plus Ribavirin for Chronic Hepatitis C Virus Infection. N Engl J Med 2002, 347:975-982.

Fukushi S, Katayama K, Kurihara C, et al. Complete 5' noncoding region is necessary for the efficient internal initiation of hepatitis C virus RNA. Biochem Biophys Res Commun 1994, 199:425-432.

Furuse M, Fujita K, Hiiragi T, et al. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 1998, 141:1539-1550.

Gardner JP, Durso RJ, Arrigale RR, et al. L-SIGN (CD209L) is a liver-specific capture receptor for hepatitis C virus. Proc Nat. Acad Sci USA 2003, 100:4498-4503.

Gastaminza P, Cheng G, Wieland S, et al. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. J Virol 2008, 82:2120-2129.

Germi R, Crance JM, Garin D, et al. Cellular glycosaminglycans and low density lipoprotein receptor are involved in hepatitis C virus adsorption. J Med Virol 68:206-215.

Grakoui A, McCourt DW, Wychowski C, et al. Characterization of hepatitis C virus-encoded serine proteinase: determination of proteinase-dependent polyprotein cleavage sites. J Virol 1993a, 67:2832–2843.

Grakoui A, McCourt DW, Wychowski C, et al. A second hepatitis C virus-encoded proteinase. Proc Natl Acad Sci USA 1993b, 90:10583-10587.

Gretton SN, Taylor AI, McLauchlan J. Mobility of the hepatitis C virus NS4B protein on the endoplasmic reticulum membrane and membrane-associated foci. J Gen Virol 2005, 86:1415-1421.

Griffin SD, Beales LP, Clarke DS, et al. The p7 protein of hepatitis C virus forms an ion channel that is blocked by the antiviral drug, Amantadine. FEBS Lett 2003, 535:34-38.

Grove J, Huby T, Stamataki Z, et al. Scavenger receptor BI and BII expression levels modulate hepatitis C virus infectivity. J Virol 2007, 81: 3162-3169.

Haqshenas G, Mackenzie JM, Dong X, et al. Hepatitis C virus p7 protein is localized in the endoplasmic reticulum when it is encoded by a replication-competent genome. J Gen Virol 2007, 88:134-142.

Heck JA, Meng X, Frick DN. Cyclophilin B stimulates RNA synthesis by the HCV RNA dependent RNA polymerase. Biochem Pharmacol 2009, 1173-1180.

Helle F, Dubuisson J. Hepatitis C virus entry into host cells. Cell Mol Life Sci 2008, 65:100-112.

Hellen CU, Pestova TV. Translation of hepatitis C virus RNA. J Viral Hepat 1999, 6:79-87.

Heller TS, Saito J, Auerbach T, et al. An in vitro model of hepatitis C virion production. Proc Nat. Acad Sci USA 102 (2005), pp. 2579–2583.

Heo TH, Chang JH, Lee JW, et al. Incomplete humoral immunity against hepatitis C virus is linked with distinct recognition of putative multiple receptors by E2 envelope glycoprotein. J Immunol 2004, 173:446-455.

Hollinger FB, Gitnick GL, Aach RD, et al. Non-A, non-B hepatitis transmission in chimpanzees: a project of the Transfusion-transmitted Viruses Study Group. Intervirology 1978, 10:60-68.

Honda M, Beard MR, Ping LH, et al. A phylogenetically conserved stem-loop structure at the 5´ border of the internal ribosome entry site of hepatitis C virus is required for cap-independent viral translation. J Virol 1999, 73:1165-1174.

Hsu M, Zhang J, Flint M, et al. Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. Proc Natl Acad Sci USA 2003, 100:7271-7276.

Hüssy P, Langen H, Mous J, et al. Hepatitis C virus core protein: carboxy-terminal boundaries of two processed species suggest cleavage by a signal peptide peptidase. Virology 1996, 224-93-104.

Ito T and Lai MMC. Determination of the secondary structure of and cellular protein binding to the 3'-untranslated region of the hepatitis C virus RNA genome. J Virol 1997, 71:8698-8706.

Jennings TA, Chen Y, Sikora D, et al. RNA unwinding activity of the hepatitis C virus NS3 helicase is modulated by the NS5B polymerase. Biochemistry 2008, 47:1126-1135.

Jeong SH, Qiao M, Nascimbeni M, et al. Immunization with hepatitis C virus-like particles induces humoral and cellular immune responses in honhuman primates. J Virol 2004, 78:6995-7003.

Jin L and Peterson DL. Expression, isolation, and characterization of the hepatitis C virus ATPase/RNA helicase. Arch Biochem Biophys 1995, 323:47-53.

Jones CT, Murray CL, Eastman DK, et al. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. J Virol 2007, 81:8374-8383.

Kaito M, Watanabe S, Tsukiyama-Kohara K, et al. Hepatitis C virus particle detected by immunoelectron microscopy study. J Gen Virol 1994, 75:1755-1760.

Kapadia SB, Barth H, Baumert T, et al. Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. J Virol 2007, 81:374-383.

Kato J, Kato N, Yoshida H, et al. Hepatitis C virus NS4A and NS4B proteins suppress translation in vivo. J Med Virol 2002, 66:187–199.

Kato N. Molecular virology of hepatitis C virus. Acta Med Okayama 2001, 55:133-159.

Kato T, Furusaka A, Miyamoto M, et al. Sequence analysis of hepatitis C virus isolated from a fulminant hepatitis patient. J Med Virol 2001, 64:334-339.

Kato T, Date T, Miyamoto M, et al. Efficient replication of the genotype 2a hepatitis C virus subgenomic replicon. Gastroenterology 2003, 125:1808-1817.

Kato T, Date T, Miyamoto M, et al. Nonhepatic cell lines HeLa and 293 support efficient replication of hepatitis C virus genotype 2a subgenomic replicon. J Virol 2005, 79:592-596.

Kim DW, Gwack Y, Han JH, et al. C-terminal domain of the hepatitis C virus NS3 protein contains an RNA helicase activity. Biochem Biophys Res Commun 1995, 215:160-166.

Kim YK, Kim CS, Lee SH, et al. Domains I and II in the 5' nontranslated region of the HCV genome are required for RNA replication. Biochem Biophys Res Commun 2002, 290:105-112.

Kolykhalov AA, Feinstone SM, Rice SM. Identification of a highly conserved sequence element at the 3´terminus of hepatitis C virus genome RNA. J Virol 1996, 70:3363-3371.

Kolykhalov AA, Mihalik K, Feinstone SM, et al. Hepatitis C virus-encoded enzymatic activities and conserved RNA elements in the 3' nontranslated region are essential for virus replication in vivo. J Virol 2000, 74:2046-2051..

Komurian-Pradel F, Rajoharison A, Berland JL, et al. Antigenic relevance of F protein in chronic hepatitis C virus infection. Hepatology 2004, 40:900-909.

Kou YH, Chou SM, Wang YM, et al. Hepatitis C virus NS4A inhibits cap-dependent and the viral IRES-mediated translation through interacting with eukaryotic elongation factor 1A. J Biomed Sci 2006, 13:861–874.

Koutsoudakis G, Kaul A, Steinmann E, et al. Characterization of the early steps of hepatitis C virus infection by using luciferase reporter viruses. J Virol 2006, 80:5308-5320.

Krieger N, Lohmann V, Bartenschlager R. Enhancement of hepatitis C virus RNA replication by cell culture-adaptive mutations. J Virol 2001, 75:4614-4624.

Kuo G, Choo QL, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989, 244:362-364.

Lavillette D, Tarr AW, Voisset C, et al. Characterization of host-range and cell entry properties of the major genotypes and subtypes of hepatitis C virus. Hepatology 2005, 41:265-274.

Lavillette D, Pécheur EI, Donot P, et al. Characterization of fusion determinants points to the involvement of three discrete regions of both E1 and E2 glycoproteins in the membrane fusion process of hepatitis C virus. J Virol 2007, 81:8752-8765.

Lesburg CA, Cable MB, Ferrari E, et al. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. Nat Struct Biol 1999, 6:937-943.

Lin C, Thomson JA, Rice CM. A central region in the hepatitis C virus NS4A protein allows formation of an active NS3-NS4A serine proteinase complex in vivo and in vitro. J Virol 1995, 69:4373–4380.

Lindenbach BD, Evans MJ, Syder AJ, et al. Complete replication of hepatitis C virus in cell culture. Science 309 (2005), pp. 623–626.

Lindenbach BD, Meuleman P, Ploss A, et al. Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro. Proc Natl Acad Sci USA 2006, 103:3805-3809.

Lohmann V, Körner F, Koch JO, et al. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 1999, 285:110-113.

Lohmann V, Körner F, Dobierzewska A, et al. Mutations in hepatitis C virus RNAs conferring cell culture adaptation. J Virol 2001, 75:1437-1449.

Lozach PY, Lortat-Jacob H, de Lacroix de Lavalette A, et al. DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. J Biol Chem 2003, 278:20358-20366.

Manns MP, McHutchinson JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001, 358:958-965.

Matsumoto M, Hwang SB, Jeng KS, et al. Homotypic interaction and multimerization of hepatitis C virus core protein. Virology 1996, 218:43-51.

McKeating JA, Zhang LQ, Logvinoff C, et al. Diverse hepatitis C virus glycoproteins mediate viral infection in a CD81-dependent manner. J Virol 2004, 78:8496-8505.

McLauchlan J, Lemberg MK, Hope G, et al. Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets. EMBO J 2002, 21:3980-3988.

Meertens L, Bertaux C, Dragic T. Hepatitis C virus entry requires a critical postinternalization step and delivery to early endosomes via clathrin-coated vesicles. J Virol. 2006, 80:11571-11578.

Meertens L, Bertaux C, Cukierman L, et al. The tight junction proteins claudin-1, -6, and -9 are entry cofactors for hepatitis C virus. J Virol 2008, 82:3555-3560.

Miyanari Y, Atsuzawa K, Usuda N, et al. The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol 2007, 9:961-969.

Molina S, Castet V, Fournier-Wirth C, et al. The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. J Hepatol 2007, 46:411-419.

Monazahian M, Böhme I, Bonk S, et al. Low-density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999, 57:223-229.

Moradpour D, Englert C, Wakita T, et al. Characterization of cell lines allowing tightly regulated expression of hepatitis C virus core protein. Virology 1996, 222:52-63.

NahmiasY, Casali M, Barbe L, et al. Liver endothelial cells promote LDL-R expression and the uptake of HCV-like particles in primary rat and human hepatocytes. Hepatology 2006, 43:257-265.

Nakagawa M, Sakamoto N, Tanabe Y, et al. Suppression of hepatitis C virus replication by cyclosporin A is mediated by blockade of cyclophilins. Gastroenterology 2005, 129-1031-1041.

Ndjomou J, Pybus OG, Matz B. Phylogenetic analysis of hepatitis C virus isolates indicates a unique pattern of endemic infection in Cameroon. J Gen Virol 2003, 84:2333-2341.

Ohba K, Mizokami M, Lau JYN, et al. Evolutionary relationship of hepatitis C, pesti-, flavi-, plantviruses, and newly discovered GB hepatitis agents. FEBS Lett 1996, 378:232-234.

Op de Beeck A, Montserret R, Duvet S, et al. The transmembrane domains of hepatitis C virus envelope glycoprotein E1 and E2 play a major role in heterodimerization. J Biol Chem 2000, 275:31428-31437.

Otto GA, Lukavsky PJ, Lancaster AM, et al. Ribosomal proteins mediate the hepatitis C virus IRES-HeLa 40S interaction. RNA 2002, 8:913–923.

Pavlovic D, Neville DC, Argaud O, et al. The hepatitis C virus p7 protein forms an ion channel that is inhibited by long-alkyl-chain iminosugar derivatives. Proc Nat. Acad Sci USA 2003, 100:6104-6108.

Pawlotsky JM, Chevalier S, McHutchinson JG. The hepatitis C virus life cycle as a target for new antiviral therapies. Gastroenterology 2007, 132:1979-1998.

Pietschmann T, Lohmann V, Kaul A, et al. Persistent and transient replication of full-length hepatitis C virus genomes in cell culture. J Virol 2002, 76:4008-4021.

Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. Science 1998, 282:938-941.

Ploss A, Evans MJ, Gaysinskaya VA, et al. Human occluding is a hepatitis C virus entry factor required for infection of mouse cells. Nature 2009, 457:882-886.

Pöhlmann S, Zhang J , Baribaud F, et al. Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. J Virol 2003, 77:4070-4080.

Poynard T, Bedossa P, Opolon P et al. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997, 349:825-833.

Prince AM, Huima-Byron T, Parker TS, et al. Visualization of hepatitis C virions and putative defective interfering particles isolated from low-density lipoproteins. J Viral Hepat 1996, 3:11-17.

Qiao M, Ashok M, Bernard KA, et al. Induction of sterilizing immunity against West Nile virus (WNV) by immunization with WNV-like particles produced in insect cells. J Infect Dis 2004, 190:2104-2108.

Roussel J, Pillez A, Montpellier C, et al. Characterization of the expression of the hepatitis C virus F protein. J Gen Virol 2003, 84:1751-1759.

Sakai A, Claire MS, Faulk K, et al. The p7 polypeptide of hepatitis C virus is critical for infectivity and contains functionally important genotype-specific sequences. Proc Nat. Acad Sci USA 2003, 100:11646-11651.

Santolini E, Migliaccio G, La Monica N. Biosynthesis and biochemical properties of the hepatitis C virus core protein. J Virol 1994, 68:3631-3641.

Santolini E, Pacini L, Fipaldini C, et al. The NS2 protein of hepatitis C virus is a transmembrane polypeptide. J Virol 1995, 69:7461-7471.

Scarselli E, Ansuini H, Cerino R, et al. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. EMBO J 2002, 21:5017-5025.

Schmidt-Mende J, Bieck E, Hugle T, et al. Determinants for membrane association of the hepatitis C virus RNA-dependent RNA polymerase. J Biol Chem 2001, 276:44052-44063.

Shavinskaya A, Boulant S, Penin F, et al. The lipid droplet binding domain of hepatitis C virus core protein is a major determinant for efficient virus assembly. J Biol Chem 2007, 282:37158-37169.

Shimizu YK, Feinstone SM, Kohara M, et al. Hepatitis C virus: detection of intracellular virus particles by electron microscopy. Hepatology 1996a, 23:205-209.

Shimizu YK, Igarashi H, Kiyohara T, et al. A hyperimmune serum against a synthetic peptide corresponding to the hypervariable region 1 of hepatitis C virus can prevent viral infection in cell cultures. Virology 1996b, 223:409-412.

Simmonds P. The origin and evolution of hepatitis viruses in humans. J Gen Virol 2001, 82:693-712.

Simmonds P. Genetic diversity and evolution of hepatitis C virus – 15 years on. J Gen Virol 2004, 85:3173-3188.

Simmonds P, Bukh J, Combet C, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005, 42:962-973.

Simons JN, Leary TP, Dawson GJ, et al. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1995, 1:564-569.

Sizova DV, Kolupaeva VG, Pestova TV, et al. Specific interaction of eukaryotic translation initiation factor 3 with the 5' nontranslated regions of hepatitis C virus and classical swine fever virus RNAs. J Virol 1998, 72:4775–4782.

Song Y, Friebe P, Tzima E, et al. The hepatitis C virus RNA 3'-untranslated region strongly enhances translation directed by the internal ribosome entry site. J Virol 2006, 80:1579-11588.

Spahn CM, Kieft JS, Grassucci RA, et al. Hepatitis C virus IRES RNA-induced changes in the conformation of the 40s ribosomal subunit. Science 2001, 291:1959–1962.

Takahashi K, Kishimoto S, Yoshizawa H, et al. p26 protein and 33-nm particle associated with nucleocapsid of hepatitis C virus recovered from the circulation of infected hosts. Virology 1992, 191:431-434.

Tanaka T, Kato N, Cho MJ, et al. A novel sequence found at the 3' terminus of hepatitis C virus genome. Biochem Biophys Res 1995, 215:744-749.

Tanaka T, Kato N, Cho MJ, et al. Structure of the 3' terminus of the hepatitis C virus genome. J Virol 1996, 70:3307-3312.

Tanji Y, Hijikata M, Satoh S, et al. Hepatitis C virus-encoded nonstructural protein NS4A has versatile functions in viral protein processing. J Virol 1995, 69:1575–1581.

Thiel HJ, Collett MS, Gould EA et al. Family Flaviviridae, In: Fauquet CM, Mayo MA, Maniloff J et al., eds. Virus Taxonomy:VIIIth Report of the International Committee on Taxonomy of Viruses. San Diego: Academic Press; 2005:979-996.

Thomssen R, Bonk S, Propfe C, et al. Association of hepatitis C virus in human sera with lipoprotein. Med Microbiol Immunol 1992, 181:293-300.

Thomssen R, Bonk S, Thiele A. Density heterogeneities of hepatitis C virus in human sera due to the binding of beta-lipoproteins and immunoglobulins. Med Microbiol Immunol 1993, 182:329-334.

Timpe JM, Stamataki Z, Jennings A, et al. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. Hepatology 2007, 47:17-24.

Tomei L, Failla C, Santolini E, et al. NS3 is a Serine protease required for processing of hepatitis C virus polyprotein. J Virol 1993, 67:4017–4026.

Tomei L, Failla C, Vitale RL, et al. A central hydrophobic domain of the hepatitis C virus NS4A protein is necessary and sufficient for the activation of the NS3 protease. J Gen Virol 1996, 77:1065-1070.

Tong MJ, el-Farra NS, Reikes AR et al. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995, 332:1463-1466.

Tsukiyama-Kohara K, Iizuka N, Kohara M, et al. Internal ribosome entry site within hepatitis C virus RNA. J Virol 1992, 66:1476-1483.

Valli MB, Serafino A, Crema A, et al. Transmission in vitro of hepatitis C virus from persistently infected human B-cells to hepatoma cells by cell-to-cell contact. J Med Virol 2006, 78:192-201.

Valli MB, Crema A, Lanzilli G, et al. Molecular and cellular determinants of cell-to-cell transmission of HCV in vitro. J Med Virol 2007, 79:1491-1499.

Varaklioti A, Vassilaki N, Georgopoulou U, et al. Alternate translation occurs within the core coding region of the hepatitis C viral genome. J Biol Chem 2002, 17713-17721.

Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005, 11:791–796.

Walewski JL, Keller TR, Stump DD, et al. Evidence for a new hepatitis C virus antigen encoded in an overlapping reading frame. RNA 2001, 7:710-721.

Wang C, Sarnow P, Siddiqui A. Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. J Virol 1993, 67:3338-3344.

Wang H, Shen XT, Ye R, et al. Roles of the polypyrimidine tract and 3' noncoding region of hepatitis C virus RNA in the internal ribosome entry site-mediated translation. Arch Virol 2005, 150:1085–1099.

Watashi K, Ishii N, Hijikata M, et al. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol Cell 2005, 19:111-122.

Webb NR, Connell PM, Graf GA, et al. SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high-density lipoprotein and cells. J Biol Chem 1998, 273:15241-15248.

Weihofen A, Binns K, Lemberg MK, et al. Identification of signal peptide peptidase, a presenelin-type aspartic protease. Science 2002, 296:2215-2218.

Weiner AJ, Brauer MJ, Rosenblatt J, et al. Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virology 1991, 180:842-848.

Witteveldt J, Evans MJ, Bitzegeio J, et al. CD81 is dispensable for hepatitis C virus cell-to-cell transmission in hepatoma cells. J Gen Virol 2009, 90:48-58.

Wölk B, Sansonno D, Kräusslich HG, et al. Subcellular localization, stability, and transcleavage competence of the hepatitis C virus NS3-NS4A complex expressed in tetracycline-regulated cell lines. J Virol 2000, 74:2293-2304.

Wünschmann S, Medh JD, Klinzmann D, et al. Characterization of hepatitis C virus (HCV) and HCV E2 interactions with CD81 and the low-density lipoprotein receptor. J Virol 2000, 74:10055-10062.

Xu Z, Choi J, Yen TS, et al. Synthesis of a novel hepatitis C virus protein by ribosomal frameshift. EMBO J 2001, 20:3840-3848.

Xu Z, Choi J, Lu W, et al. Hepatitis C virus F protein is a short-lived protein associated with the endoplasmic reticulum. J Virol 2003, 77:1578-1583.

Yanagi M, St Claire M, Emerson SU. In vivo analysis of the 3' untranslated region of the hepatitis C virus after in vitro mutagenesis of an infectious cDNA clone. Proc Natl Acad Sci USA 1999, 96:2291-2295.

Yang F, Robotham JM, Nelson HB, et al. Cyclophilin A is an essential cofactor for hepatitis C virus infection and the principal mediator of cyclosporine resistance in vitro. J Virol 2008, 82:5269-5278.

Yi M and Lemon SM. 3' Nontranslated RNA signals required for replication of hepatitis C virus RNA. J Virol 2003, 77:3557-3568.

Yi M, Villanueva RA, Thomas DL, et al. Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured hepatoma cells. Proc Natl Acad Sci USA 2006, 103:2310-2315.

You S and Rice CM. 3´ RNA elements in hepatitis C virus replication: kissing partners and long poly(U). J Virol 2008, 82:184-195.

Yu X, Qiao M, Atanasov I, et al. Cryo-electron microscopy and three-dimensional reconstructions of hepatitis C virus particles. Virology 2007, 367:126-134.

Zeisel MB, Koutsoudakis G, Schnober EK, et al. Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. Hepatology 2007, 46:1722-1731.

Zhang J, Yamada O, Yoshida H, et al. Autogenous translational inhibition of core protein: implication for switch from translation to RNA replication in hepatitis C virus. Virology 2002, 293:141–150.

Zhang LQ, Randall G, Higginbottom A, et al. CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. J Virol 2004, 78:1448-1455.

Zheng A, Yuan F, Li Y, et al. Claudin-6 and claudin-9 function as additional coreceptors for hepatitis C virus. J Virol 2007, 81:12465-12471.

Zhong J, Gastaminza P, Cheng G, et al. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci USA 2005, 102:9294–9299.

Chapter 7: Prophylaxis and vaccination of viral hepatitis

Heiner Wedemeyer

Introduction

Understanding the biology and modes of transmission of hepatitis viruses has significantly improved over the last decades. Fortunately, the incidence of hepatitis virus infections has significantly decreased in most areas around the world. Still, prophylactic vaccines are only available against HAV and HBV. Although an enormous amount of basic and clinical research has been performed to develop a vaccine against hepatitis C, it is very unlikely that a prophylactic or therapeutic HCV vaccine will be licensed within the next 5-7 years. A first phase II vaccine trial against hepatitis E has been successful; nevertheless, the completion of this vaccine development will not be in the near future. Prophylaxis for HCV, HDV (for HBV-infected patients) and HEV therefore must happen by avoiding all possible routes of exposure to the respective hepatitis viruses discussed in detail in Chapters 1-4.

Prophylaxis of hepatitis viruses

Hepatitis A and E

The hepatitis A and E viruses are usually transmitted by oral ingestion of contaminated food or water. Thus, particular caution is warranted when individuals from low endemic areas such as Western Europe and the USA travel to countries with a high prevalence of HAV and HEV infections. We must remember that hepatitis E can also be a zoonosis. A recent German case-control study identified 32% of all reported HEV infections as being autochthonous infections, meaning not associated with travelling to endemic countries (Wichmann 2008). In these patients consumption of offal and wild boar meat is independently associated with HEV infection. This may have significant implications for immunosuppressed patients as cases of chronic hepatitis E with the development of advanced fibrosis have been described in patients after organ transplantation (Kamar 2008). HEV has frequently been detected in the meat of pigs; Danish farmers show a higher prevalence of HEV antibodies. Importantly, zoonotic HEV infection is usually caused by HEV genotype 3 while HEV genotype 1 can be found in travelling-associated hepatitis E. HAV and HEV are also transmitted by blood transfusion although cases are extremely rare.

Hepatitis B and D

HBV and HDV were transmitted frequently by blood transfusion before HBsAg testing of all blood products was introduced in the 1970s. Since then, vertical transmission and sexual exposure have become the most frequent routes of HBV infection. Medical procedures still represent a potential source for HBV transmissions and thus strict and careful application of standard hygienic precautions for all medical interventions are absolutely mandatory not only in endemic areas but also in Western countries. This

holds true in particular for immunocompromised individuals who are highly susceptible to HBV infection as HBV is characterized by a very high infectivity (Wedemeyer 1998). Moreover, immunosuppressed patients are at risk for reactivation of occult HBV infection after serological recovery from hepatitis B. Treatments with high doses of steroids and rituximab have especially been identified as major risk factors for HBV reactivation (Lalazar 2007). After a new diagnosis of HBV infection, all family members of the patient need to be tested for their immune status against HBV. Immediate active vaccination is recommended for all anti-HBc-negative contact persons. HBsAg-positive individuals should use condoms during sexual intercourse if it is not known if the partner has been vaccinated.

Hepatitis C

Less than 1% of individuals who are exposed to HCV by an injury via contaminated needles develop acute HCV infection. At Hannover Medical School, not a single HCV seroconversion occurred after 166 occupational exposures with anti-HCV positive blood in a period of 6 years (2000-2005). Earlier studies published in the mid-nineties suggested higher rates of HCV transmission by needle stick injury. However, more recent and larger studies have reported significantly lower rates of acute hepatitis C after needle-stick injury. We recently performed a systematic review of the literature identifying 22 studies with a total of 6,956 injuries with HCV contaminated needles. Only 52 individuals (0.75%) became infected. The risk of acute HCV infection was lower in Europe at 0.42% compared to Eastern Asia at 1.5% (Kubitschke 2007). Thus, the risk of acquiring HCV infection after a needle-stick injury is lower than frequently reported. Worldwide differences in HCV seroconversion rates may suggest that genetic factors may provide some level of natural resistance against HCV. Factors associated with a higher risk of HCV transmission are likely to be the level of HCV viremia in the index patient, the amount of transmitted fluid and the duration between contamination of the respective needle and injury. Suggested follow-up procedures after needle stick injury are shown in Figure 1.

Sexual intercourse with HCV-infected persons has clearly been identified as a risk for HCV infection, as about 10-20% of patients with acute hepatitis C report this as a potential risk factor (Table 1). However, there is also large evidence that the risk of acquiring HCV sexually is extremely low in individuals with stable partnerships who avoid injuries. Cohort studies including >500 HCV-infected patients followed over periods of more than 4 years could not identify any cases of confirmed HCV transmission. Thus, guidelines generally do not recommend the use of condoms in monogamous relationships. However, this statement does not hold true for HIV-positive homosexual men. Recently, several outbreaks of acute hepatitis C have been described in this scenario (Fox 2008; Low 2008). Transmitted cases had more sexual partners, increased levels of high-risk sexual behaviour (in particular, fisting) and were more likely to have shared drugs via a nasal or anal route than controls (Turner 2006).

Due to the low HCV prevalence in most European countries and due to a relatively low vertical transmission rate of 1-6%, general screening of pregnant women for anti-HCV is not recommended. Interestingly, transmission may be higher for girls than for boys (European Pediatric Hepatitis C Virus Network 2005). Transmission

rates may be higher in HIV-infected women so pregnant women should be tested for hepatitis C. Other factors possibly being associated with high transmission rates are the level of HCV viremia, maternal intravenous drug use, and specific HLA types of the children. Caesarean sections are not recommended for HCV RNA positive mothers as there is no clear evidence that Caesarean sections reduce transmission rates. Children of HCV-infected mothers should be tested for HCV RNA after 1 month as maternal anti-HCV antibodies can be detected for several months after birth. Mothers with chronic hepatitis C can breast-feed their children as long as they are HIV-negative and do not use intravenous drugs (European Pediatric Hepatitis C Virus Network 2001).

The Spanish Acute HCV study group has recently identified hospital admission as a significant risk factor for acquiring HCV infection in Spain (Martinez-Bauer 2008). The data are in line with other reports from Italy (Santantonio 2006) and the USA (Corey 2006). We have recently reported data from the German Hep-Net Acute HCV studies and found 38 cases (15% of the entire cohort) of acute HCV patients who reported a medical procedure as the most likely risk factor for having acquired HCV (Deterding 2008). The majority of those were hospital admissions with surgery in 30 cases; other invasive procedures including dental treatment were present in only 4 cases. Medical procedures were significantly more often the probable cause of infection in patients older than 30 years of age ($p=0.002$) but not associated with disease severity or time from exposure to onset of symptoms. Thus, medical treatment per se represents a significant risk factor for HCV infection – even in developed countries. Strict adherence to universal precaution guidelines is urgently warranted.

Vaccination against hepatitis A

The first active vaccine against HAV was licensed in 1995. The currently available inactive vaccines are manufactured from cell culture-adapted HAV, grown either in human fibroblasts or diploid cells (Nothdurft 2008). Two doses of the vaccine are recommended. The second dose should be given between 6 and 18 months after the first dose. All vaccines are highly immunogenic and basically all vaccinated healthy persons develop protective anti-HAV antibodies. Similar vaccine responses are obtained in children and adults and no relevant regional differences in response to HAV vaccination have been observed. The weakest vaccine responses have been described for young children receiving a 0, 1, 2 months schedule (Hammitt 2008). Patients with chronic liver disease do respond to vaccination but may display lower anti-HAV titers (Keeffe 1998). Since 1996 a combined vaccine against HAV and HBV is available that needs to be administered three times, on a 0, 1, 6 months schedule. More than 80% of healthy individuals have detectable HAV antibodies by day 21 applying an accelerated vaccine schedule of 0, 7 and 21 days using the combined HAV/HBV vaccine, and all study subjects are immune against HAV by 2 months (Kallinowski 2003).

HAV vaccines are very well tolerated and no serious adverse events have been linked with the administration of HAV vaccines (Nothdurft 2008). The vaccine can safely be given together with other vaccines or immunoglobulins without compromising the development of protective antibodies.

Vaccination is recommended for different groups of individuals including non-immune individuals who plan to travel to endemic countries, medical health professionals, homosexual men, persons in contact with hepatitis A patients, and individuals with chronic liver diseases. Some studies have suggested that patients with chronic hepatitis C have a higher risk to develop fulminant hepatitis A (Vento 1998), however this finding has not been confirmed by several other investigators (Deterding 2006). The implementation of childhood vaccination programs has led to a significant and impressive declines of HAV infections in several countries, justifying further efforts aiming to control the spread of HAV in endemic countries (Hendrickx 2008). It is important to highlight that most studies have also shown that HAV vaccination is cost-effective (Rein 2008; Hollinger 2007).

Recently, long-term follow-up studies after complete HAV vaccination have been published. Interestingly, anti-HAV titers sharply decline during the first year after vaccination but remain detectable in almost all individuals for at least 10 years after vaccination. Based on these studies it was estimated that protective anti-HAV antibodies should persist for at least 27 years after successful vaccination of children or young adults (Hammitt 2008).

Vaccination against hepatitis B

The hepatitis B vaccine is the first vaccine able to reduce the incidence of cancer. In Taiwan, a significant decline in cases of childhood hepatocellular carcinoma has been observed after the implementation of programs to vaccinate all infants against HBV (Chang 1997). This landmark study impressively highlighted the usefulness of universal vaccination against HBV in endemic countries. Controversial discussions are ongoing regarding to what extent universal vaccination against HBV may be cost-effective in low-endemic places such as the UK, the Netherlands or Scandinavia (Zuckerman 2007). In 1992 the World Health Organization recommended general vaccination against hepatitis B everywhere. In the long run, hepatitis B can be eradicated by worldwide implementation of this recommendation, because humans are the only epidemiologically relevant virus host. 164 countries have introduced a hepatitis B vaccine in their national infant immunization schedules by the end of 2006 (www.who.int; accessed Nov 12th 2008).

The first plasma-derived hepatitis B vaccine was approved by the FDA in 1981. Recombinant vaccines consisting of HBsAg produced in yeast became available in 1986. In the USA, two recombinant vaccines are licensed (Recombivax® and Engerix-B®) while additional vaccines are used in other countries. The vaccines are administered three times on a 0, 1, and 6 months schedule.

Who should be vaccinated? (The German Guidelines (Cornberg 2007))

- Hepatitis B high-risk persons working in health care settings including trainees, students, cleaning personnel;
- Personnel in psychiatric facilities or comparable welfare institutions for cerebrally damaged or disturbed patients; other persons who are at risk because of blood contact with possibly infected persons dependent on the risk evaluation, e.g., persons giving first aid professionally or voluntarily, employees of ambu-

lance services, police officers, social workers, and prison staff who have contact with drug-dependent people;
- Patients with chronic kidney disease, dialysis patients, patients with frequent blood or blood component transfusions (e.g., hemophiliacs), patients prior to extensive surgery (e.g., before operations using heart-lung machine. The urgency of the operation and the patient's wish for vaccination protection are of primary importance);
- Persons with chronic liver disease including chronic diseases with liver involvement as well as HIV-positive persons without HBV markers;
- Persons at risk of contact with HBsAg carriers in the family or shared housing, sexual partners of HBsAg carriers;
- Patients in psychiatric facilities or residents of comparable welfare institutions for cerebrally damaged or disturbed persons as well as persons in sheltered workshops;
- Special high-risk groups, e.g., homosexually active men, regular drug users, sex workers, prisoners serving extended sentences;
- Persons at risk of contacting HBsAg carriers in facilities (kindergarten, children's homes, nursing homes, school classes, day care groups);
- Persons travelling to regions with high hepatitis B prevalence for an extended period of time or with expected close contact with the local population;
- Persons who have been injured by possibly contaminated items, e.g., needle puncture (see post-exposition prophylaxis);
- Infants of HbsAg-positive mothers or of mothers with unknown HBsAg status (independent of weight at birth) (see post-exposition prophylaxis).

Routine testing for previous contact with hepatitis B is not necessary before vaccination unless the person belongs to a risk group and may have acquired hepatitis B before. Pre-vaccine testing is usually not cost-effective in populations with anti-HBc prevalence below 20%. Vaccination of an HBsAg-positive individual can be performed without any danger – however, it is ineffective.

Efficacy of vaccination against hepatitis B

A response to HBV vaccination is determined by the development of anti-HBs antibodies which is detectable in 90-95% of individuals one month after a complete vaccination schedule (Wedemeyer 2007; Coates 2001). Responses are lower in elderly people and much weaker in immunocompromised persons such as organ transplant recipients, patients receiving haemodialysis and HIV-infected individuals. In case of vaccine non-response, another three courses of vaccine should be administered and the dose of the vaccine should be increased. Other possibilities to increase the immunogenicity of HBV vaccines include intrader-mal application and co-administration of adjuvants and cytokines (Cornberg 2007). The response to vaccination should be controlled in high-risk individuals such as medical health professionals and immune-compromised persons. Some guidelines also recommend to test elderly persons after vaccinations as vaccine response does decline more rapidly in the elderly (Wolters 2003).

Post-exposure prophylaxis

Non-immune persons who have been in contact with HBV-contaminated materials (e.g., needles) or who have had sexual intercourse with an HBV-infected person should undergo active-passive immunization (active immunization plus hepatitis B immunoglobulin) as soon as possible – preferentially within the first 48 hours of exposure to HBV. Individuals previously vaccinated but who have an anti-HBs titer of <10 IU/L should also be vaccinated both actively and passively. No action is required if an anti-HBs titer of >100 IU/l is documented; active vaccination alone is sufficient for persons with intermediate anti-HBs titers between 10 and 100 IU/L (Cornberg 2007).

Safety of HBV vaccines

Several hundred million individuals have been vaccinated against hepatitis B. The vaccine is very well tolerated. Injection site reactions in the first 1-3 days and mild general reactions are common, although they are usually not long lasting. Whether there is a causal relationship between the vaccination and the seldomly-observed neurological disorders occurring around the time of vaccination is not clear. In the majority of these case reports the concomitant events most likely occurred coincidentally and are independent and not causally related. That hepatitis B vaccination causes and induces acute episodes of multiple sclerosis or other demyelating diseases is repeatedly discussed (Geier 2001; Hernan 2004; Girard 2005). However, there are no scientific facts proving such a relationship. Numerous studies have not been able to find a causal relationship between the postulated disease and the vaccination (Sadovnick 2000; Monteyne 2000; Ascherio 2001; Confavreux 2001; Institute of Medicine Report 2002; CDC 2004; Schattner 2005).

What is the long-term immunogenicity of the hepatitis B vaccination?

Several studies have been published in recent years investigating the long-term efficacy of HBV vaccination. After 10-15 years, between one third and two thirds of vaccinated individuals have completely lost anti-HBs antibodies and only a minority maintain titers of >100 IU/L. However, in low/intermediate endemic countries such as Italy, this loss in protective humoral immunity did not lead to many cases of acute or even chronic HBV infection (Zanetti 2005). To what extent memory B- and T-cell responses contribute to a relative protection against HBV in the absence of anti-HBs remains to be determined. Nevertheless, in high-endemic countries such as Gambia a significant proportion of infants develop anti-HBc indicating active HBV infection (18%) and some children develop chronic hepatitis B (van der Sande 2007). Thus, persons at risk should receive booster immunization if HBs antibodies have been lost.

Prevention of vertical HBV transmission

Infants of HBsAg positive mothers should be immunized actively and passively within 12 hours of birth. This is very important as the vertical HBV transmission rate can be reduced from 95% to <5% (Ranger-Rogez 2004). Mothers with very high HBV viremia, of >50 million IU/ml, should receive in addition antiviral therapy with a potent HBV polymerase inhibitor (European Association For The Study Of The Liver 2008).

If active/passive immunization has been performed, there is no need to recommend Caesarean sec-tion. Mothers of vaccinated infants can breast feed unless oral antiviral medications are being taken by the mother, which can be detected in the breast milk.

Vaccination against hepatitis C

No prophylactic or therapeutic vaccine against hepatitis C is available. As re-infections after spontaneous or treatment-induced recovery from hepatitis C virus infection have frequently been reported, the aim of a vaccine will very likely be not to prevent completely an infection with HCV but rather to modulate immune responses in such a way that the frequency of evolution to a chronic state can be reduced.

HCV specific T-cell responses play an important role in the natural course of HCV infection. The adaptive T-cell response is mediated both by CD4+ helper T-cells and CD8+ killer T-cells. Several groups have con-sistently found an association between a strong, multispecific and maintained HCV-specific CD4+ and CD8+ T-cell response and the resolution of acute HCV infection. While CD4+ T-cells seem to be present for several years after recovery, there are conflicting data whether HCV-specific CD8+ T-cells responses persist or decline over time. However, several studies have observed durable HCV-specific T-cells in HCV-seronegative individuals who were exposed to HCV by occupational exposure or as household members of HCV-positive partners, but who never became HCV RNA positive. These observations suggest that HCV-specific T-cells may be induced upon sub-clinical exposure and may contribute to protection against clinically apparent HCV infection. T-cell responses are usually much weaker in chronic hepatitis C. The frequency of specific cells is low but also effector function of HCV-specific T-cells is impaired. Different mechanisms are discussed as being responsible for this impaired T-cell function, including higher frequen-cies of regulatory T-cells (T-regs), altered dendritic cell activity, upregulation of the inhibitory molecules PD-1 on T-cells and many others. HCV proteins can directly or indirectly contribute to altered functions of different immune cells.

To what extent humoral immune responses against HCV contribute to spontaneous clearance of acute hepatitis C is less clear. Higher levels of neutralizing antibodies early during the infection are associated with viral clearance (Pestka 2007). However, antibodies with neutralizing properties occur at high levels during chronic infection. Yet, no completely sterilizing humoural anti-HCV immunity exists in the long-term after recovery (Rehermann 2005).

Few phase I vaccine studies based either on vaccination with HCV peptides, HCV proteins or recombinant vectors expressing HCV proteins have been completed. HCV-specific T-cells or antibodies against HCV can be induced by these vaccines in healthy individuals. However, it will be difficult to prove vaccine efficacy and vaccine effectiveness. Studies in chimpanzees have shown that it is very unlikely that a vaccine will be completely protective against heterologous HCV infections. However, a reasonable approach might be the development of a vaccine that does not confer 100% protection against acute infection but prevents progression of acute hepatitis C to chronic infection. This approach has, however, to compete with antiviral treatment of acute hepatitis C. It is very unlikely that a vaccine against hepatitis C will be licensed within the next 5-7 years.

Some studies regarding therapeutic vaccination have taken place (Wedemeyer 2006; Klade 2008). These studies show that induction of HCV-specific humoural or cellular immune responses is possible even in chronically infected individuals. However, so far neither therapeutic vaccination nor other immunomodulatory attempts such as treatment with cytokines (interferon gamma; IL-2; IL-10; IL-12) or toll-like receptor agonists have shown significant clinical benefits in patients with chronic hepatitis C.

Vaccination against hepatitis E

A phase II vaccine trial performed in Nepal showed a vaccine efficacy of 95% for an HEV recombinant protein (Shrestha 2007). 2000 soldiers received three vaccines on a 0, 1, 6 months schedule or placebo and subjects were followed for a median of 800 days. Except injection site reactions side effects were similar in both groups. Importantly, of the 69 subjects who developed hepatitis E, 66 were in the placebo group. However, and unfortunately, no phase III study to complete the vaccine's development has yet started to our knowledge. Thus, no HEV vaccine will be available in the next few years. Until then, preventive hygienic measures remain the only option to avoid HEV infection.

References

Ascherio A, Zhang SM, Hernan MA, Olek MJ, Coplan PM, Brodovicz K, Walker AM. (2001). Hepatitis B vaccination and the risk of multiple sclerosis. N. Engl. J. Med. 344, 327-332.

CDC (2004). Recombinant Hepatitis B Vaccine and the Risk of Multiple Sclerosis. Electronic Citation www.cdc.gov/ncidod/diseases/hepatitis/b/hbv_ms.pdf.

Chang,M.H., Chen,C.J., Lai,M.S., Hsu,H.M., Wu,T.C., Kong,M.S., Liang,D.C., Shau,W.Y., and Chen,D.S. (1997). Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N. Engl. J. Med. 336, 1855-1859.

Coates,T., Wilson,R., Patrick,G., Andre,F., and Watson,V. (2001). Hepatitis B vaccines: assessment of the seroprotective efficacy of two recombinant DNA vaccines. Clin. Ther. 23, 392-403.

Confavreux,C., Suissa,S., Saddier,P., Bourdes,V., and Vukusic,S. (2001). Vaccinations and the risk of relapse in multiple sclerosis. Vaccines in Multiple Sclerosis Study Group. N. Engl. J. Med. 344, 319-326.

Corey,K.E., Ross,A.S., Wurcel,A., Schulze Zur,W.J., Kim,A.Y., Lauer,G.M., and Chung,R.T. (2006). Outcomes and treatment of acute hepatitis C virus infection in a United States population. Clin. Gastroenterol. Hepatol. 4, 1278-1282.

Cornberg,M., Protzer,U., Dollinger,M.M., Petersen,J., Wedemeyer,H., Berg,T., Jilg,W., Erhardt,A., Wirth,S., Schirmacher,P., Fleig,W.E., and Manns,M.P. (2007). Prophylaxis, diagnosis and therapy of hepatitis B virus (HBV) infection: the German guidelines for the management of HBV infection. Z. Gastroenterol. 45, 1281-1328.

Deterding,K., Tegtmeyer,B., Cornberg,M., Hadem,J., Potthoff,A., Boker,K.H., Tillmann,H.L., Manns,M.P., and Wedemeyer,H. (2006). Hepatitis A virus infection suppresses hepatitis C virus replication and may lead to clearance of HCV. J. Hepatol. 45, 770-778.

Deterding,K., Wiegand,J., Gruner,N., and Wedemeyer,H. (2008). Medical procedures as a risk factor for HCV infection in developed countries: Do we neglect a significant problem in medical care? J. Hepatol. 48, 1019-1020.

European Association For The Study Of The Liver (2008). EASL Clinical Practice Guidelines: Management of chronic hepatitis B. J. Hepatol.

European Paediatric Hepatitis C Virus Network. (2001) Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. BJOG. 108, 371-377.

European Paediatric Hepatitis C Virus Network. (2005). A significant sex--but not elective cesarean section--effect on mother-to-child transmission of hepatitis C virus infection. J. Infect. Dis. 192, 1872-1879.

Fox,J., Nastouli,E., Thomson,E., Muir,D., McClure,M., Weber,J., and Fidler,S. (2008). Increasing incidence of acute hepatitis C in individuals diagnosed with primary HIV in the United Kingdom. AIDS 22, 666-668.

Geier,M.R. and Geier,D.A. (2001). Immunologic reactions and hepatitis B vaccine. Ann. Intern. Med. 134, 1155.

Girard,M. (2005). Autoimmune hazards of hepatitis B vaccine. Autoimmun. Rev. 4, 96-100.

Hammitt,L.L., Bulkow,L., Hennessy,T.W., Zanis,C., Snowball,M., Williams,J.L., Bell,B.P., and McMahon,B.J. (2008). Per-sistence of antibody to hepatitis A virus 10 years after vaccination among children and adults. J. Infect. Dis. 198, 1776-1782.

Hendrickx,G., Van Herck,K., Vorsters,A., Wiersma,S., Shapiro,C., Andrus,J.K., Ropero,A.M., Shouval,D., Ward,W., and Van Damme,P. (2008). Has the time come to control hepatitis A globally? Matching prevention to the changing epidemiology. J. Viral Hepat. 15 Suppl 2, 1-15.

Hernan,M.A., Jick,S.S., Olek,M.J., and Jick,H. (2004). Recombinant hepatitis B vaccine and the risk of multiple sclerosis: a prospective study. Neurology 63, 838-842.

Hollinger,F.B., Bell,B., Levy-Bruhl,D., Shouval,D., Wiersma,S., and Van Damme,P. (2007). Hepatitis A and B vaccination and public health. J. Viral Hepat. 14 Suppl 1, 1-5.

Institute of Medicine - Report (2002). Hepatitis B vaccine and demyelinating neurological disorders. Electronic Citation www.cdc.gov/nip/vaccine/hep/hepb/iom.htm.

Kallinowski,B., Jilg,W., Buchholz,L., Stremmel,W., and Engler,S. (2003). Immunogenicity of an accelerated vaccination regime with a combined hepatitis a/b vaccine in patients with chronic hepatitis C. Z. Gastroenterol. 41, 983-990.

Kamar,N., Selves,J., Mansuy,J.M., Ouezzani,L., Peron,J.M., Guitard,J., Cointault,O., Esposito,L., Abravanel,F., Danjoux,M., Durand,D., Vinel,J.P., Izopet,J., and Rostaing,L. (2008). Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N. Engl. J. Med. 358, 811-817.

Keeffe,E.B., Iwarson,S., McMahon,B.J., Lindsay,K.L., Koff,R.S., Manns,M., Baumgarten,R., Wiese,M., Fourneau,M., Safary,A., Clemens,R., and Krause,D.S. (1998). Safety and immunogenicity of hepatitis A vaccine in patients with chronic liver disease. Hepatology 27, 881-886.

Klade,C.S., Wedemeyer,H., Berg,T., Hinrichsen,H., Cholewinska,G., Zeuzem,S., Blum,H., Buschle,M., Jelovcan,S., Buerger,V., Tauber,E., Frisch,J., and Manns,M.P. (2008). Therapeutic vaccination of chronic hepatitis C nonresponder patients with the peptide vaccine IC41. Gastroenterology 134, 1385-1395.

Kubitschke, A., Bader.C, Tillmann, H. L., Manns, M. P., Kuhn, S., and Wedemeyer H. Injury with HCV-contaminated needles: What is the true rate of serconversion? Internist (Berl), 2007. Ref Type: In Press.

Lalazar,G., Rund,D., and Shouval,D. (2007). Screening, prevention and treatment of viral hepatitis B reactivation in patients with haematological malignancies. Br. J. Haematol. 136, 699-712.

Low,E., Vogel,M., Rockstroh,J., and Nelson,M. (2008). Acute Hepatitis C in HIV-Positive Individuals. AIDS Rev. 10, 245-253.

Martinez-Bauer,E., Forns,X., Armelles,M., Planas,R., Sola,R., Vergara,M., Fabregas,S., Vega,R., Salmeron,J., Diago,M., Sanchez-Tapias,J.M., and Bruguera,M. (2008). Hospital admission is a relevant source of hepatitis C virus acquisition in Spain. J. Hepatol. 48, 20-27.

Monteyne,P. and Andre,F.E. (2000). Is there a causal link between hepatitis B vaccination and multiple sclerosis? Vaccine 18, 1994-2001.

Nothdurft,H.D. (2008). Hepatitis A vaccines. Expert. Rev. Vaccines. 7, 535-545.

Pestka,J.M., Zeisel,M.B., Blaser,E., Schurmann,P., Bartosch,B., Cosset,F.L., et al. (2007). Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. Proc. Natl. Acad. Sci. U. S. A 104, 6025-6030.

Ranger-Rogez,S. and Denis,F. (2004). Hepatitis B mother--to--child transmission. Expert. Rev. Anti. Infect. Ther. 2, 133-145.

Rehermann,B. and Nascimbeni,M. (2005). Immunology of hepatitis B virus and hepatitis C virus infection. Nature Reviews Immunology 5, 215-229.

Rein,D.B. and Weinbaum,C.M. (2008). The cost-effectiveness of using hepatitis A/B combined vaccine versus hepatitis B vaccine alone for high-risk heterosexuals. Vaccine 26, 5331-5333.

Sadovnick,A.D. and Scheifele,D.W. (2000). School-based hepatitis B vaccination programme and adolescent multiple sclerosis. Lancet 355, 549-550.

Santantonio,T., Medda,E., Ferrari,C., Fabris,P., Cariti,G., Massari,M., et al. (2006). Risk factors and outcome among a large patient cohort with community-acquired acute hepatitis C in Italy. Clin. Infect. Dis. 43, 1154-1159.

Schattner,A. (2005). Consequence or coincidence? The occurrence, pathogenesis and significance of autoimmune manifestations after viral vaccines. Vaccine 23, 3876-3886.

Shrestha,M.P., Scott,R.M., Joshi,D.M., Mammen,M.P., Jr., Thapa,G.B., Thapa,N., et al. (2007). Safety and efficacy of a recombinant hepatitis E vaccine. N. Engl. J. Med. 356, 895-903.

Turner,J.M., Rider,A.T., Imrie,J., Copas,A.J., Edwards,S.G., Dodds,J.P., and Stephenson,J.M. (2006). Behavioural predictors of subsequent hepatitis C diagnosis in a UK clinic sample of HIV positive men who have sex with men. Sex Transm. Infect. 82, 298-300.

van der Sande,M.A., Waight,P.A., Mendy,M., Zaman,S., Kaye,S., Sam,O., et al. (2007). Long-term protection against HBV chronic carriage of Gambian adolescents vaccinated in infancy and immune response in HBV booster trial in adolescence. PLoS. ONE. 2, e753.

Vento,S., Garofano,T., Renzini,C., Cainelli,F., Casali,F., Ghironzi,G., Ferraro,T., and Concia,E. (1998). Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. N. Engl. J. Med. 338, 286-290.

Wedemeyer,H., Cornberg,M., Protzer,U., Berg,T., and Dollinger,M.M. (2007). [German guidelines on diagnosis and therapy of hepatitis B]. Dtsch. Med. Wochenschr. 132, 1775-1782.

Wedemeyer,H., Pethig,K., Wagner,D., Flemming,P., Oppelt,P., Petzold,D.R., Haverich,A., Manns,M.P., and Boeker,K.H. (1998). Long-term outcome of chronic hepatitis B in heart transplant recipients. Transplantation 66, 1347-1353.

Wedemeyer,H., Van Vlierberghe,H., Blum,H., Nevens,F., Gschwantler,M., Zeuzem,S., Roskams,T., Dincq,S., Vander,S.C., De Winter,H., Maertens,G., and Horsmans,Y. (2006). E1 therapeutic vaccination in patients with chronic HCV genotype 1 infection: Results of a 15 months, placebo-controlled trial. J. Hepatol.

Wichmann,O., Schimanski,S., Koch,J., Kohler,M., Rothe,C., Plentz,A., Jilg,W., and Stark,K. (2008). Phylogenetic and case-control study on hepatitis E virus infection in Germany. J. Infect. Dis. 198, 1732-1741.

Wolters,B., Junge,U., Dziuba,S., and Roggendorf,M. (2003). Immunogenicity of combined hepatitis A and B vaccine in elderly persons. Vaccine 21, 3623-3628.

Zanetti,A.R., Mariano,A., Romano,L., D'Amelio,R., Chironna,M., Coppola,R.C., et al. (2005). Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. Lancet 366, 1379-1384.

Zuckerman,J., van Hattum,J., Cafferkey,M., Gjorup,I., Hoel,T., Rummukainen,M.L., and Weiland,O. (2007). Should hepatitis B vaccination be introduced into childhood immunisation programmes in northern Europe? Lancet Infect. Dis. 7, 410-419.

Part 2

Hepatitis B and D

Chapter 8: Diagnostic tests in acute and chronic hepatitis B

Oliver Schildgen

Introduction

Over the past three decades, laboratory diagnostics of viral infections have become more and more influenced by molecular biology, the field of technology that has grown fastest in this period of time. Classical serologic and virologic tests have advanced and sometimes been replaced by novel detection methods that rely on genome amplification procedures like PCR and NASBA.

Especially for the human hepatitis B virus this technological development has been extremely important. As mentioned in an earlier chapter, in contrast to other viruses, HBV is extremely hard to cultivate as it does not replicate in any cell line used regularly in diagnostic laboratories. Furthermore, earlier techniques were not sensitive enough to detect even small amounts of virus in blood and blood products and consequently failed to avoid unintentional transmission of virus from donors to blood product recipients.

Aims of diagnostics tests in the management of HBV-infected patients

The first diagnosis of an HBV infection has to figure out whether the infection is acute or chronic. Therefore, as standard procedure, the patient with HBV infection diagnosed by clinical symptoms or elevated alanine aminotransferase (ALT) levels needs to test positive for anti-hepatitis B core antigen (HBcAg) antibodies. HBcAg is massively expressed in both acute and chronic infections and is a clear sign of HBV infection. After a positive result for anti-HBcAg antibodies, antibodies reactant to the surface antigen (HBsAg) are looked for. If found, this indicates that the patient is cured from the infection or has been successfully vaccinated.

Based on these initial serologic diagnostics, further efforts to define the status of the infection are made. An anti-HBcAg positive but anti-HBsAg negative patient may be dealing with a chronic infection. In these cases a number of parameters should be investigated, namely early antigen (HBeAg), anti-HBeAg, HBsAg, HBcAg, and finally, the viral load measured as genome equivalents per ml in serum. HBeAg is normally expressed only in case of an acute and/or ongoing infection with active replication. Unfortunately, so-called precore mutants exist that display active replication without expressing HBeAg, still bearing a high risk for progression to hepatocellular carcinoma (HCC). It is worth noting that HBeAg seroconversion occurs in up to 98% of people and that this is not a marker for a cure of the infection, although it does act as a marker for healing.

To sum up, classical serological screenings, using the definitions for chronicity (see Chapter 2), have to be initially performed to analyze the serological status of the HBV infection before more expensive molecular methods are performed. These methods are generally used for monitoring treatment efficacy and treatment compliance, to identify resistant strains, and to identify precore mutant strains of HBV.

Molecular assays in the diagnosis and management of HBV

Utility of quantitative HBV DNA assays

Many scientific societies have published consensus papers and/or guidelines for the management of chronically-infected HBV patients (EASL 2002; de Franchis 2003; Keeffe 2006; Liaw 2005; Lok 2001; Lok 2004a; Lok 2004b). All of them recommend an initial quantification of viral load and continuous measurements during follow-up monitoring. Follow-up is considered important for deciding on initiation of treatment or changes to the patient's drug regimen. Furthermore, sensitive methods for quantification are needed for detection of even low viremia in patients infected with strains bearing a high risk for development of hepatocellular carcinoma such as HBeAg negative strains.

One agreed-upon criterion for chronic HBV infection is a detectable viral load – measured as viral DNA in serum or plasma – for a minimum of 6 months (de Franchis 2003; Keeffe 2006; Liaw 2005; Lok 2001; Lok 2004a; Lok 2004b). In this case, replication is considered to be active if >20,000 IU/ml or >100,000 copies/ml can be detected. Also, in HBeAg-negative chronic hepatitis B virus infections, HBV DNA is the only marker for viral replication that consequently needs to be monitored. A cutoff limit of 2000 IU/ml differentiates active from inactive replication (Manesis 2003; Zacharakis 2005).

Furthermore, qualitative and quantitative measurement of viral DNA is important to monitor another condition, occult hepatitis. This, by definition, is characterized as HBV infection with measureable DNA levels in the absence of detectable HBsAg. Testing for occult hepatitis B virus infection is recommended if (a) cryptogenic liver disease is observed, (b) prior to immunosuppression, and (c) in solid organ transplant donors with positive HBV serology (HBcAg antibodies) (Conjeevaram 2001; Torbenson 2004; Torbenson 2002). It is recommended that viral load should be measured every 3-6 months (EASL 2002; de Franchis 2003; Liaw 2005), although the therapeutic regimen may influence the decision on the interval lengths.

Furthermore, the measurement of viral load after starting therapy is a useful standard tool to help identify therapy non-responders (Schildgen 2004; Schildgen 2006; Sirma 2007; Volz 2007). Non-response to therapy can be induced by host factors, viral resistance, or non-compliance (reviewed by Tillman 2007). For quantification of the HBV viral load, several assays are commercially available, each having advantages and disadvantages (reviewed by Valsamakis 2007).

Utility of HBV genotyping

Genotyping of the HBV genome can be useful. First, the viral genotype influences the success of therapy, e.g., patients with an HBV genotype B infection have a better chance for a more favorable outcome than those patients infected with genotype C. Furthermore, genotype-specific response to drugs has also been observed for some antiviral compounds (Chen 2004; Colombo 2003; Enomoto 2006; Erhardt 2005; Flink 2006; Fung 2004; Guettouche 2005; Kao 2002; Kao 2003; Kao 2000; Kobayashi 2002; Liu 2002; Peteres 2004; Sanchez-Tapias 2002; Zhang 1996).

Second, genotyping is the simplest method for identification of resistance mutations known to be associated with viral non-response to nucleoside and nucleotide analogues. For those mutations that develop during treatment, genotyping is the method of choice for orientation and subsequent phenotyping (Volz 2007; Sirma 2007; Schildgen 2004; Schildgen 2006).

Third, genotyping plays an important role in the identification of infection chains in a nosocomial setting or if transmission by blood donations or blood products has occurred. Genotyping can be performed by in-house or commercial sequencing. PCR followed by INNO-LiPA hybridization has recently been developed and covers newly described mutations in the most recent product release (Hussain 2003; Hussain 2006; Osiowy 2003; Osiowy 2006). INNO-LiPA has the major advantage in being able to detect mixed infections as well.

Utility of antiviral resistance testing

With the introduction of more and more antiviral compounds into clinical practice over the last decade the option for new and combination treatments of HBV infections has increased greatly, and will continue to grow. As a side effect of the number of novel antivirals the development of resistance mutations has also started to increase, and it can only be assumed that the problem of antiviral resistance in HBV will become as complicated as what is happening for HIV treatments. After a genotypic analyses as mentioned above mutations already known can be identified and associated with resistance. Major evidence for resistance is if such mutations, like the mutations in the polymerase YMDD motif, evolve during ongoing therapy. The real question for virologists is if none of the known mutations is observed at failure. In such cases it has to be estimated how far other novel mutations not yet associated with resistance or novel mutations may contribute to the therapy failure.

In that case, in vitro phenotyping procedures established in a rather small number of HBV laboratories need to be performed (see Chapter 10). Unfortunately, known mutations can be detected by commercial methods whereas novel mutations remain speculative and thus, undetected or underestimated. Most recently, a project funded by the German Ministry for Education and Research that covers the first systematic geno2pheno approach for HBV has started. The major aim is to analyze clinical histories, corresponding genotypes, and the respective phenotypic profiles of 200-500 people with chronic HBV with respect to their individual drug regimens. By making use of a combined machine learning approach this project will result in a novel diagnostic tool that is combined with a genotypic assay and will rank the likelihood of success of a new drug or drug combination for a given RT sequence.

Host-related treatment failure, i.e., non-response despite proven compliance combined with a lack of resistance-associated mutations is an increasing problem in resistance testing. This panorama may be due to mal-functioning host mechanisms (see chapter 10) or to resistance mutations in the periphery of the postulated active site of the RT polymerase. The latter can be solely investigated by full length sequencing of a large number of clinical samples and subsequent phenotypic testing, until a crystal-structure is available that allows credible molecular modeling.

Utility of core promotor and precore mutation detection assays

Today, the diagnosis of HBeAg-negative chronic hepatitis B virus infection is based on the assessment of a combination of infection markers, namely positive HBsAg, negative HBeAg, and detectable viral DNA, in concert with anti-HBeAg antibodies and the evidence for liver injury measured by elevated liver enzymes or histopathological findings. Assays are commercially available in formats of PCR+hybridization, INNO-LiPA hybridization and sequencing, and the Affigene HBV mutant VL19 test (Olivero 2006; Qutub 2006).

Conclusion and ways forward

The major challenge for HBV diagnostics in the future will be the increasing number of mutations and the immune escape mutants, occult hepatitis and HBeAg-negative chronic hepatitis. Novel tools like those already established for HIV that help in the interpretation of laboratory results may help overcome these problems. However, the main problem will remain, namely the costs that tend to explode with every newly approved drug and the accompanying number of laboratory investigations that are essential to avoid suboptimal treatments while trying to find the optimal drug regimen.

References

Chen JD, Liu CJ, Lee PH, Chen PJ, Lai MY, Kao JH, et al. Hepatitis B genotypes correlate with tumor recurrence after curative resection of hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2004 Jan;2(1):64-71.

Colombo M, Rumi MG, Ninno ED. Treatment of chronic hepatitis C in Europe. J Hepatobiliary Pancreat Surg. 2003;10(2):168-71.

Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? Hepatology. 2001 Jul;34(1):204-6.

de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, et al. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol. 2003;39 Suppl 1:S3-25.

EASL International Consensus Conference on Hepatitis B. 13-14 September 2002: Geneva, Switzerland. Consensus statement (short version). J Hepatol. 2003 Apr;38(4):533-40.

Enomoto M, Tamori A, Nishiguchi S. Hepatitis B virus genotypes and response to antiviral therapy. Clin Lab. 2006;52(1-2):43-7.

Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, et al. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut. 2005 Jul;54(7):1009-13.

Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol. 2006 Feb;101(2):297-303.

Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology. 2004 Oct;40(4):790-2.

Guettouche T, Hnatyszyn HJ. Chronic hepatitis B and viral genotype: the clinical significance of determining HBV genotypes. Antivir Ther. 2005;10(5):593-604.

Hussain M, Chu CJ, Sablon E, Lok AS. Rapid and sensitive assays for determination of hepatitis B virus (HBV) genotypes and detection of HBV precore and core promoter variants. J Clin Microbiol. 2003 Aug;41(8):3699-705.

Hussain M, Fung S, Libbrecht E, Sablon E, Cursaro C, Andreone P, et al. Sensitive line probe assay that simultaneously detects mutations conveying resistance to lamivudine and adefovir. J Clin Microbiol. 2006 Mar;44(3):1094-7.

Kao JH. Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J Gastroenterol Hepatol. 2002 Jun;17(6):643-50.

Kao JH. Hepatitis B virus genotypes and hepatocellular carcinoma in Taiwan. Intervirology. 2003;46(6):400-7.

Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. J Hepatol. 2000 Dec;33(6):998-1002.

Keeffe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. Clin Gastroenterol Hepatol. 2006 Aug;4(8):936-62.

Kobayashi M, Arase Y, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, et al. Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. J Med Virol. 2002 Dec;68(4):522-8.

Liaw YF, Leung N, Guan R, Lau GK, Merican I, McCaughan G, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update. Liver Int. 2005 Jun;25(3):472-89.

Liu CJ, Kao JH, Chen PJ, Lai MY, Chen DS. Molecular epidemiology of hepatitis B viral serotypes and genotypes in taiwan. J Biomed Sci. 2002 Mar-Apr;9(2):166-70.

Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2001 Dec;34(6):1225-41.

Lok AS, McMahon BJ. [AASLD Practice Guidelines. Chronic hepatitis B: update of therapeutic guidelines]. Rom J Gastroenterol. 2004 Jun;13(2):150-4. a

Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. Hepatology. 2004 b Mar;39(3):857-61.

Manesis EK, Papatheodoridis GV, Sevastianos V, Cholongitas E, Papaioannou C, Hadziyannis SJ. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis B e antigen-negative chronic hepatitis B virus infection. Am J Gastroenterol. 2003 Oct;98(10):2261-7.

Olivero A, Ciancio A, Abate ML, Gaia S, Smedile A, Rizzetto M. Performance of sequence analysis, INNO-LiPA line probe assays and AFFIGENE assays in the detection of hepatitis B virus polymerase and precore/core promoter mutations. J Viral Hepat. 2006 Jun;13(6):355-62.

Osiowy C, Giles E. Evaluation of the INNO-LiPA HBV genotyping assay for determination of hepatitis B virus genotype. J Clin Microbiol. 2003 Dec;41(12):5473-7.

Osiowy C, Villeneuve JP, Heathcote EJ, Giles E, Borlang J. Detection of rtN236T and rtA181V/T mutations associated with resistance to adefovir dipivoxil in samples from patients with chronic hepatitis B virus infection by the INNO-LiPA HBV DR line probe assay (version 2). J Clin Microbiol. 2006 Jun;44(6):1994-7.

Peters MG, Hann Hw H, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology. 2004 Jan;126(1):91-101.

Qutub MO, Germer JJ, Rebers SP, Mandrekar JN, Beld MG, Yao JD. Simplified PCR protocols for INNO-LiPA HBV Genotyping and INNO-LiPA HBV PreCore assays. J Clin Virol. 2006 Nov;37(3):218-21.

Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterology. 2002 Dec;123(6):1848-56.

Schildgen O, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, et al. Variant of hepatitis B virus with primary resistance to adefovir. N Engl J Med. 2006 Apr 27;354(17):1807-12.

Schildgen O, Schewe CK, Vogel M, Daumer M, Kaiser R, Weitner L, et al. Successful therapy of hepatitis B with tenofovir in HIV-infected patients failing previous adefovir and lamivudine treatment. Aids. 2004 Nov 19;18(17):2325-7.

Sirma H, Funk A, Gerlich W, Schildgen O. Does pre-treatment with lamivudine prime for adefovir resistance of hepatitis B virus infection? J Antimicrob Chemother. 2007 Aug;60(2):448-9.

Tillmann HL. Antiviral therapy and resistance with hepatitis B virus infection. World J Gastroenterol. 2007 Jan 7;13(1):125-40.

Torbenson M, Kannangai R, Astemborski J, Strathdee SA, Vlahov D, Thomas DL. High prevalence of occult hepatitis B in Baltimore injection drug users. Hepatology. 2004 Jan;39(1):51-7.

Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis. 2002 Aug;2(8):479-86.

Valsamakis A. Molecular testing in the diagnosis and management of chronic h epatitis B. Clin Microbiol Rev. 2007 Jul;20(3):426-39, table of contents.

Volz S, Schildgen O, Muller A, Tillmann RL, Eis-Hubinger AM, Kupfer B, et al. [The human bocavirus: pathogen in airway infections?]. Dtsch Med Wochenschr. 2007 Jul 5;132(28-29):1529-33.

Zacharakis GH, Koskinas J, Kotsiou S, Papoutselis M, Tzara F, Vafeiadis N, et al. Natural history of chronic HBV infection: a cohort study with up to 12 years follow-up in North Greece (part of the Interreg I-II/EC-project). J Med Virol. 2005 Oct;77(2):173-9.

Zhang X, Zoulim F, Habersetzer F, Xiong S, Trepo C. Analysis of hepatitis B virus genotypes and pre-core region variability during interferon treatment of HBe antigen negative chronic hepatitis B. J Med Virol. 1996 Jan;48(1):8-16.

Chapter 9: Standard of care for hepatitis B virus infection

Florian van Bömmel, Johannes Wiegand, Thomas Berg

Introduction

Despite the availability of a prophylactic vaccine, chronic hepatitis B virus (HBV) infection remains a major global health concern with more than 350 million chronically infected individuals worldwide (Lavanchy 2004). These people carry a significantly increased risk of life-threatening complications such as hepatic decompensation, liver cirrhosis and hepatocellular carcinoma (HCC) (Beasley 1988). Recent studies have shown that the level of serum HBV DNA correlates with the risk of developing cirrhosis and HCC (Chen 2006; Iloeje 2006) (Figure 1). Suppressing the replication of HBV to below detection is now a major goal in HBV treatment.

There are currently two classes of approved agents: antiviral nucleoside/nucleotide analogues directly inhibiting HBV DNA replication and interferon α-based therapies that may modulate the host immune response as well as viral replication.

The number of agents approved for the treatment of chronic HBV infection continues to climb. In Europe, there are seven drugs: standard interferon α-2a and α-2b and peg-interferon α-2a, the nucleoside analogues lamivudine, telbivudine, and entecavir, and the nucleotide analogues adefovir and tenofovir. Nucleos(t)ides such as emtricitabine, torcitabine, amdoxovir and alamifovir are currently in development.

Due to this significant expansion of therapeutic options progression of HBV infections and complications can be prevented if the infection is diagnosed early and effectively treated. The early diagnosis of chronic hepatitis B by HBsAg screening in high-risk groups and in patients with elevated transaminases plays a crucial role in the management of HBV infection.

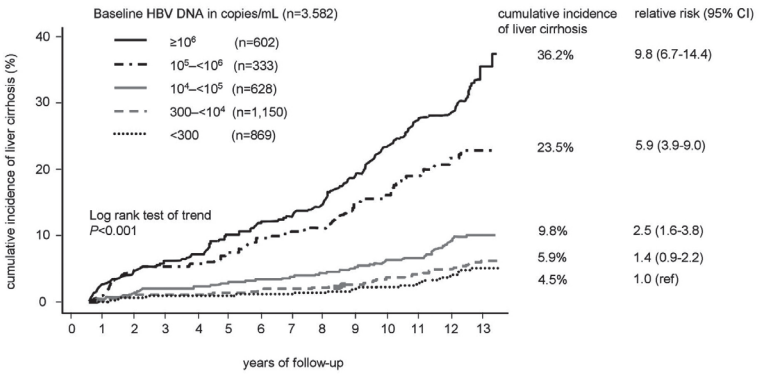


Figure 1. Cumulative incidence of liver cirrhosis in untreated HBV-infected individuals within a mean observation period of 11.4 years (REVEAL Study). The incidence of liver cirrhosis increases over time depending on baseline HBV DNA levels (Iloeje 2006).

Goals of antiviral therapy

Treatment endpoints

The aim of chronic hepatitis B therapy is to reduce the complications of HBV infection such as liver failure and HCC and to increase survival (European Association for the Study of the Liver 2009; Lok 2007; Lok 2009). To determine the success of antiviral therapy surrogate markers are used during and after treatment. These parameters include virologic (HBeAg and HBsAg status, HBV DNA level) and patient-related (aminotransferases, liver histology) criteria.

In two recent studies a close correlation between baseline HBV DNA levels and progression of the disease was demonstrated. In the REVEAL study, 3774 untreated HBV-infected individuals were followed over a mean time period of 11.4 years in Taiwan (Chen 2006; Iloeje 2006). HBV DNA levels at baseline were the strongest predictor of cirrhosis and HCC development (Figure 1). In multivariate models in this study, the relative risk of cirrhosis increased when HBV DNA reached levels greater than 300 copies/ml, independent of whether patients were HBeAg-negative or HBeAg-positive. The relative risk for developing HCC was 1.4 in patients with HBV DNA levels of 300 to 1000 and increased to 2.4 in patients with 1000-10,000, to 5.4 in patients with 10,000 to 100,000 and to 6.7 in patients with HBV DNA levels >1 million copies/ml. In addition, individuals with HBV DNA levels $\geq 10^4$ copies/ml (or ≥2,000 IU/ml) were found to have a 3-15 fold greater incidence of liver cancer as compared to those with a viral load $<10^4$ copies/ml. According to these results, a meta-analysis covering 26 prospective studies revealed a statistically significant and consistent correlation between viral load levels and histologic, biochemical, or serologic surrogate markers (Mommeja-Marin 2003). It can therefore be concluded that the complete and persistent suppression of HBV replication is a reliable endpoint for the treatment of chronic HBV infection.

In HBeAg positive patients, seroconversion from HBeAg to anti-HBe was found to be a reliable surrogate marker for prognosis of chronic HBV infection (Figure 2) leading in many cases to an inactive HBsAg carrier state. In these patients, HBsAg remains detectable but HBV replication continues at low or even undetectable levels and transaminases are generally within normal ranges.

Long-term observations revealed, however, that HBeAg seroconversion can not always be taken as a guarantee for long-term remission. A reactivation of the disease with “seroreversion” (i.e., HBeAg becoming positive again) as well as a transition to HBeAg-negative chronic hepatitis B with increased (but often fluctuating) HBV DNA levels can occur in up to 30% of patients (Hadziyannis 1995; Hadziyannis 2001; Hadziyannis 2006). Therefore, HBeAg seroconversion should be regarded as a stable treatment endpoint only in conjunction with durable and complete suppression of HBV replication.

In contrast, the endpoint of therapy for patients with HBeAg negative disease is more difficult to assess. Long-term suppression of HBV replication and ALT normalization are the only practical parameters of response to therapy. Once antiviral therapy is stopped, durability of response is not guaranteed due to the fluctuating course of HBeAg negative chronic hepatitis B.

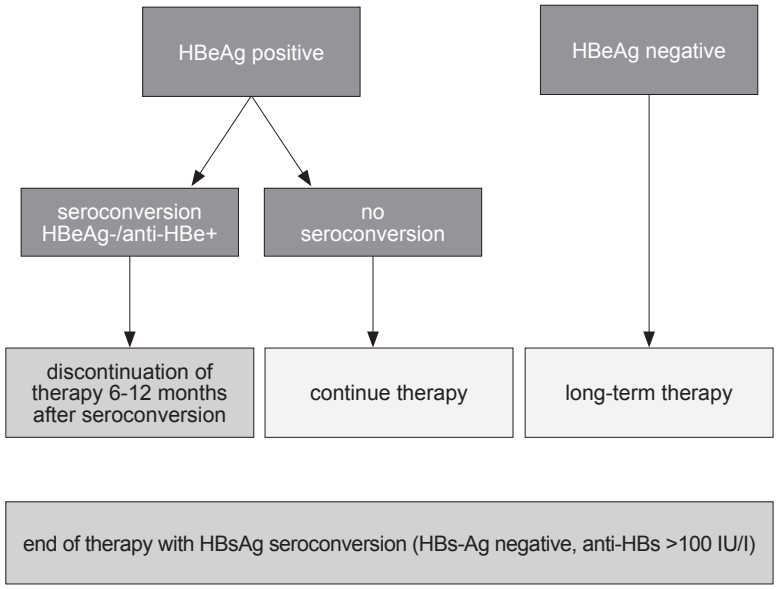


Figure 2. Possible endpoints of treatment of chronic HBV infection. After achieving HBeAg or HBsAg seroconversion, antiviral treatment can be stopped. However, it is recommended to maintain treatment for a period of 6-12 months after HBeAg or HBsAg seroconversion.

HBsAg loss or seroconversion to anti-HBs is the ultimate goal and most desirable endpoint of antiviral therapy. Because HBsAg loss or seroconversion is associated with a complete and definitive remission of the activity of chronic hepatitis B and an improved long-term outcome, it is regarded as a cure from chronic hepatitis B. However, HBsAg loss or seroconversion can be induced in only a limited number of patients after short-term treatment (<5%). Interestingly, in recent follow-up studies in PEG-IFN α as well as nucleos(t)ide analogue treated patients an increase of the rate of HBsAg loss during long-term studies was shown (Marcellin 2009b). The rate of HBsAg seroclearance during therapy with nucleos(t)ide analogues is probably linked to the duration of the period with low HBV replication. It seems therefore conclusive that with increasing treatment durations higher rates of HBsAg loss/seroconversion will be observed.

Nevertheless, due to the unique life cycle of HBV which involves nuclear deposition of episomal covalently closed circular DNA (cccDNA) a cellular template of the viral genome persists in hepatocytes even decades after HBsAg seroconversion (Rehermann 1996). Therefore, it is thought that due to cccDNA persistence complete eradication of HBV infections is impossible. Reactivation of HBV infection can occur in certain circumstances from these nuclear reservoirs even decades after HBsAg loss, for instance during immunosuppressive therapy.

Criteria for the response to therapy

Virologic:
- sustained decrease of HBV DNA, at least to <104 copies/ml (2x103 IU/ml), ideally to <300 copies/ml (60 IU/ml).
- sustained HBe seroconversion in HBeAg positive patients
- ideally, loss of HBsAg

Biochemical:
- sustained ALT normalization

Histologic:
- reduction of fibrosis (histological staging)
- reduction of inflammatory activity (histological grading)

Potential long-term effects:
- avoidance of cirrhosis, hepatocellular carcinoma (HCC), transplant, and death

Indication for antiviral therapy

Indications for acute hepatitis B

Acute hepatitis resolves spontaneously in 95-99% of cases (McMahon 1985). The possible effect of antiviral therapy has never been definitively proven and due to the high rate of spontaneous remission of acute hepatitis B in adults, therapy with the currently available drugs is not indicated. Currently, there are two randomized controlled studies, one from India (LAM 100 mg/day vs. placebo) and one from China (LAM 100 mg/day vs. standard of care) on the early initiation of therapy, looking at 71 and 80 patients, respectively, with severe acute hepatitis B (Kumar 2007; Yu 2009). Both studies saw a faster decrease of HBV DNA and of bilirubin levels in the patients treated with LAM vs. the control group, and both studies found that the kinetics of ALT levels where not affected by LAM treatment. However, in the Chinese group a significantly better clinical improvement as well as mortality was seen in patients treated with LAM, while the Indian study found no improvement in clinical outcome with LAM. Several case reports from Europe also revealed that patients with severe and fulminate hepatitis B may benefit from early antiviral therapy with LAM or other nucleos(t)ide analogues by reducing the need for high-urgency liver transplantation (Tillmann 2006).

An immediate oral antiviral therapy seems justified to prevent fulminate liver failure in cases where signs of liver synthesis impairment (drop of PT value, increase in INR) during acute hepatitis B are present.

Indications for antiviral therapy of chronic hepatitis B

Patients with HBsAg positive chronic hepatitis should be considered as possible candidates for antiviral therapy especially in situations when there is a significant level of HBV replication (Chen 2006; Iloeje 2006). Differentiation between HBeAg posi-

tive and HBeAg negative chronic hepatitis B is not necessary anymore for treatment indication, however with respect to the choice of the appropriate antiviral drug these criteria may be still useful.

Current recommendations of the different national and international societies are shown in Table 1 (Akarca 2007; Balik 2008; Carosi 2008; Colle 2007; Cornberg 2007; EASL Jury 2003; European Association for the Study of the Liver 2009; Janssen 2008; Juszczyk 2008; Keeffe 2007; Liaw 2008; Lok 2007; Lok 2009; Waked 2008). The main focus has shifted from histological proven disease activity to HBV DNA levels as the most relevant factor for a decision to initiate therapy. Thus, most of the recently published guidelines now recommend antiviral treatment for patients with HBV DNA levels >10,000 copies/ml (or >2000 IU/ml), in association with a sign of ongoing hepatitis which can either be ALT levels greater than 2 times the upper limit of normal or significant fibrosis in the liver, as demonstrated by liver histology greater than A1/F1.

In this context, a liver biopsy prior to the initiation of treatment can be useful for the treatment decision in some patients, as it provides important information on the prognosis of the disease, and helps in planning of subsequent therapeutic decisions for patients in which first line treatment fails. However, a liver biopsy is not mandatory to initiate treatment for the majority of patients (Table 1).

All patients with liver cirrhosis or high-grade liver fibrosis and any measurable HBV DNA should be considered for antiviral therapy (European Association for the Study of the Liver 2009; Lok 2007; Cornberg 2007). A flow chart showing the indication for antiviral treatment according to the recently published German guidelines is depicted in Figure 3 (Cornberg 2007). In patients with decompensated cirrhosis (Child-Pugh score B or C) IFN α is contraindicated.

Inactive chronic HBsAg carriers, characterised by negative HBeAg status (anti-HBeAg positivity), low HBV DNA levels (<10,000 copies/ml) and serum aminotransferases within normal levels do not have an indication for antiviral therapy (Cornberg 2007). However, differentiation between inactive HBsAg carriers and patients with chronic HBeAg-negative hepatitis may be difficult in some cases. Elevated transaminases are no reliable parameter for assessing the stage of liver fibrosis and long-term prognosis of HBV-infected patients. Even in patients with normal or slightly elevated aminotransferases there can be a significant risk for the development of HBV-associated complications (Chen 2006; Iloeje 2006; Kumar 2008). It is reasonable to perform a liver biopsy in these individuals and to control the level of HBV DNA at three-month intervals.

HBV immunotolerant patients are mostly under 30 years old and can be recognized by their high HBV DNA levels, HBeAg positivity, normal ALT levels and absence of significant histological changes. According to most practice guidelines immediate therapy is not required (Akarca 2007; Balik 2008; Carosi 2008; Colle 2007; Cornberg 2007; EASL Jury 2003; European Association for the Study of the Liver 2009; Janssen 2008; Juszczyk 2008; Keeffe 2007; Liaw 2008; Lok 2007; Lok 2009; Waked 2008). However, patients with elevated risk for HCC development, as those with a positive family history, and patients from high endemic areas like Asia or Africa may perhaps benefit from early antiviral therapy (Cornberg 2007). Studies are under way to further clarify this issue, especially to answer the question whether early intervention with antiviral therapy will positively influence the long-term risk for HCC.

AASLD (Lok 2007; 2009)	• Consider treatment: • HBeAg(+): HBV DNA >20,000 IU/ml + ALT ≤2x ULN + biopsy shows moderate/severe inflammation or significant fibrosis. • HBeAg(+): HBV DNA >20,000 IU/ml + ALT >2x ULN Observe for 3-6 months and treat if no spontaneous HBeAg loss. • HBeAg(-): HBV DNA >20,000 IU/ml + ALT >2x ULN • Consider biopsy: • HBeAg(+): HBV DNA >20,000 IU/ml + ALT >2x ULN + compensated • HBeAg(+): HBV DNA >20,000 IU/ml + ALT 1-2x ULN + age >40 years or family history of HCC • HBeAg(-): HBV DNA >2,000-20,000 IU/ml + ALT 1-2x ULN
APASL (Liaw 2008)	• Consider treatment: • All patients: HBV DNA detectable + advanced fibrosis/cirrhosis • HBeAg(+): HBV DNA >20,000 IU/ml + ALT >2x ULN + impending/overt decompensation • HBeAg(-): HBV DNA > 2,000 + ALT >2x ULN + impending/overt decompensation
EASL (European Association for the Study of the Liver 2009)	• Consider treatment: • HBV DNA >20,000 IU/ml + ALT >2x ULN + moderate to severe necroinflammation
Belgian (Colle 2007)	• Consider treatment: • HBeAg(+): HBV DNA >20,000 IU/ml + ALT >2x ULN (or moderate/severe hepatitis on biopsy) • HBeAg(-): HBV DNA ≥2,000 IU/ml and elevated ALT • Consider biopsy: • Fluctuating or minimally elevated ALT (especially in those older than 35-40 years)
Dutch (Janssen 2008)	• Consider treatment: • HBeAg(+) and HBeAg(-): HBV DNA ≥20,000 IU/ml and ALT ≥2x ULN or active necrotic inflammation • HBeAg(-): HBV DNA ≥2,000–20,000 IU/ml and ALT ≥2x ULN (and absence of any other cause of hepatitis)
German (Cornberg 2007)	• Consider treatment: • HBV DNA >2,000 IU/ml + minimal inflammation/low fibrosis or ALT ≥2x ULN
Italian (Carosi 2008)	• Consider treatment: • HBeAg(+): HBV DNA >20,000 IU/ml + ALT >2x ULN • HBeAg(-): HBV DNA >2,000 IU/ml + abnormal ALT and or fibrosis (Ishak ≥S2) • Consider biopsy: • HBeAg(-): HBV DNA >2,000 IU/ml + borderline ALT, or if DNA 2,000–20,000 IU/ml + high ALT
Polish (Juszczyk 2008)	• Consider treatment: • HBeAg(+): HBV DNA ≥20,000 IU/ml + raised ALT; biopsy not required • HBeAg(-): HBV DNA ≥2,000 IU/ml + raised ALT; biopsy not required • Biopsy required: • Normal ALT
Turkish VHSD (Balik 2008)	• Consider treatment: • HBeAg(+): HBV DNA >20,000 IU/ml + ALT >ULN or age >40 years (ALT 1-2x ULN) + histological indication • HBeAg(-): HBV DNA >2,000 IU/ml + histological indication
Turkish TASL (Akarca 2007)	• Consider treatment: • HBV DNA >2,000 IU/ml + histological fibrosis >2 • HBV DNA >20,000 IU/ml + any histological finding + ALT >2x ULN

Table 1. Guideline key recommendations for indication for antiviral treatment of HBV infections.

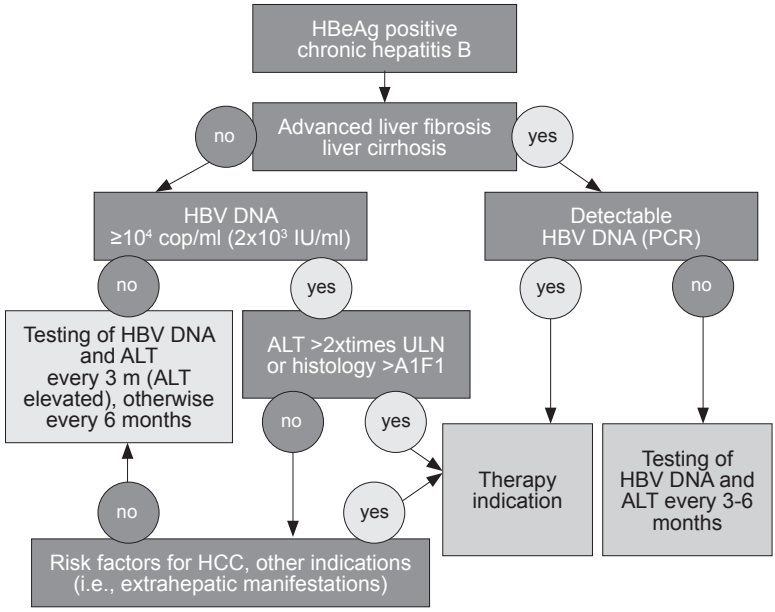


Figure 3. Indication for antiviral treatment according to the German guidelines for the treatment of chronic HBV infection. Treatment should be considered if HBV DNA levels exceed 10^4 copies/ml and if signs of ongoing hepatitis like ALT >2x upper limit of normal or liver histology >A1/F1 is present. Of note, asymptomatic carriers with family history of HCC should receive treatment even if signs of hepatitis are absent (Cornberg 2007).

Treatment indication: Summary of the recommendations of the German Guidelines

- In principal, all patients with chronic hepatitis B should be evaluated for antiviral therapy. The indication for treatment initiation depends on the level of viral replication (HBV DNA 10^4 copies/ml, corresponding to $2x10^3$ IU/ml), the histological grading and staging, and the level of serum aminotransferases.

- Especially patients with advanced fibrosis or cirrhosis need consistent antiviral therapy in case of detectable viremia.

- Reactivation of hepatitis B virus replication due to immunosuppression increases the risk of acute decompensation and cirrhosis. It should be avoided by preventive therapy.

- Alcohol and drug consumption are not a contraindication for treatment with nucleos(t)ide analogues.

- Pregnancy is usually a contraindication for all available drugs. Therapy with nucleos(t)ide analogues during pregnancy may be considered if the benefit outweighs the risk.

- Occupational and social aspects and extrahepatic complications may justify therapy in individual cases (Cornberg 2007).

Treatment options for chronic HBV infection

There are two classes available for the treatment of chronic HBV infection: interferon α (standard or pegylated (PEG)-IFN α) and inhibitors of the HBV polymerase, the nucleoside and acyclic nucleotide analogues.

While IFN α has been a mainstay in the treatment of chronic HBV infection for many years it is limited by its tolerability and side effect profile allowing administration for only a limited period of time (6-12 months, maximum 24 months). Nucleoside and nucleotide analogues have a better tolerability and are therefore applied in the long-term treatment of chronic hepatitis B. However, the efficacy of these oral agents can be hampered by the risk of the emergence of resistance. Two interferons and five oral antivirals are currently approved for the treatment of chronic HBV infections: standard IFN α-2b and PEG-IFN α-2a, lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (LdT), entecavir (ETV) and tenofovir disoproxil fumarate (TDF) (Table 2). The efficacy of the available drugs after one year of treatment, assessed by the proportion of individuals with HBV DNA below the limit of detection, normalized transaminases and HBeAg seroconversion is shown in Figure 4.

Agent	Name	Dose	Duration
Interferon α			
Standard Interferon α-2a	Roferon®	2.5-5 mi. IU per m^2 body surface 3x/week	4-6 months
Standard Interferon α-2b	Intron A®	5-10 mi. IU 3x/week	4-6 months
Pegylated Interferon α-2a	Pegasys®	180 µg/week	48 weeks
Nucleoside analogues			
Lamivudine	Zeffix®	100 mg/day	long-term*
Telbivudine	Sebivo®	600 mg/day	long-term*
Entecavir	Baraclude®	0.5 mg/day 1 mg/day for patients with lamivudine resistance	long-term* long-term*
Nucleotide analogues			
Adefovir dipivoxil	Hepsera®	10 mg/day	long-term*
Tenofovir disoproxil fumarate	Viread®	300 mg/day	long-term*

** see Figure 7*

Table 2. Overview of interferons and oral antiviral drugs currently approved for the treatment of HBV infection.

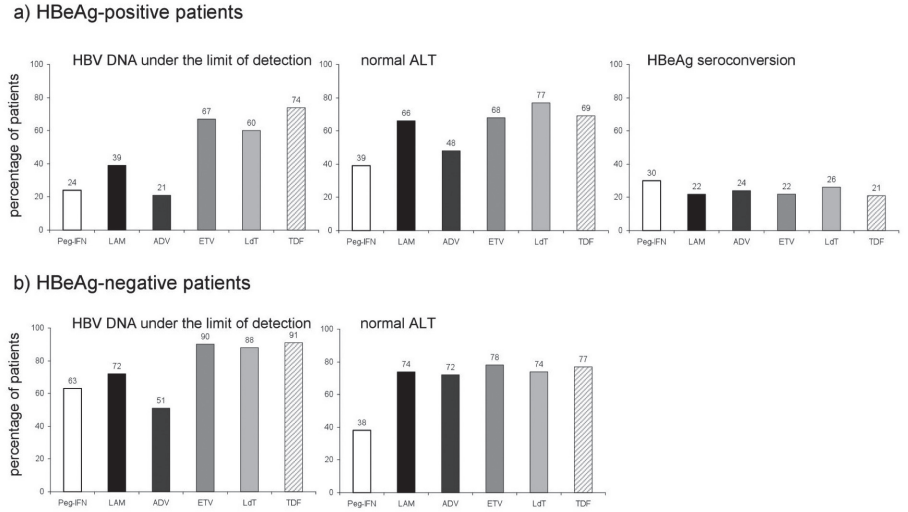


Figure 4. Efficacy of agents approved for the treatment of chronic HBV infection after one year of therapy (suppression of HBV DNA below the limit of detection, ALT normalization and HBeAg seroconversion). Differences between the different agents have to be interpreted with caution - the data does not come from head-to-head studies.

Interferons

IFN α is a natural occurring cytokine with immunomodulatory, antiproliferative and antiviral activity. The therapeutic efficacy of IFN α can often be clinically recognized by an increase of ALT levels at least twice the baseline level. These flares often precede virologic response.

The main aim of standard or PEG-IFN α treatment is to induce a long-term remission by finite treatment duration. Overall a long-term response defined by either HBeAg seroconversion or durable suppression of HBV DNA to low or undetectable levels can be achieved in approximately 30% of treated patients. In these responders the chance for HBsAg loss in the long-term is relatively high.

Standard IFN α:

Standard IFN α was approved as therapy of chronic hepatitis B in 1992. IFN α is applied in dosages ranging from 5 million units (MU) to 10 MU thrice weekly (or every other day). In a meta-analysis, a significant improvement in endpoints was shown in patients with HBeAg-positive chronic hepatitis B being treated with standard-interferon a in comparison to patients without treatment. Complete remission of fibrotic changes was observed and was often associated with the loss of HBsAg. Furthermore, there is a trend towards reduction of hepatic decompensation (treated 8.9% vs. untreated 13.3%), hepatocellular carcinoma (1.9 vs. 3.2%), and liver associated deaths (4.9 vs. 8.7%) (Craxì 2003).

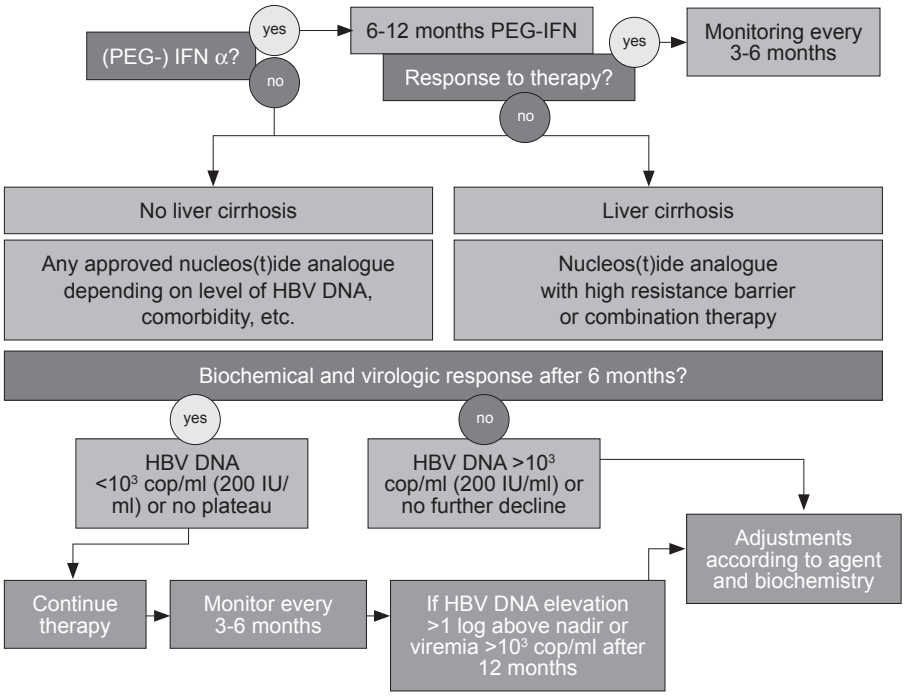


Figure 5. Treatment algorithm for chronic HBV infection according to the German Guidelines. Prior to evaluating the the best suitable nucleos(t)ide analogue, the indication for interferon therapy should always be considered (Cornberg 2007).

A significant decrease in ALT as well as HBV DNA concentration was also shown for standard interferon a for the therapy of HBeAg-negative chronic hepatitis B (Brunetto 2003). However, these patients relapse frequently after the end of treatment (25-89%) as evidenced by elevation of ALT levels and increase of HBV DNA levels. The relapse rate seems to be higher when treatment duration is short (16 to 24 weeks) compared to longer treatment (12 to 24 months). A retrospective comparison of therapies lasting for between 5 and 12 months showed that with longer treatment the chance of a long-term response was 1.64 times higher (normalization of ALT, HBV DNA $<1x10^6$ copies/ml 1-7 years after end of therapy). The overall response rates were 54% at the end of therapy, 24% at 1 year after therapy, and 18% 7 years after therapy (Manesis 2001).

In several studies, patients who had a long-term response to treatment demonstrated a more favourable course than patients who were untreated, unresponsive, or who had a relapse interferon a therapy with respect to the endpoints of progression to liver cirrhosis, liver associated deaths, and development of hepatocellular carcinoma (Brunetto 2003; Lampertico 2003).

Pegylated interferon α (PEG-IFN α)

The addition of a polyethylene glycol molecule (PEG) to the IFN has resulted in a significant increase in half-life, thereby allowing administration once weekly. Standard IFN α has now generally been replaced by PEG-IFN α. Two types of PEG-IFN α were developed: PEG-IFN α-2a and PEG-IFN α-2b, of which PEG-IFN α-2a was licensed for the treatment of chronic HBV infection in a weekly dose of 180 µg (subcutaneous) for 48 weeks in both HBeAg-positive and HBeAg-negative patients. The safety profiles of PEG-IFN α and standard IFN α are similar. Following therapy termination a relatively high relapse rate is to be expected (>50%). The questions of optimal dose of PEG-IFN α-2a (90µg vs. 180µg) and optimal duration of therapy have not been conclusively defined yet.

HBeAg-positive patients

For the treatment of HBeAg-positive chronic hepatitis B using pegylated interferon α four randomized, controlled studies have been performed (Chan 2005; Crespo 1994; Janssen 2005; Lau 2005). These studies compared PEG-IFN α to standard IFN, LAM, and/or a combination therapy with PEG-IFN α and LAM for a duration of 48-weeks course of PEG-IFN α-2a (180 µg per week) with or without LAM was compared to LAM monotherapy in HBeAg-positive patients. Sustained HBeAg seroconversion at the end of follow up (week 72) was significantly higher in patients treated with PEG-IFN α-2a alone or in combination with LAM than in patients treated with LAM alone (32% and 27% versus 19%) (Marcellin 2004).

Importantly, it was recently shown that PEG-IFN α can induce immunomodulatory effects which persist beyond the end of therapy leading to high HBsAg clearance rates in the follow-up period. In a recent study, 97 patients with chronic HBV infection who had received treatment with standard interferon were retrospectively analyzed for a median period of 14 (range, 5-20) years. During the observation period, 28 patients (29%) of this cohort lost HBsAg (Moucari 2009a). Interestingly, all of the patients who lost HBsAg in this study showed a significantly stronger decrease in HBsAg levels during treatment with PEG-IFN α as compared to patients who remained HBsAg positive. This influence of HBsAg kinetics on the outcome after treatment cessation was also found in another study (Lau 2008). In one study with 172 patients who were treated with PEG-IFN α-2b as monotherapy or in combination with LAM, the loss of HBeAg within the first 32 week of treatment was shown to be an on-treatment predictor for HBsAg loss during a mean period of 3.5 years after the end of treatment. HBsAg loss was found in 36% of the patients with early HBeAg loss and only in 4% of the patients with HBeAg loss after 32 weeks of treatment. Of note, in this study early HBeAg loss tended to occur more often in patients treated with PEG-IFN α and LAM combination therapy than in those treated with PEG-IFN α alone (35 vs. 21%; p=0.10) (Buster 2009).

HBeAg-negative patients

The efficacy and safety of PEG-IFN α-2a (180 µg once weekly) plus placebo, PEG-IFN α-2a plus LAM (100 mg daily), and LAM alone was also compared in 177, 179, and 181 patients with HBeAg-negative chronic hepatitis B, respectively. Patients were treated for 48 weeks and followed for an additional 24 weeks. After 24 weeks of

follow-up, the percentage of patients with normalization of ALT levels or HBV DNA levels below 20,000 copies/ml was significantly higher with PEG-IFN α-2a monotherapy (59% and 43%, respectively) and PEG-IFN α-2a plus LAM (60% and 44%) than with LAM monotherapy (44% and 29%); the rates of sustained suppression of HBV DNA below 400 copies/ml were 19% with PEG-IFN α-2a monotherapy, 20% with combination therapy, and 7% with LAM alone.

The rate of HBsAg clearance in HBeAg negative patients was also looked at (Marcellin 2009a). In a study evaluating the outcome of 315 patients who were treated with either PEG-IFN α-2a, LAM 100 mg or combination of both drugs for 48 months, three years after the end of treatment the level of HBsAg losses was 8.7% in patients who had been treated with a PEG-IFN α-2a alone or in combination with LAM while no patient treated with LAM as monotherapy cleared HBsAg. Of the patients who had received a PEG-IFN α-2a and who still had undetectable HBV DNA three years after treatment, 44% had lost their HBsAg. In another study comprising 48 patients who were treated with PEG-IFN α-2a, a decrease in serum HBsAg levels of 0.5 and 1 log IU/ml at weeks 12 and 24 of therapy was associated with a positive predictive value for HBsAg loss of 90% and 97% at week 96 after treatment, respectively (Moucari 2009).

Although combination of LAM plus PEG-IFN α failed to demonstrate benefit when evaluated at the end of follow-up in most studies, a more pronounced on-treatment virologic response (week 48) was observed with combination therapy as compared to LAM or PEG-IFN α alone. This more profound HBV DNA suppression induced by the combination regimen was associated with a lower incidence of LAM resistance (presence of YMDD mutants in 1% vs. 18% at the end of therapy). The use of a fixed combination with LAM plus PEG-IFN α is presently not recommended.

However, combination therapies between PEG-IFN α and more potent nucleos(t)ide analogues may be attractive. Preliminary data indicate that PEG-IFN α plus ADV can lead to HBsAg loss in 22% of cases. Although the number of treated patients is low (n=45) it has to be admitted that similar rates of HBsAg loss have never been reported before (Takkenberg 2009).

Nucleoside and nucleotide analogues

Nucleos(t)ide analogues inhibit viral replication by blocking the nucleoside binding site of the viral polymerase and competing with the natural substrate deoxyadenosine triphosphate (dATP) and by terminating DNA chain prolongation after incorporation into viral DNA. Nevertheless, these agents' detailed mechanisms of action for inhibiting HBV DNA synthesis vary greatly. Nucleoside and acyclic nucleotide analogues represent different subclasses of reverse transcriptase inhibitors: while both are based on purines or pyrimidines, acyclic nucleotide analogues possess an open (acyclic) ribose ring which confers greater binding capacity to resistant HBV polymerase strains (Figure 6).

In contrast to IFN-based treatment strategies where finite treatment duration of 24-48 weeks is established, treatment duration for nucleos(t)ide analogues is not well defined but has to be administered for extended periods in order to control viral replication long-term. Short-term application of these agents for only 48 weeks is associated with prompt relapse in viremia.

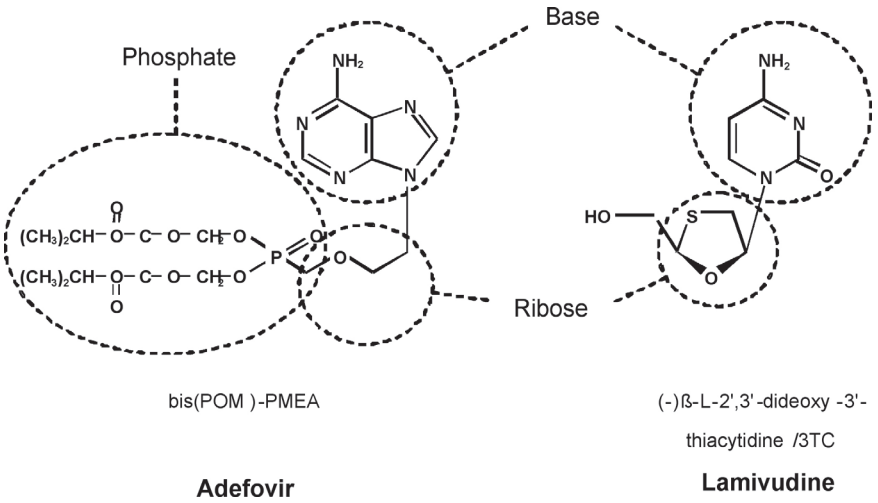


Figure 6. Chemical structure of the nucleoside analogue LAM and the acyclic nucleotide analogue ADV as an example for purine- or pyrimidine-based viral transcription inhibitors. LAM is based on cytosine while adefovir is based on adenine. In contrast to LAM, ADV possesses an acyclic ribose ring which gives a higher flexibility to the molecule.

Studies with nucleoside and nucleotide analogues have clearly demonstrated that suppression of HBV viremia is associated with a significant decrease in histologic inflammatory activity and fibrosis, including partial reversion of earlier stages of liver cirrhosis (Chen 2006; Iloeje 2006; Mommeja-Marin 2003). The HBeAg seroconversion rate also increases with increasing treatment duration (Liaw 2000; Lok 2000). Most importantly, effective long-term control of HBV replication by nucleoside or nucleotide analogues is associated with a reduction of long-term complications such as HCC and development of liver cirrhosis (Toy 2009). There is also evidence that effective inhibition of HBV replication can reduce HBV cccDNA, possibly running parallel to the decline in serum HBsAg levels (Werle-Lapostolle 2004; Wursthorn 2006). These findings hopefully indicate that long-term antiviral therapy may lead to a complete response in a significant number of patients.

A central aspect of HBV polymerase inhibitor treatment is the prevention and management of HBV resistance to these drugs (see Chapter 10). Resistance against nucleoside or nucleotide analogues can occur during suboptimal treatment and often leads to aggravation of liver disease. Therefore nucleoside-naive and nucleoside-experienced patients have to be distinguished. Since several nucleoside analogues have overlapping resistance profiles, prior nucleoside experience should be taken into account when selecting a second-line therapy.

Lamivudine (LAM)

Lamivudine is a synthetic nucleoside analogue that was approved for the treatment of chronic hepatitis B in 1998. LAM is the (-) enantiomer of 2' -3' dideoxy-3'-thiacytidine. The phosphorylated form (3TC-TP) exerts its therapeutic action by competing with dCTP for incorporation into the growing viral DNA chains, causing chain termination. By inhibiting both the RNA- and DNA-dependent DNA polymerase activities, the synthesis of both the first strand and the second strand of HBV DNA are interrupted. LAM is an oral medication and its dose for chronic hepatitis B is 100 mg daily. This dose was chosen based on a preliminary trial that randomly assigned 32 patients to receive 25, 100, or 300 mg of LAM daily for a total of 12 weeks (Dienstag 1995). In this study the dose of 100 mg was more effective than 25 mg and was similar to 300 mg in reducing HBV DNA levels, therefore the dose of 100 mg daily was chosen for hepatitis B therapy. The efficacy data of LAM after 1 year of treatment are summarized in Figure 4.

Long-term LAM treatment is associated with an increasing rate of antiviral drug resistance reaching approximately 70% after 5 years in patients with HBeAg positive, high-level replication HBV infection. Therefore, in many guidelines LAM is not considered a first-line agent in the treatment of chronic HBV infection any more. However, LAM still may play a role in combination regimens or in patients with mild chronic hepatitis B expressing low levels of HBV DNA ($<10^5$ copies/ml). The early and complete virologic response to LAM within 6 months of therapy (<400 copies/ml) hereby constitutes a prerequisite for long-term control of HBV infection without the risk of developing resistance.

Adefovir dipivoxil (ADV)

Adefovir dipivoxil was approved for treatment of chronic hepatitis B in the US in 2002 and in Europe in 2003. It is an oral diester prodrug of adefovir, a nucleotide adenosine analogue that, in its active form (adefovir diphosphate), inhibits the HBV DNA polymerase. Because the acyclic nucleotide already contains a phosphate-mimetic group, it needs only two, instead of three, phosphorylation steps to reach the active metabolite stage. It does not depend on the virus-induced kinase to exert its antiviral action. Adefovir dipivoxil was the first substance with simultaneous activity against wild-type, pre-core, and LAM-resistant HBV variants. It is active *in vitro* against a number of DNA viruses, in addition to HBV, and retroviruses (i.e., HIV). The dose of 10 mg per day was derived from a study comparing 10 mg versus 30 mg/d. The higher dosage leads to stronger suppression of HBV DNA levels but also to renal toxicity with an increase of creatinine levels that limits use (Hadziyannis 2003).

ADV was the first acyclic nucleotide that was widely used in the treatment of LAM-resistant HBV infections. However, the antiviral effect of ADV in the licensed dosage of 10 mg/d is rather low as compared to other available antivirals (Figure 4); this disadvantage makes ADV vulnerable to HBV resistance (Hadziyannis 2006a). Today, the add-on therapy in LAM resistance is the preferred strategy for the use of ADV, as discussed below (Lampertico 2007). With the approval of TDF, ADV should not be used as first-line monotherapy any more.

Telbivudine (LdT)

Telbivudine is a thymidine analogue which is active against HBV but at least *in vitro* not active against other viruses, including HIV and hepatitis C virus (HCV). It was reported to be non-mutagenic, non-carcinogenic, non-teratogenic, and to cause no mitochondrial toxicity. A favourable safety profile at a daily dose of 600 mg was demonstrated (Hou 2008; Lai 2007). However, CK elevations were observed more often as compared to the group treated with LAM and neurotoxicity may be an issue when LdT is administered in combination with PEG-IFN α (Fleischer 2009).

LdT at 600 mg/day expresses higher antiviral activity compared to either LAM at 100 mg/day or ADV at 10 mg/day (Figure 4). More patients achieved HBeAg loss within 48 weeks of treatment as compared to other nucleoside/nucleotide analogues.

However, resistance to LdT has been found to occur in up to 21% after 2 years of treatment (Tenney 2009). Resistance was predominantly observed in patients who did not achieve undetectable HBV DNA level after 24 weeks of treatment (Zeuzem 2009). LdT should be used quite cautiously in HBeAg positive patients with high HBV DNA levels ($>10^9$ copies/ml) because the risk of incomplete virologic response at week 24 is especially high in this patient population. Furthermore, treatment with LdT should be modified in all patients when HBV DNA levels do not reach undetectable by 24 weeks of treatment (switch to a more potent drug or add-on a second non-cross-resistant drug). LdT shows cross resistance to LAM and ETV. As a consequence LdT should not be used in LAM or ETV refractory patients.

In patients who received LdT treatment neuropathy and myopathy have been reported. In the GLOBE trial, during a period of 104 weeks grades 3/4 elevations in CK levels were observed in 88 of 680 (12.9%) patients who received LdT and in 28 of 687 (4.1%) patients who received LAM ($p<0.001$) (Liaw 2009). However, rhabdomyolysis was not observed in anyone. In combination with PEG-IFN, LdT has also been reported to be associated with peripheral neuropathy. Peripheral neuropathy was described in 9 of 48 (18.75%) patients who received combination therapy of PEG-IFN and LdT and only in 10 of 3500 (0.28%) patients who received LdT monotherapy (Goncalves 2009).

Entecavir (ETV)

Entecavir, a cyclopentyl guanosine nucleoside analogue, is a selective inhibitor of HBV replication and was licensed in 2006. Entecavir blocks all three polymerase steps involved in the replication process of the hepatitis B virus: first, base priming; second, reverse transcription of the negative strand from the pre-genomic messenger RNA; third, synthesis of the positive strand of HBV DNA. In comparison to all other nucleoside and nucleotide analogues, ETV is more efficiently phosphorylated to its active triphosphate compound by cellular kinases. It is a potent inhibitor of wild-type HBV but is less effective against LAM-resistant HBV mutants. Therefore, ETV was approved at a dose of 0.5 mg per day for treatment naïve HBeAg-positive and -negative patients and in the dose of 1 mg per day for patients with prior treatment with LAM (Lai 2005; Sherman 2008).

ETV has a favourable tolerability profile and can be easily adjusted to renal function. Besides LAM it is the only nucleos(t)ide analogue which is available as tablet and as an oral solution. However, ETV may cause severe lactic acidosis in patients with impaired liver function and a MELD score of >20 points (Lange 2009).

Treatment-naïve HBeAg positive patients achieved undetectable HBV DNA levels in 67% and 74% after one and two years of ETV treatment, respectively (Figure 4) (Gish 2007; Sherman 2008). Long-term studies in ETV responder patients demonstrated that response can be maintained in nearly all patients over an observation period of up to five years. So far, the rate of resistance in the long-term is estimated to be approximately 1% for treatment-naïve patients (Tenney 2009). Loss of HBsAg occurs in 5% of treatment-naïve individuals after two years of ETV therapy (Gish 2010).

In LAM resistant patients ETV is less potent. Only 19% and 40% of these patients achieved undetectable HBV DNA after one and two years, respectively, despite an increased dose of 1 mg/day (Gish 2007; Sherman 2008). Due to cross-resistance up to 45% of patients with LAM resistance develop resistance against ETV after 5 years of treatment (Tenney 2009).

Although ETV is not cross-resistant to and more potent than ADV, recent data indicate impaired efficacy of ETV after treatment failure of ADV (Cho 2009; Reijnders 2009; Shim 2009). The mechanisms of this are not clear. Insufficient intracellular drug levels or presence of viral strains with reduced susceptibility against ETV at baseline are discussed as possible explanations. Interestingly, ADV-related mutations (A181V and N236T) persist during ETV therapy although they should be susceptible to the second-line treatment (Reijnders 2009).

Tenofovir (TDF)

Tenofovir disoproxil fumarate, an ester prodrug form of tenofovir (PMPA; (R)-9-(2-phosphonylmethoxypropyl)), is an acyclic nucleoside phosphonate (or nucleotide analogue). TDF has selective activity against retroviruses and hepadnaviruses and is currently approved for the treatment of human immunodeficiency virus (HIV) infections and for therapy of chronic hepatitis B. TDF shows strong activity against both HBeAg-positive and HBeAg–negative HBV infections in treatment-naïve patients (Heathcote 2008; Marcellin 2008). *In vitro* studies have clearly demonstrated that LAM resistant HBV strains (mutations rtM204I/V, rtL180M and rtL173M) remain susceptible to TDF (Lada 2004). These observations are consistent with clinical studies showing a high efficacy of TDF in LAM resistant HBV infections irrespectively of the kind of mutation mediating LAM resistance (van Bömmel 2010). Due to possibly existing cross-resistance to ADV, the efficacy of TDF might be hampered by the presence of ADV resistance; however, a breakthrough of HBV DNA during TDF treatment in patients with previous ADV failure was not observed (van Bömmel 2007).

TDF is generally well-tolerated and not associated with severe side effects. For HBV mono-infected, treatment-naïve patients, renal safety during TDF monotherapy was investigated in three studies. In a randomized study comprising HBeAg negative patients, none of 212 patients treated with TDF for three years and non of 112 patients who were treated with ADV for one year who then switched to TDF for two years had a decrease in GFR to levels <50 ml/min or an increase of serum creatinine levels >0.5 mg/dl (Marcellin 2009). In a similar study in HBeAg positive patients, of 130 patients treated with TDF for 3 years and of 76 patients treated with ADV for one year and consecutively with TDF for 2 years, only one patients showed an increase in serum creatinine levels >0.5 mg/dl starting at year two (Heathcote 2009). In a sub-analysis of both studies in 152 HBeAg-positive and

-negative Asian patients, no increase of serum creatinine >0.5 mg/dl or of eGFR <50 ml/min was found in up to 3 years of TDF treatment (Liaw 2009a).

In large randomized clinical trials in HBV/HIV coinfected patients the use of TDF was associated with an excellent renal safety profile. However, mild impairment of renal function up to acute renal failure (Coca 2002; Créput 2003; Gallant 2005; Karras 2003; Peyriere 2004; Verhelst 2002) and Fanconi syndrome was reported in some HBV/HIV coinfected patients on TDF treatment (Gaspar 2004). From a recent long-term study in 102 HIV/HBV coinfected patients treated with TDF for a mean duration of 55 months (range 8-88 months), a statistically significant decrease in mean eGFR estimated from 105±30 ml/min to 94±26 ml/min at the end of observation as assessed by the MDRD formula was shown (Reijenders 2009). Nine (9%) patients developed renal impairment, which persisted in three subjects for more than 3 months. In three patients TDF treatment had to be stopped due to renal dysfunction. In these patients the decline of creatinine clearance was significantly greater than in those being exposed to other nucleoside-based antiviral drugs. Regular monitoring of renal function is therefore recommended during TDF therapy.

Three year efficacy data are available for TDF in (mostly) treatment-naïve HBeAg positive and -negative patients. TDF showed marked antiviral efficacy with complete virologic response rates (HBV DNA <400 copies/ml) reaching nearly 100% and 90% in HBeAg-negative and -positive patients respectively, after three years of therapy. In the study in HBeAg-positive patients, 6% of patients experienced HBsAg loss, of whom 4% had received TDF continuously and 5% had switched from ADV to TDF after 6 months (Heathcote 2009; Marcellin 2009).

No drug resistance has been observed so far. Similar efficacy without development of resistance was also demonstrated when TDF was administered to LAM-refractory patients or those showing incomplete response to ADV (van Bömmel 2010).

Nucleos(t)ide analogue combination therapy as first-line treatment

As of now, nucleoside and nucleotide analogues are not indicated for first-line combination therapy.

There is only one study comparing a combination therapy with LAM and ADV to LAM monotherapy in untreated patients (Sung 2008). In this study, there was no difference in the virologic and biochemical response between both groups. The rate of LAM resistance was much lower in the combination group. However, the development of resistance could not be completely avoided even with the use of an additional dose of ADV.

Another study analyzing the combination of LAM with LdT also showed no benefit for combination therapy (Lai 2005). No data are available on other combination therapies.

The risk of teratogenicity of nucleos(t)ide analogues is assessed by a classification based on any data gathered in clinical trials as well as through the FDA Pregnancy Registry. TDF and LdT are listed as pregnancy category B drugs and LAM, ADV and ETV as category C drugs. In pregnant women with high levels of HBV viremia, LAM treatment during the last trimester of pregnancy was reported to reduce the risk of intra-uterine and perinatal transmission of HBV if given in addition to passive and active vaccination by HBIg and HBV (van Zonneveld 2003). During treatment with TDF, the birth defect prevalence was recently shown to be as high as during treatment

with LAM (Brown 2009). However, treatment with nucleos(t)ide analogues during pregnancy should be carefully monitored and should be limited to the second and third trimester. As exacerbations of chronic hepatitis B may occur, women with HBV should be monitored closely after delivery (ter Borg 2008).

Prognostic factors for therapeutic success

Several factors are positively associated with long-term remission and may help to guide treatment decisions. Pre-treatment factors predictive of HBeAg seroconversion are low viral load, high ALT levels (above 2-5 times ULN) and high histological grading (Flink 2006; Hadziyannis 2006; Lai 2007; Perrillo 1990; Perrillo 2002; Wong 1993; Yuen 2007; Zoulim 2008). These general baseline predictors are relevant especially for treatment regimens with PEG-IFN α but may in part be relevant also for nucleos(t)ide analogues (Table 3).

	Nucleoside/nucleotide analogues	Peg-interferon α
Before treatment	Low viral load (HBV DNA ≤ 107 IU/ml), high serum ALT levels (above 3 times ULN), high activity scores on liver biopsy (at least A2)	
During treatment	Undetectable HBV DNA in a real-time PCR assay at 24 or 48 weeks is associated with HBeAg seroconversion in HBeAg-positive patients and lower incidence of resistance	HBV DNA decrease <20,000 IU/ml at 12 weeks is associated with a 50% chance of HBeAg seroconversion in HBeAg-positive patients and with a 50% chance of sustained response in HBeAg-negative paitents
HBeAg decrease		HBeAg decrease at week 24 may predict HBeAg seroconversion
HBV genotype	HBV genotype shows no influence on response	Association with HBV genotype A and B and than with genotypes C response to IFN α is higher than genotypes C and D; however the association is weak and HBV genotype should not be the only argument for treatment decision

Table 3. Predictors of response to antiviral therapy.

HBV genotypes have been shown to be associated only with viral response to interferon a. Patients with HBV genotype A, prevalent in Northern Europe and USA, showed a much higher rate of HBeAg and HBsAg seroconversion than patients with HBV genotype D, prevalent in the South of Europe, or the HBV genotypes B or C originating from Asia (Keeffe 2007; Wiegand 2008).

A pooled analysis from the two largest trials using PEG-IFN α-2a or -2b in chronic hepatitis B tried to calculate a score predicting successful interferon therapy based on an individual patient's characteristics (viral load, ALT level, HBV genotype, age, gender). However, this approach may only be feasible in HBeAg-positive patients (Buster 2009).

How to treat

One can choose either to treat with PEG-IFN α in order to induce a long-term control by finite treatment or with nucleos(t)ide analogues to inhibit HBV replication long-term (Figure 3).

At first, interferon therapy should be evaluated. However, if a patient does not fulfil the criteria for PEG-IFN α, has contraindications, or is intolerant, long-term therapy with nucleos(t)ide analogues is recommended. If a nucleos(t)ide analogue is chosen several parameters have to be considered prior to therapy: the antiviral efficacy of the drug, the durability of response, the resistance barrier, and the stage of liver disease.

If the initial viral load is low and liver cirrhosis has been excluded, any approved drug may be used. The use of LAM, however, should be restricted to patients with mild fibrosis and HBV DNA levels $<10^5$ copies/ml. For patients with high-level HBV replication ($>10^9$ copies/ml) only drugs with a high genetic barrier should be used (i.e., ETV or TDF, see Table 4).

Drug	Advantage	Disadvantage	Recommendation
Lamivudine (LAM)	- low treatment costs - oral solution available for individual dosage in case of renal impairment	- high risk of resistance in long-term monotherapy - cross-resistance to ETV and LdT	- use as first-line therapy only in selected patients with low viral load - prevention of exacerbation in HBsAg +, HBV DNA - patients with immunosuppression - pre-emptive therapy in case of HBsAg negative, anti-HBc positive patients with immunosuppression
Adefovir dipivoxil (ADV)	- a lot of experience in combination with LAM - no cross-resistance to LAM	- moderate antiviral activity - primary non-response in 10-20% of cases - slow viral kinetics during therapy - risk of viral resistance in long-term monotherapy - nephrotoxicity	- not to use as first-line therapy
Telbivudine (LdT)	- high antiviral efficacy - potentially no cross-resistance to entecavir as potential concerns	- moderate risk for viral resistance in long-term monotherapy - neuropathy and myopathy	- first-line therapy possible - can be combined with TDF
Entecavir (ETV)	- high antiviral efficacy - low risk for viral resistance in long-term monotherapy in lamivudine-naïve patients - combination therapy with TDF as rescue therapy - oral solution available for individual dosage in case of renal impairment	- in LAM-experienced patients high risk for viral resistance in long-term monotherapy	- first-line therapy possible - can be combined with TDF
Tenofovir disoproxil fumarate (TDF)	- high antiviral efficacy - low risk for viral resistance in long-term monotherapy	- nephrotoxicity and bone density decline as potential concerns	- first line therapy possible - can be combined with ETV, LdT or LAM if needed

Table 4. Recommendations for the use of nucleos(t)ide analogues in clinical practice.

Monitoring of patients before and during antiviral therapy

Before therapy, HBV DNA levels should be measured with a highly sensitive assay. These results should be confirmed 1-2 months after initiation of therapy. In addition, ALT levels reflecting the inflammatory activity as well as creatinine levels should be determined. HBV genotyping is only recommended in patients who are considered candidates for treatment with interferon. HBV resistance testing can be useful in patients with prior failure to more than one nucleoside/nucleotide analogue, but this is not yet a standard diagnostic approach. HBV resistance has to be expected when an increase of HBV DNA of >1 log during antiviral treatment is observed. In cases of primary treatment failure an appropriate second line treatment can be chosen without resistance testing.

During therapy, HBV DNA, ALT and creatinine levels should be measured initially, after 4 to 6 weeks and then every 3 months. The early identification of viral resistance and an early adjustment of therapy are crucial. Patients with suppression of HBV replication <300 copies/ml (60 IU/ml) for at least 2 years may perhaps be scheduled at 6 month intervals. However, no studies have been performed that support this procedure.

In HBeAg-positive patients, HBeAg and anti-HBe as well as HBsAg and anti-HBs should be also measured if HBV DNA levels become undetectable to identify seroconversion as an endpoint of HBV therapy (Table 5).

Tests before antiviral treatment	**Interval**
HBV DNA quantitative	
HBeAg, anti-HBe	
HBV genotype	If IFN-based treatment is planned
ALT level	
Creatinine level	
Other chemistry tests	
Tests during antiviral treatment	**Interval**
HBV DNA quantitative	After 4-6 weeks, after 12 weeks, then every 3-6 months
HBeAg, anti-HBe	3-6 months, if HBV DNA is undetectable
HBsAg, anti-HBs	3-6 months, in HBeAg-positive patients after HBeAg seroconversion in and HBeAg-negative patients if
HBV	DNA is undetectable
HBV resistance test	If HBV DNA increases >1 log during antiviral treatment and pre-treatment history is not tractable, but first check for treatment adherence!
ALT level	Initially every month, than every 3-6 months
Creatinine level*	Every 3-6 months
Other chemistry tests	Every 3-6 months

** patients treated with TDF should initially be monitored every 4 weeks to watch for decrease of kidney function*

Table 5. Recommendation for laboratory tests for monitoring antiviral therapy.

HBV DNA as a parameter of response to antiviral therapy

During antiviral therapy, the decrease of HBV DNA levels from baseline is the most important tool in monitoring treatment efficacy. Complete response to antiviral therapy is defined as suppression of HBV DNA to below the limit of detection as measured by a sensitive real time PCR assay (Figure 7). Incomplete suppression is characterized by persistent HBV replication despite antiviral therapy. Ongoing HBV replication should be avoided to prevent the selection of resistant HBV strains by replication of the virus in the presence of drug in the so called "plateau phases". An HBV DNA breakthrough despite continuous antiviral therapy is often caused by viral resistance. Measuring of HBV DNA kinetics early during therapy will help to guide antiviral treatment and to establish early stopping rules or add-on strategies to avoid antiviral failure (Figure 7).

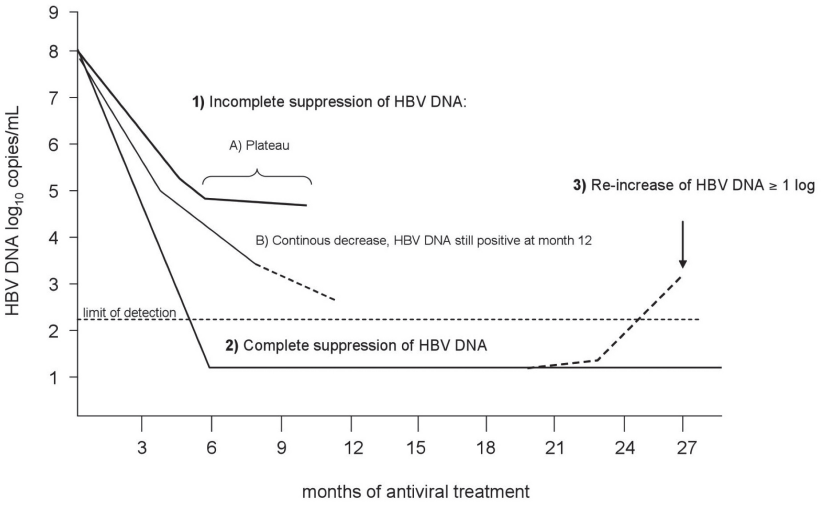


Figure 7. Possible courses of antiviral treatment with nucleoside/nucleotide analogues. Incomplete suppression of HBV DNA results in either a "plateau phase" or in a continuous slow decline. A plateau phase represents a high risk for selection of resistant HBV variants, therefore treatment should be changed to a more effective agent or combination therapy. A continuous slow decline should induce a treatment change after 6 months if drugs with a low genetic barrier like LAM or LdT are used. If drugs with a high genetic barrier like ETV or TDF are applied, a continuous slow decline can be monitored for at least 12 months without increased risk of consecutive HBV resistance.

Incomplete or partial virologic response to oral nucleoside or nucleotide analogues is defined as a decrease of HBV DNA >1 log but remaining measurable (Lavanchy 2004) (Figure 7). The definition of partial response depends on the type of treatment; thus, for agents with a high genetic barrier against resistance like ETV, ADV or TDF partial response is defined after 12 months and for substances with a low genetic barrier against HBV resistance like LAM or LdT, after 6 months of monotherapy. In case

of partial response to a drug with a low genetic barrier, an appropriate rescue therapy should be initiated. By current guidelines, a combination treatment with a nucleotide analogue is recommended for these patients. However, it was recently shown that patients with partial response to LAM or to ADV have a high probability of responding to TDF monotherapy, without risking the development of resistance (Heathcote 2009; Marcellin 2009; van Bömmel 2010). Patients with a partial response to ADV were also shown to have a high probability of responding to a following monotherapy with ETV, irrespective of the presence of mutations associated with HBV resistance to ADV (Leung 2009; Leung 2009a) (Figure 8).

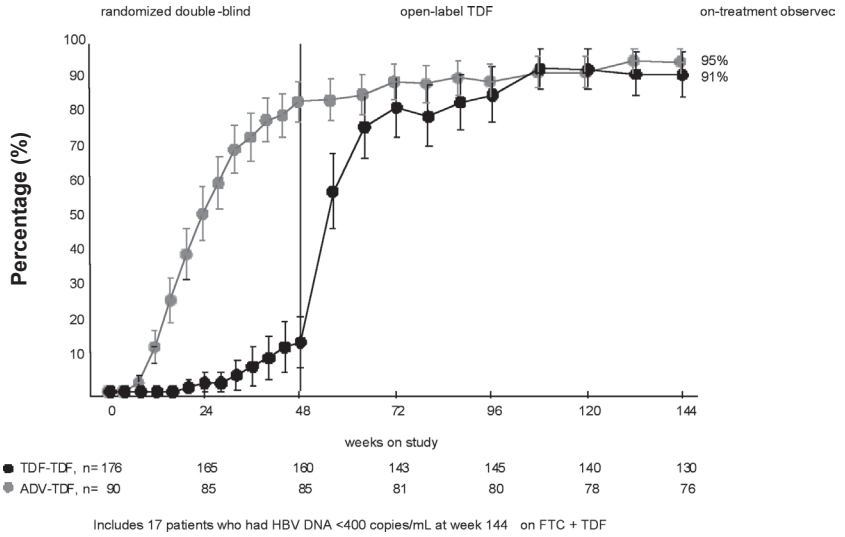


Figure 8. Percentage of HBeAg-positive patients achieving HBV DNA <400 copies/ml over 4 years of monotherapy with TDF 300 mg. Patients who were treated with adefovir during the first 48 weeks were changed to TDF. Of note, in 17 patients with undetectable HBV DNA at week 144, treatment was changed to a combination of TDF and emtricitabine due to incomplete response (van Bömmel 2010).

For patients with partial response to a drug with a high genetic barrier, current guidelines also recommend the initiation of a combination treatment. Recently published long-term studies have however shown that the continuation of a new monotherapy in these patients does increase the percentage of patients with undetectable HBV DNA without increasing the risk of development of resistance. Thus, for monotherapy with TDF in HBeAg-positive and HBeAg-negative patients, an increase of patients with complete suppression of HBV DNA between the end of the first and the end of the third year of treatment from 81% to 95% and from 90% to 99% was shown (Gallant 2005; Verhelst 2002) (Figure 9).

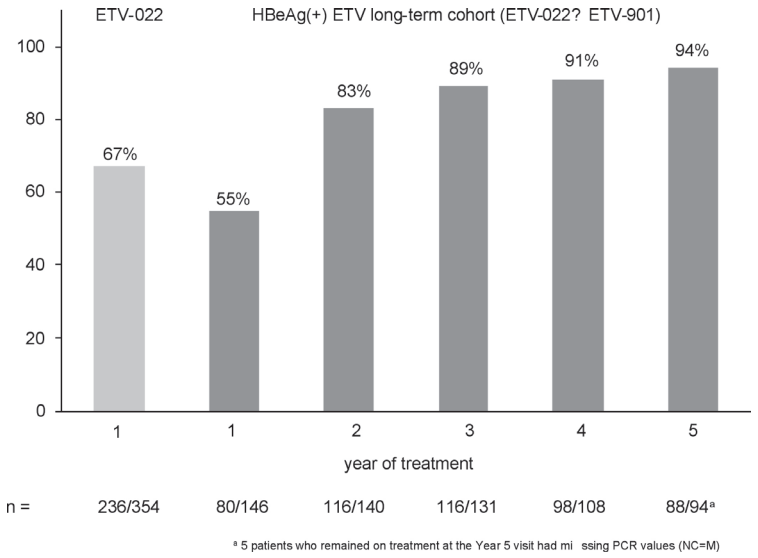


Figure 9. Percentage of patients with HBV DNA levels <400 copies/ml during long-term treatment with ETV 1 mg. The long-term cohort ETV-901 consists of HBeAg positive patients initially treated in the study ETV-022 (ETV 0.5 mg/day), which was designed for a duration of one year. Of note, 5 patients who remained on treatment at the year 5 visit had missing PCR values (Han 2008).

For monotherapy with ETV at 1 mg/day, an increase from 55% to 91% and 94% after the fourth and fifth years was demonstrated (Figure 10) (Han 2008). In case of incomplete viral suppression at week 48, a continuation of monotherapy with TDF or ETV 1 mg is advisable as long as HBV DNA levels decrease continuously. However, the debate on whether switching or adding on a second drug as optimal management is still unanswered.

Even though prolongation of monotherapy with ETV or TDF will probably lead to undetectable HBV DNA in the long term in most patients, a fast suppression of HBV replication is mandatory in some patients (e.g., those with liver cirrhosis) to stop the progression of liver disease. For these patients, no definite therapeutic strategies have been evaluated yet. Preliminary results of a study assessing the efficacy of a rescue combination therapy with ETV and TDF have recently been able to induce suppression to undetectable levels in most patients with partial response; however, data on long-term efficacy and safety are not available (Petersen 2009).

In any case of treatment failure, adherence to therapy should be evaluated prior to treatment modification. Elimination of HBV DNA during TDF-based therapeutic regimes can drop from 87% to 71% of cases if adherence is not ensured, which is also important in preventing drug resistance (Berg 2008).

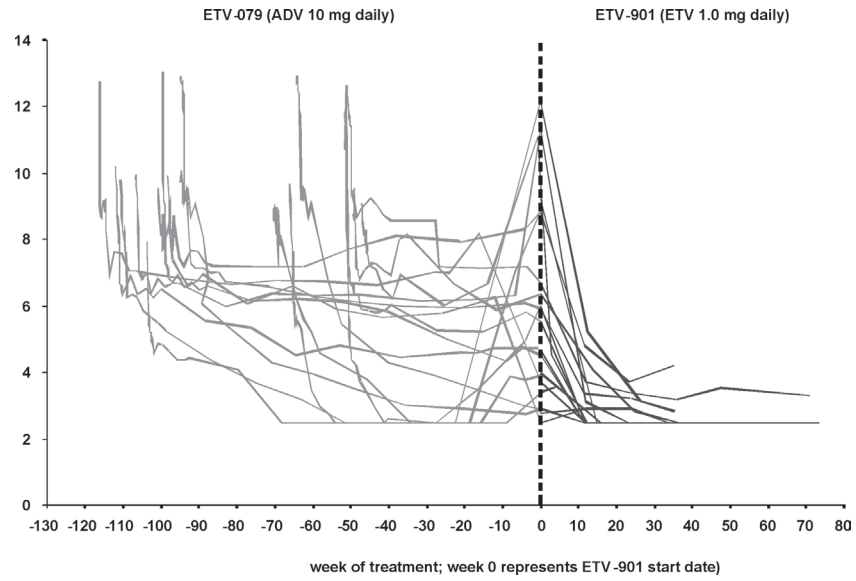


Figure 10. Kinetics of HBV DNA levels in 18 patients who were switched to ETV monotherapy after treatment failure or non-response to ADV treatment. The efficacy of ETV treatment with 1 mg/day independent from the presence of mutations associated with HBV resistance to ADV (Leung 2009).

Treatment duration and stopping rules

In HBeAg-positive patients continuous treatment with nucleos(t)ide analogues is necessary as long as HBeAg seroconversion is not achieved. Even after seroconversion occurred antiviral therapy should be continued for at least another 12 months to avoid the risk of "sero-reversion" after stopping the nucleos(t)ide analogue therapy.
Since only 30-35% of all patients treated with PEG-IFN α reach HBeAg seroconversion after 48 weeks, studies have been conducted recently to predict the probability of seroconversion in relation to viral kinetics. In one retrospective analysis early prediction of stable seroconversion was possible by week 12 of therapy if HBV DNA had reached levels below 5 log/ml within this short treatment period (Fried 2005).

In 53% of these patients HBeAg seroconversion was observed while patients with HBV DNA levels of 5 to 9 log copies/ml or levels above 9 log/ml achieved HBeAg seroconversion in only 17% and 14%, respectively. Thus, individualized PEG-IFN α strategies are certainly an interesting option for the future.

Criteria for optimal treatment duration are still lacking in patients with HBeAg-negative chronic hepatitis B. PEG-IFN α should be administered for 48 weeks, and unlimited long-term use of nucleos(t)ide analogues is recommended.

The effect of stopping therapy after a long-term ADV treatment of 4 to 5 years with complete viral suppression was recently evaluated (Hadziyannis 2008). Despite the fact that all patients suffered a slight virologic relapse within 3 months of

stopping therapy, most patients went below detection over the following 4 years without any therapy. Moreover, 28% of the patients lost HBsAg. Thus, final recommendations about the treatment period with defined stopping rules do not exist for HBeAg negative patients.

In patients with liver cirrhosis oral antiviral treatment should not be discontinued at any time point because of the risk of liver decompensation during a virologic rebound.

References

Balik I, Tabak F. Viral Hepatitis (VHSD) Guidelines 2008.

Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61:1942–1956.

Berg T, Moller B, Trinh H, Chan S, Marcellin P, Suarez E, Snow-Lampart A, Frederick D, Oldach D, Sorbel J. Tenofovir Disoproxil Fumarate (TDF) versus Emtricitabine plus TDF for Treatment of Chronic Hepatitis B (CHB) in Subjects with Persistent Viral Replication Receiving Adefovir Dipivoxil (ADV). J Hepatol 2008; 48:S34–S34.

Brown R, Goodwin D, Peschell K, Zhang S, Fagan E. Tenofovir Disoproxil Fumarate-Containing Regimens in Pregnancy: Report From the Antiretroviral Pregnancy Registry. 60th Annual Meeting of the American Association for the Study of Liver Diseases October 30 - November 3, 2009 Boston, Massachusetts, USA. Abstract 407.

Buster EH, Flink HJ, Simsek H, Heathcote EJ, Sharmila S, Kitis GE, Gerken G, Buti M, de Vries RA, Verhey E, Hansen BE, Janssen HL. Early HBeAg loss during peginterferon alpha-2b therapy predicts HBsAg loss: results of a long-term follow-up study in chronic hepatitis B patients. Am J Gastroenterol 2009;104:2449–2457.

Carosi G, Rizzetto M. Italian Guidelines 2008.

Chan HL, Leung NW, Hui AY, Wong VW, Liew CT, Chim AM, Chan FK, Hung LC, Lee YT, Tam JS, Lam CW, Sung JJ. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alpha2b and lamivudine with lamivudine alone. Ann Intern Med 2005;142:240–250.

Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65–73.

Cho SW, Koh KH, Cheong JY, Lee MH, Hong SP, Yoo WD, Kim SO. Low efficacy of entecavir therapy in adefovir-refractory hepatitis B patients with prior lamivudine resistance. J Viral Hepat 2009 Aug 12.

Coca S, Perazella MA. Acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. Am J Med Sci 2002;324:342–344.

Colle I, Adler M, Brenard R, et al. Acta Gastroenterol Belg 2007;70:389–420.

Cornberg M, Protzer U, Dollinger MM, Petersen J, Wedemeyer H, Berg T, Jilg W, Erhardt A, Wirth S, Schirmacher P, Fleig WE, Manns MP. Prophylaxis, diagnosis and therapy of hepatitis B virus (HBV) infection: the German guidelines for the management of HBV infection. Z Gastroenterol 2007;45:1281–1328.

Créput C, Gonzales-Canali G, Hill G, et al. Renal lesions in HIV-1-positive patient treated with tenofovir. AIDS 2003;17:935–937.

Crespo J, Lozano JL, Echevarría S, San Miguel G, Artiñano E, López Arias MJ, Pons Romero F. Influence of hepatitis C virus infection and human immunodeficiency on the natural history of chronic delta hepatitis. Rev Esp Enferm Dig. 1994;85:185–191.

Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med 1995;333:1657–1661.

EASL Jury. J Hepatol 2003;39:S3-25.

European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol 2009;50:227–242.

Fleischer RD, Lok AS. Myopathy and neuropathy associated with nucleos(t)ide analog therapy for hepatitis B. J Hepatol 2009;51:787–791.

Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL; HBV 99-01 Study Group. Treatment with Peginterferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol 2006;101:297–303.

Fried M, Liaw YF, Luo KX, Lau GK, et al. Role of baseline and on-treatment quantitative HBeAg in predicting response to peginterferon α-2a (40kD) (Pegasys) monotherapy in a large, multinational trial of patients with chronic hepatitis B. Hepatology 2005;42:268A.

Gallant JE, Parish MA, Keruly JC, Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. Clin Infect Dis 2005;40:1194–1198.

Gaspar G, Monereo A, Garcia-Reyne A, de Guzman M. Fanconi syndrome and acute renal failure in a patient treated with tenofovir: a call to action. AIDS 2004;18:351–352.

Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, Han KH, Chao YC, Lee SD, Harris M et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. Gastroenterology 2007;133:1437-1444.

Gish RG, Chang TT, Lai CL, de Man R, Gadano A, Poordad F, Yang J, Brett-Smith H, Tamez R. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naïve HBeAg-positive patients with chronic hepatitis B. J Viral Hepat 2010;17:16-22.

Goncalves J, Laeufle R and Avila C. Increased risk with combination of telbivudine and pegylated-interferon Alfa-2A in study CLDT600A2406, compared to uncommon rate with telbivudine monotherapy from the novartis global database. J Hepatol 2009;50:A329.

Hadziyannis S. Hepatitis B e antigen negative chronic hepatitis B: from clinical recognition to pathogenesis and treatment. Viral Hepat Rev 1995;1:7–13.

Hadziyannis SJ, Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: natural history and treatment. Semin Liver Dis 2006;26:130–141.

Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2001;34:617–624.

Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800–807.

Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang T-T, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 2006a;131:1743–1751.

Hadziyannis SJ, Costamena A, Laras A, Hadziyannis E. Predictors/determinants of sustained virological response (SVR) and of HBsAg loss following long term adefovir dipivoxil (ADV) therapy in HBeAg-negative chronic hepatitis (CHB). Program and abstracts of the 59th Annual Meeting of the American Association for the Study of Liver Diseases; October 31 - November 4, 2008; San Francisco, California. Abstract 874.

Han S, Chang T-T, Chao Y-C, et al. Four-year Entecavir treatment in nucleoside-naïve HBeAg(+) patients: Results from studies ETV-022 and -901. 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2008); 31 October – 4 November 2008; San Francisco, CA, USA. Abstract 893.

Heathcote E, Gane EJ, De Man RA, Lee SS, Filisiak R, Manns MP, et al. Three years of tenofovir disoproxil (TDF) treatment in HBeAg-positive patients with chronic hepatitis B (study 103), preliminary analysis. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston 2009. Abstract 483.

Heathcote J, George J, Gordon S, Bronowicki J-P, Sperl J, Williams RR, et al. Tenofovir disoproxil fumarate (TDF) for the treatment of HBeAg positive chronic hepatitis B: week 72 TDF data and week 24 adefovir dipivoxil switch data (study 103). 43rd Annual Meeting of the European Association for the Study of the Liver (EASL) April 23-27; Milan, Italy: 2008. Abstract 1593.

Hou J, Yin YK, Xu D, Tan D, Niu J, Zhou X, et al. Telbivudine versus lamivudine in Chinese patients with chronic hepatitis B: Results at 1 year of a randomized, double-blind trial. Hepatology 2008;47:447–454.

Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678–686.

Janssen HL, Buster EH, Bakker CM, et al. Dutch Guidelines 2008.

Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alpha-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123–129.

Juszczyk J, Boro_-Kaczmarska A, Cianciara J, et al. Polish Guidelines 2008.

Karras A, Lafaurie M, Furco A, et al. Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. Clin Infect Dis 2003;36:1070–1073.

Keeffe EM, Zeuzem S, Koff RS, et al. Clin Gastroenterol Hepatol 2007;5:890–897.

Kumar M, Satapathy S, Monga R, Das K, Hissar S, Pande C, Sharma BC, Sarin SK. A randomized controlled trial of lamivudine to treat acute hepatitis B. Hepatology 2007;45:97–101.

Lada O, Benhamou Y, Cahour A, Katlama C, Poynard T, Thibault V. In vitro susceptibility of lamivudine-resistant hepatitis B virus to adefovir and tenofovir. Antivir Ther 2004;9:353-363.

Lai CL, Leung N, Teo EK, Tong M, Wong F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown NA. Telbivudine Phase II Investigator Group. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528–536.

Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients. N Engl J Med 2007;357:2576–2588.

Lampertico P, Del Ninno E, Viganò M, Romeo R, Donato MF, Sablon E, et al. Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003;37:756–763.

Lampertico P, Viganò M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. Gastroenterology 2007;133:1445–1451.

Lange CM, Bojunga J, Hofmann WP, Wunder K, Mihm U, Zeuzem S, Sarrazin C. Severe lactic acidosis during treatment of chronic hepatitis B with entecavir in patients with impaired liver function. Hepatology 2009;50:2001-6.B. Gastroenterology 2009;136:486–495.

Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon α-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682–2695.

Lau GKK, et al. On-treatment HBsAg decline during peginterferon alfa-2a (40KD) ± lamivudine in patients with HBeAg-positive CHB as a potential predictor of durable off-treatment response. 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2008). San Francisco 2008. Abstract 910

Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97–107.

Leung NJ. Entecavir (ETV) therapy in chronic hepatitis B patients previously treated with adefovir (ADV) with incomplete response on-treatment or relapse off-treatment. Hepatology 2009;50:S334.

Leung N, Peng CY, Hann HW, Sollano J, Lao-Tan J, Hsu CW, et al. Early hepatitis B virus DNA reduction in hepatitis B e antigen-positive patients with chronic hepatitis B: A randomized international study of entecavir versus adefovir. Hepatology 2009a;49:72–79.

Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, et al. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. Gastroenterology 2000;119:172–180.

Liaw Y-F, Suh DJ, Omata M. Available at: www.apasl.info/guidelinesHBV.html (provisional). Accessed 13 April 2008.

Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, et al. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology 2009;136:486–495.

Liaw Y-F, Lee CM, Akarca U, Papatheodoridis G, Wong F, Chang TT, et al. A Phase 2, Double-Blind, Randomized Study Comparing the Safety of Tenofovir Disoproxil Fumarate (TDF), Emtricitabine Plus TDF (Truvada, TVD) and Entecavir (ETV) in Subjects with Decompensated Chronic Hepatitis B Liver Disease. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston 2009a. Abstract 222.

Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology 2000;119:172–180.

Lok ASF, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507–539.

Lok ASF, McMahon BJ. Chronic Hepatitis B: Update 2009. Hepatology 2009;50:1–35.

Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. Gastroenterology 2001;121:101–109.

Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon α-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206–1217.

Marcellin P, Jacobson I, Habersetzer F, Senturk H, Andreone P, et al. Tenofovir disoproxil fumarate (TDF) for the treatment ofHBeAg-negative chronic hepatitis B: week 72 TDF data and week 24 adefovir dipivoxil switch data (study 102) [oral presentation #1602]. 43rd Annual Meeting of the European Association for the Study of the Liver (EASL); Milan, Italy: 2008.

Marcellin P, Buti M, Krastev Z, Germanidis G, Kaita KD, Kotvez I, Buggisch P et al. Three years of tenofovir disoproxil (TDF) treatment in HBeAg-negative patients with chronic hepatitis B (study 103), preliminary analysis. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston 2009. Abstract 481.

Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon α-2a. Gastroenterology 2009a;136:2169–2179.

Marcellin P, Piratvishut T, Brunetto MR, Bonino F, Farci P, Yurdaydin C, et al. A finite course of peginterferon alfa-2a results in inactive chronic hepatitis B and HBsAg cleacance 5 years post-treatment in patients with HBeAg-negative disease: baseline characteristica and predictive factors of long-term response. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston 2009b. Abstract 387.

McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. J Infect Dis 1985;151:599–603.

Mommeja-Marin H, Mondou E, Blum MR, Rousseau F. Serum HBV DNA as a marker of efficacy during therapy for chronic HBV infection: analysis and review of the literature. Hepatology 2003;37:1309–1319.

Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, Dauvergne A, Asselah T, Boyer N, Bedossa P, Valla D, Vidaud M, Nicolas-Chanoine MH, Marcellin P.Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon α-2a in HBeAg-negative patients. Hepatology. 2009;49:1151–1157.

Moucari R, Korevaar A, Lada O, Martinot-Peignoux M, Boyer N, Mackiewicz V, et al. High rates of HBsAg seroconversion in HBeAg-positive chronic hepatitis B patients responding to interferon: a long-term follow-up study. J Hepatol 2009a;50:1084–1092.

Perrillo RP, Lai CL, Liaw YF, Dienstag JL, Schiff ER, Schalm SW, et al. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36:186–194.

Perrillo RP, Schiff ER, Davis GL, Bodenheimer HC, Lindsay K, Payne J, et al. A randomized, controlled trial of interferon alpha-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. N Engl J Med 1990;323:295–301.

Petersen J, Lutgehetmann M, Zoulim F, et al. Entecavir and Tenofovir combination therapy in chronic Hepatitis B: Rescue therapy in patients with advanced fibrosis and multiple previous treatment failures. Results from an international multicenter cohort study. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston. October 30-November 1, 2009. Abstract 405.

Peyriere H, Reynes J, Rouanet I, et al. Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. J Acquir Immune Defic Syndr 2004;35:269–273.

Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 1996;2:1104–1108.

Reijnders JG, Pas SD, Schutten M, de Man RA, Janssen HL. Entecavir shows limited efficacy in HBeAg-positive hepatitis B patients with a partial virologic response to adefovir therapy. J Hepatol 2009;50:674–683.

Reijnders JG, de Vries-Sluijs T, Hansen BE, Zaaijer HL, Prins JM, Schutten M, et al. Fife years tenofovir therapy is associated with maintained virologic response, but significant decline in renal function in HIV/HBV coinfected patients. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston 2009a. Abstract 425.

Sherman M, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, et al. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. Hepatology 2008;48:99–108.

Shim JH, Suh DJ, Kim KM, Lim YS, Lee HC, Chung YH, Lee YS. Efficacy of entecavir in patients with chronic hepatitis B resistant to both lamivudine and adefovir or to lamivudine alone. Hepatology 2009;50:1064–1071.

Snow-Lampart A, Chappell B, Curtis M, Zhu Y, Heathcote J, Marcellin P, Borroto-Esoda K. Resistance Surveillance for up to 144 Weeks in HBeAg+ and HBeAg-Hepatitis B Patients Treated with Tenofovir DF Showed No Relationship between Virologic Breakthrough and Emergence of Genotypic Changes in HBV Polymerase. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston 2009. Abstract 480.

Sung JJ, Lai JY, Zeuzem S, Chow WC, Heathcote EJ, Perrillo RP, et al. Lamivudine compared with lamivudine and adefovir dipivoxil for the treatment of HBeAg-positive chronic hepatitis B. J Hepatol 2008;48:728–735.

Takkenberg B, Zaaijer H, Weegink C, Terpstra V, Dijkgraaf M, Jansen P, et al. Baseline HBsAg level predict HBsAg loss in chronic hepatitis B patients treatd with a combination of Peginterferon alfa-2a and adefovir: an interim analysis. J Hepatol 2009;50:S8.

ter Borg MJ, Leemans WF, de Man RA, Janssen HL. Exacerbation of chronic hepatitis B infection after delivery. J Viral Hepat 2008;15:37–41.

Tillmann HL, Hadem J, Leifeld L, Zachou K, Canbay A, Eisenbach C, et al. Safety and efficacy of lamivudine in patients with severe acute or fulminant hepatitis B, a multicenter experience. J Viral Hepat 2006;13:256–263.

Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. Hepatology 2009;49:1503–1514.

Toy M, Veldhuijzen IK, de Man RA, Richardus JH, Schalm SW. Potential impact of long-term nucleoside therapy on the mortality and morbidity of active chronic hepatitis B. Hepatology 2009;50:743–751.

van Bömmel F, Trojan J, Feucht HH, Möller B, et al. Tenofovir shows limited efficacy in treatment of HBV infections resistant against adefovir. Hepatology 2007;46:664A.

van Bömmel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology 2010;51:73–80.

van Zonneveld M, van Nunen AB, Niesters HG, de Man RA, Schalm SW, Janssen HL. Lamivudine treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. J Viral Hepat 2003;10:294–297.

Verhelst D, Monge M, Meynard JL, et al. Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 2002;40:1331–1333.

Waked I, Salama M, Zayed N, et al. Egyptian Guidelines 2008.

Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126:1750–1758.

Wiegand J, Karlas T, Schiefke I, Krasselt U, Bock T, Mössner J, Tillmann HL. Resistance management in chronic hepatitis B complicated by renal failure. Clin Nephrol 2010, in press.

Wiegand J, Wedemeyer H, Finger A, Heidrich B, Rosenau J, Michel G, et al. A decline in hepatitis B virus surface antigen (HBsAg) predicts clearance, but does not correlate with quantitative HBeAg or HBV DNA levels. Antivir Ther 2008;13:547–554.

Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312–323.

Wursthorn K, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, Longerich T, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. Hepatology 2006;44:675–684.

Yu JW, Sun LJ, Zhao YH, Kang P, Li SC. The Study of Efficacy of Lamivudine in Patients with Severe Acute Hepatitis B. Dig Dis Sci 2009 Dec 3.

Yuen MF, Fong DY,Wong DK, Yuen JC, Fung J, Lai CL. Hepatitis B virus DNA levels at week 4 of lamivudine treatment predict the 5-year ideal response. Hepatology 2007;46:1695–1703.

Zeuzem S, Gane E, Liaw YF, Lim SG, DiBisceglie A, Buti M, et al. Baseline characteristics and early on-treatment response predict the outcomes of 2 years of telbivudine treatment of chronic hepatitis B. J Hepatol 2009;51:11–20.

Zoulim F, Perrillo R. Hepatitis B: reflections on the current approach to antiviral therapy. J Hepatol 2008;48:S2–S19.

Chapter 10: Resistance and management of resistance in HBV therapy

Stefan Mauss, Heiner Wedemeyer

Introduction

Interferon monotherapy has been the standard of care for chronic hepatitis B since the mid-1990s. Primary resistance to interferon presents as HBe or HBsAg loss; seroconversion is less frequently reported for HBV genotypes B, C and D compared to HBV genotype A (Erhardt 2005; Flink 2006). However, the development of resistance to interferon while on therapy has not been reported to date. Recently, in patients with chronic hepatitis C, a genetic polymorphism at the locus IL28B has been identified as a host factor associated with response to therapy (Ge 2009). If similar host factors exist for response to interferon therapy in chronic hepatitis B, they are not known. However, a recent paper did demonstrate an association of the natural history of hepatitis B infection and genetic variants in the HLA-DP locus (Kamatami 2009).

Since the introduction of lamivudine, treatment of chronic hepatitis B has been characterised by a rapid increase in the number of available antiviral drugs, all belonging to the class of HBV polymerase inhibitors (Figure 1). Due to better tolerance and more convenient administration compared to interferon, HBV polymerase inhibitors today account for the vast majority of prescribed therapies for chronic hepatitis B in Western countries. However, long-term suppression of HBV is needed, particularly in HBeAg-negative patients harbouring the precore mutant. This is due to the high relapse rate after discontinuation of antiviral therapy in the absence of HBsAg seroconversion, a rare event in the first years of treatment.

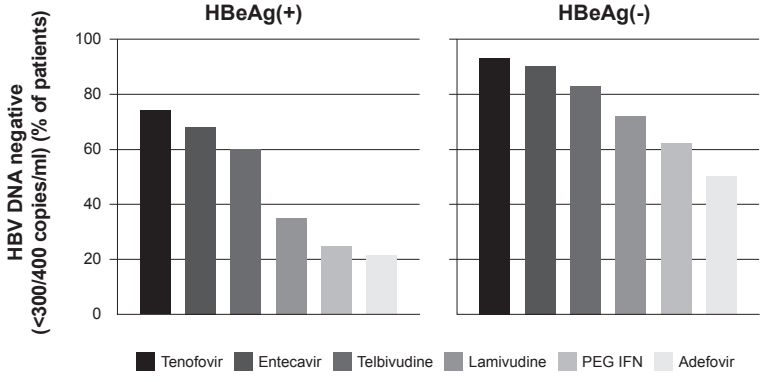


Figure 1: Proportion of patients with undetectable HBV DNA after 48 or 52 weeks of treatment. Data does not represent "head-to-head" trials (based on Heathcote 2007; Lai 2006; Liaw 2009; Marcellin 2003; Marcellin 2007).

For this reason, the understanding of resistance and cross-resistance of HBV polymerase inhibitors is relevant in long-term treatment strategies. Suboptimal antiviral therapy resulting in the development of early resistance will harm future treatment options and lead to progressive liver disease, especially in those with few treatment options (Brunelle 2005; Kurashige 2009). In addition, some HBV polymerase variants may interact with immunologically relevant epitopes of the envelope resulting in immune escape mutants. These mutants may be able to successfully infect vaccinated individuals. Although this finding is currently an *in vitro* observation, the confirmation of this phenomenon in patients will result in a serious public health concern, particularly in countries with a high prevalence of hepatitis B.

Principles of antiviral HBV therapy – how to avoid resistance

Treatment of HBV is relatively safe and easy compared to hepatitis C treatment or HIV therapy. But avoiding the induction of resistance is one of the critical efforts that needs to be made by physicians and patients. They need to choose the right therapy and monitoring schedule, and pay close attention to good adherence.

Entecavir and tenofovir have proven efficacy and very little or no resistance in treatment naïve patients in the first years of therapy (Heathcote 2009; Lampertico 2009). In patients with limited HBV replication, telbivudine has also shown good results, although in patients with high viral load treatment results can be compromised by the development of resistance, which is also true for adefovir and lamivudine (Zeuzem 2009).

As previously stated, treating patients for long periods suboptimally with HBV polymerase inhibitors can result in the development of viral resistance - particularly in patients with less than optimal viral suppression (Lai 2006). In particular, lamivudine and telbivudine are prone to developing resistance rapidly. Therapy with HBV polymerase inhibitors needs to fully suppress viral replication (HBV DNA <300 copies/ml). HBV DNA should be monitored after the first 4-6 weeks of therapy to assess adherence and then every 3-6 months while on therapy. If complete viral suppression determined by an ultrasensitive assay is not achieved on polymerase monotherapy within 6 months with lamivudine, telbivudine or adefovir, treatment should be switched to tenofovir or entecavir. In patients on either tenofovir or entecavir, combination therapy with non-cross-resistant HBV polymerase inhibitors should be considered after 12 months in case a plateau of viral replication is reached. Resistance to nucleoside polymerase inhibitors eliminates or markedly reduces antiviral efficacy of all other nucleosides and may affect even nucleotide polymerase inhibitors due to cross resistance.

Resistance can also be associated with significant flares of hepatitis and has been associated with a higher rate of clinical complications in one Asian study (Liaw 2004) and with a lower overall survival in an Italian cohort (DiMarco 2004). Therefore, resistance needs to be avoided, particularly in patients with cirrhosis. Based on these severe consequences of treatment failure, we would recommend selecting a drug with a high genetic barrier for antiviral resistance in cirrhotic individuals.

Treatment endpoints

In HBeAg-positive patients infected with wild type HBV strains HBeAg seroconversion has been shown to be associated with a reduction in liver-associated morbidity and increased survival (Niederau 1996). Thus, HBeAg seroconversion is considered a clinical endpoint in this patient population and discontinuation of HBV polymerase inhibitors is recommended 6-12 months after HBeAg seroconversion in patients who have not developed liver cirrhosis (Cornberg 2007). HBeAg loss is reported in up to 50% of patients treated with HBV polymerase inhibitors after prolonged periods of therapy of several years (Hadziyannis 2006). Recent cohort data sheds some doubt on the durability of HBeAg seroconversion on therapy with polymerase inhibitors (Perquin 2009), but well structured studies assessing this important aspect still need to be carried out.

Treatment with pegylated interferon a for 48 weeks results in HBeAg seroconversion in about a third of patients (Lau 2005).

Discontinuation of HBV polymerase inhibitor therapy in patients without HBeAg seroconversion usually results in relapse of chronic hepatitis B. With interferon a the situation may become more complex and is at least partially dependent on the HBV genotype in addition to the HBeAg status (Erhardt 2005).

Treatment endpoints in HBeAg-negative hepatitis B in most cases are restricted to sustained normalisation of ALT levels and suppression of HBV DNA, as HBsAg seroconversion is rare with current treatment options. Consequently, treatment duration and endpoints are more difficult to define in these patients. Re-appearance of HBV DNA after stopping HBV polymerase inhibitor treatment is observed in almost all patients even after treatment of 2-5 years. Most guidelines therefore recommend indefinite treatment of patients with HBeAg-negative chronic hepatitis B. PEG-IFN *a*-2a has also been studied in HBeAg-negative hepatitis B leading to a 6-month off-treatment response (HBV DNA <400 copies/ml) in up to 20% of patients (Marcellin 2004).

HBsAg seroconversion happens in about 5% of patients after a year of treatment with pegylated interferon. In addition, about 20% of patients reach a low replicative status of their chronic hepatitis B, at least temporarily, after interferon discontinuation (Bonino 2007). After an observational period of five years after one year of interferon-based therapy, the seroconversion rate increases to 12% (Marcellin 2009). For HBV polymerase inhibitors HBsAg seroconversion has been reported for HBeAg negative patients in less than 5% of patients in published prospective studies.

Resistance patterns of HBV polymerase inhibitors

Lamivudine was the first approved HBV polymerase inhibitor. It is characterized by good clinical tolerability, moderate antiviral efficacy and rather quick development of resistance in cases of not fully suppressive antiviral therapy (Figure 2). Within the first year of therapy up to 20% of patients may develop mutations in the YMDD motif associated with loss of activity against HBV. About 70-80% of patients without HBeAg seroconversion develop lamivudine-resistant variants after four or more years (Figure 2).

Lamivudine mutations may confer cross-resistance to telbivudine, emtricitabine and entecavir. Preliminary data indicate that the development of multiple lamivudine associated mutations may even reduce the efficacy of tenofovir therapy (Lada 2008).

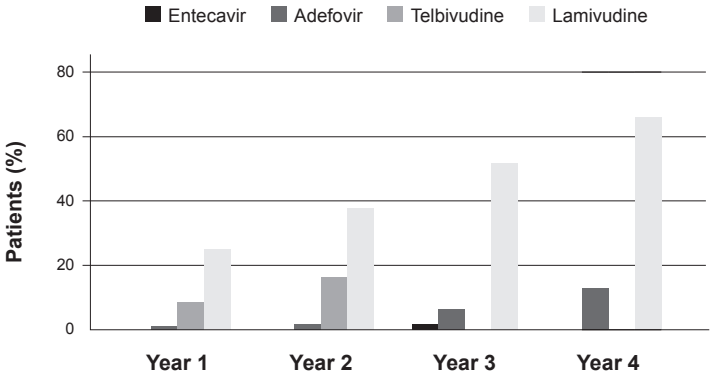


Figure 2: Cumulative incidence of HBV polymerase inhibitor resistance. These numbers are average estimates based on numerous studies. Resistance rates differ between trials and cohorts. Overall, resistance rates have been higher in HBeAg-positive patients than in HBeAg-negative patients. Long-term data for adefovir has only been reported for HBeAg-negative patients and thus resistance rates may be even higher for HBeAg-positive individuals. Data for entecavir is biased since both patients with best responses (e.g., HBeAg seroconversion) and patients with suboptimal virological responses (>700,000 copies/ml after one year of treatment) were withdrawn from the study after one year.

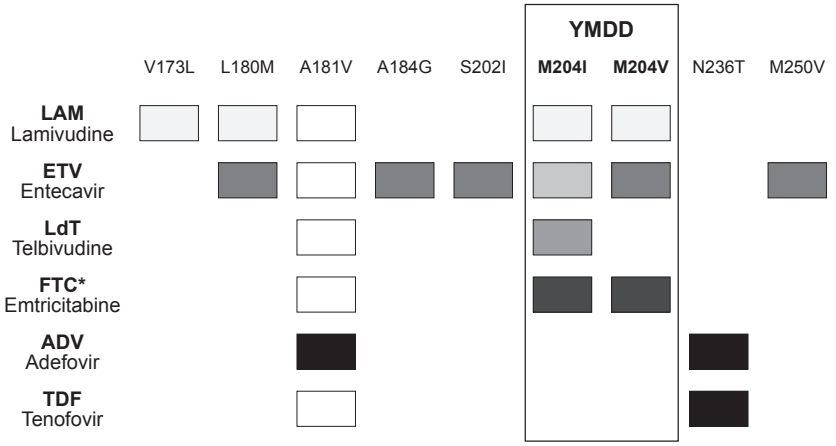


Figure 3: Resistance patterns of different antiviral drugs used for the treatment of chronic hepatitis B. The numbers indicate the respective aminoacid position in the HBV polymerase gene. For entecavir resistance at positions 204/180 plus an additional mutation at position 184, 202 or 250 is required to lead to clinically significant drug resistance. Most but not all variants have been shown to be associated with drug resistance both *in vitro* and *in vivo*.

Emtricitabine has comparable antiviral properties and a similar resistance profile to lamivudine (Lim 2006). However it is approved as an antiretroviral medication only for HIV, not for the treatment of chronic hepatitis B. In HBV, its use is mainly limited as part of combination therapy with tenofovir in HIV-coinfected patients with an indication for antiretroviral therapy.

Telbivudine, a recently approved HBV polymerase inhibitor, has shown superior antiviral efficacy compared to lamivudine in HBeAg positive and negative patients. However, development of resistance is considerable in naïve patients with highly replicative hepatitis B and the resistance pattern is essentially the same as that of lamivudine, resulting in complete cross-resistance of the two compounds (Lai 2006; Zeuzem 2009) (Table 1). Outcomes are better and antiviral efficacy more sustained in patients with an HBV DNA of less than 10^6 IU/ml (Zeuzem 2009). Combination therapy of telbivudine and lamivudine does not improve the antiviral efficacy nor does it delay the development of resistance compared to telbivudine monotherapy (Lai 2005).

Resistance against nucleoside analogues	**Recommended Therapeutic Option - "add-on"**
Lamivudine	Adefovir, tenofovir
Telbivudine	Adefovir, tenofovir
Entecavir	Adefovir, tenofovir
Resistance against nucleotide analogues	
Adefovir (LAM-naive)	Entecavir, telbivudine, lamivudine (tenofovir)
Adefovir (LAM-resist.)	Entecavir + tenofovir
Tenofovir (No *in vivo* data available)	Entecavir, telbivudine, lamivudine

Table 1: Recommendations in secondary treatment failure of HBV polymerase inhibitors based on the German Hepatitis B Treatment Guidelines.

Adefovir was the second approved HBV polymerase inhibitor. It has full activity in lamivudine-resistant patients. However, its antiviral potency is limited by its nephrotoxicitiy. Due to tubular damage of the kidney, the approved dose is limited to 10 mg/day, although 30 mg/day showed superior antiviral efficacy (Marcellin 2003). The reduced antiviral potency is counterbalanced, however, by a favourable resistance profile. Development of resistance occurs later and to a lesser extent compared to lamivudine or telbivudine (Figure 3), although resistance to adefovir may occur more often in patients with pre-existing lamivudine resistance (Lee 2006). No association of response to treatment with HBV genotypes was evident in its registrational trials (Westland 2003). Reports on a limited number of patients from Spain suggest a reduced efficacy of adefovir in HBV genotype A2 (Chueca 2007).

Adefovir-resistant or non-responding HBV strains seem to respond to tenofovir with a slower viral decline, but without signs of true cross-resistance (Berg 2008; Van Bömmel 2010).

The combination of adefovir plus lamivudine in the presence of lamivudine resistance delays the development of lamivudine resistance considerably compared to switching to adefovir monotherapy (Lampertico 2006; Lampertico 2007).

Entecavir is an HBV nucleoside polymerase inhibitor with good antiviral efficacy and slow development of resistance in treatment-naïve patients (Chang 2006; Lai 2006; Lampertico 2009). This is due to the fact that more than one mutation in the HBV polymerase gene is required to confer resistance to entecavir. However, entecavir shares some resistance mutations with lamivudine and telbivudine. The presence of lamivudine resistance mutations at L180M, M204I, L180M + M204V facilitates the development of resistance to entecavir because only one additional mutation is required for the development of full resistance. As a result, in contrast to treatment of naïve patients where entecavir is clearly superior to lamivudine, its antiviral potency is markedly reduced in patients with lamivudine resistance and up to 40% of lamivudine-resistant patients develop full entecavir resistance after 3 years of treatment (Tenney 2007; Colonno 2007).

Patients with resistance only to adefovir have favourable treatment results with entecavir, while patients with combined adefovir and lamivudine resistance do not respond well to entecavir monotherapy (Reijnders 2007; Nguyen 2009; Chloe 2009; Shim 2009).

Tenofovir is approved for the treatment of HIV and HBV. Early data from HBV/HIV-coinfected patients showed a strong antiviral potency and slow development of resistance (Núñez 2002; Nelson 2003; van Bommel 2004). In its registrational trials tenofovir was superior to adefovir resulting in substantially higher rates of full viral suppression in HBeAg positive (tenofovir 69% vs. adefovir 9%, HBV DNA <40 IU/ml) and HBeAg negative patients (tenofovir 91% vs. adefovir 56% HBV DNA <40 IU/ml) at 52 weeks of therapy (Heathcote 2009; Marcellin 2008). In HIV-positive patients anecdotal cases of renotubular dysfunction were reported. Otherwise tenofovir is well tolerated. It is active in lamivudine-resistant patients (Schmutz 2006; Manns 2009). So far, no obvious resistance patterns to tenofovir have been observed associated with antiviral failure in trials and cohorts.

The acquistion of adefovir resistance mutations and multiple lamivudine resistance mutations may impair the activity of tenofovir (Fung 2005; Lada 2008; van Bömmel 2010), although even in these situations tenofovir retains activity against HBV (Berg 2008; Petersen 2009).

Combination therapy of chronic hepatitis B to delay development of resistance

Combination therapy is thought to be superior to monotherapy, particularly in patients with highly replicative hepatitis B (HBV DNA $>10^9$ copies/ml). However, trials assessing *de novo* combination therapy versus monotherapy are limited. The experience with combining telbivudine and lamivudine suggests that combinations of two nucleoside analogues with an overlapping resistance profile do not have an additive antiviral effect (Lai 2005). In contrast, combining a nucleoside with a nucleotide

polymerase inhibitor with different resistance profiles may be of benefit (Sung 2008). Trials that will provide more evidence on how to best use the current antiviral options are currently underway or are being designed. However, these trials may require larger patient numbers than currently included and may need longer observational periods due to agents like entecavir and tenofovir having such considerable efficacy as monotherapy. In theory, *de novo* combination therapy in treatment naïve patients should be superior to sequential monotherapy. Unfortunately, strategic trials assessing these different therapeutic approaches are not being conducted. However, it should be remembered that – in contrast to HIV – immune control of HBV is possible, limiting the duration of therapy. With the availability of HBV polymerase inhibitors with high resistance barriers, even treatment-naive patients with high levels of HBV replication may be treated initially with one drug. The key issue, however, is to adapt as early as possible if sufficient suppression of replication is not achieved, in order to avoid development of resistance.

Management of drug resistance

Primary and secondary treatment failure has to be distinguished in the treatment of hepatitis B. A clinically sufficient primary response after 6 months is defined by a reduction of HBV DNA to at least $<10^3$ copies/ml (200 IU/ml) or by a continuous drop of HBV DNA through month 12. In contrast, if a rise in HBV DNA by one log or more is observed while on antiviral therapy, a secondary resistance is very likely to be present. HBV resistance usually arises several months before biochemical relapse with elevation of transaminases, thus regular HBV DNA monitoring is required during antiviral therapy (e.g., every 3 months) (Cornberg 2007). Testing for variants associated with resistance might be useful if HBV DNA levels rise during treatment.

Most viral breakthroughs in treatment-naïve patients on entecavir or tenofovir are the result of adherence issues. Therefore, patient adherence should be assessed before genotypic resistance testing is done.

Additional compensatory mutations can develop if monotherapy is continued despite HBV resistance, thereby broadening the possibilities of cross-resistance (Locarnini 2004). Knowledge of the antiviral efficacy, the resistance barrier, and the resistance profile of each available oral antiviral is a prerequisite for the rational use of nucleos(t)ide analogues for hepatitis B. In the case of resistance to a nucleoside analogue (lamivudine, telbivudine, emtricitabine, entecavir), early add-on treatment with a nucleotide analogue (adefovir, tenofovir) is recommended in most cases. In the opposite scenario, a nucleoside addition to current nucleotide treatment should happen if adefovir or tenofovir treatment failure begins to occur (Figure 4). In the case of adefovir, switching from adefovir to tenofovir should be assessed as an additional measure.

Historically, most data generated has been from patients with lamivudine resistance. In this setting the advantage of adding adefovir rather than switching to adefovir is well established (Lampertico 2005; Lampertico 2007). Moreover, adefovir should be added early at low HBV DNA levels, when a rise in HBV DNA has been confirmed but before a biochemical relapse has occurred. Today, the most appropriate strategy may be a switch to tenofovir with or without continuation of lamivudine (Manns 2009).

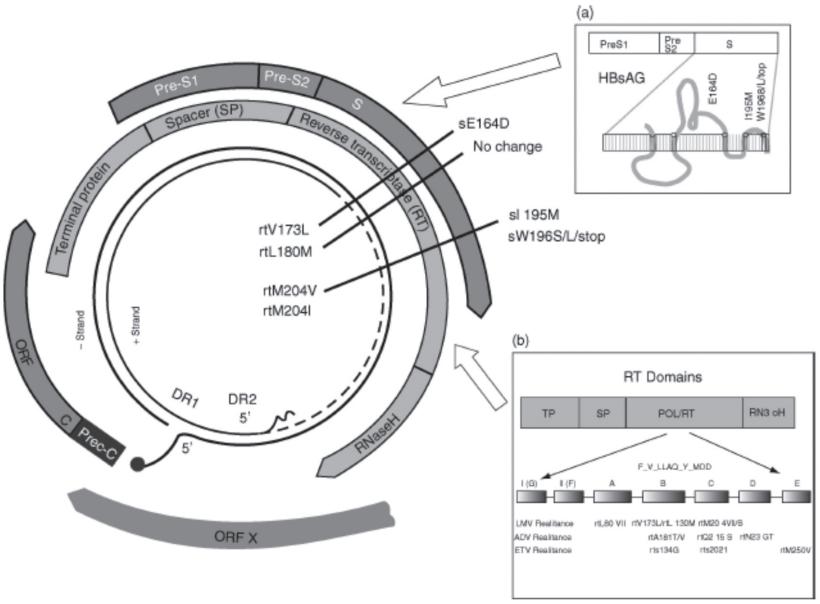


Figure 4: Due to the overlapping reading frame between HBV polymerase and envelope sequences, mutations in the HBV polymerase, in particular at codons 173, 180 and 204, may lead to changes in the conformation of immunodominant domains of the HBV envelope.

Special considerations in HIV/HBV coinfection

In patients with chronic hepatitis B and HIV, the first question to ask is if there is an indication for antiretroviral therapy. In patients with no such indication (>350 CD4 cells), interferon or an HBV polymerase inhibitor without HIV activity are options. The initially recommended monotherapy with entecavir is now considered obsolete - the anti-HIV activity of entecavir has recently been described (M184V) in anecdotal cases (MacMahon 2007). Currently, adefovir and telbivudine are recommended, based on limited *in vivo* data for adefovir or *in vitro* data in combination with anecdotal case reports for telbivudine (Delaugerre 2002; Sheldon 2005; Avilla 2009; Milazzo 2009). As both drugs have limitations in the setting of HBV-monoinfected patients the initiation of antiretroviral therapy allowing the use of tenofovir plus lamivudine/emtricitabine should be considered in particular in patients with advanced liver fibrosis.

In patients with an indication for antiretroviral therapy, a regimen containing tenofovir plus lamivudine or emtricitabine is favored in order to delay development of lamivudine or emtricitabine resistance in HBV. The incidence of HBV resistance in patients treated with lamivudine after two years is about 50% in HIV/HBV-coinfected patients (Benhamou 1999). In patients who have already developed lamivudine-resistant HBV, tenofovir should be added to or replace lamivudine for HBV treatment (Schmutz 2006). Whether entecavir should be added in patients on tenofovir +/- emtricitabine/lamivudine should be decided on an individual basis.

A change of antiretroviral regimen in HBV/HIV-coinfected patients due to the development of HIV resistance must take the HBV infection into consideration, as the chronic hepatitis B may be exacerbated in the absence of an active HBV polymerase inhibitor.

Immune escape and polymerase inhibitor resistance

Another relevant but unexpected consequence of lamivudine resistance is the induction of conformational changes in the HBsAg due to an overlapping reading frame in the genetic sequence of the HBV polymerase and the HBsAg (Figure 4). Because of this, mutations in the HBV polymerase may induce changes in the envelope of the virus resulting in an altered immunogenicity. This may result in vaccine escape mutants. In vitro and ex vivo studies support this hypothesis, which may have important public health implications (Mathews 2006; Sheldon 2007). Studies in chimpanzees have indeed confirmed that infections with drug-induced HBV variants are possible despite the presence of high anti-HBs levels that were considered protective in the vaccinated host (Kamili 2009).

In addition to humoral escape, lamivudine resistance may also affect cellular immunity against HBV. Suboptimal antiviral therapy, e.g., with lamivudine, especially in high prevalence countries, could undermine the success of vaccination efforts leading to a spread of HBV vaccine escape mutants.

The YMDD motif is also part of an MHC class I restricted CTL epitope. YMDD-specifc cytotoxic T lymphocytes may partially cross-react with YVDD and YIDD variants (Lin 2005) and thereby contribute to a prevention of emergence of resistance. However, more studies are needed to explore in detail the consequences of the development of viral resistance to polymerase inhibitors for T cell immunity against HBV.

Conclusion

In summary, therapy with HBV polymerase inhibitors to date is limited to two active subclasses with different resistance profiles. In consequence, resistance due to suboptimal treatment on only one agent can eliminate or reduce the effect of newer agents due to partial or complete cross-resistance. This sequence is well documented for lamivudine, telbivudine and entecavir. There are some data indicating the possibility of partial cross-resistance between entecavir and tenofovir. The potential of adefovir to induce partial resistance to tenofovir is emerging in newly published studies.

The superiority of *de novo* combination therapy for HBV over sequential monotherapy is likely for patients with very high HBV viremia, but still has to be proven in prospective clinical trials. In patients with low or intermediate viremia, the risk for development of resistance is rather low when using drugs with a high genetic barrier and when a rapid suppression of HBV replication is achieved (Figure 5).

In conclusion, the choice of first-line treatment strategy will determine future treatment options; being judicious is paramount as it can, in cases of suboptimal therapeutic approaches, result in a rapid exhaustion of options within just a few years.

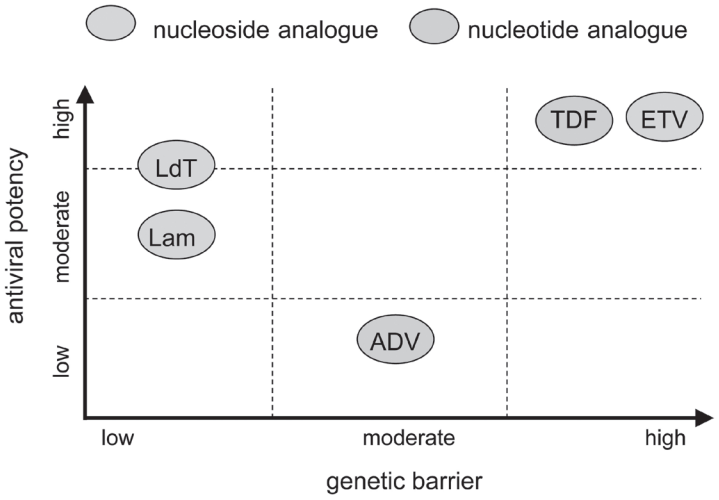


Figure 5: Antiviral potency and genetic resistance barrier of currently approved HBV polymerase inhibitors.

References

Avila C, Karwowska S, Lai C, Evans T. Telbivudine has no in vitro activity against laboratory and clinical HIV-1, including 5 clades and drug-resistant clinical isolates. Proceedings of the 12th Conference on Retroviruses and Opportunistic Infections; Montréal, Canada; 2009 February 8-11; poster 813b.

Benhamou Y, Bochet M, Thibault V, Di Martino V, Caumes E, Bricaire F, et al. Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients. Hepatology. 1999;30(5):1302-6.

Berg T, Moller B, Trinh H, Chan S, Marcellin P, Suarez E, et al. Tenofovir disoproxil fumarate (TDF) versus emtricitabine plus TDF for treatment of chronic Hepatitis B (CHB) in subjects with persistent viral replication receiving adefovir dipivoxil (ADV). Journal of Hepatology. 2008; 48(Suppl.2): S34 (abstract 76).

Bonino F, Marcellin P, Lau GK, Hadziyannis S, Jin R, Piratvisuth T, et al. Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group. Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B. Gut. 2007; 56(5): 699-705.

Brunelle MN, Jacquard AC, Pichoud C, Durantel D, Carrouée-Durantel S, Villeneuve JP, et al. Susceptibility to antivirals of a human HBV strain with mutations conferring resistance to both lamivudine and adefovir. Hepatology. 2005; 41(6): 1391-8.

Chang T, Gish R, De Man R. A comparison of entecavir and lamivudine for HBeAg- positive chronic hepatitis B. N Engl J Med 2006; 354: 1001-1010.

Chloe WH, Hong SP, Kim BK, Ko SY, Jung YK, Kim JH, et al. Evolution of hepatitis B virus mutation during entecavir rescue therapy in patients with antiviral resistance to lamivudine and adefovir. Antivir Ther. 2009;14(7): 985-93.

Chueca N, Nogales C, Rodriguez F, et al. Hepatitis B Virus genotyping may influence therapeutic decisions. 5th European HIV Drug Resistance Workshop, Cascais 2007, abstract 88.

Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology. 2006;44(6):1656-65.

Cornberg M, Protzer U, Dollinger MM, Petersen J, Wedemeyer H, Berg T, et al. Prophylaxis, Diagnosis and Therapy of Hepatitis-B-Virus-(HBV-) Infection: upgrade of the guideline. Z Gastroenterol. 2007; 45(6): 525-74.

Delaugerre C, Marcelin AG, Thibault V, Peytavin G, Bombled T, Bochet MV, et al. Human immunodeficiency virus (HIV) Type 1 reverse transcriptase resistance mutations in hepatitis B virus (HBV)-HIV-coinfected patients treated for HBV chronic infection once daily with 10 milligrams of adefovir dipivoxil combined with lamivudine. Antimicrob Agents Chemother. 2002;46(5):1586-8.

Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Italian Association for the Study of the Liver (AISF) Lamivudine Study Group, Italy. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology. 2004; 40(4):883-91.

Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, Haussinger D. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut. 2005; 54(7):1009-13.

Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. HBV 99-01 Study Group. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol. 2006;101(2):297-303.

Fung SK, Andreone P, Han SH et al. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. J Hepatol 2005; 43(6):937-43.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009; 461(7262):399-401.

Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Adefovir Dipivoxil 438 Study Group. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology. 2006;131(6):1743-51.

Heathcote JE, Gane EJ, De Man RA, Lee SS, Fisiak R, Manns M, et al. Three years of tenofovir disoproxil (TDF) treatment in HBeAg-positive patients with chronic hepatitis B(study 1003), preliminary analysis. Hepatology 2009; 50,4:533A

Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet. 2009; 41(5): 591-5.

Kamili S, Sozzi V, Thompson G, Campbell K, Walker CM, Locarnini S, Krawczynski K. Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model. Hepatology. 2009; 49(5): 1483-91.

Kurashige N, Ohkawa K, Hiramatsu N, Oze T, Yakushijin T, Mochizuki K, et al. Two types of drug-resistant hepatitis B viral strains emerging alternately and their susceptibility to combination therapy with entecavir and adefovir. Antivir Ther. 2009;14(6): 873-7.

Lada O, Gervais A, Branger M, Peytavin G, Colin G, Fraqueiro G, et al. Low rate of delayed response in lamivudine experienced HIV/HBV co-infected patients treated with enofovir disoproxil fumarate (TDF). Journal of Hepatology. 2008; 48(Suppl.2): S259 (abstract 695).

Lai CL, Leung N, Teo EK, Tong M, Wong F, Hann HW, et al. Telbivudine Phase II Investigator Group. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology. 2005;129(2):528-36.

Lai CL, Gane E, Hsu CW et al. Two-year results from the GLOBE trial in patients with hepatitis B: Greater clinical and antiviral efficacy for telbivudine (LdT) vs.lamivudine. Hepatology, 2006; 44, 222A.

Lai C, Shouval D, Lok A. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006; 354: 1011-1020.

Lampertico P, Vigano M, Manenti E et al. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. Hepatology 2005; 42: 1414-1419.

Lampertico P, Marzano A, Levriero M et al. Adefovir and lamivudine combination therapy is superior to adefovir monotherapy for lamivudine-resistant patients with HBeAg-negative hepatitis B. Hepatology, 2006; 44, 693A.

Lampertico P, Viganò M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with Lamivudine: a 3-year study of 145 Lamivudine-resistant hepatitis B patients. Gastroenterology. 2007; 133(5):1445-51.

Lampertico P, Vigano M, Facchetti F, Suter F, Minola E, Fracessetti O, et al. Entecavir for NUC-naive chronic epatiti B patients in clinical practice: Long-term effectiveness from a large multi center color study in 376 patients. Hepatology 2009; 50,4:408A

Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon Alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005; 30;352(26):2682-95.

Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology. 2006; 43(6):1385-91.

Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Cirrhosis Asian Lamivudine Multicentre Study Group. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004; 351(15):1521-31.

Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, et al. GLOBE Study Group. 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology. 2009;136(2):486-95.

Lim SG, Ng TM, Kung N, Krastev Z, Volfova M, Husa P, et al. Emtricitabine FTCB-301 Study Group. A double-blind placebo-controlled study of emtricitabine in chronic hepatitis B. Arch Intern Med. 2006; 166(1):49-56.

Lin CL, Tsai SL, Lee TH, Chien RN, Liao SK, Liaw YF. High frequency of functional anti-YMDD and -mutant cytotoxic T lymphocytes after in vitro expansion correlates with successful response to lamivudine therapy for chronic hepatitis B. Gut. 2005; 54 (1): 152-61.

Locarnini S, Hatzakis A, Heathcote J, Keeffe EB, Liang TJ, Mutimer D, et al. Management of antiviral resistance in patients with chronic hepatitis B. Antivir Ther. 2004; 9 (5): 679-93.

Manns M, Jeffers L, Dalekos G, Berg T, Trepo C, et al. Safety and efficacy of 96 weeks of tenofovir disoproxil fumarate therapy in lamivudine experienced patients. J Hepatol 2009; 50(1) : S335.

Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir Dipivoxil 437 Study Group. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003; 27;348(9):808-16.

Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2004; 351(12):1206-17.

Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med. 2008; 359(23):2442-55.

Marcellin P, Piratvisuth T, Brunetto MR, Bonino F, Farci P, et al. A finite course of peginterferon alfa-2a results in inactive chronic hepatitis B and HBsAg clearance 5 years Post treatment in patients with HBeAg-negative disease: baseline characteristics and predictive factors of long-term response. Hepatology 2009; 50 (4): 487A.

Matthews GV, Bartholomeusz A, Locarnini S, et al. Characteristics of drug resistant HBV in an international collaborative study of HIV-HBV-infected individuals on extended lamivudine therapy. AIDS. 2006;20(6):863-70.

McMahon MA, Jilek BL, Brennan TP, et al. The HBV drug entecavir - effects on HIV-1 replication and resistance. N Engl J Med 2007;356:2614-2621.

Milazzo L, Caramma I, Lai A, Violin M, De Maddalena C, Cesari M, et al. Telbivudine in the treatment of chronic hepatitis B: experience in HIV type-1-infected patients naive for antiretroviral therapy. Antivir Ther. 2009; 14(6):869-72.

Nguyen MH, Huy NT, Nguyen T, Do ST, Tran P, Nguyen KK, et al. Complete viral suppression and ALT normalization on entecavir (ETV) therapy in patients who were previously treated with adefovir (ADV): A multicenter study. Hepatology 2009; 50,4: 540A.

Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, Haussinger D. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med. 1996; 30;334(22):1422-7.

Nelson M, Portsmouth S, Stebbing J. An open-label study of tenofovir in HIV-1 and hepatitis B virus co-infected individuals. AIDS 2003;17:F7-F10.

Núñez M, Pérez-Olmeda M, Díaz B, Ríos P, González-Lahoz J, Soriano V. Activity of tenofovir on hepatitis B virus replication in HIV-co-infected patients failing or partially responding to lamivudine. AIDS 2002; 16:2352-2354.

Perquin M, Reijinders JG, Zhang NP, Hansen BE, Janssen HL. HbeAg seroconversion during nucleos(t)ide analogue treatment does not lead to durable remission of chronic hepatitis B. Hepatology 2009; 50,4: 407A.

Petersen J, Lutgehetmann M, Zoulim F, Sterneck M, Janssen HL, Berg T, et al. Entecavir and tenofovir combination therapy in chronic hepatitis B: rescue therapy in patients with advanced fibrosis and multiple previous treatment failures. Hepatology 2009; 50,4: 496A.

Reijnders JG, De Man RA, Pas SD, Schutten M, Janssen HL. Entecavir: A rescue therapy for chronic hepatitis B patients with a limited virological response to adefovir? Hepatology 2007; 46, No. 4, (Suppl.1): 660A, Abstract No. 951.

Schmutz G, Nelson M, Lutz T, Sheldon J, Bruno R, von Boemmel F, et al. Combination of tenofovir and lamivudine versus tenofovir after lamivudine failure for therapy of hepatitis B in HIV-coinfection. AIDS. 2006;20(15):1951-4.

Sheldon JA, Corral A, Rodés B, Mauss S, Rockstroh J, Berger F, et al. Risk of selecting K65R in antiretroviral-naive HIV-infected individuals with chronic hepatitis B treated with adefovir. AIDS 2005;19(17):2036-8.

Sheldon J, Ramos B, Garcia-Samaniego J et al. Selection of Hepatitis B Virus (HBV) Vaccine Escape Mutants in HBV-Infected and HBV/HIV-Coinfected Patients Failing Antiretroviral Drugs With Anti-HBV Activity. J Acquir Immune Defic Syndr. 2007; 46(3):279-82.

Shim JH, Suh DJ, Kim KM, Lim YS, Lee HC, Chung YH, Lee YS. Efficacy of entecavir in patients with chronic hepatitis B resistant to both lamivudine and adefovir or to lamivudine alone. Hepatology. 2009; 50(4): 1064-71.

Sung JJ, Lai JY, Zeuzem S, Chow WC, Heathcote EJ, Perrillo RP, et al. Lamivudine compared with lamivudine and adefovir dipivoxil for the treatment of HBeAg-positive chronic hepatitis B. J Hepatol. 2008; 48(5):728-35.

Tenney DJ, Rose RE, Baldick CJ, Levine SM, Pokornowski KA, Walsh AW, et al. Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. Antimicrob Agents Chemother. 2007;51(3):902-11.

Van Bommel F, Wunsche T, Mauss S. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004; 40: 1421-1425.

Van Bömmel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology. 2010; 51(1): 73-80.

Westland C, Delaney W 4th, Yang H, Chen SS, Marcellin P, Hadziyannis S, et al. Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. Gastroenterology. 2003;125(1):107-16.

Zeuzem S, Gane E, Liaw YF, Lim SG, DiBisceglie A, Buti M, et al. Baseline characteristics and early on-treatment response predict the outcomes of 2 years of telbivudine treatment of chronic hepatitis B. J Hepatol. 2009; 51(1): 11-20.

Chapter 11: Hepatitis D
Diagnostic procedures and therapy

Heiner Wedemeyer

Introduction

Hepatitis delta is considered the most severe form of viral hepatitis in humans. The hepatitis delta virus (HDV) is a defective RNA virus which requires the hepatitis B virus (HBV) surface antigen (HBsAg) for complete replication and transmission, while the full extent of the HBV helper function is unexplored (Rizzetto 1983; Taylor 2006). Hence, hepatitis delta occurs only in HBsAg-positive individuals either as acute coinfection or as superinfection in patients with chronic hepatitis B (Farci 2003) (Figure 1). Several studies have shown that chronic HDV infection leads to more severe liver disease than chronic HBV mono-infection with an accelerated course of fibrosis progression, an increased risk of hepatocellular carcinoma and early decompensation in the setting of established cirrhosis (Farci 2003; Fattovich 2000; Fattovich 1987). Simultaneous HBV and HDV infection has also been shown to be more severe than infection with HBV alone in chimpanzees (Dienes 1990). So far, only interferon alpha treatment has been shown to exert significant antiviral activity against HDV and has been linked to improve the long-term outcome. Recent data on the use of pegylated interferon confirm earlier findings - PEG-IFN leads to sustained virological response rates in about one quarter of patients.

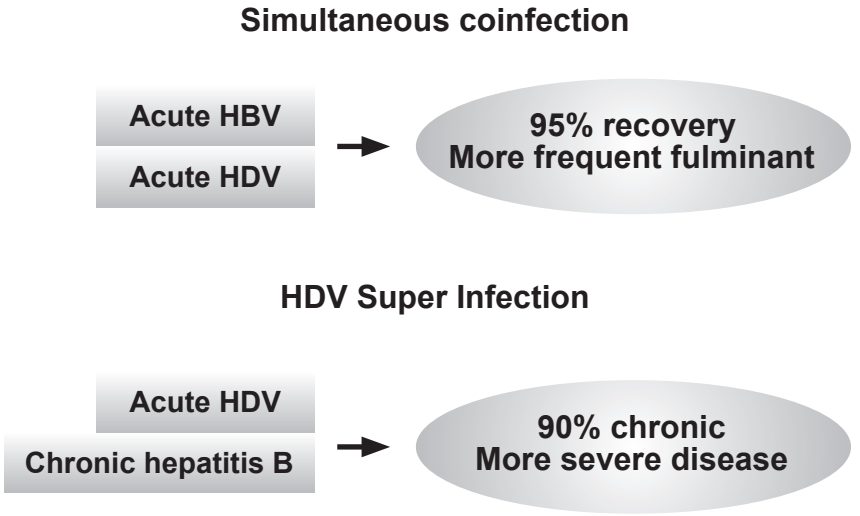


Figure 1. Courses of delta hepatitis.

Virology of delta hepatitis

The HDV virion is approximately 36 nm large containing HDV RNA and delta antigen. HDV RNA is single-stranded, highly base-paired, circular and by far the smallest genome of any animal virus, containing close to 1700 nucleotides (Taylor 2006). It is coated with the envelope protein derived from the pre-S and S antigens of the hepatitis B virus. The HDV RNA has six open reading frames (ORFs), three on the genomic and three on the antigenomic strand. One ORF codes for the hepatitis delta antigen (HDAg), while the other ORFs do not appear to be actively transcribed. Two HDAgs exist: the small HDAg (24 kD) is 155 amino acids long and the large HDAg (27 kD) is 214 amino acids long. A single nucleotide change (A-G) in the small HDAg sequence leads to the synthesis of the large HDAg. The small HDAg accelerates genome synthesis, while the large HDAg that inhibits HDV RNA synthesis is necessary for virion morphogenesis (Taylor 2006). Replication of HDV RNA occurs through a 'double rolling circle model' in which the genomic strand is replicated by a host RNA polymerase to yield a multimeric linear structure that is then autocatalytically cleaved to linear monomers and ligated into the circular HDV RNA viral progeny.

Genetic analysis has revealed the presence of at least seven HDV genotypes (Radjef 2004) (Figure 2). Genotype 1 is the most frequently seen genotype and is distributed throughout the world, especially in Europe, the Middle East, North America and North Africa. Genotype 2 is seen in East Asia, and genotype 3 is seen exclusively in the northern part of South America. Genotype 1 is associated with both severe and mild disease whereas genotype 2 causes a milder disease over a long-term course (Su 2006). All patients who have been included in the large European HIDT-I treatment trial in Germany, Turkey and Greece were proven to be infected with HDV genotype I (Zachou 2006).

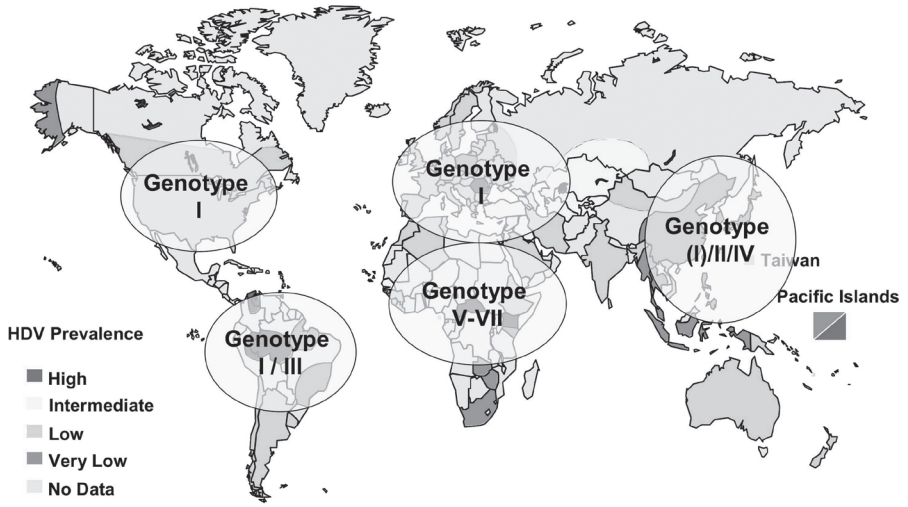


Figure 2. Prevalence of HDV genotypes.

Epidemiology of delta hepatitis

Delta hepatitis is not an uncommon disease. Being linked to HBV, HDV is spread in the same way as HBV, mainly through parenteral exposure (Niro 1999). It is highly endemic in Mediterranean countries, the Middle East, Central Africa, and northern parts of South America (Radjef 2004) (Figure 2). In Western countries, high prevalence can be found in HBsAg-positive intravenous drug users (Wedemeyer 2007; Gaeta 2000). Worldwide, more than 350 million people are considered to be chronically infected with HBV and 15-20 million of those are estimated to be anti-HDV positive (Hadziyannis 1997). Delta hepatitis is highly endemic in Southern Europe. Several studies performed in the 1980s and 1990s showed a prevalence of anti-HDV among HBsAg-positive individuals of more than 20% (Farci 2003). In Turkey, HDV prevalences in HBsAg-positive patients ranged between <5% in Western Turkey to >27% in South East Turkey (Degertekin 2008). Another country with a particular high prevalence of delta hepatitis is Mongolia with up to one third of chronic hepatitis cases being caused by HDV infection (Tsatsralt-Od 2005).

As a result of the implementation of HBV vaccination programs, the incidence of HDV infections significantly decreased in Southern Europe in the 1990s (Gaeta 2000) (Figure 3).

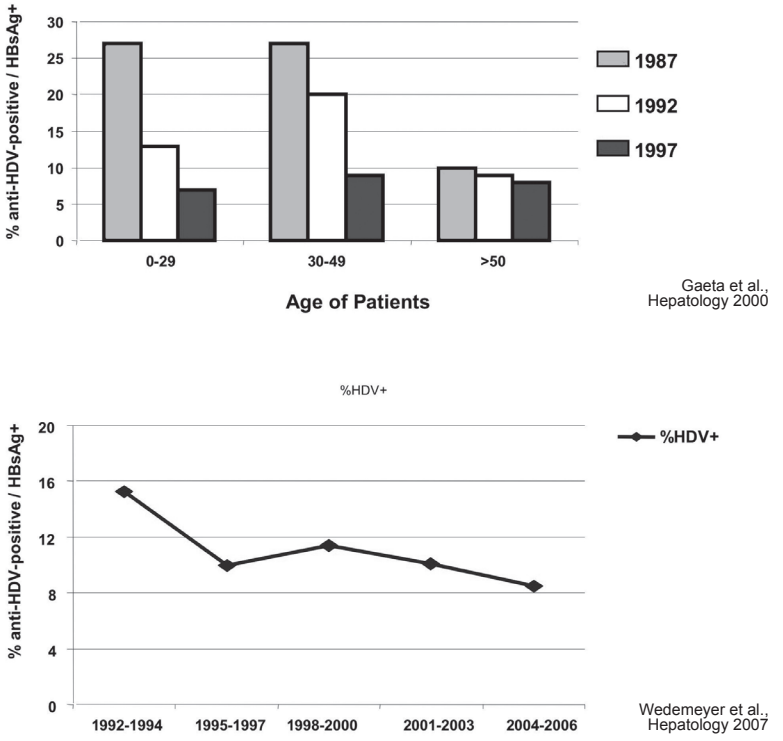


Figure 3. Prevalence of hepatitis D virus in Italy and Germany.

Chronic delta hepatitis still represents a significant health burden in Central Europe – in particular due to immigration from highly endemic areas (Wedemeyer 2007; Erhardt 2003) (Figure 4) (Table 1). In our experience at a referral center for liver disease, about 8-10% of HBsAg-positive patients still test positive for anti-HDV (Figure 3). More than three quarters of our delta hepatitis patients were not born in Germany. However, the geographical origin of our patients has changed during the last decade. While until the mid-1990s the majority of HDV-positive patients was born in Turkey, the proportion of Eastern European patients has significantly increased in recent years (Wedemeyer 2007) (Table 1).

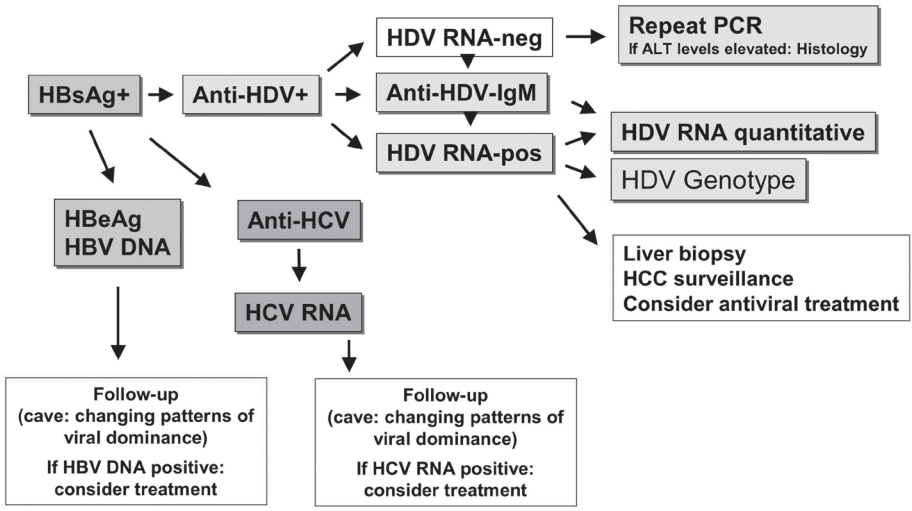


Figure 4. Diagnostic steps in delta hepatitis.

Origin of patients	HDV diagnosis 1992 - 1996 N=3	HDV diagnosis 1997 - 2006 N=101	p-value
Germany (%)	23.2 (N=10)	17.8 (N=18)	n.s.
Turkey (%)	**41.8 (N=18)**	19.8 (N=20)	0.006
Eastern Europe/NIS (%)	16.6 (N=5)	**34.6 (N=35)**	0.003

Table 1. Country of birth in patients with delta hepatitis at Hannover Medical School.

Limited data is available on the epidemiology of delta hepatitis in the USA. Earlier studies published between 1985 and 1993 reported HDV prevalences of 2% in homosexual men (Weisfuse 1989), around 20% in haemophiliacs (Rizzetto 1983) and female prostitutes (Troisi 1993) and up to 30% in hepatitis B carriers in Illinois (Hershow 1989). However, no study including a significant number of individuals has been published since 1993. In particular, the prevalence of HDV in high-risk populations such as IV drug users is unknown in US populations.

Pathogenesis of HDV infection

Knowledge about the pathogenesis of delta hepatitis infection is limited. Clinical observations have provided examples of mostly an immune-mediated process in delta hepatitis disease. However, patterns suggesting a cytopathic viral disease have occasionally been observed. A typical example of the latter were outbreaks of severe hepatitis in the northern part of South America (Nakano 2001). These mostly fulminant hepatitis cases were induced by genotype 3 delta virus. However, in the usual case of delta hepatitis the liver histology is not different from a patient with hepatitis B or hepatitis C with accompanying necroinflammatory lesions. Importantly, HDV viremia is not directly associated with the stage of liver disease (Zachou 2006). Cellular immune responses against the hepatitis D virus have been described by few investigators (Nisini 1997; Aslan 2003; Huang 2004) suggesting that the quantity and quality of T cell responses may be associated with some control of the infection. We have recently shown that the frequency of cytotoxic CD4+ T cells is higher in delta hepatitis patients than in individuals with HBV or HCV infection (Aslan 2006). This limited information taken together suggests that HDV is mainly an immune-mediated disease, at least in HDV genotype 1 infection. Antiviral therapies should therefore also aim to enhance anti-HDV immunity to confer long-term control of the infection. Interestingly, we have seen that the quality of the HDV-specific T cell response is able to predict the response to PEG-IFN α-2a treatment (Wedemeyer 2007).

Coinfections with multiple hepatitis viruses are associated with diverse patterns of reciprocal inhibition of viral replication (Raimondo 2006). HDV has frequently been shown to suppress HBV replication (Jardi 2001; Sagnelli 2000). Between 70% and 90% of delta hepatitis patients are HBeAg negative with low levels of HBV DNA. However, the course of HBeAg-positive delta hepatitis has not been well studied. It is of importance to note that even HBeAg-positive patients may be negative for HBV DNA in the context of HDV coinfection. On the other hand, HBV pre-core stop codons may also develop in delta hepatitis patients and thus HBeAg-negative patients can display significant levels of HBV DNA requiring antiviral treatment against hepatitis B.

There is also increasing evidence that HDV can not only suppress HBV replication but also HCV replication in tri-infected patients (Wedemeyer 2001). In our experience, less than one fifth of anti-HCV/HBsAg/anti-HDV-positive individuals are positive for HCV RNA. It is not clear how many of the anti-HCV-positive/HCV RNA-negative patients have recovered from HCV infection and how many patients just show a suppressed HCV replication in the context of viral coinfections. It is

interesting to note that viral dominance may change over time. Thus triple infected patients should be followed closely and, if indicated, treatment of the dominant virus needs to be considered.

Clinical course of delta hepatitis

Acute HBV/HDV coinfection

Acute HBV/HDV coinfection leads to recovery in more than 90% of cases but frequently causes severe acute hepatitis with a high risk for developing a fulminant course (Rizzetto 2000). In contrast, HDV is cleared spontaneously only in a minority of patients with HDV superinfection of chronic HBsAg carriers (Figure 1). The observation that histopathology of simultaneous HBV and HDV infection is more severe than in infection with HBV alone, has also been documented in experiments with chimpanzees (Dienes 1990). Several outbreaks of very severe courses of acute delta hepatitis in patients have been described in different regions of the world (Casey 1996; Flodgren 2000; Tsatsralt-Od 2006). However, fortunately, acute delta hepatitis has become rather infrequent over the last two decades in Western countries due to the introduction of vaccination programs.

Chronic delta hepatitis

Several studies have shown that chronic HDV infection leads to more severe liver disease than chronic HBV monoinfection, with an accelerated course of fibrosis progression, an increased risk of hepatocellular carcinoma and early decompensation in the presence of cirrhosis (Fattovich 1987; Jardi 2001; Sagnelli 2000; Rizzetto 2000; Uzunalimoglu 2001; Wedemeyer 2007). HDV accounts for almost half of all cases of liver cirrhosis and hepatocellular carcinoma in South East Turkey (Degertekin 2008; Uzunalimoglu 2001; Yurdaydin 2006a). A recent long-term observational study from Taiwan has reported a cumulative survival of HDV genotype 1-infected patients of as low as 50% after 15 years (Su 2006). HDV infection has also been associated with a higher risk of developing liver cirrhosis in HIV-coinfected patients as 66% of HIV/HBV/HCV/HDV-infected patients but only 6% of HBV/HCV/HIV-infected patients present with liver cirrhosis in a Spanish cohort (Castellares 2008). Similarly, delta hepatitis was associated with poorer survival in HIV-infected patients in Taiwan (Sheng 2007).

Diagnosis of delta hepatitis

Every HBsAg-positive patient must be tested for anti-HDV antibodies at least once. There is currently no evidence that direct testing for HDV RNA in the absence of anti-HDV is of any use. A positive result for anti-HDV does not necessarily indicate "active" delta hepatitis as HDV RNA can become negative indicating recovery from HDV infection. Over the long term as well, anti-HDV antibodies can be lost after recovery. However, anti-HDV may persist for years even when the patient has experienced HBsAg seroconversion (Wedemeyer 2007).

"Active" replicative delta hepatitis should be confirmed by the detection of HDV RNA. If HDV RNA is positive, subsequent evaluation of grading and staging of liver disease, surveillance for hepatocellular carcinoma and consideration of antiviral treat-

ment is indicated. HDV RNA quantification is offered by some laboratories. However, so far there is no evidence that HDV RNA levels correlate with any clinical marker of liver disease (Zachou 2006). Thus, HDV RNA quantification is currently only useful if antiviral treatment is indicated. Stopping rules during antiviral treatment depending on the level of antiviral decline are currently being evaluated. Patients with less than a 3 log decline of HDV RNA after 24 weeks of treatment will not benefit from antiviral treatment with PEG-IFN α-2b (Erhardt 2006).

HDV genotyping is performed by some research labs and may help to identify patients with a higher or lower risk of developing end-stage liver disease (Su 2006). In western countries almost all patients are infected with HDV-genotype 1, thus, genotyping may be considered only in immigrants or populations with mixed genotype prevalences.

In the 1980s and 1990s the diagnosis of active delta hepatitis was dependent on anti-HDV IgM testing. Anti-HDV-IgM testing might still be useful in patients who test HDV RNA negative but have evidence of liver disease which cannot be explained by other reasons. Due to the variability of the HDV genome and the lack of standardization of HDV RNA assays, HDV RNA may test false negative or be under the detection limit of the assay in the case of fluctuating viral load. In these cases, HDV RNA testing should be repeated and anti-HDV-IgM testing might be performed, if available.

As delta hepatitis only occurs in the context of HBV coinfection, a solid work-up of HBV infection including HBV DNA quantification and HBeAg/anti-HBe determination is warranted. Similarly, testing for anti-HCV and anti-HIV is mandatory. In our experience, up to one third of anti-HDV positive patients also test positive for anti-HCV.

Treatment of Delta Hepatitis

Nucleoside and nucleotide analogues

Several nucleoside and nucleotide analogues used for the treatment of HBV infection have been shown to be ineffective against HDV (Table 2). Famcyclovir, used in the 1990s to treat HBV infection (Wedemeyer 1999), had no significant antiviral activity against HDV in a Turkish trial (Yurdaydin 2002). Similarly, lamivudine was ineffective in trials of delta hepatitis (Wolters 2000; Niro 2005; Yurdaydin 2008; Lau 1999b). Ribavirin alone or in combination with interferon also did not lead to increased rates of HDV RNA clearance (Niro 2006a; Gunsar 2005; Garripoli 1994). However, a long-term observational study of HIV-infected individuals receiving HAART followed HBV/HDV/HIV-coinfected individuals for a median of more than 6 years; over this time, a decline of HDV RNA from 7 $\log_{10}$ to 5.8 $\log_{10}$ was observed and 3 out of 16 patients became HDV RNA negative (Sheldon 2008). Thus, very long treatment with HBV polymerase inhibitors may lead to beneficial effects in delta hepatitis possibly due to a reduction of HBsAg levels. Future long-term trials will need to confirm these data in triple-infected individuals.

Another promising and surprising alternative to the currently approved HBV polymerase inhibitors may be clevudine. Clevudine, a nucleoside analogue currently in

development for the treatment of hepatitis B, has recently been shown to inhibit delta virus viremia in woodchucks. No data are available yet in humans treated with clevudine for HDV (Casey 2005).

Nucleos(t)ide analogues	
Famciclovir ineffective	*Yurdaydin et al., Hepatol 2002*
Lamivudine ineffective	*Wolters et al., J Viral Hepatitis 2000; Lau et al., Hepatology 1999; Niro, Aliment Pharmacol Ther. 2005; Niro et al., J Viral Hepatitis 2008 Yurdaydin et al., J Viral Hepatitis 2008*
Ribavirin ineffective	*Niro et al., Hepatology 2006 Garripoli et al., Liver 1994 Gunsar et al., Antiv Therapy 2005*
Interferon α	
Sustained biochemical responses in 0-36% of patients Few studies with virologic endpoints Treatment for>12 months may be requiered	*Farci et al., NEJM 1994 Di Marco et al., J Viral Hepatitis 1996 Niro et al., J Viral Hepatitis 2005; Yurdaydin et al., J Viral Hepatitis 2008*
Higher IFN doses were associated with better survival in small cohort study	*Gunsar et al., Antiv Therapy 2005*

Table 2. Treatment options in delta hepatitis.

Recombinant interferon alpha

Interferon α has been used for the treatment of delta hepatitis since the mid 1980s (Rizzetto 1986). Since then, many trials have explored different durations and doses of interferon alpha in HDV-infected patients. However, data are difficult to compare as endpoints are different in the trials and few studies have followed HDV RNA levels over time (Niro 2005).

One randomized Italian study on the use of high dose interferon alpha is especially important as interferon treatment has been associated with a beneficial long-term outcome in delta hepatitis patients (Farci 1994; Farci 2004). Some studies have used extended doses of interferon treatment and it seems that two years of treatment is superior in terms of HDV RNA clearance (Niro 2005). In one case report from NIH, 12 years of interferon treatment led finally to resolution of both HDV infection and HBsAg clearance (Lau 1999a). High doses of interferon and extended treatment are tolerated by only a minority of patients and treatment option are very limited for the majority of patients (Manns 2006).

Pegylated interferon alpha

Recently, pegylated interferon has also been used in small trials to treat delta hepatitis with sustained virological response rates of about 20% (Castelnau 2006; Niro 2006; Erhardt 2006) (Table 3).

Castelnau, Gault et al. Hepatology 2006	
14 patients, 12 months of PEG-IFN α-2b	**SVR in 6 patients (43%)**
Niro, Rizzetto et al. Hepatology 2006	
38 patients, 72 weeks PEG-IFN α-2b	
16 patients monotherapy	**SVR: 8 patients (21%)**
22 patients + ribavirin (first 48 weeks)	**Ribavirin had no additional effect**
Erhardt, Häussinger et al. Liver Int 2006	
12 patients, 48 weeks of PEG-IFN α-2b	**SVR in 2 patients (17%)**

Table 3. Pegylated interferon in delta hepatitis.

In 2004, the Hep-Net International Delta hepatitis Intervention Trial (HIDIT-1) began. 90 patients (42 in Germany, 39 in Turkey and 9 in Greece) with chronic HDV infection and compensated liver disease were randomized to receive either 180 µg PEG-IFN α-2a QW plus 10 mg adefovir dipivoxil QD (group A, N=31), 180 µg PEG-IFN α-2a QW plus placebo (group B, N = 29) or 10 mg adefovir dipivoxil qd alone (group C, N=30) for 48 weeks. HBV DNA and HDV RNA were investigated by real-time PCR. Ten patients did not complete 48 weeks of therapy because of disease progression (N=6) or interferon-associated side effects (N=4). Both PEG-IFN groups showed a significantly higher reduction in mean HDV RNA levels than the adefovir monotherapy group by week 48. HDV RNA became negative in 21%, 30% and 8% of patients, respectively (PEG-IFN vs. adefovir, p=0.06). While patients receiving PEG-IFN α-2a alone or adefovir monotherapy had similar mean HBsAg levels at week 0 and week 48, the PEG-IFN α-2a/adefovir combination group showed a 1.1 $\log_{10}$ IU/ml decline of HBsAg levels by week 48 ($p<0.001$). These data are in line with a report from Greece of a significant decline in HbsAg levels in delta hepatitis patients receiving long-term treatment with interferon alpha (Manesis 2007).

Overall the HIDIT-1 study showed that (i) PEG-IFN α-2a displays a significant antiviral efficacy against HDV in more than 40% of patients with 25% becoming HDV RNA negative after 48 weeks; (ii) adefovir dipivoxil has little efficacy in terms of HDV RNA reduction but may be considered for patients with significant HBV replication; (iii) combination therapy of PEG-IFN α-2a plus adefovir has no advantages for HBV DNA or HDV RNA reduction; (iv) a combination therapy of pegylated interferon with a nucleotide is superior to either monotherapy in reducing HBsAg levels in HBV-infected patients (Wedemeyer 2007; Yurdaydin 2006b).

Currently, additional trials are ongoing to investigate the efficacy of PEG-IFN α-2a in combination with tenofovir for the treatment of delta hepatitis. Moreover, alternative treatment options need to be explored. Among these, prenylation inhibitors may be promising (Bordier 2003). HDV replication depends on a prenylation step and prenylation inhibitors have already been developed for the treatment of malignancies.

References

Aslan N, Yurdaydin C, Bozkaya H, Baglan P, Bozdayi AM, Tillmann HL et al. Analysis and function of delta-hepatitis virus-specific cellular immune responses. Journal of Hepatology 2003; 38:15-16.

Aslan N, Yurdaydin C, Wiegand J, Greten T, Ciner A, Meyer MF et al. Cytotoxic CD4 T cells in viral hepatitis. J Viral Hepat 2006; 13(8):505-514.

Bordier BB, Ohkanda J, Liu P, Lee SY, Salazar FH, Marion PL et al. In vivo antiviral efficacy of prenylation inhibitors against hepatitis delta virus. J Clin Invest 2003; 112(3):407-414.

Casey J, Cote PJ, Toshkov IA, Chu CK, Gerin JL, Hornbuckle WE et al. Clevudine inhibits hepatitis delta virus viremia: a pilot study of chronically infected woodchucks. Antimicrob Agents Chemother 2005; 49(10):4396-4399.

Casey JL, Niro GA, Engle RE, Vega A, Gomez H, McCarthy M et al. Hepatitis B virus (HBV)/hepatitis D virus (HDV) coinfection in outbreaks of acute hepatitis in the Peruvian Amazon basin: the roles of HDV genotype III and HBV genotype F. J Infect Dis 1996; 174(5):920-926.

Castellares C, Barreiro P, Martin-Carbonero L, Labarga P, Vispo ME, Casado R et al. Liver cirrhosis in HIV-infected patients: prevalence, aetiology and clinical outcome. J Viral Hepat 2008; 15(3):165-172.

Castelnau C, Le Gal F, Ripault MP, Gordien E, Martinot-Peignoux M, Boyer N et al. Efficacy of peginterferon alpha-2b in chronic hepatitis delta: relevance of quantitative RT-PCR for follow-up. Hepatology 2006; 44(3):728-735.

Dienes HP, Purcell RH, Popper H, Ponzetto A. The significance of infections with two types of viral hepatitis demonstrated by histologic features in chimpanzees. J Hepatol 1990; 10(1):77-84.

Degertekin H, Yalcin K, Yakut M, Yurdaydin C. Seropositivity for delta hepatitis in patients with chronic hepatitis B and liver cirrhosis in Turkey: a meta-analysis. Liver Int 2008; 28(4):494-498.

Erhardt A, Knuth R, Sagir A, Kirschberg O, Heintges T, Haussinger D. Socioepidemiological data on hepatitis delta in a German university clinic--increase in patients from Eastern Europe and the former Soviet Union. Z Gastroenterol 2003; 41(6):523-526.

Erhardt A, Gerlich W, Starke C, Wend U, Donner A, Sagir A et al. Treatment of chronic hepatitis delta with pegylated interferon-alpha2b. Liver Int 2006; 26(7):805-810.

Farci P, Mandas A, Coiana A, Lai ME, Desmet V, Van Eyken P et al. Treatment of chronic hepatitis D with interferon alfa-2a. N Engl J Med 1994; 330(2):88-94.

Farci P. Delta hepatitis: an update. J Hepatol 2003; 39 Suppl 1:S212-S219.

Farci P, Roskams T, Chessa L, Peddis G, Mazzoleni AP, Scioscia R et al. Long-term benefit of interferon alpha therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. Gastroenterology 2004; 126(7):1740-1749.

Fattovich G, Boscaro S, Noventa F, Pornaro E, Stenico D, Alberti A et al. Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. J Infect Dis 1987; 155(5):931-935.

Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). Gut 2000; 46(3):420-426.

Flodgren E, Bengtsson S, Knutsson M, Strebkova EA, Kidd AH, Alexeyev OA et al. Recent high incidence of fulminant hepatitis in Samara, Russia: molecular analysis of prevailing hepatitis B and D virus strains. J Clin Microbiol 2000; 38(9):3311-3316.

Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Stornaiuolo G, Lobello S et al. Chronic hepatitis D: a vanishing Disease? An Italian multicenter study. Hepatology 2000; 32(4 Pt 1):824-827.

Garripoli A, Di M, V, Cozzolongo R, Costa C, Smedile A, Fabiano A et al. Ribavirin treatment for chronic hepatitis D: a pilot study. Liver 1994; 14(3):154-157.

Gunsar F, Akarca US, Ersoz G, Kobak AC, Karasu Z, Yuce G et al. Two-year interferon therapy with or without ribavirin in chronic delta hepatitis. Antivir Ther 2005; 10(6):721-726.

Hadziyannis SJ. Review: hepatitis delta. J Gastroenterol Hepatol 1997; 12(4):289-298.

Hershow RC, Chomel BB, Graham DR, Schyve PM, Mandel EJ, Kane MA et al. Hepatitis D virus infection in Illinois state facilities for the developmentally disabled. Epidemiology and clinical manifestations. Ann Intern Med 1989; 110(10):779-785.

Huang YH, Tao MH, Hu CP, Syu WJ, Wu JC. Identification of novel HLA-A*0201-restricted CD8+ T-cell epitopes on hepatitis delta virus. J Gen Virol 2004; 85(Pt 10):3089-3098.

Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, Galimany R et al. Role of hepatitis B, C, and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. Hepatology 2001; 34(2):404-410.

Lau DT (a), Kleiner DE, Park Y, Di Bisceglie AM, Hoofnagle JH. Resolution of chronic delta hepatitis after 12 years of interferon alfa therapy. Gastroenterology 1999; 117(5):1229-1233.

Lau DT (b), Doo E, Park Y, Kleiner DE, Schmid P, Kuhns MC et al. Lamivudine for chronic delta hepatitis. Hepatology 1999; 30(2):546-549.

Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. Gut 2006; 55(9):1350-1359.

Manesis EK, Schina M, Le Gal F, Agelopoulou O, Papaioannou C, Kalligeros C et al. Quantitative analysis of hepatitis D virus RNA and hepatitis B surface antigen serum levels in chronic delta hepatitis improves treatment monitoring. Antivir Ther 2007; 12(3):381-388.

Nakano T, Shapiro CN, Hadler SC, Casey JL, Mizokami M, Orito E et al. Characterization of hepatitis D virus genotype III among Yucpa Indians in Venezuela. J Gen Virol 2001; 82(Pt 9):2183-2189.

Niro GA, Casey JL, Gravinese E, Garrubba M, Conoscitore P, Sagnelli E et al. Intrafamilial transmission of hepatitis delta virus: molecular evidence. J Hepatol 1999; 30(4):564-569.

Niro GA, Rosina F, Rizzetto M. Treatment of hepatitis D. J Viral Hepat 2005; 12(1):2-9.

Niro GA (a), Ciancio A, Tillman HL, Lagget M, Olivero A, Perri F et al. Lamivudine therapy in chronic delta hepatitis: a multicentre randomized-controlled pilot study. Aliment Pharmacol Ther 2005; 22(3):227-232.

Niro GA, Ciancio A, Gaeta GB, Smedile A, Marrone A, Olivero A et al. Pegylated interferon alpha-2b as monotherapy or in combination with ribavirin in chronic hepatitis delta. Hepatology 2006; 44(3):713-720.

Nisini R, Paroli M, Accapezzato D, Bonino F, Rosina F, Santantonio T et al. Human CD4+ T-cell response to hepatitis delta virus: identification of multiple epitopes and characterization of T-helper cytokine profiles. J Virol 1997; 71(3):2241-2251.

Radjef N, Gordien E, Ivaniushina V, Gault E, Anais P, Drugan T et al. Molecular phylogenetic analyses indicate a wide and ancient radiation of African hepatitis delta virus, suggesting a deltavirus genus of at least seven major clades. J Virol 2004; 78(5):2537-2544.

Raimondo G, Brunetto MR, Pontisso P, Smedile A, Maina AM, Saitta C et al. Longitudinal evaluation reveals a complex spectrum of virological profiles in hepatitis B virus/hepatitis C virus-coinfected patients. Hepatology 2006; 43(1):100-107.

Rizzetto M. The delta agent. Hepatology 1983; 3(5):729-737.

Rizzetto M, Rosina F, Saracco G, Bellando PC, Actis GC, Bonino F et al. Treatment of chronic delta hepatitis with alpha-2 recombinant interferon. J Hepatol 1986; 3 Suppl 2:S229-S233.

Rizzetto M. Hepatitis D: virology, clinical and epidemiological aspects. Acta Gastroenterol Belg 2000; 63(2):221-224.

Sagnelli E, Coppola N, Scolastico C, Filippini P, Santantonio T, Stroffolini T et al. Virologic and clinical expressions of reciprocal inhibitory effect of hepatitis B, C, and delta viruses in patients with chronic hepatitis. Hepatology 2000; 32(5):1106-1110.

Sheldon J, Ramos B, Toro C, Rios P, Martinez-Alarcon J, Bottecchia M et al. Does treatment of hepatitis B virus (HBV) infection reduce hepatitis delta virus (HDV) replication in HIV-HBV-HDV-coinfected patients? Antivir Ther 2008; 13(1):97-102.

Sheng WH, Hung CC, Kao JH, Chang SY, Chen MY, Hsieh SM et al. Impact of hepatitis D virus infection on the long-term outcomes of patients with hepatitis B virus and HIV coinfection in the era of highly active antiretroviral therapy: a matched cohort study. Clin Infect Dis 2007; 44(7):988-995.

Su CW, Huang YH, Huo TI, Shih HH, Sheen IJ, Chen SW et al. Genotypes and viremia of hepatitis B and D viruses are asso-ciated with outcomes of chronic hepatitis D patients. Gastroenterology 2006; 130(6):1625-1635.

Taylor JM. Hepatitis delta virus. Virology 2006; 344(1):71-76.

Tsatsralt-Od B, Takahashi M, Nishizawa T, Endo K, Inoue J, Okamoto H. High prevalence of dual or triple infection of hepatitis B, C, and delta viruses among patients with chronic liver disease in Mongolia. J Med Virol 2005; 77(4):491-499.

Tsatsralt-Od B, Takahashi M, Endo K, Buyankhuu O, Baatarkhuu O, Nishizawa T et al. Infection with hepatitis A, B, C, and delta viruses among patients with acute hepatitis in Mongolia. J Med Virol 2006; 78(5):542-550.

Troisi CL, Hollinger FB, Hoots WK, Contant C, Gill J, Ragni M et al. A multicenter study of viral hepatitis in a United States hemophilic population. Blood 1993; 81(2):412-418.

Uzunalimoglu O, Yurdaydin C, Cetinkaya H, Bozkaya H, Sahin T, Colakoglu S et al. Risk factors for hepatocellular carcinoma in Turkey. Dig Dis Sci 2001; 46(5):1022-1028.

Wedemeyer H, Boker KH, Pethig K, Petzold DR, Flemming P, Tillmann HL et al. Famciclovir treatment of chronic hepatitis B in heart transplant recipients: a prospective trial. Transplantation 1999; 68(10):1503-1511.

Wedemeyer H, Tillmann HL, Tegtmeyer B, Cornberg M, Schuler A, Liermann H et al. Infection with multiple hepatitis viruses: Evidence for suppression of HCV replication by HDV and HBV. Hepatology 2001; 34(4):223A.

Wedemeyer H., Yurdaydin C. Delta Hepatitis. In: Tillman HL, editor. Handbuch Hepatitis B: Diagnostik, Verlauf, Therapie. Bremen: Uni-Med, 2007: 96-103.

Wedemeyer H, Yurdaydin C, Dalekos G, Erhardt A, Cakaloglu Y, Degertekin H et al. 72 week data of the HIDIT-1 trial: A multicenter randomised study comparing peginterferon alpha-2a plus adefovir vs. peginterferon alpha-2a plus placebo vs. adefovir in chronic delta hepatitis. Journal of Hepatology 2007; 46:S4.

Wedemeyer H, Heidrich B, Manns MP. Hepatitis D virus infection - Not a vanishing disease in Europe! Hepatology 2007; 45(5):1331-1332.

Wedemeyer H, Ciner A, Yurdaydin C, Zachou K, Aslan N, Meyer S et al. Differential cytokine pattern of HDV-specific cellular immune responses distinguishes treatment responder and nonresponder to peg-IFN alpha-2a treatment: Results from the HEP-NET/international HIDIT-1 study. Journal of Hepatology 2007; 46:S13-S14.

Weisfuse IB, Hadler SC, Fields HA, Alter MJ, O'Malley PM, Judson FN et al. Delta hepatitis in homosexual men in the United States. Hepatology 1989; 9(6):872-874.

Wolters LM, van Nunen AB, Honkoop P, Vossen AC, Niesters HG, Zondervan PE et al. Lamivudine-high dose interferon combination therapy for chronic hepatitis B patients co-infected with the hepatitis D virus. J Viral Hepat 2000; 7(6):428-434.

Yurdaydin C, Bozkaya H, Gurel S, Tillmann HL, Aslan N, Okcu-Heper A et al. Famciclovir treatment of chronic delta hepatitis. J Hepatol 2002; 37(2):266-271.

Yurdaydin C. Delta hepatitis in Turkey: decreasing but not vanishing and still of concern. Turk J Gastroenterol 2006; 17(1):74-75.

Yurdaydin C (a), Wedemeyer H, Dalekos G, Erhardt A, Cakaloglu Y, Degertekin H et al. A multicenter randomised study comparing the efficacy of pegylated interferon-alfa-2a plus adevofir dipivoxil vs. pegylated interferon-alfa-2a plus placebo vs. adevofir dipivoxil for the treatment of chronic delta hepatitis: The hep-net/international delta hepatitis intervention trial (HID-IT). Hepatology 2006; 44(4):230A.

Yurdaydin C (b), Bozkaya H, Onder FO, Senturk H, Karaaslan H, Akdogan M et al. Treatment of chronic delta hepatitis with lamivudine vs lamivudine + interferon vs interferon. J Viral Hepat 2008; 15(4):314-321.

Zachou K, Yurdaydin C, Dienes HR, Dalekos GN, Erhardt A, Cakaloglu Y et al. Significance of HDV-RNA and HBsAg levels in delta hepatitis: First data of the HEP-NET/International HDV Intervention Trial. Journal of Hepatology 2006; 44:S178.

Part 3

Hepatitis C

Chapter 12: Diagnostic tests in acute and chronic hepatitis C

Christian Lange, Christoph Sarrazin

Introduction

Common symptoms of hepatitis C like fatigue, muscle ache, loss of appetite or nausea are unspecific and, in many cases, mild or even not present. Consequently, hepatitis C is often diagnosed accidentally and, unfortunately, remains heavily under-diagnosed. It is estimated that only one out of four individuals infected with HCV is aware of their disease and so can not take advantage of treatment options and risk further transmission of the virus (McHutchison 2004). Untreated hepatitis C advances to a chronic state in up to 80% of people, which leads to liver cirrhosis in 20-40% with an accompanying risk of hepatic decompensation, hepatocellular carcinoma and death (McHutchison 2004). In light of these facts, HCV diagnostics should be performed thoroughly in all patients presenting with increased aminotransferase levels, with chronic liver disease of unclear aetiology and with a history of enhanced risk of HCV transmission (i.e., past IV or nasal drug dependency, transmission of blood or blood products before the year 1990, major surgery before 1990, needle stick injuries, non-sterile tattoos or piercings, enhanced risk of sexual transmission).

For the diagnosis of hepatitis C both serologic and nucleic acid-based molecular assays are available (Scott 2007). Serologic tests are sufficient when chronic hepatitis C is expected, with a sensitivity of more than 99% in the 3rd generation assays. Positive serologic results require HCV RNA measurement in order to discriminate between chronic hepatitis C and resolved HCV infection from the past. When acute hepatitis C is considered, serologic screening alone is insufficient because anti-HCV antibodies may develop late after transmission of the virus. In contrast, HCV RNA is detectable within a few days of infection, making nucleic acid-based tests mandatory in diagnosing acute hepatitis C. HCV RNA measurement is furthermore essential in the determination of treatment indication, duration and success (Terrault 2005). The latter has to be confirmed at clearly defined times during treatment to decide whether therapy should be continued or not. It should be repeated 24 weeks after treatment completion to assess whether a sustained virologic response (SVR) has been achieved. Both qualitative and quantitative HCV RNA detection assays are available. Qualitative tests are highly sensitive and are used for diagnosing hepatitis C for the first time, for the screening of blood and organ donations and for confirming SVR after treatment completion. Quantitative HCV RNA detection assays offer the possibility of measuring the viral load exactly over a wide range of copies and are essential in treatment monitoring. Qualitative and quantitative HCV RNA assays are now being widely replaced by real-time PCR-based assays that can detect HCV RNA over a very wide range, from low levels of approximately 10 IU/ml up to 10 million IU/ml.

After diagnosing hepatitis C, the HCV genotype should be determined by nucleic acid-based techniques in every patient considered for HCV therapy because the currently recommended treatment duration and ribavirin doses differ among the genotypes.

Morphological methods like immunohistochemistry, in situ-hybridization or PCR from liver specimens play only an accessory role in the diagnosis of hepatitis C because of their low sensitivity, poor specificity and low efficacy compared to serologic and nucleic acid-based approaches.

Serologic assays

In current clinical practice, antibodies against multiple HCV epitopes are detected by commercially available 2nd and 3rd generation enzyme-linked immunoassays (EIAs). In these tests, HCV-specific antibodies from serum samples are captured by recombinant HCV proteins and are then detected by secondary antibodies against IgG or IgM. These secondary antibodies are labeled with enzymes that catalyse the production of coloured, measurable compounds.

The first applied EIAs for the detection of HCV-specific antibodies were based on epitopes derived from the NS4 region (C-100) and had a sensitivity of 70–80% and a poor specificity (Scott 2007). C-100-directed antibodies occur approximately 16 weeks after viral transmission. 2nd generation EIAs additionally detect antibodies against epitopes derived from the core region (C-22), NS3 region (C-33) and NS4 region (C-100), which leads to an increased sensitivity of approximately 95% and to a lower rate of false-positive results. With these assays HCV-specific antibodies can be detected approximately 10 weeks after HCV infection (Pawlotsky 2003). To narrow the diagnostic window from viral transmission to positive serological results, a 3rd generation EIA has been completed by an antigen from the NS5 region and the substitution of a highly immunogenic NS3 epitope. This innovation allows the detection of anti-HCV antibodies approximately four to six weeks after infection with a sensitivity of more than 99% (Colin 2001). The clinical specificity, however, is slightly decreased compared to the 2nd generation assays. Anti-HCV IgM measurement can narrow the diagnostic window in only a minority of patients. Anti-HCV IgM detection is also not sufficient to discriminate between acute and chronic hepatitis C because some chronically infected patients produce anti-HCV IgM intermittently and not all patients respond to acute HCV infection by producing anti-HCV IgM.

The specificity of serologic HCV diagnostics in general is difficult to define since an appropriate gold standard is lacking. It is evident, however, that false-positive results are more frequent in patients with rheuma-factors and in populations with a low hepatitis C prevalence, for example in blood and organ donors. Although several immunoblots for the confirmation of positive HCV EIA results are available, these tests have lost their clinical importance since the development of highly sensitive methods for HCV RNA detection. Immunoblots are mandatory to make the exact identification of serologically false-positive-tested individuals possible. Importantly, the sensitivity of immunoblotting is lower compared to EIAs, which bears the risk of the false-negatively-classifying of HCV-infected individuals.

False-negative HCV antibody testing may occur in patients on hemodialysis or in severely immunosuppressed patients like in HIV infection or in haematological malignancies.

HCV core antigen assays

In principle, detection of the HCV core antigen could be a cheaper alternative to nucleic acid testing for the diagnosis and management of hepatitis C. However, the introduction of a reliable and sensitive HCV core antigen assay was burdened with a number of difficulties like the development of specific monoclonal antibodies recognizing all different HCV subtypes and the need for accumulation and dissociation of HCV particles from immune complexes to increase sensitivity. The first HCV core antigen detection system (trak-C‘, Ortho Clinical Diagnostics) became commercially available in the US and Europe several years ago. In this assay, HCV core proteins were bound to coated monoclonal antibodies in a microwell after dissociation of the HCV particles from immune complexes. Bound core antigen was incubated with an anti-core-specific Fab antibody fragment conjugated with horseradish peroxidase followed by quantitative detection performed by addition of o-phenylenediamine (OPD) / hydrogen peroxide and measurement of the optical density. The HCV core antigen assay proved highly specific (99.5%), genotype independent, and had a low inter- and intra-assay variability (coefficient of variation 5–9%) (Veillon 2003). HCV core antigen is measurable 1–2 days after HCV RNA becomes detectable. The limit of detection is 1.5 pg/ml which corresponds to an HCV RNA level of approximately 10,000–50,000 IU/ml. In a study of anti-HCV antibody and HCV RNA positive patients presenting in an outpatient clinic, 6/139 people (4%) were HCV core antigen negative. In these patients, HCV RNA concentrations were 1300–58,000 IU/ml highlighting the limitations of the HCV core antigen assay as confirmation of ongoing hepatitis C in anti-HCV-positive patients. As a consequence, this first HCV core antigen assay was withdrawn from the market.

Most recently, another quantitative HCV core antigen assay (Architect HCV Ag, Abbott Diagnostics), a further development of the previous assay, was approved by the European Union. This assay comprises 5 different antibodies to detect HCV core antigen, is highly specific (99.8%) and shows equivalent sensitivity for determination of chronic hepatitis C as HCV RNA measurement (Morota 2009). The detection limit corresponds to HCV RNA levels of 600–1000 IU/ml. Further studies are ongoing to show the utility of this more sensitive HCV core antigen assay for diagnosis and management of patients with HCV infection.

Nucleic acid testing for HCV

Until 1997, HCV quantitative results derived from various HCV RNA detection systems did not represent the same concentration of HCV RNA in a clinical sample. Because of the importance of an exact HCV RNA load determination for management of patients, the World Health Organization (WHO) established the HCV RNA international standard based on international units (IU) which is used in all clinically applied HCV RNA tests. Other limitations of earlier HCV RNA detection assays were false-negative results due to polymerase inhibition, for example by drug interference, false-positive results due to sample contaminations because the reaction tubes had to be opened frequently, or due to under- and over-quantification of samples of certain HCV genotypes (Pawlotsky 2003; Morishima 2004). Currently, several HCV RNA assays are commercially available (Table 1).

Assay	Distributor	Technology	Approval status
Qualitative HCV RNA detection assays			
Amplicor™ HCV 2.0	Roche Molecular Systems	PCR	FDA, CE
Versant™ HCV	Siemens Medical Solutions Diagnostics	TMA	FDA, CE
Quantitative HCV RNA detection assays			
Amplicor™ HCV Monitor 2.0	Roche Molecular Systems	PCR	CE
HCV SuperQuant™	National Genetics Institute	PCR	
Versant™ HCV RNA 3.0	Siemens Medical Solutions Diagnostics	bDNA	FDA, CE
Cobas Ampliprep/ Cobas TaqMan	Roche Molecular Systems	Real-time PCR	FDA, CE
Abbott RealTime™ HCV	Abbott Diagnostics	Real-time PCR	CE

Table 1. Commercially available HCV RNA detection assays.

Qualitative assays for HCV RNA detection

Until recently qualitative assays for HCV RNA had substantially lower limits of detection in comparison with quantitative HCV RNA assays. The costs of a qualitative assay are also lower compared to a quantitative assay. Therefore, qualitative HCV RNA tests are used for the first diagnosis of acute hepatitis C, in which HCV RNA concentrations are fluctuating and may be very low, as well as for confirmation of chronic hepatitis C infection in patients with positive HCV antibodies. In addition, they are used for the confirmation of virologic response during, at the end of, and after antiviral therapy, as well as in screening blood and organ donations for presence of HCV.

Qualitative RT-PCR

In reverse transcriptase-PCR- (RT-PCR-) based assays, HCV RNA is used as a matrix for the synthesis of a single-stranded complementary cDNA by reverse transcriptase. The cDNA is then amplified by a DNA polymerase into multiple double-stranded DNA copies. Qualitative RT-PCR assays are expected to detect 50 HCV RNA IU/ml or less with equal sensitivity for all genotypes.

The Amplicor™ HCV 2.0 (Roche Molecular Systems, USA) is an FDA- and CE-approved RT-PCR system for qualitative HCV RNA testing that allows detection of HCV RNA concentrations down to 50 IU/ml of all genotypes (Nolte 2001) (Table 1). The DNA polymerase of Thermus thermophilus used in this assay provides both DNA polymerase and reverse transcriptase activity and allows HCV RNA amplification and detection in a single step, single tube procedure.

Transcription-mediated amplification (TMA) of HCV RNA

TMA-based qualitative HCV RNA detection has a very high sensitivity (Sarrazin 2002; Hendricks 2003). TMA is performed in a single tube in three steps: target capture, target amplification and specific detection of target amplicons by a hybridization protection

assay. Two primers, one of which contains a T7 promoter, one T7 RNA polymerase and one reverse transcriptase, are necessary for this procedure. After RNA extraction from 500µl serum, the T7 promoter-containing primer hybridises with the viral RNA with the result of reverse transcriptase-mediated cDNA synthesis. The reverse transcriptase also provides an RNase activity which degrades the RNA of the resulting RNA/DNA hybrid strand. The second primer then binds to the cDNA already containing the T7 promoter sequence from the first primer, and a DNA/DNA double-strand is synthesised by the reverse transcriptase. Next, the RNA polymerase recognizes the T7 promoter and produces 100-1000 RNA transcripts, which are subsequently returned to the TMA cycle leading to exponential amplification of the target RNA. Within one hour, approximately 10 billion amplicons are produced. The RNA amplicons are detected by a hybridisation protection assay with amplicon-specific labeled DNA probes. The unhybridised DNA probes are degraded during a selection step and the labeled DNA is detected by chemiluminescence.

A commercially available TMA assay is the Versant™ HCV RNA Qualitative Assay (formerly Bayer, now Siemens Medical Solutions Diagnostics, Germany). This system is accredited by the FDA and CE and provides an extremely high sensitivity, which is superior to RT-PCR-based qualitative HCV RNA detection assays (Sarrazin 2000; Sarrazin 2001; Hofmann 2005). The lower detection limit is 5-10 IU/ml with a sensitivity of 96-100%, and a specificity of more than 99.5%. These performance characteristics are independent of the HCV genotype.

Quantitative HCV RNA detection

HCV RNA quantification can be achieved either by target amplification techniques (competitive and real-time PCR) or by signal amplification techniques (branched DNA (bDNA) assay) (Table 1). Several FDA- and CE-approved standardised systems are commercially available. The Cobas Amplicor™ HCV Monitor from Roche Diagnostics is based on a competitive PCR technique whereas the Versant™ HCV RNA Assay (Siemens Medical Solutions Diagnostics) is based on a bDNA technique. More recently, the Cobas TaqMan assay and the Abbott RealTime™ HCV test, both based on real-time PCR technology, have been introduced. The technical characteristics, detection limits and linear dynamic detection ranges of these systems are summarized below. Due to their very low detection limit and their broad and linear dynamic detection range, they have already widely replaced the previously used qualitative and quantitative HCV RNA assays.

Competitive PCR: Cobas Amplicor™ HCV Monitor 2.0 (Roche Diagnostics)

The Cobas Amplicor™ HCV Monitor 2.0 is a semi-automated quantitative detection assay based on a competitive PCR technique. Quantification is achieved by the amplification of two templates in a single reaction tube, the target and the internal standard. The latter is an internal control RNA with nearly the same sequence as the target RNA with a clearly defined initial concentration. The internal control is amplified by the same primers as the HCV RNA. Comparison of the final amounts of both templates allows calculation of the initial amount of HCV RNA. The dynamic range of the CE-labeled Amplicor™ HCV Monitor 2.0 assay is 500 to approximately 500,000 IU/ml

with a specificity of almost 100%, independent of the HCV genotype (Lee 2000; Konnick 2002). For higher HCV RNA concentrations pre-dilution of the original sample is required.

Branched DNA Hybridization Assay (Versant™ HCV RNA Assay 3.0, Siemens)

Branched DNAHybridization Assay is based on signal amplification technology. After reverse transcription of the HCV RNA, the resulting single-stranded complementary DNA strands bind to immobilised capture oligonucleotides with a specific sequence from conserved regions of the HCV genome. In a second step, multiple oligonucleotides bind to the free ends of the bound DNA strands and are subsequently hybridised by multiple copies of an alkaline phosphatase-labeled DNA probe. Detection is achieved by incubating the alkaline phosphatase-bound complex with a chemiluminescent substrate (Sarrazin 2002).

The Versant™ HCV RNA assay is at present the only FDA- and CE-approved HCV RNA quantification system based on a branched DNA technique. The lower detection limit of the current version 3.0 is 615 IU/ml and linear quantification is ensured between 615–8,000,000 IU/ml, independent of the HCV genotype (Morishima 2004; Ross 2002). The bDNA assay only requires 50µl serum for HCV RNA quantification and is currently the assay with the lowest sample input.

Real-time PCR-based HCV RNA detection assays

Real-time PCR technology provides optimal features for both HCV RNA detection and quantification because of its very low detection limit and broad dynamic range of linear amplification (Sarrazin 2006) (Figure 1). Distinctive for real-time PCR technology is the ability of simultaneous amplification and detection of the target nucleic acid allowing direct monitoring of the PCR process (Higuchi 1992). RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by a DNA polymerase-mediated cDNA amplification.

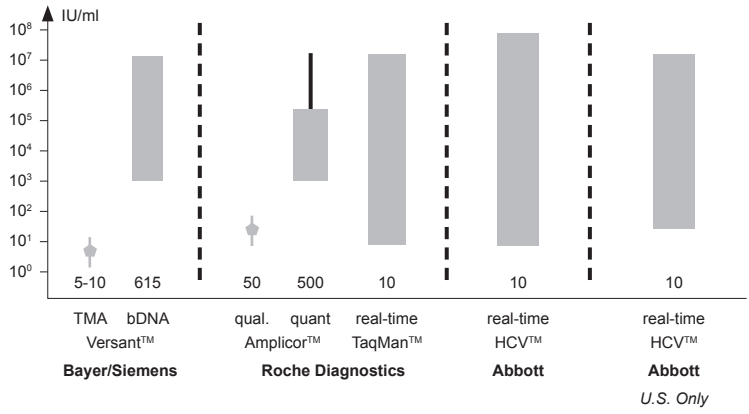


Figure 1. Detection limits and linear dynamic ranges of commercially available HCV RNA detection assays.

DNA detection simultaneous to amplification is preferentially achieved by the use of target sequence-specific oligonucleotides linked to two different molecules, a fluorescent reporter molecule and a quenching molecule. These probes bind the target cDNA between the two PCR primers and are degraded or released by the DNA polymerase during DNA synthesis. In case of degradation the reporter and quencher molecules are released and separated, which results in the emission of an increased fluorescence signal from the reporter. Different variations of this principle of reporter and quencher are used by the different commercially available assays. The fluorescence signal, intensified during each round of amplification, is proportional to the amount of RNA in the starting sample. Quantification in absolute numbers is achieved by comparing the kinetics of the target amplification with the amplification kinetics of an internal control of a defined initial concentration.

Highly effective and almost completely automated real-time PCR-based systems for HCV RNA measurement have been introduced by Roche Molecular Systems (US) and Abbott Laboratories (US). For replacement of the qualitative TMA and the quantitative bDNA-based assays, Siemens Diagnostics has also developed a real-time based PCR, scheduled to be launched in 2010.

All commercially available HCV RNA assays are calibrated to the WHO standard based on HCV genotype 1. Significant differences between different RT-PCR assays and other quantitative HCV RNA tests have been reported that in the case of the real-time PCR-based assays represent a slight under-quantification by one assay (real-time HCV) and a slight over-quantification by the other (Cobas TaqMan). In addition, it has been shown that results may vary significantly between assays with different HCV genotypes despite standardisation to IU (Chevaliez 2007; Vehrmeren 2008).

Cobas TaqMan HCV Test (Roche Diagnostics)

The CE-accredited Cobas TaqMan (CTM) assay uses reporter- and quencher-carrying oligonucleotides specific to the 5'UTR of the HCV genome and to the template of the internal control, a synthetic RNA for binding the same primers as for HCV RNA. Reverse transcription and cDNA amplification is performed by the Z05 DNA polymerase. For HCV RNA extraction from serum or plasma samples, a Cobas TaqMan assay was developed either in combination with the fully automated Cobas Ampliprep (CAP) instrument using magnetic particles, or in combination with manual HCV RNA extraction with glass fiber columns using the high pure system (HPS) viral nucleic acid kit. The current versions of both combinations have a lower detection limit of approximately 10 IU/ml and a linear amplification range of HCV RNA from approximately 40 to 10,000,000 IU/ml. Samples from HCV genotypes 2-5 have been shown to be under-quantified by the first version of the HPS-based Cobas TaqMan assay. The recently released second version of this assay has now demonstrated equal quantification of all HCV genotypes (Colluci 2007). For the Cobas Ampliprep Cobas TaqMan (CAP/CTM) assay significant under-quantification of HCV genotype 4 samples has been shown (Konnick 2005; Gelderblom 2006; Colucci 2007; Sizmann 2007; Vermehren 2008) and a second version of this assay is in preparation. Taken together, with the exception of HCV genotype 4 samples with the CAP/CTM assay, the Cobas TaqMan assay makes both highly sensitive qualitative and linear quantitative HCV RNA detection feasible with excellent performance in one system with complete automation.

RealTime™ HCV Test (Abbott Diagnostics)

The CE-accredited Abbott RealTime™ HCV Test uses reporter- and quencher-carrying oligonucleotides specific for the 5'UTR as well. HCV RNA concentrations are quantified by comparison with the amplification curves of a cDNA from the hydroxypyruvate reductase gene from the pumpkin plant Curcurbita pepo, which is used as an internal standard. This internal standard is amplified with different primers from those of the HCV RNA, which may be the reason for the linear quantification of very low HCV RNA concentrations. The Abbott RealTime™ HCV Test provides a lower detection limit of 12 IU/ml, a specificity of more than 99.5% and a linear amplification range from 12 to 10,000,000 IU/ml independent of the HCV genotype (Michelin 2007; Sabato 2007; Schutten 2007; Vermehren 2008).

HCV genotyping

HCV is heterogeneous with an enormous genomic sequence variability, developed by the rapid replication cycle with the production of 10^{12} virions per day and the low fidelity of the HCV RNA polymerase. Six genotypes (1-6), multiple subtypes (a, b, c…) and most recently a seventh HCV genotype have been characterized. These genotypes vary in approximately 30% of their RNA sequence with a median variability of approximately 33%. HCV subtypes are defined by differences in their RNA sequence of approximately 10%. Within one subtype, numerous quasispecies exist and may emerge during treatment with specific antivirals. These quasispecies are defined by a sequence variability of less than 10% (Simmonds 2005). Because the currently recommended treatment durations and ribavirin doses depend on the HCV genotype, HCV genotyping is mandatory in every patient who is considered for antiviral therapy (Bowden 2006).

Both direct sequence analysis and reverse hybridisation technology allows HCV genotyping. Initial assays were designed to analyse exclusively the 5' untranslated region (5'UTR), which is burdened with a high rate of misclassifications especially on the subtype level. Current assays were improved by additionally analyzing the coding regions, in particular the genes encoding the non-structural protein NS5B and core protein, both of which provide non-overlapping sequence differences between the genotypes and subtypes (Bowden 2006).

Genotyping by reverse hybridising assay (Versant™ HCV Genotype 2.0 System (LiPA), Siemens Medical Solutions Diagnostics)

In reverse hybridising, biotinylated cDNA clones from HCV RNA are produced by reverse transcriptase and then transferred and hybridised to immobilised oligonucleotides specific to different genotypes and subtypes. After removing unbound DNA by a washing step, the biotinylated DNA fragments can be detected by chemical linkage to coloured probes.

The Versant™ HCV Genotype 2.0 System (Siemens Medical Solutions Diagnostics) is suitable for indentifying genotypes 1-6 and more than 15 different subtypes and is currently the preferentially used assay for HCV genotyping. By simultaneous

analyses of the 5'UTR and core region, a high specificity is achieved especially to differentiate the genotype 1 subtypes. In a study evaluating the specificity of the Versant™ HCV Genotype 2.0 System, 96.8% of all genotype 1 samples and 64.7% of all genotype samples were correctly subtyped. No misclassifications at the genotype level were observed. Difficulties in subtyping occurred in particular in genotypes 2 and 4. Importantly, none of the misclassifications would have had clinical consequences, which qualifies the Versant™ HCV Genotype 2.0 System as highly suitable for clinical decision-making (Bouchardeau 2007).

Genotyping by direct sequence analysis (TRUGENE™ HCV 5'NC Genotyping Kit, Siemens)

The TruGene assay determines the HCV genotype and subtype by direct analysis of the nucleotide sequence of the 5'UTR region. Incorrect genotyping rarely occurs with this assay. However, the accuracy of subtyping is poor because of the exclusive analyses of the 5'UTR. Currently, the TRUGENE™ NS5B HCV genotyping assay, which additionally analyzes the NS5B region, is under development (Pawlotsky 2003).

Genotyping by real-time PCR technology (Abbott RealTime™ HCV Genotype II assay)

The current Abbott RealTime™ HCV Genotype II assay is based on real-time PCR technology, which is less time consuming than direct sequencing. Preliminary data revealed a 96% concordance at the genotype level and a 93% concordance on the genotype 1 subtype level when compared to direct sequencing of the NS5B and 5'UTR regions. Nevertheless, single genotype 2, 3, 4, and 6 isolates were misclassified at the genotype level, indicating a need for assay optimization (Vaghefi 2009).

Implications for diagnosing and managing acute and chronic hepatitis C

Diagnosing acute hepatitis C

When acute hepatitis C is suspected, the presence of both anti-HCV antibodies and HCV RNA should be tested. For HCV RNA detection, sensitive qualitative techniques with a lower detection limit of 50 IU/ml or less are required, for example TMA, qualitative RT-PCR or the newly developed real-time PCR systems. Testing for anti-HCV alone is insufficient for the diagnosis of acute hepatitis C because HCV specific antibodies appear only weeks after viral transmission. In contrast, measurable HCV RNA serum concentrations emerge within the first days after infection. However, HCV RNA may fluctuate during acute hepatitis C, making a second HCV RNA test necessary several weeks later in all negatively tested patients with a suspicion of acute hepatitis C. When HCV RNA is detected in seronegative patients, acute hepatitis C is very likely. When patients are positive for both anti-HCV antibodies and HCV RNA, it may be difficult to discriminate between acute and acutely exacerbated chronic hepatitis C. Anti-HCV IgM detection will not clarify because its presence is common in both situations.

Diagnosing chronic hepatitis C

Chronic hepatitis C should be considered in every patient presenting with clinical, morphological or biological signs of chronic liver disease. When chronic hepatitis C is suspected, screening for HCV antibodies by 2nd or 3rd generation EIAs is adequate because their sensitivity is <99%. False-negative results may occur rarely in immunosuppressed patients (i.e., HIV) and in patients on dialysis. When anti-HCV antibodies are detected, the presence of HCV RNA has to be determined in order to discriminate between chronic hepatitis C and resolved HCV infection. The latter cannot be distinguished by HCV antibody tests from rarely occurring false-positive serological results, the exact incidence of which is unknown. Serological false-positive results can be identified by the additional performance of an immunoblot assay. Many years after disease resolution, anti-HCV antibodies may become undetectable via commercial assays in some patients.

Diagnostic tests in the management of hepatitis C therapy

The current treatment recommendations for acute and chronic hepatitis C are based on HCV genotyping and on HCV RNA load determination before, during and after antiviral therapy. When HCV RNA has been detected, exact genotyping and HCV RNA load determination is necessary in every patient considered for antiviral therapy. Exact subtyping might gain increased importance for future STAT-C therapies because some subtypes behave differently regarding the development of resistance. Low HCV RNA concentrations (<600,000–800,000 IU/ml) is a positive predictor of SVR. Genotyping is mandatory before treatment initiation, as the dose of ribavirin and optimal treatment duration is determined specifically on the underlying HCV genotype (McHutchison 2004; Terrault 2005). For HCV genotype 1 (and 4) treatment can be shortened to 24 weeks in patients with low baseline viral load (<600,000–800,000 IU/ml) and rapid virologic response with undetectable HCV RNA at week 4 of treatment (RVR). In slow responders with a 2log decline but still detectable HCV RNA levels at week 12 and undetectable HCV RNA at week 24 treatment should be extended to 72 weeks, and in patients with complete early virologic response with undetectable HCV RNA at week 12 (cEVR) standard treatment is continued out to 48 weeks. Genotypes 5 and 6 are treated the same as genotype 1 infected patients due to the lack of adequate clinical trials whereas genotypes 2 and 3 generally allow treatment duration of 24 weeks, which may be shortened to 16 weeks (depending on RVR and [low] baseline viral load) or extended to 36-48 weeks depending on the initial viral decline (Layden-Almer 2006; Manns 2006). Independent of the HCV genotype, proof of HCV RNA load decrease is necessary to identify patients with little chance of achieving SVR. HCV RNA needs to be quantified before and 12 weeks after treatment initiation and antiviral therapy should be discontinued if a decrease of less than 2log HCV RNA levels is observed (negative predictive value 88-100%). In a second step, HCV RNA should be tested with highly sensitive assays after 24 weeks of treatment because patients with detectable HCV RNA at this time point only have a 1-2% chance of achieving SVR. SVR, defined as the absence of detectable HCV RNA 24 weeks after treatment completion, should be assessed by an HCV RNA detection assay with a lower limit of 50 IU/ml or less to evaluate long-lasting treatment success (Layden-Almer 2006; Manns 2006).

Due to the differences in HCV RNA concentrations of up to a factor of 4 between the different commercially available assays, despite standardisation of the results to IU, and due to intra- and interassay variability of up to a factor of 2, it is recommended to always use the same assay in a given patient before, during and after treatment and to repeat HCV RNA measurements at baseline in cases with HCV RNA concentrations between 400,000 and 1,000,000 IU/ml.

References

Bouchardeau F, Cantaloube JF, Chevaliez S, Portal C, et al. (2007). “Improvement of hepatitis C virus (HCV) genotype determination with the new version of the INNO-LiPA assay.” J Clin Microbiol 45(4): 1140-5.

Bowden DS, Berzsenyi MD (2006). “Chronic hepatitis C virus infection: genotyping and its clinical role.” Future Microbiol 1: 103-12.

Castera L, Vergniol J, Foucher J, Le Bail B, et al. (2005). “Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C.” Gastroenterology 128(2): 343-50.

Chevaliez S, Bouvier-Alias M, Brillet RPawlotsky JM (2007). “Overestimation and underestimation of hepatitis C virus RNA levels in a widely used real-time polymerase chain reaction-based method.” Hepatology 46(1): 22-31.

Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, et al. (2001). “Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature.” J Viral Hepat 8(2): 87-95.

Colucci G, Ferguson J, Harkleroad C, Lee S, et al. (2007). “Improved COBAS TaqMan hepatitis C virus test (Version 2.0) for use with the High Pure system: enhanced genotype inclusivity and performance characteristics in a multisite study.” J Clin Microbiol 45(11): 3595-600.

Gelderblom HC, Menting SBeld MG (2006). “Clinical performance of the new rRoche COBAS TaqMan HCV Test and High Pure System for extraction, detection and quantitation of HCV RNA in plasma and serum.” Antivir Ther 11(1): 95-103.

Hendricks DA, Friesenhahn M, Tanimoto L, Goergen B, et al. (2003). “Multicenter evaluation of the VERSANT HCV RNA qualitative assay for detection of hepatitis C virus RNA.” J Clin Microbiol 41(2): 651-6.

Higuchi R, Dollinger G, Walsh PSGriffith R (1992). “Simultaneous amplification and detection of specific DNA sequences.” Biotechnology (N Y) 10(4): 413-7.

Hofmann WP, Dries V, Herrmann E, Gartner B, et al. (2005). “Comparison of transcription mediated amplification (TMA) and reverse transcription polymerase chain reaction (RT-PCR) for detection of hepatitis C virus RNA in liver tissue.” J Clin Virol 32(4): 289-93.

Konnick EQ, Erali M, Ashwood ERHillyard DR (2002). “Performance characteristics of the COBAS Amplicor Hepatitis C Virus (HCV) Monitor, Version 2.0, International Unit assay and the National Genetics Institute HCV Superquant assay.” J Clin Microbiol 40(3): 768-73.

Konnick EQ, Williams SM, Ashwood ERHillyard DR (2005). “Evaluation of the COBAS Hepatitis C Virus (HCV) TaqMan analyte-specific reagent assay and comparison to the COBAS Amplicor HCV Monitor V2.0 and Versant HCV bDNA 3.0 assays.” J Clin Microbiol 43(5): 2133-40.

Layden-Almer JE, Cotler SJLayden TJ (2006). “Viral kinetics in the treatment of chronic hepatitis C.” J Viral Hepat 13(8): 499-504.

Lee SC, Antony A, Lee N, Leibow J, et al. (2000). “Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics.” J Clin Microbiol 38(11): 4171-9.

Manns MP, Wedemeyer HCornberg M (2006). “Treating viral hepatitis C: efficacy, side effects, and complications.” Gut 55(9): 1350-9.

McHutchison JG (2004). “Understanding hepatitis C.” Am J Manag Care 10(2 Suppl): S21-9.

Michelin BD, Muller Z, Stelzl E, Marth E, et al. (2007). “Evaluation of the Abbott RealTime HCV assay for quantitative detection of hepatitis C virus RNA.” J Clin Virol 38(2): 96-100.

Morishima C, Chung M, Ng KW, Brambilla DJ, et al. (2004). “Strengths and limitations of commercial tests for hepatitis C virus RNA quantification.” J Clin Microbiol 42(1): 421-5.

Morota K, Fujinami R, Kinukawa H, Machida T, et al. (2009). “A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen.” J Virol Methods 157(1): 8-14.

Nolte FS, Fried MW, Shiffman ML, Ferreira-Gonzalez A, et al. (2001). “Prospective multicenter clinical evaluation of AMPLICOR and COBAS AMPLICOR hepatitis C virus tests.” J Clin Microbiol 39(11): 4005-12.

Pawlotsky JM (2003). “Diagnostic testing in hepatitis C virus infection: viral kinetics and genomics.” Semin Liver Dis 23 Suppl 1: 3-11.

Pawlotsky JM (2003). “Use and interpretation of hepatitis C virus diagnostic assays.” Clin Liver Dis 7(1): 127-37.

Ross RS, Viazov S, Sarr S, Hoffmann S, et al. (2002). “Quantitation of hepatitis C virus RNA by third generation branched DNA-based signal amplification assay.” J Virol Methods 101(1-2):159-68.

Sabato MF, Shiffman ML, Langley MR, Wilkinson DS, et al. (2007). “Comparison of performance characteristics of three real-time reverse transcription-PCR test systems for detection and quantification of hepatitis C virus.” J Clin Microbiol 45(8): 2529-36.

Sarrazin C (2002). “Highly sensitive hepatitis C virus RNA detection methods: molecular backgrounds and clinical significance.” J Clin Virol 25 Suppl 3: S23-9.

Sarrazin C, Gartner BC, Sizmann D, Babiel R, et al. (2006). “Comparison of conventional PCR with real-time PCR and branched DNA-based assays for hepatitis C virus RNA quantification and clinical significance for genotypes 1 to 5.” J Clin Microbiol 44(3): 729-37.

Sarrazin C, Hendricks DA, Sedarati FZeuzem S (2001). “Assessment, by transcription-mediated amplification, of virologic response in patients with chronic hepatitis C virus treated with peginterferon alpha-2a.” J Clin Microbiol 39(8): 2850-5.

Sarrazin C, Teuber G, Kokka R, Rabenau H, et al. (2000). “Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays.” Hepatology 32(4 Pt 1): 818-23.

Schutten M, Peters D, Back NK, Beld M, et al. (2007). “Multicenter evaluation of the new Abbott RealTime assays for quantitative detection of human immunodeficiency virus type 1 and hepatitis C virus RNA.” J Clin Microbiol 45(6): 1712-7.

Scott JDGretch DR (2007). “Molecular diagnostics of hepatitis C virus infection: a systematic review.” Jama 297(7): 724-32.

Simmonds P, Bukh J, Combet C, Deleage G, et al. (2005). “Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes.” Hepatology 42(4): 962-73.

Sizmann D, Boeck C, Boelter J, Fischer D, et al. (2007). “Fully automated quantification of hepatitis C virus (HCV) RNA in human plasma and human serum by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol 39(4): 326-7.”

Terrault NA, Pawlotsky JM, McHutchison J, Anderson F, et al. (2005). “Clinical utility of viral load measurements in individuals with chronic hepatitis C infection on antiviral therapy.” J Viral Hepat 12(5): 465-72.

Vahefi P, Marchadier E, Dussaix E, Roque-Afonso AM. (2009). Hepatitis C virus genotyping: Comparison of the Abbott RealTime HCV Genotype II assay and NS5B sequencing. Pathol Biol (Paris), epub.

Veillon P, Payan C, Picchio G, Maniez-Montreuil M, et al. (2003). Comparative evaluation of the total hepatitis C virus core antigen, branched DNA, and amplicor monitor assays in determining viremia for patients with chronic hepatitis C during interferon plus ribavirin combination therapy. J Clin Microbiol 41(7):3121-20.

Vehrmeren J, Kau A, Gärtner B, Göbel R, Zeuzem S, Sarrazin C (2008). “Differences between two real-time PCR based assays (Abbott RealTime HCV, COBAS AmpliPrep/COBAS TaqMan) and one signal amplification assay (VERSANT HCV RNA 3.0) for HCV RNA detection and quantification.” J Clin Microbiol 46(12): 880-91.

Chapter 13: Hepatitis C
Standard of care

Markus Cornberg, Michael P. Manns, Heiner Wedemeyer

Therapy of acute hepatitis C

The goal of acute hepatitis C treatment is the prevention of persistent HCV infection. The natural rate of HCV evolution to a chronic state is 50-90%. As a vaccine is not yet available, early treatment of acute HCV infection with interferon alpha (IFN α) is the only option to prevent persistent HCV infection; however, the diagnosis of acute primary HCV infection may be difficult and its distinction from exacerbation of an underlying unrecognized chronic HCV infection may be difficult.

The immediate treatment of patients with symptomatic acute hepatitis C with recombinant IFN or PEG-IFN α monotherapy for 24 weeks can prevent the development of chronic hepatitis C in approximately 90% of cases (Jaeckel 2001; Wiegand 2006; Broers 2005; Santantonio 2005; Vogel 1996). However, good patient adherence to therapy is necessary to achieve these response rates (Wiegand 2006) (Table 1). Coadministration with ribavirin does not seem to be necessary. Symptomatic patients also have a good chance of clearing HCV spontaneously (Gerlach 203; Hofer 2003), occurring usually in the first 12 weeks after the onset of symptoms. The treatment of only those patients who remain HCV RNA positive 12 weeks after the onset of symptoms results in an overall sustained virological response (self-limited and treatment-induced) in 91% of patients (Gerlach 2003). Asymptomatic patients, however, should probably be treated immediately since these patients have a higher risk for evolution to a chronic state.

However, early treatment of acute HCV infection to prevent chronic disease does have its limitations. A main problem is that primary HCV infection is usually asymptomatic and most patients cannot be identified in this early stage of disease. Another reason is that a number of patients have medical contraindications for treatment with interferon (IFN) or are not ready for therapy because they are still IV drug users. There are two concerns in treating active drug users with IFN. In case of successful therapy there is a risk of re-infection with HCV (Manns 2009). The second reason is the side effect profile of IFN, especially the neuropsychiatric problems, that may result in a worsening of addictive behavior (Wiegand 2006). In addition, it has been shown that the acceptance of and adherence to antiviral therapy by these patients is low due to the side effects of IFN (Broers 2005) (Table 1).

There are many important questions on the treatment of acute hepatitis C that may be answered in ongoing clinical studies. For example, a study coordinated by the German Competence Network for Viral Hepatitis (Hep-Net) is underway to test if a wait-and-see strategy is as effective as immediate treatment (www.kompetenznetz-hepatitis.de/study_house/hcv_III_studie.htm).

The first preliminary data from this trial were presented at the 2009 EASL Annual Meeting. Early treatment was superior in the intent-to-treat analysis, although this was caused mainly by higher drop out rates in the delayed treatment arm. Of note,

all patients who started treatment later and who completed treatment and follow-up responded to treatment. The trial also showed for the first time that early treatment is as effective in patients with asymptomatic acute hepatitis C (Deterding 2009).

Also, highly effective antiviral drugs with fewer side effects may be on the horizon, which may allow for short-term treatment for all acute HCV patients.

Study	n	Treatment	Start of therapy	Duration	Efficacy
(Jaeckel 2001)	44 recruited in 24 centers	IFN α–2b (4 weeks 5 MU daily, a 20 weeks 5 MU TIW)	89 days after infection (range 30-112 days)	24 wks	43/44 (98%)
(Santantonio 2005)	28	PEG-IFN α-2b (1.5 µg/kg/week)	12 weeks after onset of disease (17/28 chronic, 16 treated)	24 wks	15/16 (94%)
(Broers 2005)	27 (22 IVDU)	PEG-IFN α-2b (1.5 µg/kg/week)	100±82 days after onset of symptoms, 63±82 days after diagnosis (asymptomatic) (22/27 chronic, 14 treated)	24 wks	8/14 (57%) 7/8 (88%) of adherent pts.
(Wiegand 2006)	89 recruited in 53 centers	PEG-IFN α-2b (1.5 µg/kg/week)	76 days after infection range 14-150 days), 27 days after onset of symptoms (range 5-131)	24 wks	63/89 (71%) 58/65 (89%) of adherent pts.

Table 1. Pivotal studies investigating early PEG-IFN therapy in patients with acute HCV infection.

Standard therapy for chronic hepatitis C

Goal of antiviral therapy

The importance of an effective treatment against hepatitis C can be seen simply by the numbers – globally, there are approximately 130 million people chronically infected with the virus. Despite the implementation of blood-donor screening in the early ‘90s, there is still an anticipated increase of HCV-related cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) over the next decade (Davis 2009). The goal of antiviral therapy is to cure the hepatitis C via a sustained elimination of the virus. Long-term benefits will be the reduction of HCV-related morbidity and mortality (Veldt 2007). A sustained elimination of HCV is achieved if the HCV RNA remains negative six months after the end of treatment (a sustained virological response, SVR). Follow-up studies document that more than 99% of patients who achieve an SVR remain HCV RNA negative 5 years after the end of treatment and no signs of hepatitis have been documented (Manns 2008; Swain 2007).

Several extrahepatic manifestations, such as cryoglobulinemia, non-Hodgkin lymphoma, membranoproliferative glomerulonephritis or porphyria cutanea tarda have been reported in the natural history of hepatitis C virus infection (HCV). Antiviral treatment may improve clinical symptoms even without achieving an SVR. On the other hand, antiviral therapy may worsen extrahepatic manifestations (Pischke 2008; Zignego 2007).

Basic therapeutic concepts and medication

Before the identification of HCV as the infectious agent for non-A, non-B hepatitis (Choo 1989) IFN led to a normalization of transaminases and an improvement of liver histology (Hoofnagle 1986). After the identification of HCV it became possible to measure success of therapy as a long-lasting disappearance of HCV RNA from serum, the SVR. Since then, SVR rates have increased from 5-20% with IFN monotherapy up to 40%-50% with the combination of IFN and ribavirin (Poynard 1998; McHutchinson 1998) (Figure 1). The development of pegylated interferon α (PEG-IFN) added a new milestone to the treatment of chronic hepatitis C. Two PEG-IFN are available; PEG-IFN α-2b (PEG-Intron™, Schering-Plough) and PEG-IFN a-2a (PEGASYS™, Hofmann La-Roche). Pegylation of the IFN allows once weekly administration due to an improved pharmacokinetic profile. PEG-IFN/ribavirin combination therapy improves the overall SVR to 54-63% (Manns 2001; Fried 2002; Hadziyannis 2004) (Figure 1, Table 2).

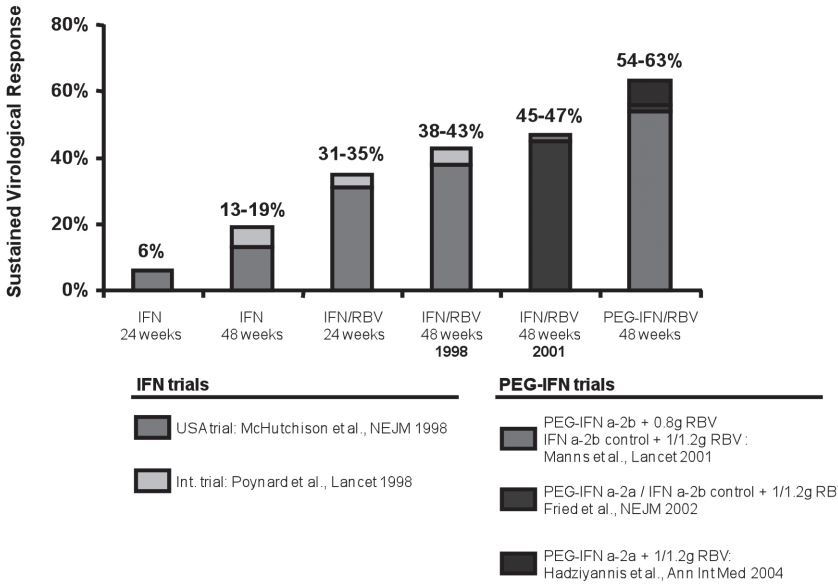


Figure 1. Development of chronic hepatitis C therapy. The sustained virologic response rates have been improved from around 5% with interferon monotherapy in the early 90s to >60% today with an optimized standard therapy of PEG-IFN plus ribavirin.

Although some smaller trials from Southern Europe suggested slightly higher SVR rates in patients treated with PEG-IFN α-2a (Rumi; Ascione), a large US multicenter study did not detect any significant difference between the two PEG-IFNs + ribavirin regarding SVR (McHutchinson 2007). The two PEG-IFNs do have different pharmacokinetic profiles due to their different polyethylene glycol moieties. PEG-IFN α-2b is bound to a single linear 12 kDa polyethylene glycol molecule, whereas PEG-IFN

α-2a is covalently attached to a 40 kDa branched chain polyethylene glycol moiety. The distinct sizes of the PEG-IFN influence the volume of distribution. PEG-IFN a-2b is given adjusted for body weight (1.5 µg/kg once weekly), while the larger PEG-IFN α-2a is given in a fixed dose of 180 µg once weekly (reviewed in Cornberg 2002; Pedder 2003) (Table 3). PEG-IFN α-2b may also be dosed at 1.0 µg/kg once patients became negative for HCV RNA without major declines in SVR rates (Mchutchinson 2009; Manns 2009).

Study	Treatment	HCV genotype	Duration	SVR
(Manns 2001)	1.5µg/kg PEG-IFN α-2b 800 mg ribavirin	HCV-1 HCV-2/3	48 weeks 48 weeks	42% 82%
	1.5µg/kg PEG-IFN α-2b >10.6 mg/kg ribavirin	HCV-1 HCV-2/3	48 weeks 48 weeks	48% (retrospective) 88% (retrospective)
(Fried 2002)	180µg PEG-IFN α-2a 1000/1200 mg ribavirin	HCV-1 HCV-2/3	48 weeks 48 weeks	46% 76%
(Hadziyannis 2004)	180µg PEG-IFN α-2a 800 mg ribavirin	HCV-1 HCV-2/3 	24 weeks 48 weeks 24 weeks 48 weeks	29% 40% 78% 73%
	180µg PEG-IFN α-2a 1000/1200 mg ribavirin	HCV-1 HCV-2/3 HCV-2/3 	24 weeks 48 weeks 24 weeks 48 weeks	41% 51% 78% 77%
(Zeuzem 2004)	1.5µg/kg PEG-IFN α-2b 800-1400 mg ribavirin	HCV-2 HCV-3	24 weeks	93% 79%
(Kamal 2005)	1.5µg/kg PEG-IFN α-2b 1000/1200 mg ribavirin	HCV-4	24 weeks 36 weeks 48 weeks	29% 66% 69%

Table 2. Efficacy of antiviral treatment with PEG-IFN plus ribavirin in patients with chronic hepatitis C. SVR depends on HCV genotype, dose and duration of treatment.

Ribavirin should be administered according to the bodyweight of the patient. A retrospective analysis of the large PEG-IFN α-2b/ribavirin pivotal trial revealed that the optimal ribavirin (Rebetol™, Schering-Plough) dose is at least 11 mg/kg (Manns 2001). A prospective, multicenter, open-label, investigator-initiated study confirmed that PEG-IFN α-2b plus weight-based ribavirin is more effective than flat-dose ribavirin, particularly in HCV genotype 1 patients (Jacobson 2007). A ribavirin dose of 15 mg/kg would be ideal, although higher doses are associated with higher rates of anemia (Snoeck 2006). When combined with PEG-IFN α-2a, a ribavirin (Copegus™, Hofmann La-Roche) dose of 1000 mg if <75 kg or 1200 mg if ≥75 kg is recommended for HCV genotype 1 patients while a flat dose of 800 mg ribavirin was suggested for patients with HCV genotypes 2 and 3 (Table 3) (Hadziyannis 2004). There was no additional benefit of higher ribavirin doses in HCV genotypes 2/3 patients. However, relapse rates may increase with increasing body weight of the patient (Jacobson 2007). Therefore, for HCV genotype 2/3 patients a weight-based dose of ribavirin (12-15

mg/kg) may be preferred, especially when reducing the treatment duration, i.e., to 16 weeks (Schiffman 2007). The 24-week schedule for HCV genotype 2/3 patients was confirmed whereas patients with HCV genotype 1 require 48 weeks of therapy (Table 3) (Hadziyannis 2004). The 24-week regimen for patients with HCV genotypes 2 and 3 has also been confirmed for the combination of PEG-IFN α-2b and ribavirin (Zeuzem 2004; Cornberg 2003) (Table 2).

Early HCV RNA kinetics predicts outcome and success of the treatment. Patients with HCV genotype 1 who do not show an HCV RNA decline of more than 2 $\log_{10}$ or who have serum concentrations of more than 30,000 IU/ml HCV RNA after 12 weeks of therapy, have no chance of achieving an SVR (Davis 2003; Berg 2003). Thus, therapy should be discontinued in these patients.

Drug	Dosing
Type I Interferons	
Pegylated Interferon α-2a (Pegasys®)	180 µg once weekly (QW)
Pegylated Interferon α-2b (PEG-Intron®)	1.5 µg/kg QW
Interferon α-2a (Roferon®)	3–4.5 mill IU three times weekly (TIW)
Interferon α-2b (Intron A®)	3 mill IU TIW
Consensus Interferon (Infergen®)	9 µg TIW
Ribavirin	
Ribavirin (Copegus®)	800–1200 mg daily
Ribavirin (Rebetol®)	600–1400 mg daily

Table 3. Approved drugs for the treatment of chronic hepatitis C (2010).

Individualization and optimization strategies

Adherence to therapy

Adherence to therapy is one of the most important factors associated with the success of therapy (McHutchinson 2002). The definition of adherence used here is the "80/80/80 rule", that is, patients who receive more than 80% of the IFN, more than 80% of the ribavirin, and are treated for more than 80% of the planned duration of treatment are considered adherent. One of the first studies investigating the effect of adherence demonstrated that patients who fulfilled the 80/80/80 rule had a 63% SVR compared to 52% of those with less than 80% adherence (McHutchinson 2002). This was statistically significant for HCV genotype 1 patients. Another study showed that a cumulative ribavirin dose of more than 60% is important to achieve an SVR (Reddy 2007). Therefore, it is important to reduce side effects and motivate the patients to adhere to treatment in order to optimize treatment responses especially in the difficult-to-treat genotype 1 patients.

Optimal treatment duration

Optimal treatment duration may also improve the management of chronic hepatitis C. While some patients with unfavorable baseline factors regarding SVR may need a longer treatment time to improve the response, patients with favorable baseline factors may be treated for a shorter period of time to reduce costs and side effects.

HCV genotypes 2 and 3

Many studies have investigated the reduction of treatment duration for HCV genotypes 2 and 3 to 16, 14, or even 12 weeks. Overall, reducing the treatment duration to less than 24 weeks increases the number of relapsers (Shiffman 2007; Mangia 2005; Dalgard 2008; Andriulli 2008). However, some HCV genotype 2/3 patients may indeed be treated for 12-16 weeks if certain prerequisites are fulfilled, especially the rapid virologic response (RVR) by wk 4 of therapy (HCV RNA negative). Only patients with RVR at week 4 had high SVR rates after 16 weeks (Poustchi 2008), 14 weeks (Dalgard 2008; Dalgard 2004), or even 12 weeks of therapy (Mangia 2005) (Table 4).

Study	Treatment	Subgroups	Therapy weeks	SVR
(Poustchi	180 µg PEG-IFN α-2a	>600 IU/ml TW4	24	36%
2008)	800-1200 mg ribavirin	<600 IU/ml TW4	24	80%, 84% if HCV RNA<800,000 IU/ml
N=153		<600 IU/ml TW4	16	82%, 93% if HCV RNA<800,000 IU/ml
(Shiffman	180 µg PEG-IFN α-2a	all patients	24	70%
2007)	800 mg ribavirin	all patients	16	62%
N=1469		<50IU/ml TW4 (RVR)	24	85%
		<50IU/ml TW4 (RVR)	16	79%
		<400,000IU/ml TW0 (LVL)	24	81%
		<400,000IU/ml TW0 (LVL)	16	82%
(Mangia	1.0 µg PEG-IFN α-2b	Standard group	24	76%
2005)	1000-1200 mg ribavirin	Standard group	24	91% if TW4 HCV RNA <50 IU/ml
		>50 IU/ml TW4 (no RVR)	24	64%
		<50 IU/ml TW4 (RVR)	12	85%
(Dalgrard	1.5 µg PEG-IFN α-2b	<50 IU/ml TW4 (RVR)	24	91% ITT, 93% with F24 HCV RNA test
2008)	800-1400 mg ribavirin	<50 IU/ml TW4 (RVR)	14	81% ITT, 86% with F24 HCV RNA test
		>50 IU/ml TW4 (no-RVR)	24	55% ITT, 59% with F24 HCV RNA test
(Manns	1.0 µg PEG-IFN α-2b	All patients	24 (1.5)	67% ITT, 82% as treated
2009)	1.5 µg PEG-IFN α-2b	All patients	24 (1.0)	64% ITT, 80% as treated
N=682	800-1400 mg ribavirin	All patients	16 (1.5)	57% ITT, 68% as treated

Table 4. Optimization of treatment duration in patients with HCV genotypes 2 and 3.

In addition to the RVR, the specific HCV genotype and the baseline viral load are associated with response. Patients with genotype 2 respond better to PEG-IFN + RBV therapy than those infected with genotype 3 (Zeuzem 2004) (Table 2). Furthermore, the shorter treatment schedules reveal that genotype 3 patients with low baseline viremia (<400-800.000 IU/ml) had a much better chance of responding than those with high viral load (>400-800.000 IU/ml) (Shiffman 2007; Poustchi 2008). Patients with

genotype 2 and patients with genotype 3 plus low viral load with an RVR by 4 weeks of therapy can be treated for less than 24 weeks. However, reducing treatment duration is not recommended in patients with advanced liver fibrosis or cirrhosis (Aghemo 2006), diabetes mellitus (Poustchi 2008b) or BMI >30 kg/m^2 (45). In contrast, HCV genotype 2/3 patients without an RVR (especially HCV genotype 3 and high viral load) may be treated for longer than 24 weeks (i.e., 48 weeks) (Figure 2B). However, so far only retrospective analyses exist to support this (Willems 2007). Prospective studies investigating treatment extension to 36 or 48 weeks are ongoing. Depending on the assay used to determine RVR, around 25-30% of HCV genotype 2/3 patients belong to this difficult-to-treat population not achieving RVR (Table 5).

Tailoring treatments individually for patients with HCV genotypes 2 and 3 will reduce costs and side effects and further optimize the response rates.

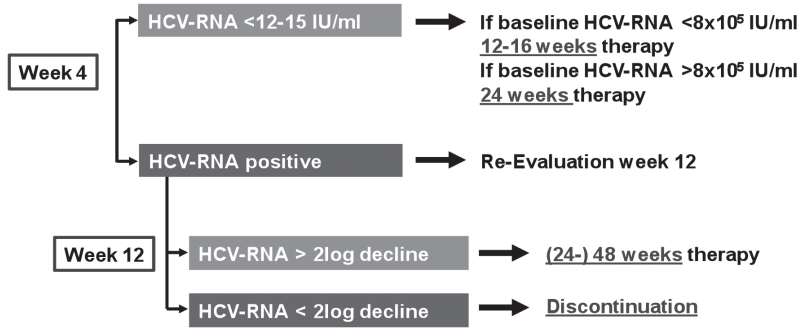


Figure 2B. Recommendation for a treatment algorithm for patients with HCV genotypes 2 and 3. Sensitive HCV RNA assays (limit of detection 12-15 IU/ml or 50 IU/ml) at weeks 4 and 12 may determine treatment duration. Reducing treatment duration is not recommended in patients with liver cirrhosis, insulin resistance or hepatic steatosis.

Study	Frequency of patients SVR without RVR	SVR without RVR (24 wks therapy)
(von Wagner 2005) 180µg PEG-IFN α-2a 800-1200 mg ribavirin	7% (HCV RNA >600 IU/ml TW4)	36%
(Shiffman 2007) 180µg PEG-IFN α-2a 800 mg ribavirin	36% (HCV RNA >50 IU/ml TW4) (24 wk group)	45%
(Mangia 2005) 1.0µg/kg PEG-IFN α-2b 1000-1200 mg ribavirin	36%-38% (HCV RNA >50 IU/ml TW4)	48%-64%
(Dalgard 2004) 1.5µg/kg PEG-IFN α-2b 800-1400 mg ribavirin	22% (HCV RNA >50 IU/ml TW4/TW8)	56%
(Dalgard 2008) 1.5µg/kg PEG-IFN α-2b 800-1400 mg ribavirin	29% (HCV RNA >50 IU/ml TW4)	55%

Table 5. SVR of patients with HCV genotypes 2 or 3 not achieving RVR.

HCV genotype 1

We face the opposite problem with genotype 1. Extending the treatment duration beyond 48 weeks is a strategy that may improve response rates in some patients. The rationale is to extend the time of HCV RNA negativity, especially in patients with a slow viral decline (no RVR, or first HCV RNA negative between weeks 12 and 24), to reduce the relapse rate in these so-called "slow responders". Several studies have investigated the efficacy and safety of 48 weeks versus 72 weeks of treatment with PEG-IFN + RBV in chronic hepatitis C (Berg 2006; Mangia 2008; Pearlman 2007; Sanchez Tapias 2006) (Table 6). Some studies report a benefit of extended therapy in patients who are HCV RNA positive at treatment week 4. The relapse rate after 72 weeks of therapy was significantly reduced (Sanchez Tapias 2006; Ferenci 2009). However, treatment duration beyond one year may lead to higher drop out rates (Table 6), resulting in lower ITT responses (Sanchez Tapias 2006). One study demonstrated that patients who achieve EVR (>2 log decline of HCV RNA at week 12) but are still HCV RNA positive at week 12 achieved significantly higher SVR rates when treated for 72 instead of 48 weeks (29% vs 17%, p=0.04). A particular benefit was seen in patients with low-level viremia (<6000 IU/ml) at week 12 (Berg 2006). One recent international trial with 1427 patients did not demonstrate a significant benefit for slow responding patients treated for 72 weeks. This study showed a trend toward declining relapse rates in the subgroup of slow responders with extended treatment duration and a delta of 13% SVR in patients who were 80/80/80 adherent (Buti 2009) (Table 6). Extension of therapy to 72 weeks is likely to improve response rates for patients with a slow viral response (>2$\log_{10}$ decline but >50 IU/ml at TW12) (Figure 2A) but high motivation and compliance of the patient is mandatory (the 80/80/80 rule).

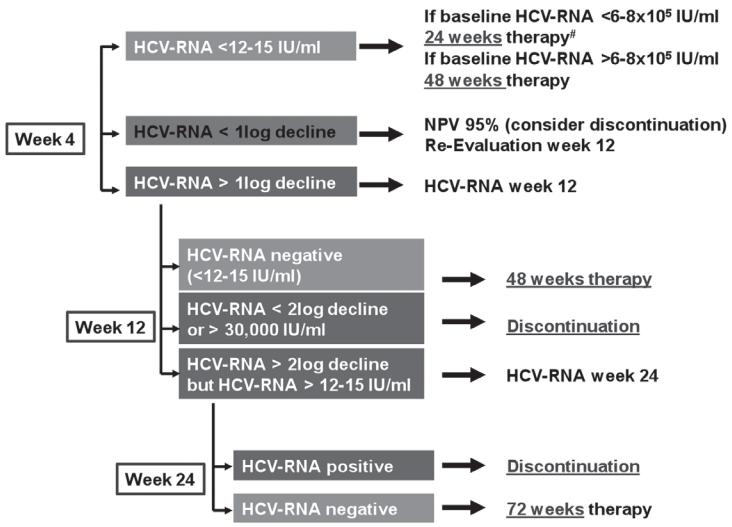


Figure 2A. Recommendation for a treatment algorithm for patients with HCV genotype 1. We also recommend this for genotypes 4-6 because of limited data in those patients. Sensitive assays (limit of detection 12-15 or 50 IU/ml) at wks 4,12, 24 may determine treatment duration. Reduction is not recommended in patients with liver cirrhosis, insulin resistance or hepatic steatosis.

On the other hand, it is possible to reduce the treatment duration to 24 weeks in patients with genotype 1 who have low viral load at baseline and a rapid virologic response (RVR) after 4 weeks of therapy (Ferenci 2008; Jensen 2006; Zeuzem 2006) (Table 6, Figure 2A).

Study	Treatment	Subgroups (Slow responders)	Therapy (weeks)	SVR, (relapse), discontinuation of therapy
(Sanchez Tapias 2006)	180µg PEG-IFN α-2a 800mg ribavirin	>50 IU/ml TW4 (no RVR)	48 72	28% (53% relapse), 18% discontinuation 44% (17% relapse), 36% discontinuation
(Berg 2006)	180µg PEG-IFN α-2a 800mg ribavirin	>50 IU/ml TW12	48 72	17%, 24% discontinuation 29%, 41% discontinuation
(Mangia 2008)	180 PEG-IFN α-2a or 1.5µg/kg PEG-IFN α-2b 1000-1200mg ribavirin	>600 IU/ml TW8 & <600 IU/ml TW12	48 72	38%, (43% relapse) 63%, (15% relapse)
(Pearlman 2007) (Ferenci	1.5µg/kg PEG-IFN α-2b 800-1400mg ribavirin 180µg PEG-IFN α-2a	≥2 log decline TW12 & >10 IU/ml TW12 >50 IU/ml TW4 (no RVR)	48 72 48	18%, (59% relapse), 14% discontinuation 38%, (20% relapse), 15% discontinuation 51%, (34% relapse)
2009)	1000-1200mg ribavirin	≥2 log decline TW12	72	59%, (19% relapse) (135µg PEG-IFN after week 48)
(Buti 2009)	1.5µg/kg PEG-IFN α-2b 800-1400mg ribavirin	≥2 log decline TW12 & detectable HCV RNA TW12	48 72	43% ITT, 44% (80/80/80 rule) 48% ITT, 57% (80/80/80 rule)

Study	Treatment	Subgroups (fast resp onder)	Therapy (wks)	SVR, (relapse), discontinuation of therapy
(Zeuzem 2006)	1.5µg/kg PEG-IFN α-2b 800-1400 mg ribavirin	<600,000 IU/ml TW0 & <29 IU/ml TW4 (RVR) <600,000 IU/ml TW0	24 24	50% 89%
(Jensen 2006)	180 PEG-IFN α-2a or 800mg or 1000-1200 mg ribavirin	<50 IU/ml TW 4 (RVR)	24	89%
(Ferenci 2008)	180 PEG-IFN α-2a or 1000-1200mg ribavirin	<50 IU/ml TW 4 (RVR)	24	74% ITT, 79% OT (N=120 HCV G1)

RVR= rapid virologic response

Table 6. Optimization of treatment duration in patients with HCV genotype 1.

The failure of other combination partners, i.e., amantadine

Many other compounds have been tested in combination with IFN, but so far only ribavirin has shown efficacy. One example of an initially promising drug that in the end failed was amantadine. In the year 2000, IFN/ribavirin and amantadine was shown to be promising in prior IFN non-responders (Brillanti 2000). While some studies confirmed the results, others demonstrated no additional benefit of amantadine in combination with IFN or IFN/ribavirin. A large German placebo-controlled multicenter study treated 400 naïve patients with IFN/ribavirin/placebo or with IFN/ribavirin/amantadine. Triple therapy increased SVR by 8% in HCV genotype 1 patients but this was not statistically significant (Berg 2003). A placebo-controlled study with

more than 700 patients coordinated by Hep-Net tested the addition of amantadine to PEG-IFN/ribavirin therapy in treatment naïve patients and gave an answer that triple therapy with amantadine offers no additional benefit (von Wagner 2008).

Other interferons

There are other type 1 interferons in development. Albinterferon α-2b (Alb-IFN) (Human Genome Sciences, Novartis), an 85.7 kilodalton protein consisting of interferon α-2b genetically fused to human serum albumin, further extends the half-life of IFN to approximately 148 hours. The pharmacokinetic profile of Alb-IFN allows dosing at intervals of 2-4 weeks compared to one week with either of the current PEG-IFN. Results of phase III trials (ACHIEVE 1 and ACHIEVE 2/3) testing multiple doses of Alb-IFN versus PEG-IFN α-2a demonstrated comparable antiviral efficacy of Alb-IFN (Zeuzem 2009; Nelson 2009). Approval of Alb-IFN is awaited sometime in 2011.

Study	Treatment	Therapy (weeks)	SVR, rates of serious or severe adverse events (AE), discontinuation
(Zeuzem 2009) N=1331 HCV G1	180µg PEG-IFN α-2a q1week 1-1.2g ribavirin daily	48	51% SVR 23.1% AE, 4.1% discontinued
	900µg alb-IFN q2weeks 1-1.2g ribavirin daily	48	48.2% SVR 24% AE, 10.4% discontinued
	1200µg alb-IFN q2weeks 1-1.2g ribavirin daily	48	47.3% SVR 28.2% AE, 10% discontinued
(Nelson 2009) N=933 HCV G2/3	180µg PEG-IFN α-2a q1week 0.8g ribavirin daily	24	84.4% SVR 3.6% discontinued
	900µg alb-IFN q2weeks 0.8g ribavirin daily	24	79.8% SVR 4.8% discontinued
	1200µg alb-IFN q2weeks 0.8g ribavirin daily	24	80% SVR 5.5% discontinued

Table 7: Results of the ACHIEVE 1 and ACHIEVE 2/3 studies.

Consensus interferon (CIFN) or interferon alphacon-1 (Infergen; Valeant) is another type 1 interferon already approved for the treatment of chronic hepatitis C (Table 4). The "consensus" molecule, composed of conserved amino acids of the type 1 interferons, shows a greater biological activity than other type 1 interferons *in vitro* (Blatt 1996; Ozes 1992). Despite this *in vitro* advantage, head-to-head studies comparing CIFN to standard IFN monotherapy reveal only minor differences in efficacy (Jong 1997). A recent study reported similar SVR in naïve patients with chronic hepatitis C when treated with CIFN in combination with ribavirin compared to pegylated IFN plus ribavirin (Sjogren 2007). Some studies investigating the effect of high and daily dosing of CIFN, in combination with ribavirin in naïve, as well as in nonresponder patients, demonstrate a promising SVR (Cornberg 2006). However, daily dosing requires high levels of motivation and compliance since adherence to therapy is an important factor influencing treatment outcome.

Other long-lasting IFNs that are currently under investigation include locteron (OctoPlus, Biolex Therapeutics) and omega-IFN with a subcutaneous delivery device (Intarcia Therapeutics) that lasts 12 weeks.

Side effects and complications

Severe side effects may reduce adherence to therapy and may result in dose modifications that result in a less-than-optimal response. Both IFN and ribavirin induce side effects that have to be managed with patients (Table 8). The IFN-related side effects can be divided into IFN-induced bone marrow suppression, flu-like symptoms, neuropsychiatric disorders, and autoimmune syndromes. The main problem of ribavirin is hemolytic anemia. Overall, side effects result in 10-20% premature withdrawals from therapy and an additional 20-30% of patients who require dose modifications. These numbers are lower in recent studies, suggesting an improved understanding and management of adverse events (Fried 2002) that may potentially lead to a better SVR. However, these percentages are seen in registration trials that use carefully selected patients. This may differ in general clinical practice, where patients with, e.g., history of depression, low platelets, or thyroid disease are being treated.

IFN side effects

The effect of IFN on bone marrow results in decreased granulocytes and thrombocytes during treatment. These are usually moderate if normal counts are present initially. However, dose modifications are necessary, especially in patients with initially low counts. This limits the use of IFN in patients with advanced liver cirrhosis who often have low platelets and are also more vulnerable to infections. Therapeutic concepts in order to raise platelet levels safely would have a significant effect on the effective management of patients, especially those with advanced liver disease. A promising novel agent is the oral thrombopoietin receptor agonist eltrombopag that has been tested in patients with chronic hepatitis C and liver cirrhosis (McHutchinson 2007). Eltrombopag was able to increase platelet levels in 75-95% of patients depending on the dose, and antiviral therapy was then initiated. Twelve weeks of antiviral therapy were then taken by 36-65% of patients receiving 30-75 mg of eltrombopag, vs only 6% of patients in the placebo group (McHutchinson 2007). Neutropenia is another of the most common reasons for dose modification. Granulocyte macrophage colony stimulating factor (GM-CSF, Filgrastim) could potentially be used to stabilize neutrophil counts during IFN therapy (Shiffman 1998; Van Thiel 1997; Younossi 2008). Cost-benefit analyses and further trials are required to recommend routine use of these agents. However, our own experience and other reports suggest that IFN-induced neutropenia is generally not associated with an increased risk for bacterial infections (Soza 2002).

Flu-like symptoms usually occur during the first weeks of treatment and severity declines over time. These symptoms include fever, chills, headache, arthralgia, and myalgia (Table 8). Antipyretic drugs such as paracetamol can help to prevent or reduce these side effects.

Neuropsychiatric side effects such as irritability, severe fatigue, and apathy are frequent (Table 8) and pose a great problem for many patients and their family members. Severe depression can occur and suicide has been reported (Janssen 1994). Psychiatric

care and the use of antidepressants, especially serotonin uptake inhibitors (SSRIs) may help reduce IFN-induced depression (Musselman 2001) and consequently improve adherence to hepatitis C therapy (Schaefer 2005). A double-blind placebo-controlled study in 100 patients with chronic hepatitis C was terminated prematurely due to a significant superiority of SSRIs over placebo in terms of decreasing scores on the Hospital Anxiety and Depression Scale (HADS). All SSRI treated patients were able to complete IFN treatment (Krauss 2008). SSRI treatment is highly effective in HCV patients during IFN-based therapies, when starting early after the onset of clinically relevant depression.

IFN has immunomodulatory properties, and treatment can induce autoimmune phenomena (Wesche 2001). The most frequent problem is the development of autoimmune thyroiditis. In most cases thyroiditis starts with hyperthyroidism that later turns into hypothyroidism. Autoimmune thyroiditis has been reported in up to 20% of patients on or after IFN-based therapies. This may not be reversible upon stopping therapy (Lisker-Melman 1992). Predisposed patients with pre-existing thyroid antibodies have a higher risk and it is possible that hepatitis C itself may be a cause of autoimmune thyroiditis (Marazuela 1996).

Other autoimmune diseases can also be aggravated by IFN therapy (e.g., diabetes or autoimmune hepatitis). Patients with documented hepatitis C infection may get worse during IFN treatment if an underlying autoimmune hepatitis is present. This has been observed particularly in LKM antibody-positive individuals. These patients require careful monitoring if IFN is considered as first-line treatment. However, IFN therapy seems to be safe in most HCV/anti-LKM-1-positive patients (Dalekos 1999; Todros 1995).

Ribavirin side effects

The main side effect of ribavirin is hemolytic anemia that frequently results in ribavirin dose reduction or even discontinuation, which may significantly affect the overall SVR, especially in patients with HCV genotype 1 (Reddy 2007).

Treatment with erythropoetin can effectively reverse ribavirin-associated anaemia and allow full adherence to ribavirin therapy (Afzahl 2004). Although the use of erythropoetin can reduce the incidence and severity of ribavirin induced anaemia, it remains to be seen whether this will affect SVR. A prospective, randomized, controlled trial has evaluated the effect of erythropoetin on SVR. Patients receiving PEG-IFN α-2b plus 13.3 mg/kg/day ribavirin were compared to patients receiving PEG-IFN α-2b, 13.3 mg/kg/day ribavirin and 40,000 U/week erythropoetin. Although there were significantly fewer ribavirin dose reductions in those patients who received erythropoetin, no improvement in SVR was shown. A third group received a higher starting dose of 15.2 mg/kg/day in combination with erythropoetin and they did show a significantly higher SVR. There was no control group in this trial (Shiffman 2007b).

Overall, erythropoetin may improve quality of life, and in some individuals it may also improve the chance of achieving an SVR (Younossi 2008; Falasca), but the treatment is expensive and off-label in many countries. This emphasizes the need for alternative ribavirin-like drugs with less toxicity and/or higher antiviral efficacy. Unfortunately, the mechanism by which ribavirin enhances the efficacy of IFN treatment and prevents relapse remains largely unknown. Proposed mechanisms are immunmodulatory effects, inhibition of the inosine monophosphate dehydrogenase (IMPDH)

activity and the induction of RNA mutagenesis (Lau 2002; Perelson 2005). More potent IMPDH inhibitors such as mycophenolate mofetil (MMF, Cellcept), VX-497 or merimepodip have been studied, but with limited effects (Rustgi 2009; Sintchak 2000; Cornberg 2002). Another approach is the development of a ribavirin pro-drug. Viramidine is the amidine version of ribavirin and is converted by the enzyme adenosine deaminase into ribavirin mainly in hepatocytes. Therefore there is less uptake of ribavirin into red blood cells after the administration of viramidine and consequently less hemolytic anaemia (Watson 2002). First results of a phase II study demonstrated that viramidine in combination with PEG-IFN α-2a led to significantly less anemia compared to ribavirin plus PEG-IFN (Gish 2007). However, phase III studies with both PEG-IFN in combination with fixed doses of viramidine (VISER-1, VISER-2) were inferior to the combination with ribavirin (Benhamou 2009; Marcellin 2009).

Drug monitoring of ribavirin could be an option to optimize the ribavirin dose without losing efficacy (Svensson 2000). The pharmacokinetics of ribavirin suggests that not only body weight but also renal function (glomerular filtration rate) should be considered when selecting the ribavirin dose (Bruchfeld 2002).

Side effects	Incidence with PEG-IFN α and ribavirin (Reddy 2007; Andriulli 2008; Zeuzem 2009)
Headache	47-62%
Pyrexia	40-46%
Myalgia	37-56%
Rigor	24-48%
Arthralgia24-34%	
Nausea	35-43%
Loss of appetite	21%
Weight loss	29%
Diarrhea	22%
Alopecia	21-36%
Rash/Dermatitis	20-24%
Injection site inflammation	25%
Pruritus	25-29%
Dyspnea	26%
Fatigue	48-64%
Insomnia	33-40%
Irritability	24-35%
Depression	22-31%

Table 8. Common side effects (>20% of patients) recorded in the major PEG-IFN/ribavirin trials. The incidences of side effects between different studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds. There were methodological differences in assessing side effects as well. Patients were selected on the basis of well-defined inclusion and exclusion criteria. Normal TSH levels pretreatment were a prerequisite.

Treatment of hepatitis C in special populations

Patients with normal aminotransferase levels

Approximately 30% of patients with chronic hepatitis C maintain persistently normal alanine aminotransferase (ALT) levels despite having detectable HCV RNA in serum. A treatment indication for these patients is unclear. First, these patients have generally mild liver disease and show a slow progression to cirrhosis. Second, treatment with IFN has been shown to be associated with ALT flares (reviewed in Tassopoulos 1999). Third, the efficacy of therapy may be lower, since patients with elevated transaminases seem to respond better (Zeuzem 2004). However, up to one third of patients with normal ALT can present with significant liver fibrosis necessitating an effective treatment (Bacon 2002; Zeuzem 2004b). 48 weeks of PEG-IFN α-2a plus ribavirin has been shown to lead to SVR rates of 52% in patients with chronic hepatitis C and persistently normal ALT levels. Treatment-related flares in ALT activity were not observed (Zeuem 2004b). The efficacy and tolerability of PEG-IFN/ribavirin combination therapy in patients with persistently normal ALT levels seems to be comparable to that seen in patients with elevated ALT levels. The decision to treat or not to treat patients with chronic hepatitis C and persistently normal ALT levels should be made on an individual basis independent of the ALT levels.

HCV and liver transplantation

HCV re-infection occurs in almost all patients after liver transplantation. While the course of hepatitis C in liver transplant recipients was believed to be rather benign in the late ‘80s and early ‘90s (Boker 1997), HCV has led to a more rapid progression post-transplant in recent years (Berenguer 2005) with cirrhosis within the first 5-10 years in 20-30% of patients. HCV definitely takes a more rapid course post-transplant than in immunocompetent individuals and treatment needs are obvious.

Antiviral therapy of HCV may be started before transplant to prevent re-infection of the graft. If this approach is successful, re-infection can be prevented in two-thirds of patients (Forns 2003). However, treatment with IFN + ribavirin is poorly tolerated in decompensated cirrhosis and this approach is feasible in only a minority of patients (Everson 2004). Preemptive treatment within the first 4-6 weeks post transplant has been disappointing with SVR between 0% and 33% for different regimens including IFN monotherapy and IFN + ribavirin (Chalansi 2005; Terrault 2004). There is more experience on the treatment of established recurrent hepatitis C. The most recent studies using PEG-IFN in combination with ribavirin led to an initial virological response rate of up to 55% (Dumortier 2004). Treatment duration should be at least similar to non-transplanted patients considering early viral kinetics and the HCV genotype. However, bone marrow toxicity, depression, and rejection are limiting factors that require aggressive management (e.g., growth factors) (Neff 2004; Rodriguez Luna 2004). The ribavirin dose may have to be adjusted since many patients have some degree of renal insufficiency. Interestingly, the risk for IFN-induced graft rejection seems to be higher if ribavirin is not used.

Overall, several issues in the sometimes rather complicated management of post-transplant hepatitis C are not yet resolved. Patients with established graft hepatitis should be treated with PEG-IFN and ribavirin. Whether re-infection can be prevented

by the new oral antivirals inhibiting HCV replication or by a combination of anti-HCV antibodies with neutralizing properties will have to be addressed in future studies.

Dialysis patients

Treatment needs for dialysis patients with hepatitis C are obvious especially if patients are considered for kidney transplantation. The outcome of HCV post-kidney transplantation is worse than for HCV negative patients after renal transplantation. However, IFN-based therapies are contraindicated post-transplantation since they may induce rejection. Thus, if possible, HCV should be eliminated before transplantation. There have been several smaller reports on the treatment of HCV with IFN monotherapy in patients with end-stage renal disease (Fabrizi 2002). Surprisingly, the results for IFN monotherapy on dialysis were better than in patients not undergoing dialysis, with SVR results of 21-64%. Data on combination with ribavirin are limited since ribavirin has been contraindicated in this setting. However, ribavirin can be given at lower doses in dialysis patients, usually between 200-400 mg daily (Bruchfeld 2001). Several trials on the use of PEG-IFN plus ribavirin in dialysis patients are ongoing. However, it has to be considered that there may be significant differences between the two pegylated interferons in the setting of dialysis since PEG-IFN α-2a is eliminated mainly by the liver while PEG-IFN α-2b is cleared via the kidney (Cornberg 2002). Thus, only PEG-IFN α-2a has been approved in this setting. Future studies need to evaluate the potential of viramidine for this special patient population.

Treatment for the future and the drug pipeline

The common desire for the future is to develop a treatment beyond IFN with less side effects and higher efficacy. Knowledge of the molecular structure of the hepatitis C proteins has allowed the design of new drugs that directly target the sites of HCV-encoded enzymes that are important for the replication of the virus. This treatment concept is defined as specifically targeted antiviral therapy for HCV (STAT-C). The HCV protease and the HCV polymerase are currently the main targets for STAT-C (see Chapter 14). Phase III trials investigating the protease inhibitors telaprevir and boceprevir are ongoing. Approval of the first STAT-C drugs is expected for 2011/2012. However, initial studies have shown that PEG-IFN and ribavirin will still be the backbone of the standard therapy for chronic hepatitis C at least for the next five years.

Another approach to the treatment of HCV infection is the induction of HCV-specific immune responses. Spontaneous recovery after acute HCV infection is associated with a strong and broad immune response, while the development of chronic hepatitis C is associated with an impaired immune response (Wedemeyer 2002; Thimme 2001). The aim of a therapeutic vaccination is to stimulate the hepatitis C-specific immune responses to control viral replication. The first therapeutic vaccines have been tested in phase I/II studies (Nevens 2003; Wedemeyer 2009). However, there is still a long way to go for an immune-mediated treatment.

Other advances include the development of small molecules such as ribozymes, antisense oligonucleotides, and small interfering RNAs (siRNAs) that have been designed to control viral gene expression. There are many new approaches to fighting hepatitis C and its complications (Chapter 14).

Treatment of patients with prior antiviral treatment failure

As more patients are treated, the size of the population of patients who fail to achieve SVR has expanded. Many non-responder patients have advanced liver disease and successful treatment may extend life expectancy (Veldt 2007). STAT-C drugs are still some years from approval and recent data indicate that STAT-C drugs plus PEG-IFN and ribavirin may not be as effective to achieve SVR in many non-responders. Thus, re-treatment of patients with previous treatment failure is one of the most important topics in the treatment of chronic hepatitis C.

Definition of treatment failure

Definition of response or treatment failure to antiviral therapy is very important when considering re-treating patients with chronic hepatitis C. Patients may have been treated with different treatment regimens and the compliance during the previous therapy was probably very varied. Most importantly, HCV RNA kinetics and the response profile during the previous therapy have to be taken into account before starting a new treatment. It is crucial to screen the patient's records and check treatment duration, drug dosing and HCV RNA of the previous therapy. Non-response is the failure of a patient to clear HCV RNA at any point during treatment. Definitions used for trials assessing novel therapy approaches, i.e., STAT-C (Chapter 14) have generally defined non-response as the failure to achieve a ≥2 log10 reduction in HCV RNA after 12 weeks (EVR). Classifications of non-response include null-response, which is used as a <2 log10 decline in HCV RNA at any time. A partial virologic response is defined as a ≥2 log10 decline in HCV RNA during therapy without clearing HCV RNA after 24 weeks of therapy. Relapse is HCV RNA negativity at the end of therapy with recurrence of viremia after discontinuation of treatment. Breakthrough reflects the attainment of HCV RNA negativity during treatment with the recurrence of viremia while treatment is ongoing (Figure 3).

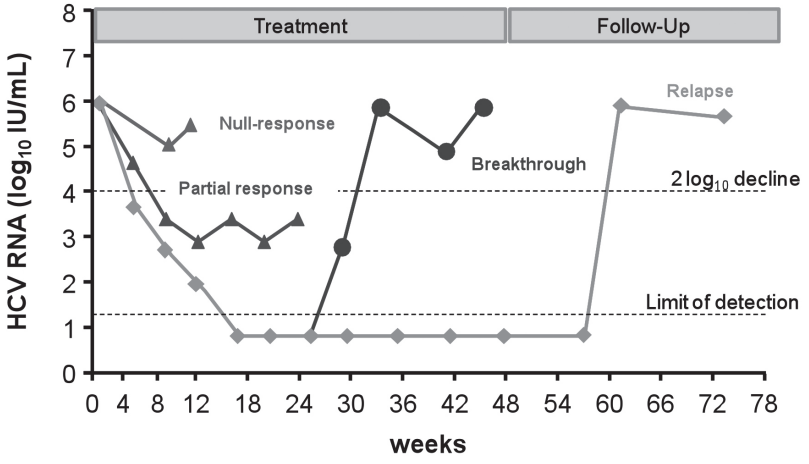


Figure 3: Different scenarios of treatment failure to antiviral therapy in chronic hepatitis C.

Re-treatment of patients with relapse after standard therapy

Re-treatment with PEG-IFN/ribavirin of relapse patients after IFN-based or PEG-IFN-based combination therapy with ribavirin resulted in SVR of 32-50% (Table 9). Patients with HCV genotype 1 and higher fibrosis scores have lower chances of achieving an SVR (Poynard 2009; Jacobson 2005). After relapse, patients should be treated at least 48 weeks, independent of the genotype. Studies have shown that patients who do not achieve HCV RNA negativity at week 12 have only a 5% chance of achieving an SVR. Thus, a more stringent stopping rule should be considered when re-treating patients after relapse. Other reasons for relapse, i.e., slow response during previous treatment without receiving 72 weeks treatment or low adherence to treatment should be evaluated before and improved during the therapy.

Study	Patient population	Treatment regimen	SVR
EPIC[3] (Poynard 2009)	Relapse after PEG-IFN α-2a/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800-1400mg ribavirin	34%
EPIC[3] (Poynard 2009)	Relapse after PEG-IFN α-2b /ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800-1400mg ribavirin	32%
EPIC[3] (Poynard 2009)	Relapse after IFN/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800-1400mg ribavirin	43%
(Jacobson 2005)	Relapse after IFN/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800 mg ribavirin	50%
(Jacobson 2005)	Relapse after IFN/ribavirin	48 weeks 1.0 µg/kg PEG-IFN α-2b + 1000-1200 mg ribavirin	32%

Table 9: SVR of IFN/ribavirin or PEG-IFN/ribavirin relapse patients.

Re-treatment of non-responders to the current standard therapy

Patients who are non-responders to standard PEG-IFN/ribavirin combination therapy have demonstrated SVRs ranging between 2-12% with a standard PEG-IFN/ribavirin re-treatment (Poynard 2009; Jacobson 2005; Shiffman 2004; Schiff 2008; Marcellin 2008). Thus, indication for re-treatment is limited. Retreatment is justified if adherence was a major problem during the previous treatment regimen. Previous HCV RNA reduction predicts the likelihood of SVR. Patients with previous partial response may benefit from re-treatment with optimized treatment regimen, i.e., extended treatment duration.

If a patient is a previous non-responder to IFN-based or PEG-IFN-based combination therapy and they have detectable HCV RNA at Week 12, treatment should be discontinued. The EPIC3 trial demonstrated that any HCV RNA level >750 IU/mL at week 12 was associated with a 0% chance of subsequent SVR (Poynard 2009). On the other hand, if a previous non-responder has undetectable HCV RNA by Week 12, treatment can be continued with a significant chance of SVR. Based on the results of the REPEAT study, treatment duration of 72 weeks should be considered. The SVR rate among patients who received 72 weeks of therapy was double that of patients who received 48 weeks of therapy (Table 10). A multivariate analysis of critical predictors of response identified a treatment duration of 72 weeks vs 48 weeks as the best predictor of response in this trial. Induction therapy did not result in a significant difference (Marcelin 2008) (Table 10), confirming previous data (Cornberg 2006).

Study	Patient population	Treatment regimen	SVR
Boceprevir Nonresponder control arm (Schiff 2008)	Nonresponder (null-responder) to PEG-IFN/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800-1400mg ribavirin	2%
EPIC[3] (Poynard 2009)	Nonresponder to PEG-IFN α-2a/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800-1400mg ribavirin	6%
EPIC[3] (Poynard 2009)	Nonresponder to PEG-IFN α-2b/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800-1400mg ribavirin	7%
REPEAT (Marcellin 2008)	Nonresponder to PEG-IFN α-2b/ribavirin	48 weeks 180µg PEG-IFN α-2a + 1000/1200 mg ribavirin	9%
REPEAT (Marcellin 2008)	Nonresponder to PEG-IFN α-2b/ribavirin	72 weeks 180µg PEG-IFN α-2a + 1000/1200 mg ribavirin	14%
REPEAT (Marcellin 2008)	Nonresponder to PEG-IFN α-2b/ribavirin	48 weeks (Induction) 360/180µg PEG-IFN α-2a + 1000/1200 mg ribavirin	7%
REPEAT (Marcellin 2008)	Nonresponder to PEG-IFN α-2b/ribavirin	72 weeks (Induction) 360/180µg PEG-IFN α-2a + 1000/1200 mg ribavirin	16%
(Jacobson 2005)	Nonresponder to IFN/ribavirin	48 weeks 1.5 µg/kg PEG-IFN α-2b + 800 mg ribavirin	10%
(Jacobson 2005)	Nonresponder to IFN/ribavirin	48 weeks 1.0 µg/kg PEG-IFN α-2b + 1000-1200 mg ribavirin	6%
HALT-C (Shiffman 2004)	Nonresponder to IFN/ribavirin	48 weeks 180µg PEG-IFN α-2a + 1000-1200 mg ribavirin	12%

Table 10. SVR of PEG-IFN/ribavirin nonresponders.

PEG-IFN maintenance therapy

There has been much interest concerning the use of low-dose PEG-IFN maintenance therapy since data has suggested that IFN may halt the progression of liver disease (Nishiguchi 1995). There are two major trials that have analyzed if maintenance treatment with IFN may alter the natural course of chronic hepatitis C. The COlchicine vs. Peg-Interferon α-2b LOng-Term (COPILOT) study compared low-dose 0.5 µg/kg PEG-IFN α-2b to colchicine in nonresponders to previous standard IFN-based or PEG-IFN-based combination therapy with ribavirin who had an Ishak fibrosis stage >3. In a comparison of event-free survival between the PEG-IFN group and the colchicine group, there was no significant difference. However, the intent-to-treat analysis revealed that among patients with portal hypertension (varices or portal hypertensive gastropathy), there was a trend toward superiority for PEG-IFN versus colchicine treatment (Afdahl 2008). The authors conclude that maintenance therapy may have a role in patients with portal hypertension. Analyses of other issues such as the extent of beta-blocker use are still awaited and there are other questions that must be answered before any definitive conclusions can be drawn.

The other study, the HALT-C trial, was a long-term maintenance study supported by the National Institutes of Health, which evaluated a large cohort of chronic HCV-infected patients who failed previous IFN-based therapy and had METAVIR stage F2-F4. Patients received 90 µg PEG-IFN α-2a maintenance treatment if they did not respond during the first 20 weeks with standard therapy. Despite the fact that there were greater reductions in viremia, decrease in alanine aminotransferase, and necroinflammation in the patients who received PEG-IFN, none of the important clinical outcomes (rates of death, decompensation, hepatocellular carcinoma, and increase in fibrosis) were favorably affected by PEG-IFN therapy (Di Bisceglie 2008). In conclusion, long-term treatment with low-dose PEG-IFN cannot be recommended for nonresponder patients.

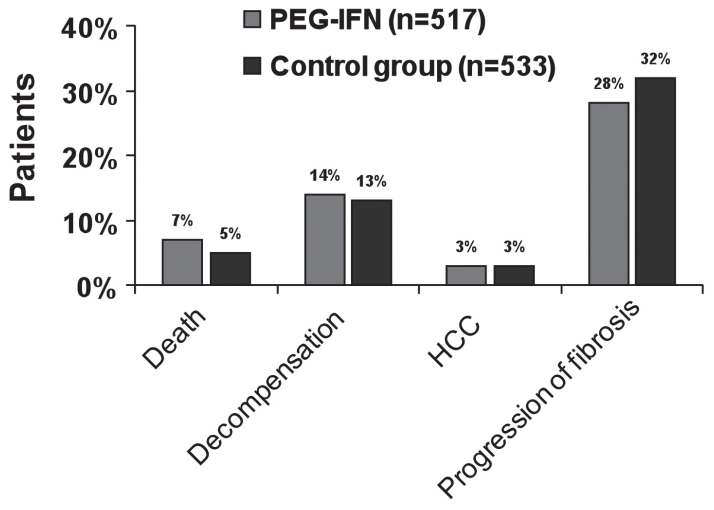


Figure 4: Results of the HALT-C study. Efficacy of a low-dose (90µg/week) PEG-IFN α-2a long-term (3.5 years) treatment in nonresponders (Di Bisceglie 2008).

References

Afdhal NH, Levine R, Brown R, Freilich B, O'Brien M, Brass C. Colchicine versus PEG-interferon alfa 2b long term therapy: Results of the 4 year copilot trial. Journal of Hepatology 2008; 48:S4-S4.

Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. Gastroenterology 2004;126:1302-1311.

Aghemo A, Rumi MG, Soffredini R, D'Ambrosio R, Ronchi G, Del Ninno E, et al. Impaired response to interferon-alpha2b plus ribavirin in cirrhotic patients with genotype 3a hepatitis C virus infection. Antivir Ther 2006; 11:797-802.

Andriulli A, Mangia A, Iacobellis A, Ippolito A, Leandro G, Zeuzem S. Meta-analysis: the outcome of anti-viral therapy in HCV genotype 2 and genotype 3 infected patients with chronic hepatitis. Aliment Pharmacol Ther 2008; 28:397-404.

Ascione A, De Luca M, Tartaglione MT, Lampasi F, Di Costanzo GG, Lanza AG, et al. Peginterferon alfa-2a plus ribavirin is more effective than peginterferon alfa-2b plus ribavirin for treating chronic hepatitis C virus infection. Gastroenterology 138:116-122.

Bacon BR. Treatment of patients with hepatitis C and normal serum aminotransferase levels. Hepatology 2002; 36:S179-184.

Benhamou Y, Afdhal NH, Nelson DR, Shiffman ML, Halliman DG, Heise J, et al. A phase III study of the safety and efficacy of viramidine versus ribavirin in treatment-naive patients with chronic hepatitis C: ViSER1 results. Hepatology 2009; 50:717-726.

Berenguer M. What determines the natural history of recurrent hepatitis C after liver transplantation? J Hepatol 2005; 42:448-456.

Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, et al. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. Hepatology 2003; 37:600-609.

Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, et al. Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. Gastroenterology 2006; 130:1086-1097.

Berg T, Kronenberger B, Hinrichsen H, Gerlach T, Buggisch P, Herrmann E, et al. Triple therapy with amantadine in treatment-naive patients with chronic hepatitis C: a placebo-controlled trial. Hepatology 2003; 37:1359-1367.

Blatt LM, Davis JM, Klein SB, Taylor MW. The biologic activity and molecular characterization of a novel synthetic interferon-alpha species, consensus interferon. J Interferon Cytokine Res 1996; 16:489-499.

Boker KH, Dalley G, Bahr MJ, Maschek H, Tillmann HL, Trautwein C, et al. Long-term outcome of hepatitis C virus infection after liver transplantation. Hepatology 1997; 25:203-210.

Brillanti S, Levantesi F, Masi L, Foli M, Bolondi L. Triple antiviral therapy as a new option for patients with interferon nonresponsive chronic hepatitis C. Hepatology 2000; 32:630-634.

Broers B, Helbling B, Francois A, Schmid P, Chuard C, Hadengue A, Negro F. Barriers to interferon-alpha therapy are higher in intravenous drug users than in other patients with acute hepatitis C. J Hepatol 2005; 42:323-328.

Bruchfeld A, Stahle L, Andersson J, Schvarcz R. Ribavirin treatment in dialysis patients with chronic hepatitis C virus infection--a pilot study. J Viral Hepat 2001; 8:287-292.

Bruchfeld A, Lindahl K, Schvarcz R, Stahle L. Dosage of ribavirin in patients with hepatitis C should be based on renal function: a population pharmacokinetic analysis. Ther Drug Monit 2002; 24:701-708.

Buti M, Lurie Y, Zakharova NG, Blokhina NP, Horban A, Sarrazin C, et al. Extended Treatment Duration in Chronic Hepatitis C Genotype 1-Infected Slow Responders: Final Results of the Success Study. Journal of Hepatology 2009; 50:S58-S58.

Chalasani N, Manzarbeitia C, Ferenci P, Vogel W, Fontana RJ, Voigt M, et al. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. Hepatology 2005; 41:289-298.

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244:359-362.

Cornberg M, Wedemeyer H, Manns MP. Treatment of chronic hepatitis C with PEGylated interferon and ribavirin. Curr Gastroenterol Rep 2002; 4:23-30.

Cornberg M, Huppe D, Wiegand J, Felten G, Wedemeyer H, Manns MP. Treatment of chronic hepatitis C with PEG-interferon alpha-2b and ribavirin: 24 weeks of therapy are sufficient for HCV genotype 2 and 3. Z Gastroenterol 2003; 41:517-522.

Cornberg M, Hadem J, Herrmann E, Schuppert F, Schmidt HH, Reiser M, et al. Treatment with daily consensus interferon (CIFN) plus ribavirin in non-responder patients with chronic hepatitis C: a randomized open-label pilot study. J Hepatol 2006; 44:291-301.

Cornberg M, Hinrichsen H, Teuber G, Berg T, Naumann U, Falkenberg C, et al. Mycophenolate mofetil in combination with recombinant interferon alfa-2a in interferon-nonresponder patients with chronic hepatitis C. J Hepatol 2002; 37:843-847.

Dalgard O, Bjoro K, Ring-Larsen H, Bjornsson E, Holberg-Petersen M, Skovlund E, et al. Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. Hepatology 2008; 47:35-42.

Dalgard O, Bjoro K, Hellum KB, Myrvang B, Ritland S, Skaug K, et al. Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 2004; 40:1260-1265.

Dalekos GN, Wedemeyer H, Obermayer-Straub P, Kayser A, Barut A, Frank H, Manns MP. Epitope mapping of cytochrome P4502D6 autoantigen in patients with chronic hepatitis C during alpha-interferon treatment. J Hepatol 1999; 30:366-375.

Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of the Hepatitis C Virus (HCV)-Infected Persons in the United States: A Multiple Cohort Model of HCV Prevalence and Disease Progression. Gastroenterology 2009.

Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003; 38:645-652.

Deterding K, Gruener N, Wiegand J, Buggisch P, Galle P, Spengler U, et al. Early Versus Delayed Treatment of Acute Hepatitis C: The German Hep-Net Acute Hcv-Iii Study - a Randomized Controlled Trial. Journal of Hepatology 2009; 50:S380-S380.

Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med 2008; 359:2429-2441.

Dumortier J, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. J Hepatol 2004; 40:669-674.

Everson GT. Treatment of chronic hepatitis C in patients with decompensated cirrhosis. Rev Gastroenterol Disord 2004; 4 Suppl 1:S31-38.

Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. Hepatology 2002; 36:3-10.

Falasca K, Ucciferri C, Mancino P, Gorgoretti V, Pizzigallo E, Vecchiet J. Use of epoetin beta during combination therapy of infection with hepatitis c virus with ribavirin improves a sustained viral response. J Med Virol 82:49-56.

Ferenci P, Laferl H, Scherzer TM, Maieron A, Hofer H, Stauber R, et al. Peginterferon Alfa-2a/Ribavirin for 48 or 72 Weeks in Hepatitis C Types 1 and 4 Patients With Slow Virologic Response. Gastroenterology 2009.

Ferenci P, Laferl H, Scherzer TM, Gschwantler M, Maieron A, Brunner H, et al. PEG-IFN α-2a / RBV for 24 wks in HCV type 1 and 4 patients with rapid virological response. Gastroenterology 2008; 135:451-458.

Forns X, Garcia-Retortillo M, Serrano T, Feliu A, Suarez F, de la Mata M, et al. Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. J Hepatol 2003; 39:389-396.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347:975-982.

Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2002; 36:S237-244.

Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 2003; 125:80-88.

Gish RG, Arora S, Rajender Reddy K, Nelson DR, O'Brien C, Xu Y, Murphy B. Virological response and safety outcomes in therapy-nai ve patients treated for chronic hepatitis C with taribavirin or ribavirin in combination with pegylated interferon alfa-2a: a randomized, phase 2 study. J Hepatol 2007; 47:51-59.

Hadziyannis SJ, Sette Jr H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004; 140:346-355.

Hofer H, Watkins-Riedel T, Janata O, Penner E, Holzmann H, Steindl-Munda P, et al. Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. Hepatology 2003; 37:60-64.

Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, et al. Treatment of chronic non-A,non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986; 315:1575-1578.

Jacobson IM, Brown Jr RS, Freilich B, Afdhal N, Kwo PY, Santoro J, et al. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. Hepatology 2007; 46:971-981.

Jacobson IM, Gonzalez SA, Ahmed F, Lebovics E, Min AD, Bodenheimer Jr HC, et al. A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. Am J Gastroenterol 2005; 100:2453-2462.

Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, et al. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001; 345:1452-1457.

Janssen HL, Brouwer JT, van der Mast RC, Schalm SW. Suicide associated with alfa-interferon therapy for chronic viral hepatitis. J Hepatol 1994; 21:241-243.

Jensen DM, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. Hepatology 2006; 43:954-960.

Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA, et al. Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral. 2005.

Kraus MR, Schafer A, Schottker K, Keicher C, Weissbrich B, Hofbauer I, Scheurlen M. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. Gut 2008; 57:531-536.s

Lau JY, Tam RC, Liang TJ, Hong Z. Mechanism of action of ribavirin in the combination treatment of chronic HCV infection. Hepatology 2002; 35:1002-1009.

Lisker-Melman M, Di Bisceglie AM, Usala SJ, Weintraub B, Murray LM, Hoofnagle JH. Development of thyroid disease during therapy of chronic viral hepatitis with interferon alfa. Gastroenterology 1992; 102:2155-2160.

Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. 2005.

Mangia A, Minerva N, Bacca D, Cozzolongo R, Agostinacchio E, Sogari F, et al. Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. Hepatology 2009; 49:358-363.

Mangia A, Minerva N, Bacca D, Cozzolongo R, Ricci GL, Carretta V, et al. Individualized treatment duration for hepatitis C genotype 1 patients: A randomized controlled trial. Hepatology 2008; 47:43-50.

Manns M, Lindsay KL, Gordon SC, Pockros PJ, Haeussinger D, Hadziyiannis S, et al. Sustained virologic response after peginterferon alfa-2b and ribavirin treatment predicts long-term clearance of HCV at 5-year follow-up. Journal of Hepatology 2008; 48:S300-S300.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358:958-965.

Manns M, Zeuzem S, Sood A, Lurie Y, Cornberg M, Klinker H, et al. Reduced Dose and Duration of Peginterferon Alfa-2b+Weight-Based Ribavirin in European and Asian Genotype 2 and 3 Chronic Hepatitis C Patients (Redd 2/3 Trial). Journal of Hepatology 2009; 50:S59-S59.

Marazuela M, Garcia-Buey L, Gonzalez-Fernandez B, Garcia-Monzon C, Arranz A, Borque MJ, Moreno-Otero R. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-alpha therapy. Clin Endocrinol (Oxf) 1996; 44:635-642.

Marcellin P, Gish RG, Gitlin N, Heise J, Halliman DG, Chun E, Rodriguez-Torres M. Safety and efficacy of viramidine versus ribavirin in ViSER2: Randomized, double-blind study in therapy-naive hepatitis C patients. J Hepatol 2009.

Marcellin P, Craxi A, Brandao-Mello CE, Di Bisceglie AM, Andreone P, Freilich B, et al. A 72-Week Treatment Duration with Peginterferon Alfa-2a (40kd) (Pegasys®) Plus Ribavirin (Copegus®) Has a Favorable Risk:Benefit Ratio in Non-Responders to Pegylated Interferon Alfa-2b (12kd) Plus Ribavirin: Findings of the Multinational Repeat Study. Hepatology 2008; 48:1147A-1147A.

McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998; 339:1485-1492.

McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009; 361:580-593.

McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002; 123:1061-1069.

McHutchison JG, Dusheiko G, Shiffman ML, Rodriguez-Torres M, Sigal S, Bourliere M, et al. Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. N Engl J Med 2007; 357:2227-2236.

Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001; 344:961-966.

Neff GW, Montalbano M, O'Brien CB, Nishida S, Safdar K, Bejarano PA, et al. Treatment of established recurrent hepatitis C in liver-transplant recipients with pegylated interferon-alfa-2b and ribavirin therapy. Transplantation 2004; 78:1303-1307.

Nelson D, Benhamou Y, Chuang WL, Lawitz E, Flisiak R, Rodriguez-Torres M, et al. Efficacy and Safety Results of Albinterferon Alfa-2b in Combination with Ribavirin in Interferon-Alfa Treatment Naive Patients with Genotype 2 or 3 Chronic Hepatitis C. Journal of Hepatology 2009; 50:S378-S378.

Nevens F, Roskams T, Van Vlierberghe H, Horsmans Y, Sprengers D, Elewaut A, et al. A pilot study of therapeutic vaccination with envelope protein E1 in 35 patients with chronic hepatitis C. Hepatology 2003; 38:1289-1296.

Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995; 346:1051-1055.

Ozes ON, Reiter Z, Klein S, Blatt LM, Taylor MW. A comparison of interferon-Con1 with natural recombinant interferons-alpha: antiviral, antiproliferative, and natural killer-inducing activities. J Interferon Res 1992; 12:55-59.

Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. Hepatology 2007; 46:1688-1694.

Pedder SC. Pegylation of interferon alfa: structural and pharmacokinetic properties. Semin Liver Dis 2003; 23 Suppl 1:19-22.

Perelson AS, Ribeiro RM. Mutagenic effects of ribavirin in vivo. J Hepatol 2005; 43:553-555.

Pischke S, Cornberg M, Manns MP. [Hepatitis associated cryoglobulinemia]. Internist (Berl) 2008; 49:297-304.

Poustchi H, Negro F, Hui J, Cua IH, Brandt LR, Kench JG, George J. Insulin resistance and response to therapy in patients infected with chronic hepatitis C virus genotypes 2 and 3. J Hepatol 2008; 48:28-34.

Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998; 352:1426-1432.

Poynard T, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, et al. Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. Gastroenterology 2009; 136:1618-1628 e1612.

Reddy KR, Shiffman ML, Morgan TR, Zeuzem S, Hadziyannis S, Hamzeh FM, et al. Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. Clin Gastroenterol Hepatol 2007; 5:124-129.

Rodriguez-Luna H, Khatib A, Sharma P, De Petris G, Williams JW, Ortiz J, et al. Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alpha2b and ribavirin: an open-label series. Transplantation 2004; 77:190-194.

Rumi MG, Aghemo A, Prati GM, D'Ambrosio R, Donato MF, Soffredini R, et al. Randomized study of peginterferon-alpha2a plus ribavirin vs peginterferon-alpha2b plus ribavirin in chronic hepatitis C. Gastroenterology 138:108-115.

Rustgi VK, Lee WM, Lawitz E, Gordon SC, Afdhal N, Poordad F, et al. Merimepodib, pegylated interferon, and ribavirin in genotype 1 chronic hepatitis C pegylated interferon and ribavirin nonresponders. Hepatology 2009; 50:1719-1726.

Santantonio T, Fasano M, Sinisi E, Guastadisegni A, Casalino C, Mazzola M, et al. Efficacy of a 24-week course of PEG-interferon alpha-2b monotherapy in patients with acute hepatitis C after failure of spontaneous clearance. J Hepatol 2005; 42:329-333.

Sanchez-Tapias JM, Diago M, Escartin P, Enriquez J, Romero-Gomez M, Barcena R, et al. Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. Gastroenterology 2006; 131:451-460.

Schaefer M, Schwaiger M, Garkisch AS, Pich M, Hinzpeter A, Uebelhack R, et al. Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. J Hepatol 2005; 42:793-798.

Schiff E, Poordad E, Jacobson I, Flamm S, Bacon B, Lawitz E, et al. Boceprevir (B) combination therapy in null responders (NR): Response dependent on interferon responsiveness. Journal of Hepatology 2008; 48:S46-S46.

Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, et al. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med 2007; 357:124-134.

Shiffman ML, Hofmann CM, Luketic VA, Sanyal AJ. Use of granulocyte macrophage colony stimulating factor alone or in combination with interferon-alpha-2b for treatment of chronic hepatitis C. J Hepatol 1998; 28:382-389.

Shiffman ML, Salvatore J, Hubbard S, Price A, Sterling RK, Stravitz RT, et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. Hepatology 2007; 46:371-379.

Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, et al. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. Gastroenterology 2004; 126:1015-1023; discussion 1947.

Sintchak MD, Nimmesgern E. The structure of inosine 5'-monophosphate dehydrogenase and the design of novel inhibitors. Immunopharmacology 2000; 47:163-184.

Sjogren MH, Sjogren Jr R, Lyons MF, Ryan M, Santoro J, Smith C, et al. Antiviral response of HCV genotype 1 to consensus interferon and ribavirin versus pegylated interferon and ribavirin. Dig Dis Sci 2007; 52:1540-1547.

Snoeck E, Wade JR, Duff F, Lamb M, Jorga K. Predicting sustained virological response and anaemia in chronic hepatitis C patients treated with peginterferon alfa-2a (40KD) plus ribavirin. Br J Clin Pharmacol 2006; 62:699-709.

Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, et al. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. Hepatology 2002; 36:1273-1279.

Svensson JO, Bruchfeld A, Schvarcz R, Stahle L. Determination of ribavirin in serum using highly selective solid-phase extraction and high-performance liquid chromatography. Ther Drug Monit 2000; 22:215-218.

Swain MG, Lai MY, Shiffman ML, Cooksley WGE, Abergel A, Lin A, et al. Durable sustained virological response after treatment with peginterferon alpha-2a (PEGASYS (R)) alone or in combination with ribavirin (COPEGUS (R)): 5-year follow-up and the criteria of a cure. Journal of Hepatology 2007; 46:S3-S3.

Tassopoulos NC. Treatment of patients with chronic hepatitis C and normal ALT levels. J Hepatol 1999; 31 Suppl 1:193-196.

Terrault NA. Prophylactic and preemptive therapies for hepatitis C virus-infected patients undergoing liver transplantation. Liver Transpl 2003; 9:S95-S100.

Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001; 194:1395-1406.

Todros L, Saracco G, Durazzo M, Abate ML, Touscoz G, Scaglione L, et al. Efficacy and safety of interferon alfa therapy in chronic hepatitis C with autoantibodies to liver-kidney microsomes. Hepatology 1995; 22:1374-1378.

Tong MJ, Reddy KR, Lee WM, Pockros PJ, Hoefs JC, Keeffe EB, et al. Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial. Consensus Interferon Study Group. Hepatology 1997; 26:747-754.

Van Thiel DH, Friedlander L, Kania RJ, Molloy PJ, Hassanein T, Faruki H. A preliminary experience with GM-CSF plus interferon in patients with HBV and HCV resistant to interferon therapy. J Viral Hepat 1997; 4 Suppl 1:101-106.

Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med 2007; 147:677-684.

Vogel W, Graziadei I, Umlauft F, Datz C, Hackl F, Allinger S, et al. High-dose interferon-alpha2b treatment prevents chronicity in acute hepatitis C: a pilot study. Dig Dis Sci 1996; 41:81S-85S.

von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005; 129:522-527.

von Wagner M, Hofmann WP, Teuber G, Berg T, Goeser T, Spengler U, et al. Placebo-controlled trial of 400 mg amantadine combined with peginterferon alfa-2a and ribavirin for 48 weeks in chronic hepatitis C virus-1 infection. Hepatology 2008; 48:1404-1411.

Watson J. Prospects for hepatitis C virus therapeutics: levovirin and viramidine as improved derivatives of ribavirin. Curr Opin Investig Drugs 2002; 3:680-683.

Wedemeyer H, He XS, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, et al. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 2002; 169:3447-3458.

Wedemeyer H, Schuller E, Schlaphoff V, Stauber RE, Wiegand J, Schiefke I, et al. Therapeutic vaccine IC41 as late add-on to standard treatment in patients with chronic hepatitis C. Vaccine 2009; 27:5142-5151.

Wesche B, Jaeckel E, Trautwein C, Wedemeyer H, Falorni A, Frank H, et al. Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C. Gut 2001; 48:378-383.

Wiegand J, Buggisch P, Boecher W, Zeuzem S, Gelbmann CM, Berg T, et al. Early monotherapy with pegylated interferon alpha-2b for acute hepatitis C infection: the HEP-NET acute-HCV-II study. Hepatology 2006; 43:250-256.

Willems B, Hadziyannis SJ, Morgan TR, Diago M, Marcellin P, Bernstein DE, et al. Increased SVR rate with 48 wks' treatment and higher RBV dose in HCV genotype 2/3 pts without a rapid virologic response (Rvr) treated with peginterferon alfa-2a (40KD) (Pegasys®) plus RBV (Copegus®). Gastroenterology 2007; 132:A728-A728.

Younossi ZM, Nader FH, Bai C, Sjogren R, Ong JP, Collantes R, et al. A phase II dose finding study of darbepoetin alpha and filgrastim for the management of anaemia and neutropenia in chronic hepatitis C treatment. J Viral Hepat 2008; 15:370-378.

Zeuzem S, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, et al. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. J Hepatol 2004; 40:993-999.

Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol 2006; 44:97-103.

Zeuzem S, Sulkowski M, Lawitz E, Rustgi V, Lurie Y, Grigorescu M, et al. Efficacy and Safety of Albinterferon Alfa-2b in Combination with Ribavirin in Treatment-Naive, Chronic Hepatitis C Genotype 1 (Chc G1) Patients. Journal of Hepatology 2009; 50:S377-S377.

Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000; 343:1666-1672.

Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, et al. Peginterferon alfa-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology 2004; 127:1724-1732.

Zignego AL, Ferri C, Pileri SA, Caini P, Bianchi FB. Extrahepatic manifestations of Hepatitis C Virus infection: a general overview and guidelines for a clinical approach. Dig Liver Dis 2007; 39:2-17.

Chapter 14: New agents for treating hepatitis C

Christian Lange, Christoph Sarrazin

Introduction

Hepatitis C therapy has continually improved since the hepatitis C virus (HCV) was first isolated in 1989. From the introduction of interferon IFN α monotherapy to the current standard of care, combination therapy with pegylated (peg) interferon a plus ribavirin, the efficacy of achieving a sustained virologic response (SVR), defined by undetectable HCV RNA 24 weeks after treatment completion, has improved significantly (McHutchison 1998; Manns 2001; Fried 2002). However, still almost half of all patients with chronic hepatitis C do not achieve a sustained virologic response. The success of the current standard treatment strongly depends on the HCV genotype with SVR rates of only 40-50% in patients infected with genotype 1, contrasted with SVR rates of approximately 80% in those infected with genotypes 2 or 3 (Manns 2001; Fried 2002; Hadziyannis 2004; McHutchison 2004). In addition, treatment with interferon-a and ribavirin is long (up to 72 weeks) and associated with numerous side effects that lead to early discontinuation in up to 20% of patients. Furthermore, a significant proportion of patients have contraindications to IFN-based therapy due to concomitant diseases and other circumstances (Fried 2002).

The exploding knowledge of the HCV life cycle and of structural features of the HCV proteins, obtained by replicative cell culture systems and crystallographic analysis, has spurred the development of many promising "directly acting antiviral agents" (DAA), also called "specifically targeted antiviral therapy for hepatitis C" (STAT-C) compounds (Kim 1996; Lohmann 1999; Lindenbach 2005; Wakita 2005) (Figure 1; Table 1).

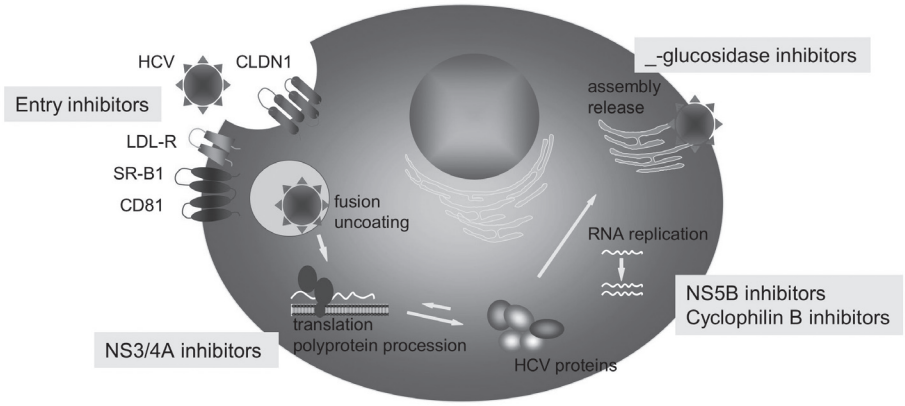


Figure 1. HCV life cycle and targets for STAT-C.

Drug name	Company	Target / Active site	Dev't phase
NS3/4A protease inhibitors			
Ciluprevir (BILN 2061)	Boehringer Ingelheim	Active site / macrocyclic	Stopped
Telaprevir (VX-950)	Vertex	Active site / linear	Phase III
Boceprevir (SCH503034)	Schering-Plough	Active site / linear	Phase III
TMC435350	Tibotec / Medivir	Active site / macrocyclic	Phase II
R7227 / ITMN-191	InterMune / Roche	Active site / macrocyclic	Phase II
MK-7009	Merck	Active site / macrocyclic	Phase II
BI201335	Boehringer Ingelheim	Active site / macrocyclic?	Phase II
Narlaprevir (SCH900518)	Schering-Plough	Active site / linear	Halted
BMS-650032	Bristol-Myers Squibb	Active site	Phase I
PHX1766	Phenomix	Active site	Phase I
ACH-1625	Achillion	Active site / macrocyclic?	Phase I
Nucleoside analogue NS5B polymerase inhibitors			
Valopicitabine (NM283)	Idenix/ Novartis	Active site / NM107	Stopped
R7128	Roche / Pharmasset	Active site / PSI-6130	Phase II
R1626	Roche	Active site / R1479	Stopped
PSI-7851	Pharmasset	Active site	Phase I
IDX184	Idenix	Active site	Phase I
Non-nucleoside NS5B polymerase inhibitors (NNI)			
BILB 1941	Boehringer Ingelheim	NNI site 1 / thumb 1	Stopped
BI207127	Boehringer Ingelheim	NNI site 1 / thumb 1	Phase II
MK-3281	Merck	NNI site 1 / thumb 1	Phase I
Filibuvir (PF-00868554)	Pfizer	NNI site 2 / thumb 2	Phase II
VCH759	ViroChem Pharma	NNI site 2 / thumb 2	Phase I
VCH916	ViroChem Pharma	NNI site 2 / thumb 2	Phase I
VCH222	ViroChem Pharma	NNI site 2 / thumb 2	Phase I
ANA598	Anadys	NNI site 3 / palm 1	Phase I
HCV-796	ViroPharma / Wyeth	NNI site 4 / palm 2	Stopped
GS-9190	Gilead	NNI site 4 / palm 2	Phase I
ABT-333	Abbott	NNI site 4 / palm 2	Phase I
NS5A inhibitor			
BMS-790052	Bristol-Myers Squibb	NS5A domain 1 inhibitor	Phase II
Indirect inhibitors / unknown mechanism of action			
Debio-025	Debiopharm	Cyclophilin inhibitor	Phase I
NIM811	Novartis	Cyclophilin inhibitor	Phase I
SCY-635	Scynexis	Cyclophilin inhibitor	Phase I
Nitazoxanide		PKR induction (?)	Phase II
Celgosivir	Migenix	Alpha-glucosidase inhibitor	Phase II

Table 1. Antivirals in the pipeline.

Several antiviral drugs are currently in phase I-III development and will significantly change standard treatment options for HCV infection in the near future. In the following section, the HCV life cycle, the resulting STAT-C targets and compounds currently under development are presented. In addition, novel developments for optimising efficacy and safety of IFN α and ribavirin will be described.

HCV life cycle and targets for STAT-C

HCV is a positive-sense single-stranded RNA virus of approximately 9600 nucleotides. The HCV genome contains a single large open reading frame encoding for a polyprotein of about 3100 amino acids. From this initially translated polyprotein, the structural HCV protein core (C) and envelope 1 and 2 (E1, E2); p7; and the six non-structural HCV proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B, are processed by both viral and host proteases. The core protein forms the viral nucleocapsid carrying E1 and E2, which are receptors for viral attachment and host cell entry. The non-structural proteins are mainly enzymes essential for the HCV life cycle (Bartenschlager 2004; Pawlotsky 2007). P7 is a small hydrophobic protein that oligomerises into a circular hexamer, most likely serving as an ion channel through the viral lipid membrane (Carrere-Kremer 2002; Clarke 2006). The large translated section of the HCV genome is flanked by the strongly conserved HCV 3´ and 5´ untranslated regions (UTR). The 5´ UTR is comprised of four highly structured domains forming the internal ribosome entry site (IRES), which plays an important role in HCV replication (Figure 2).

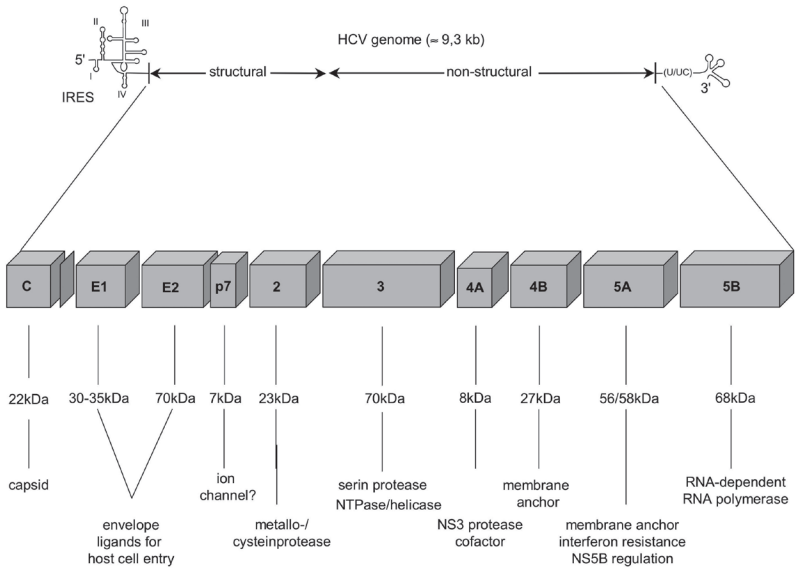


Figure 2. Genomic organisation of HCV.

Viral attachment and entry

Molecular biology of viral attachment and entry

HCV internalisation occurs through clathrin-mediated endocytosis initiated by specific interactions between E1 and E2 and their receptors on the host cell surface (Barth 2006; Bartosch 2006; Moradpour 2007). The tetraspanin protein CD81, claudin-1, occludine, scavenger receptor class B type 1 (SR-B1), the low density lipoprotein (LDL) receptor, glycosaminoglycans and the dendritic cell- / lymph node-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN/L-SIGN) have been identified as putative ligands for E1 and E2 (Pileri 1998; Bartosch 2003; Bartosch 2003; Lozach 2004; Diedrich 2006; Evans 2007).

Compounds targeting viral attachment and entry

HCV entry inhibition might enrich future hepatitis C treatment opportunities and can be theoretically achieved by the use of specific antibodies or small molecule compounds either blocking E1 and E2 or their cellular receptors. So far, only results from clinical trials using polyclonal (e.g., Civacir) (Davis 2005) or monoclonal (e.g., HCV-Ab68) (Schiano 2006) HCV-specific antibodies are available. The clinical benefit of these antibodies has been poor, however. The development of small molecule entry inhibitors is in a preclinical stage and is complicated by difficulties in the crystallographic characterization of HCV envelope proteins (VanCompernolle 2003).

HCV RNA translation and posttranslational protein processing

Molecular biology of translation and protein processing

After receptor-mediated endocytosis, the fusion of HCV with cellular membranes, and uncoating the viral nucleocapsid, the single-stranded positive-sense RNA genome of the virus is released into the cytoplasm to serve as a messenger RNA for the HCV polyprotein precursor. HCV mRNA translation is under the control of the internal ribosome entry site (IRES), which is formed by domains II-IV of the HCV 5´ UTR (Collier 2002; Gallego 2002). IRES mediates HCV polyprotein translation by forming a stable complex with the 40S ribosomal subunit, eukaryotic initiation factors and viral proteins. From the initially translated HCV polyprotein the three structural and seven non-structural HCV proteins are processed by both host and viral proteases (Bartenschlager 2004). The two viral proteases NS2 and NS3 are promising targets for STAT-C. NS2 is a metalloproteinase that cleaves itself from the NS2/NS3 protein, leading to its own loss of function and to the release of the NS3 protein (Lorenz 2006). NS3 provides a serine protease activity and a helicase/NTPase activity. The serine protease domain comprises two b-barrels and four a-helices. The serine protease catalytic triad – histidine 57, asparagine 81 and serine 139 – is located in a small groove between the two b-barrels (Kim 1996; Kim 1998). NS3 forms a tight, non-covalent complex with its obligatory cofactor and enhancer NS4A, which is essential for proper protein folding (Kim 1996) (Figure 3). The NS3/4A protease cleaves the junctions between NS3/NS4A, NS4A/NS4B, NS4B/

NS5A and NS5A/NS5B. Besides its essential role in protein processing, NS3 is integrated into the HCV RNA replication complex, supporting the unwinding of viral RNA by its helicase activity. Moreover, NS3 might play an important role in HCV persistence by inhibiting innate immune mechanisms via blocking toll-like receptor- (TRIF, Cardif) and subsequently interferon-signaling pathways (Meylan 2005; Kaukinen 2006; Malcolm 2006). Thus, pharmacologic NS3 inhibition might support viral clearance by restoring the innate immune response.

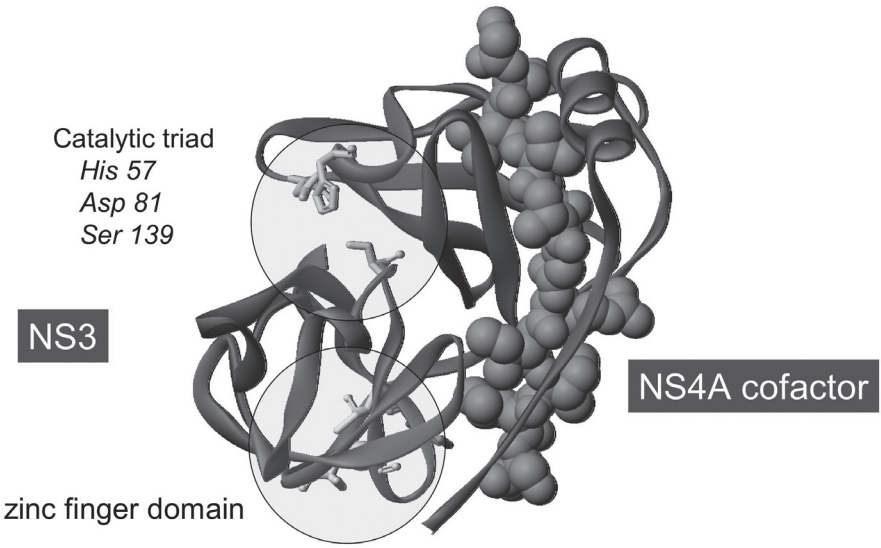


Figure 3. Molecular structure of the HCV NS3/4A protease.

Compounds targeting viral translation and protein processing

NS3/4A protease inhibitors

The active site of the NS3/4A protease is located in a shallow groove between the two b-barrels of the protease making the design of compound inhibitors relatively difficult. Nevertheless, many NS3/4A protease inhibitors are under development and can be divided into two classes, the macrocyclic inhibitors and linear tetra-peptide a-ketoamide derivatives. In general, NS3/4A protease inhibitors have been shown to strongly inhibit HCV replication during monotherapy, but also may cause the selection of resistant mutants, which is followed by viral breakthrough. The additional administration of pegylated interferon and ribavirin, however, was shown to reduce the frequency of development of resistance. Future strategies aim for combination therapies with different antiviral drugs to prevent the development of resistance. The most advanced compounds are telaprevir and boceprevir, which are expected to be approved in 2011 or 2012.

Ciluprevir (BILN 2061)

The first clinically tested NS3/4A inhibitor was ciluprevir (BILN 2061), an orally bioavailable, peptidomimetic, macrocyclic drug binding non-covalently to the active center of the enzyme (Lamarre 2003). Ciluprevir monotherapy was evaluated in a double-blind, placebo-controlled pilot study in treatment-naïve genotype 1 patients with liver fibrosis and compensated liver cirrhosis (Hinrichsen 2004). In this study ciluprevir, administered twice daily for two days at doses ranging from 25 to 500 mg, led to a mean 2-3 $\log_{10}$ decrease of HCV RNA serum levels in most patients. Importantly, the stage of disease did not affect the antiviral efficacy of ciluprevir. To assess the influence of the HCV genotype on treatment with protease inhibitors, the tolerability and efficacy of ciluprevir in genotype 2- and 3-infected individuals was examined in an equivalent study design. Compared to genotype 1 patients, the antiviral activity of ciluprevir was less pronounced and more variable in patients infected with genotypes 2 or 3 (Reiser 2005). Due to the pronounced genetic variability of HCV it is highly likely that other protease inhibitors designed for HCV genotype 1 will also not be equally effective with other genotypes.

Although the development of ciluprevir was stopped because of serious cardiotoxicity observed in an animal model, ciluprevir provided the proof-of-principle for successful suppression of HCV replication by NS3/4A inhibitors in patients with chronic hepatitis C.

Telaprevir (VX-950)

Telaprevir is an orally bioavailable, peptidomimetic NS3/4A protease inhibitor. Telaprevir is an a-ketoamide derivative binding the enzyme covalently but reversibly, with a half-life of 58 minutes of the enzyme-inhibitor complex (Lin 2006) (Figure 4). Currently, approval studies of telaprevir for both treatment-naïve patients and relapsers/non-responders are underway.

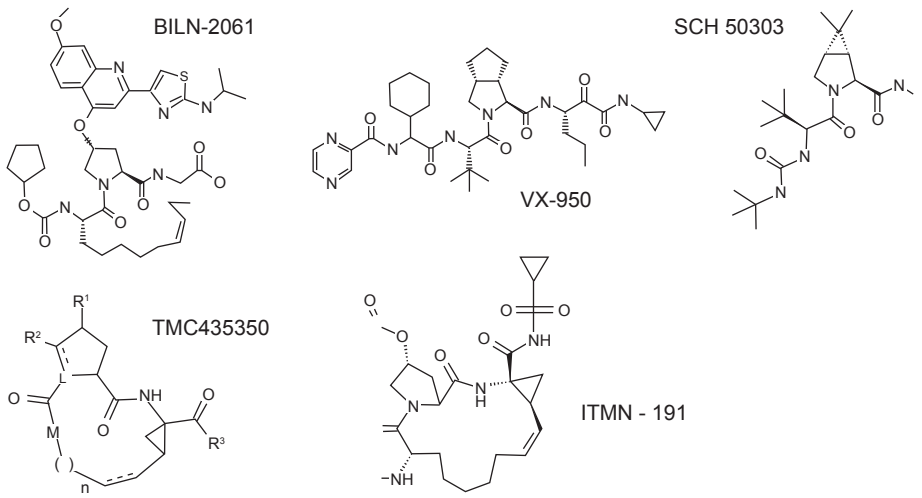


Figure 4. Molecular structure of selected NS3/4A inhibitors.

Telaprevir monotherapy phase I study

A double-blind, randomized placebo-controlled phase Ib clinical trial evaluating telaprevir monotherapy for 14 days was performed in patients with chronic genotype 1 infection (Reesink 2006). In this study, antiviral activity, safety, optimal dosage, and pharmacokinetics were assessed in treatment-naïve patients, relapsers or non-responders to standard treatment. Telaprevir was given at doses of 450 mg or 750 mg every 8 hours or 1250 mg every 12 hours for 14 days. It was well-tolerated and led to a rapid decline of HCV RNA serum levels in all groups. The best results were obtained in the 750 mg dose group with a median reduction of HCV RNA of 4.4 $\log_{10}$ after 14 days of treatment. However, viral rebound due to selected mutants occurred in all patients after treatment completion and in some patients during therapy, especially when treated with suboptimal doses (Sarrazin 2007).

Telaprevir / PEG IFN α-2a combination phase I study

A second phase I study investigated the safety, viral kinetics and the development of telaprevir-resistant mutants of telaprevir monotherapy and in combination with PEG-IFN α-2a in treatment-naïve genotype 1 patients (Forestier 2007). Telaprevir was used at a dose of 750 mg every 8 hours after an initial loading dose of 1250 mg either alone or in combination with PEG-IFN α-2a in comparison to PEG-IFN α-2a monotherapy. Treatment was given for 14 days and caused a median reduction of HCV RNA of 1.09 $\log_{10}$ in the PEG-IFN α-2a/placebo group, of 3.99 $\log_{10}$ in the telaprevir/placebo group and of 5.49 $\log_{10}$ in the telaprevir/PEG-IFN α-2a group on day 15. Although selection of telaprevir-resistant mutants occurred during telaprevir monotherapy and to a lesser extent during combination therapy with PEG-IFN α-2a, no viral breakthrough was seen during the combination therapy in the 14-day treatment period (Kieffer 2007).

Telaprevir / PEG-IFN α-2a and ribavirin triple therapy phase I study

A parallel study evaluated the safety and efficacy of telaprevir (750 mg every 8 hours) in combination with PEG-IFN α-2a and weight-based ribavirin in treatment-naive genotype 1 patients for 28 days (Lawitz 2008). After the 28-day treatment period, all patients had undetectable HCV RNA serum levels and 8 of 12 patients achieved an SVR.

Telaprevir and PEG-IFN with and without ribavirin, phase II studies

Treatment naïve studies (PROVE 1 and 2, C208, C209, C210)

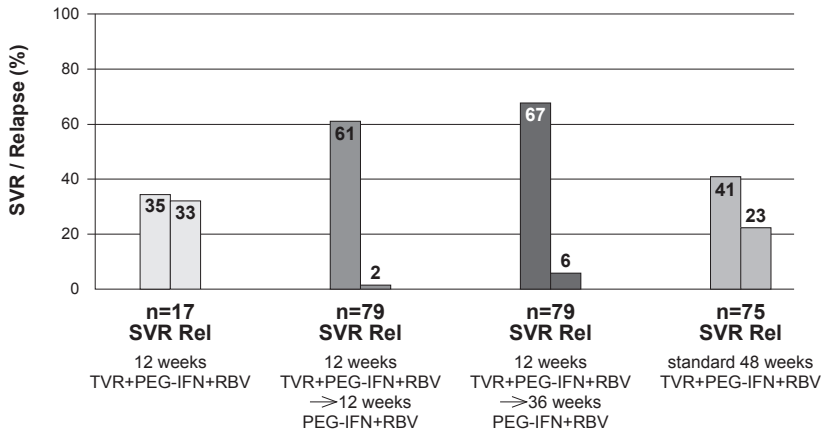
To answer the question whether the addition of telaprevir to PEG-IFN α-2a and ribavirin would reduce the treatment duration and improve the SVR rates in treatment-naïve genotype 1 patients, larger phase II clinical trials (PROVE 1 & 2) were initiated (Table 2). In addition, a study comparing two versus three times daily administration of telaprevir in combination with either pegylated interferon alfa-2a or 2b (C208) and studies in genotype 2, 3 and 4 infected patients were performed (C209, C210).

In PROVE 1, telaprevir, PEG-IFN α-2a and ribavirin were administered for 12 weeks in combination, followed by PEG-IFN α-2a and ribavirin alone for 0, 12 or 36 weeks in comparison to standard treatment. SVR rates were 35%, 61% and 67%, respectively, compared to 41% with standard treatment (Figure 5). According to the

study protocol, treatment was only stopped after 12 or 24 weeks when a rapid virological response (RVR) was achieved. Serious adverse effects, however, led to premature treatment termination in 18% of all subjects treated with telaprevir in contrast to 4% of all standard-treated patients. Most common adverse events were skin rash, anemia and gastrointestinal disorders (McHutchison 2009).

Study arm	Treatment	Number of patients PROVE 1 (USA)	PROVE 2 (Europe)
Treatment-naïve patients (TVR 3x750mg/day, PEG-IFN α-2a 180µg/week, ribavirin 1000/1200mg/day) Stopping rules: PROVE 1: Stop only if RVR was achieved, PROVE 2: Stop independently from viral response			
12 weeks triple	12 weeks: TVR + PEG-IFN α–2a + RBV	17	80
12 weeks combination	12 weeks: TVR + PEG-IFN α-2a	0	80
24 weeks (12 weeks triple + 12 weeks standard)	12 weeks: TVR + PEG-IFN α-2a + RBV 12 weeks: PEG-IFN α-2a + RBV	80	80
48 weeks (12 weeks triple + 36 weeks standard	12 weeks: TVR + PEG-IFN α-2a + RBV 36 weeks: PEG-IFN α-2a + RBV	82	0
Standard treatment	48 weeks: PEG-IFN α-2a + RBV	81	80

Table 2. Study designs of PROVE 1 and 2.



Main side effects:
Skin rash: 53-61% TVR, 41% standard
Premature treatment termination: 18% TVR, 4% standard
Resistance: 7% TVR, 0% standard

Figure 5. Results of PROVE 1 (USA).
Combination therapy of telaprevir (TVR) and PEG-IFN α-2a +/- ribavirin (RBV) in treatment-naive genotype 1 patients. SVR, sustained virologic response; Rel, relapse.

The study design of PROVE 2 is equivalent to PROVE 1 with the main difference being that treatment termination after 12 or 24 weeks was independent of achieving an RVR and one of the groups did not receive ribavirin (Table 2). The recently published final results showed SVR rates of 30%, 60% and 69% for patients treated with telaprevir and PEG-IFN alone for 12 weeks, telaprevir and PEG-IFN and ribavirin for 12 weeks and with telaprevir, PEG-IFN and ribavirin for 12 weeks followed by 12 weeks of PEG-IFN and ribavirin alone, respectively (Figure 6). SVR rates after standard treatment was 46%. However, the rate of relapse in the groups treated for 12 weeks was relatively high (Hézode 2009).

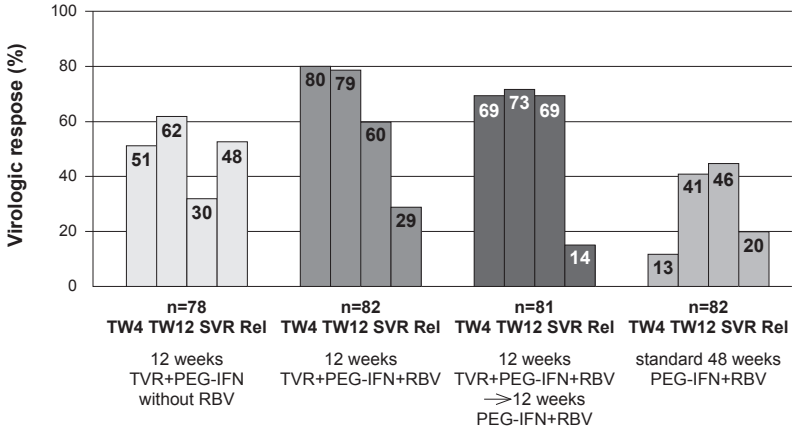


Figure 6. Results of PROVE 2 (Europe).

Combination therapy of telaprevir (TVR) and PEG-IFN α-2a +/- ribavirin (RBV) in treatment-naive genotype 1 patients.

In a small study (n=161) comparing three times daily 750mg with two times daily 1125mg telaprevir combined with pegylated interferon α-2a or -2b plus ribavirin comparable SVR rates in all 4 treatment arms were observed (81-85%). The high overall SVR rates show the potential of the triple therapy approach and are explained by experienced study centers with very low discontinuation rates (5%) in comparison with the PROVE studies. In addition, in this study the response guided therapy approach was investigated. Only in patients achieving an RVR was treatment duration shortened to 24 weeks, while the remaining patients received 48 weeks therapy. Based on the RVR rates of the different arms of the study 69-83% of all patients benefitted from a reduced treatment duration of 24 weeks.

As one would expect from the experiences of ciluprevir or telaprevir alone or in combination with PEG-IFN and ribavirin was less effective in treatment-naïve patients infected with other genotypes. For HCV genotype 2 a somewhat weaker antiviral activity in comparison with genotype 1 with a mean viral decline of 3.9 $\log_{10}$ IU/ml during 14 days monotherapy with telaprevir was observed while in genotype 3 and 4 infected patients no significant antiviral activity was detectable (0.5-0.9 $\log_{10}$ decline) (Foster 2009; Benhamou 2009).

In conclusion, PROVE 1 and 2 show that 12 weeks of triple therapy is too short and there is a high rate of relapse after treatment completion. Moreover, ribavirin is necessary in therapies with telaprevir to achieve high SVR rates. However, 12 weeks of triple therapy with telaprevir followed by 12 weeks of standard treatment in treatment-naïve genotype 1 patients greatly improve SVR rates compared to standard treatment. Thus, telaprevir is a promising candidate for future standard treatment recommendations. The RVR during triple therapy is an important predictor for treatment success and will be used for the determination of individualised treatment duration. For genotype 2-infected patients a significant antiviral activity of telaprevir was shown and future studies are required to determine a potential benefit of triple therapy for this genotype, while in genotypes 3- and 4-infected patients telaprevir was not effective.

Phase III clinical trials evaluating the safety and efficacy of telaprevir in combination with PEG-IFN α and ribavirin for 24 to 48 weeks in genotype 1 infected patients have been initiated (the ADVANCE and ILLUMINATE Studies).

Non-responder and relapser phase II studies (PROVE 3)

The study design of PROVE 3 is shown in Table 3. Telaprevir in combination with PEG-IFN α-2a (and) ribavirin was administered for 12 to 24 weeks followed by PEG-IFN α-2a and ribavirin alone for 0 to 24 weeks.

Study arm	Treatment	Number of patients
Genotype 1 non-responders and relapsers (TVR 3x750mg/day, PEG-IFN α-2a 180µg/week, ribavirin 1000/1200mg/day)		
24 weeks (12 weeks triple + 12 weeks standard)	12 weeks: TVR + PEG-IFN α-2a + RBV 12 weeks: PEG-IFN α-2a + RBV	110
48 weeks (24 weeks triple + 24 weeks standard)	24 weeks: TVR + PEG-IFN α–2a + RBV 24 weeks: PEG-IFN α-2a + RBV	110
48 weeks (24 weeks triple + 24 weeks PEG-IFN monotherapy)	24 weeks: TVR + PEG-IFN α-2a + RBV 24 weeks: PEG-IFN α-2a	110
Standard treatment	48 weeks: PEG-IFN α-2a + RBV	110

Table 3. Study design of PROVE 3.

Combination therapy of telaprevir (TVR) and PEG-IFN α-2a +/- ribavirin (RBV) in genotype 1 non-responders and relapsers.

Retreatment of non-responders with 12 weeks of triple therapy followed by 12 weeks of standard treatment led to an SVR rate of 51% (79% relapser, 39% non-responder), which is significantly higher than the SVR rate in the standard treatment arm (14%). Retreatment of non-responders with 24 weeks of triple therapy followed by 24 weeks of standard treatment led to an SVR rate of 53% (76% relapser, 38% non-responder). Retreatment of non-responders with 24 weeks of telaprevir and PEG-IFN α-2a followed by 24 weeks of PEG-IFN α-2a alone led to a SVR rate of only 24% (42% relapser, 11% non-responder), which indicates that ribavirin is also required for a successful treatment of non-responders with telaprevir (Figure 7). As in the PROVE 1 and 2 studies viral breakthrough was observed more frequently in patients infected with genotype 1a than in patients infected with genotype 1b (McHutchison 2009). A phase III clinical trial (REALIZE) evaluating telaprevir in non-responders was recently initiated.

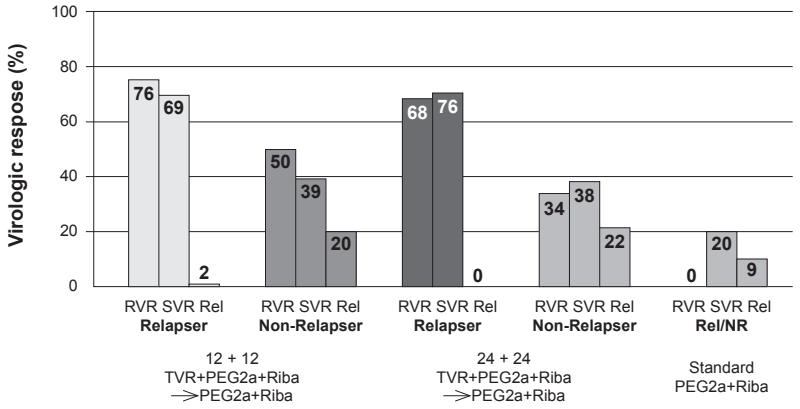


Figure 7. Results of PROVE 3.

Combination therapy of telaprevir (TVR) and PEG-IFN α-2a +/- ribavirin (RBV) genotype 1 non-responders and relapsers.

Boceprevir (SCH 503034)

Boceprevir is another novel peptidomimetic orally bioavailable a-ketoamide HCV protease inhibitor that forms a covalent but reversible complex with the NS3 protein (Malcolm 2006) (Figure 4). Currently, boceprevir is in phase III evaluation.

Boceprevir monotherapy phase I study

An initial phase I trial evaluated safety, tolerability and antiviral efficacy of boceprevir monotherapy (100 to 400 mg daily) in genotype 1 patients who had failed to respond to previous standard treatment (Zeuzem 2005). After the 14-day treatment period, a mean $\log_{10}$ reduction in HCV RNA load of 2.06 was achieved in patients treated with 400 mg boceprevir daily. Boceprevir was well tolerated at all doses without significant adverse effects. However, viral breakthrough with selection of resistant variants occurred in some patients, especially in those treated with lower doses (Susser 2009).

Boceprevir plus pegylated interferon α-2b combination phase I study

A subsequent phase Ib study evaluated the combination of boceprevir and PEG-IFN α-2b in genotype 1-infected non-responders to standard therapy (Sarrazin 2007). In this randomized, double-blind crossover study, boceprevir was administered at doses of 200 or 400 mg every eight hours either alone for seven days or in combination with PEG-IFN α-2b for 14 days in comparison to 14 days of PEG-IFN α-2b monotherapy. Because genotype 1 non-responders to standard treatment are heterogeneous, the study design intended each patient to receive boceprevir alone, in combination with PEG-IFN α-2b and PEG-IFN α-2b alone with washout-periods in between in a randomized crossover sequence. Mean maximum reductions in HCV RNA load were 2.45 and 2.88 $\log_{10}$ for boceprevir 200 mg and 400 mg plus PEG-IFN α-2b, 1.08 and 1.61 $\log_{10}$ for boceprevir monotherapy and 1.08 and 1.26 $\log_{10}$ for PEG-IFN α-2b monotherapy. Boceprevir was well-tolerated alone and in combination with PEG-IFN α-2b. The most common adverse events were headache, myalgia, rigor and fever. In some patients, especially during boceprevir monotherapy, viral breakthrough due to selection of preexisting resistant mutants was seen (Vermehren 2009).

Boceprevir and pegylated interferon with and without ribavirin, phase II

Treatment naïve phase II study (SPRINT-1)

The SPRINT-1 trial looked at the safety, tolerability and antiviral efficacy of boceprevir (800 mg three times a day) in combination with PEG-IFN α-2b and ribavirin in treatment-naïve genotype 1 patients (Table 4) (Kwo 2009).

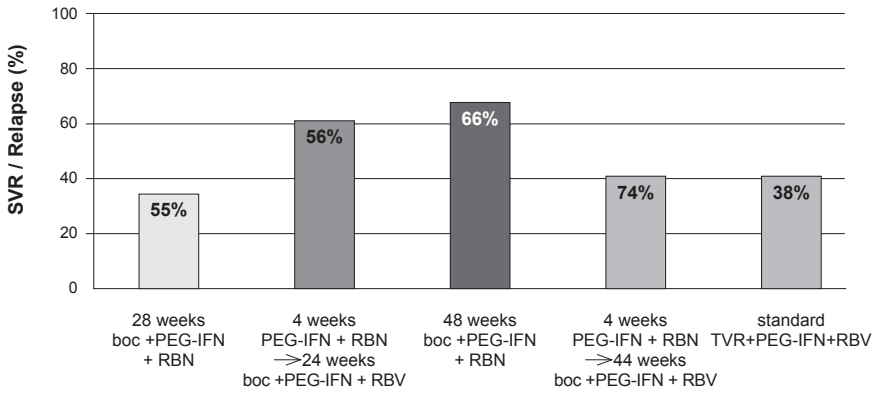
Study arm	Treatment	Number of patients
Treatment-naïve patients (Boceprevir 3 x 800 mg/day, PEG-IFN α-2b 1.5 µg/kg, ribavirin 800/1200 mg/day)		
28 weeks triple	28 weeks: boceprevir + PEG-IFN α-2b + RBV	80
4 weeks +24 weeks triple	4 weeks: PEG-IFN α-2b + RBV 24 weeks: boceprevir + PEG-IFN α-2b + RBV	80
4 weeks + 44 weeks triple	4 weeks: PEG-IFN α-2b + RBV 44 weeks: boceprevir + PEG-IFN α-2b + RBV	80
48 weeks triple	48 weeks: boceprevir + PEG-IFN α-2b + RBV	80
48 weeks triple (low dose ribavirin)	48 weeks: boceprevir + PEG-IFN α-2b + RBV (400 – 1000 mg/day)	80
Standard treatment	48 weeks: PEG-IFN α-2b + RBV	80

Table 4: Study design of SPRINT-1 (USA, Canada, Europe).
Combination therapy of boceprevir and PEG-IFN α-2a +/- ribavirin (RBV) in treatment-naive genotype 1 patients.

Treatment with boceprevir in combination with PEG-IFN α-2b and ribavirin was taken continuously for 28 or 48 weeks or for 24 or 44 weeks after a previous 4-week treatment period with PEG-IFN α-2b and ribavirin alone. This study design was

chosen to determine whether pretreatment with PEG-IFN α-2 and ribavirin could avoid the development of resistance while have positive effects on antiviral efficacy. The control group was treated with PEG-IFN α-2b and ribavirin for 48 weeks. SVR rates after 28 weeks of triple therapy were 54% and 56% after 24 weeks with an additional 4 weeks of pretreatment lead-in with PEG-IFN α-2 and ribavirin (Figure 8). SVR rates after 48 weeks of triple treatment were 67% and 75% after 44 weeks with an additional 4 weeks of pretreatment lead in with PEG-IFN α-2 and ribavirin. After 4 weeks of triple therapy with boceprevir, PEG-IFN and ribavirin 38% of patients achieved a rapid virologic response. The most common side-effects related to boceprevir were anemia, nausea, vomiting and dysgeusia. In general, SPRINT-1 has shown a higher antiviral efficacy of combination therapies with boceprevir in comparison to the standard of care with slightly better results with the 4 week lead-in phase, especially for the long treatment duration of 48 weeks. However, with 38% RVR rates, boceprevir triple therapy seems to be less potent then telaprevir triple therapy with an RVR rate of approximately 70%.

A phase III clinical trial (SPRINT-2) evaluating boceprevir in treatment-naïve patients was recently initiated.



Premature treatment termination: 28% boceprevir, 14% standard
Resistance: 5-11% TVR, 0% standard

Figure 8. Results of SPRINT-1.
Combination therapy of boceprevir and PEG-IFN α-2b + ribavirin (RBV) in treatment-naive genotype 1 patients.

Non-responder and relapser phase II studies

In a complex study of genotype 1 non-responders, the addition of boceprevir to PEG-IFN α-2b and ribavirin resulted in only slightly increased SVR rates compared to standard treatment (14% vs. 2%) (Schiff 2008). A phase III clinical trial (RESPOND-2) evaluating boceprevir in relapsers and partial non-responders was initiated recently.

Other NS3 protease inhibitors

Other NS3 protease inhibitors are currently in phase I or II development (R7227/ITMN-191, MK7009, BI 201335, TMC435350, SCH 900518, BMS-650032, PHX1766, ACH-1625). Comparable antiviral activity to that of telaprevir and boceprevir in genotype 1 infected patients were observed (Figure 10) and triple therapy studies for a number of compounds have initiated. Different resistance profiles between linear tetrapeptide and macrocyclic inhibitors binding to the active site of the NS3 protease have been outlined. However, R155 is the main overlapping position for resistance and different mutations at this amino acid site within the NS3 protease confer resistance to all protease inhibitors which are currently in advanced clinical development (Sarrazin and Zeuzem 2010).

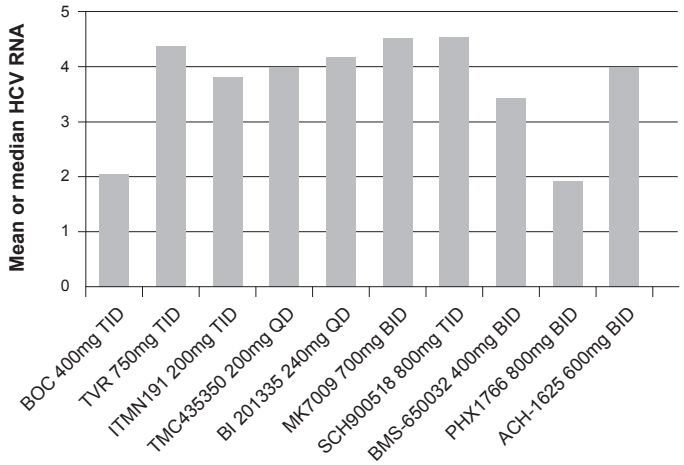


Figure 9. Antiviral activity of NS3/4A protease inhibitors.

Resistance to NS3/4A inhibitors

Because of the high replication rate of HCV and the poor fidelity of its RNA-dependent RNA polymerase, numerous variants (quasispecies) are continuously produced during HCV replication. Among them, variants carrying mutations altering the conformation of the binding sites of STAT-C compounds can develop. During treatment with specific antivirals, these drug-resistant variants have a fitness advantage and can be selected to become the dominant viral quasispecies. Many of these resistant mutants exhibit an attenuated replication with the result that, after stopping exposure to specific antivirals, the wild type may displace the resistant variants (Tong 2006; Sarrazin 2007). Nevertheless, HCV quasispecies resistant to NS3/4A protease inhibitors or non-nucleoside polymerase inhibitors can be detected at low levels in some patients who were never treated with specific antivirals before (Gaudieri 2009; Kuntzen 2008; Rodriguez-Frias 2009; Le Pogam 2008). The clinical relevance of

these pre-existing mutants is not completely understood, although there is evidence that they may reduce the chance of achieving an SVR after treatment with STAT-C compounds.

Ciluprevir

Exposure of genotype 1 replicon cells to ciluprevir and subsequent sequence analyses of the NS3 region has led to the identification of several mutations conferring ciluprevir resistance: A156T, R155Q and D168V/A. These mutations result in a 357-fold, 24-fold and 144-fold reduced susceptibility to ciluprevir, respectively, compared to wild type (Lin 2004; Lu 2004; Lin 2005). The A156T mutant confers varying levels of cross-resistance to ciluprevir, telaprevir and boceprevir. This mutation causes a significantly reduced enzymatic function attenuating the HCV life cycle, which, however, can be overcome by additional mutations at P89L, Q86R or G162R. No data are available on clinically-selected resistance mutations after administration of ciluprevir in patients with chronic hepatitis C.

Telaprevir

To date, mutations conferring telaprevir-resistance have been identified at four positions, V36A/M/L, T54A, R155K/M/S/T and A156S//T (Lin 2005; Lin 2007; Sarrazin 2007; Welsch 2008; Zhou 2008) (Table 5). The A156 mutation was revealed by *in vitro* analyses in the replicon while the other mutations were detected *in vivo* by a clonal sequencing approach during telaprevir administration in patients with chronic hepatitis C. A detailed kinetic analysis of telaprevir-resistant variants was performed in genotype 1 patients during 14 days of telaprevir monotherapy and combination therapy with PEG-IFN α-2a (Sarrazin 2007). Telaprevir monotherapy initially led to a rapid HCV RNA decline in all patients due to a strong reduction in wild type virus. In patients who developed a viral rebound during telaprevir monotherapy, mainly the single mutation variants R155K/T and A156T were uncovered by wild type reduction and became dominant after day 8. These single mutant variants were selected from preexisting quasispecies. During the viral rebound phase these variants typically were replaced by highly resistant double-mutation variants (e.g., V36M/A +R155K/T). The combination of telaprevir and PEG-IFN α-2a was sufficient to inhibit the breakthrough of resistant mutations in a 14-day study (Forestier 2007). It is important to note that after up to 3 years of telaprevir treatment low to medium levels of V36 and R155 variants were observed in single patients (Forestier 2008).

As shown also for other NS3/4A protease inhibitors (e.g., ITMN-191), the genetic barrier to telaprevir resistance differs significantly between HCV subtypes. In all clinical studies of telaprevir alone or in combination with PEG-IFN α and ribavirin, viral resistance and breakthrough occurred much more frequently in patients infected with HCV genotype 1a compared to genotype 1b. This difference was shown to result from nucleotide differences at position 155 in HCV subtype 1a (aga, encodes R) versus 1b (cga, also encodes R). The mutation most frequently associated with resistance to telaprevir is R155K; changing R to K at position 155 requires 1 nucleotide change in HCV subtype 1a and 2 nucleotide changes in subtype 1b isolates (McCown 2009).

	V36A/M	T54S/A	V55A	Q80R/K	R155K/ T/Q	A156S	A156T/ V	D168A/ V/T/H	V170A /T
Telaprevir (linear)			*						*
Boceprevir (linear)							*		
SCH 900518 (linear)									
BILN-2061 ** (macrocyclic)									
R7227/ITMN-191 (macrocyclic)						*	*		
MK7009 (macrocyclic)									
TMC435350 (macrocyclic)									
BI 201335 (macrocyclic?)									

** mutations associated with resistance* in vitro *but not described in patients.*

Table 5. Resistance mutations to HCV NS3 protease inhibitors.

Boceprevir

In the replicon system, mutations have been seen at three positions that confer boceprevir resistance (Table 5). T54A, A156S and V170A confer low level resistance to boceprevir whereas A156T, which also confers telaprevir and ciluprevir resistance, exhibits greater levels of resistance (Tong 2006). In patients with chronic hepatitis C three additional mutations were detected during boceprevir monotherapy (V36G/M/A, V55A, R155K) (Susser 2009). In a number of these patients at one year and in single patients at even 4 years after stopping boceprevir treatment resistant variants could still be detected in the HCV quasispecies by clonal sequence analysis (Susser 2009). However, another study revealed that the antiviral activity of boceprevir was not different in people whether they had or had not been previously treated with PEG-IFN α (Vermehren 2009).

NS4A inhibitors

ACH-806

ACH-806 inhibits the NS3/4A protease by a different mechanism than peptidomimetic NS3 inhibitors. ACH-806 binds to newly synthesized NS4A molecules, which leads to the blockade of their assembly with NS3 proteins. A phase Ib trial in genotype 1-infected patients demonstrated that ACH-806 has a significant inhibitory impact on HCV replication (Pottage 2007). Although the development of ACH-806 was halted because of reversible serum creatinine elevations during treatment, the concept of NS4A inhibi-

tion was proven. Importantly, no cross-resistance between ACH-806 and peptidomimetic NS3/4A protease inhibitors was observed *in vitro* (Wyles 2008; Yang 2008). Novel NS4A inhibitors (e.g., ACH-1095) are currently in preclinical development.

HCV replication

Molecular biology of HCV replication

HCV replication predominantly takes place in hepatocytes and is initiated by the formation of the replication complex. The replication complex is a highly structured association of viral proteins and RNA, of cellular proteins and cofactors, and of rearranged intracellular lipid membranes derived from the endoplasmic reticulum (Moradpour 2007; Pawlotsky 2007) (Figure 10).

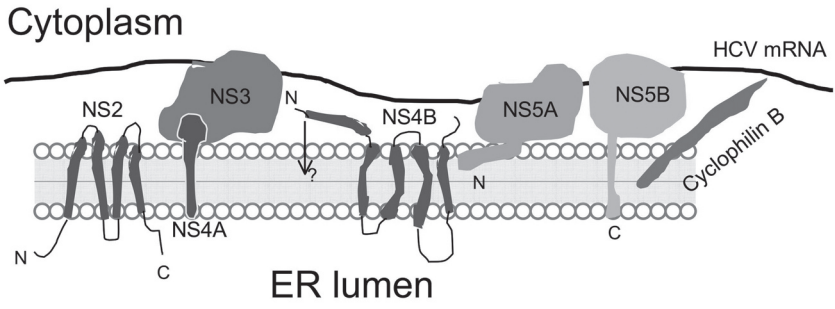


Figure 10. The HCV replication complex.

NS5B is an RNA-dependent RNA polymerase that catalyzes the synthesis of a complementary negative-strand RNA by using the positive-strand RNA genome as a template (Lesburg 1999; Bartenschlager 2004) (Figure 11). From this newly synthesized negative-strand RNA, numerous RNA strands of positive polarity are produced by NS5B activity which serve as templates for further replication and polyprotein translation. Because of poor fidelity leading to a high rate of errors in its RNA sequencing, numerous different isolates are generated during HCV replication in a given patient, termed HCV quasispecies. It is thought that due to the lack of proof-reading of the NS5B polymerase together with the high replication of HCV, every possible mutation is generated each day (Weiner 1991).

NS5A seems to play a manifold role in HCV replication (Lindenbach 2005). It has been shown that NS5A is involved in the early formation of the replication complex by interacting with intracellular lipid membranes (Lindenbach 2005; Appel 2008). In this context NS5A seems to serve as a channel that helps to protect and direct viral RNA within the membranes of the replication complex (Tellinghuisen 2005). It has also been demonstrated that NS5A is able to interact with NS5B, which results in an enhanced activity of the HCV RNA polymerase. Besides its regulatory impact on HCV replication, NS5A has been shown to modulate host cell signaling pathways,

which, for example, has been associated with interferon resistance (Wohnsland 2007). Mutations within the NS5A protein have been clinically associated with resistance and/or sensitivity to IFN-based antiviral therapy (Pascu 2004; Wohnsland 2007).

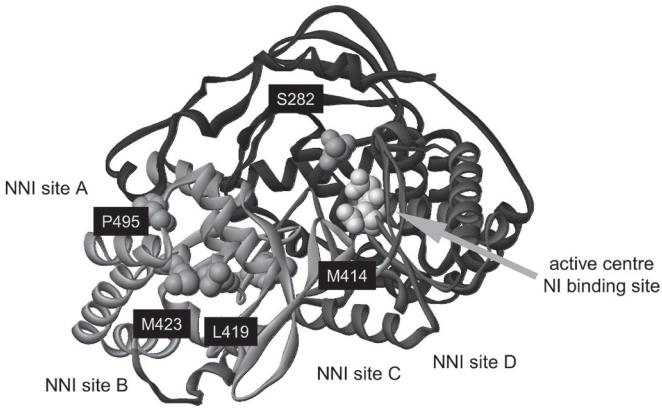


Figure 11. Structure of the HCV NS5B RNA polymerase and binding sites.

NS4B is another protein involved in the formation of the replication complex. NS4B comprises at least four transmembrane domains serving as membrane anchors in the membranous web of the replication complex (Elazar 2004). However, details of the NS4B function remain to be elucidated.

Besides these viral proteins, the cellular protein cyclophilin B has been found to be involved in HCV replication. Cyclophilin B is expressed in many human tissues and provides a cis-trans isomerase activity that supports the folding and function of many proteins. It was shown that cyclophilin B positively regulates NS5B and thus facilitates HCV replication (Flisiak 2007). The observation that cyclosporin A has an antiviral impact on HCV mediated by cyclophilin B inhibition *in vitro* has led to current developments of non-immunosuppressive cyclophilin B inhibitors.

Compounds targeting HCV replication

NS5B polymerase inhibitors

NS5B RNA polymerase inhibitors can be divided into two distinct categories. Nucleoside analogue inhibitors (NIs) like valopicitabine (NM283), R7128, R1626, PSI-7851 or IDX184 mimic the natural substrates of the polymerase and are incorporated into the growing RNA chain, thus causing direct chain termination by blocking the active site of NS5B (Koch 2006; Koch 2007). Because the active centre of NS5B is a highly conserved region of the HCV genome, NIs are potentially effective against different genotypes. Single amino acid substitutions in every position of the active centre may result in loss of function. Thus, there is a relatively high genetic barrier in the development of resistances to NIs.

In contrast to NIs, the heterogeneous class of non-nucleoside inhibitors (NNIs) achieves NS5B inhibition by binding to different allosteric enzyme sites, which results in conformational protein change before the elongation complex is formed (Beaulieu 2007). For allosteric NS5B inhibition high chemical affinity is required. NS5B is structurally organized in a characteristic "right hand motif", containing finger, palm and thumb domains, and offers at least four NNI binding sites, a benzimidazole-(thumb 1)-, thiophene-(thumb 2)-, benzothiadiazine-(palm 1)- and benzofuran-(palm 2)-binding site (Lesburg 1999; Beaulieu 2007) (Figure 12). Because of their distinct binding sites, different polymerase inhibitors can theoretically be used in combination or in sequence to manage the development of resistance. Because NNIs bind distantly to the active centre of NS5B, their application may rapidly lead to the development of resistant mutants *in vitro* and *in vivo*. Moreover, mutations at the NNI binding sites do not necessarily lead to impaired function of the enzyme.

Below, selected nucleoside, non-nucleoside and NS5A inhibitors are described (Figure 12).

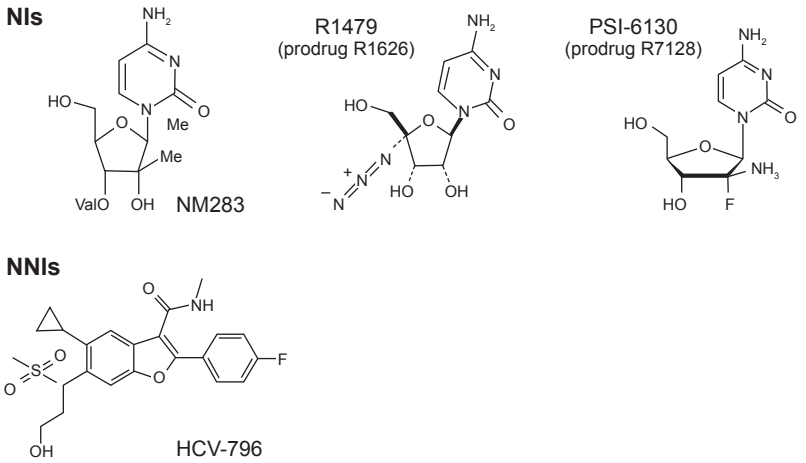


Figure 12. Molecular structure of selected NS5B polymerase inhibitors.

Nucleoside analogs

Valopicitabine

Valopicitabine (NM283, 2'-C-methylcytidine/NM107), the first nucleoside inhibitor investigated in patients with chronic hepatitis C, showed little antiviral activity. Due to gastrointestinal side effects its clinical development was stopped.

The second nucleoside inhibitor to be reported on in patients with chronic hepatitis C was R1626 (4'-azidocytidine/PSI-6130). A phase I study in genotype 1-infected patients observed a high antiviral activity at high doses of R1626 (Pockros 2008). No viral breakthrough with selection of resistant variants was reported from monotherapy or

combination studies with pegylated interferon ± ribavirin. Due to severe lymphopenia and infectious disease adverse events further development of R1626 was stopped.

R7128, another nucleoside polymerase inhibitor with antiviral activity in genotype 1 infected patients in phase I monotherapy studies, is currently being investigated in phase II clinical trials in genotypes 1, 2, and 3-infected patients in combination with PEG-IFN and ribavirin as well as in combination with the protease inhibitor R7227/ITMN-191 (Gane 2009; Lalezari 2008; Sarrazin 2009). In these studies no viral breakthrough via selection of resistant variants was reported.

Recently, two other polymerase inhibitors (PSI-7851 and IDX184) were evaluated in phase I clinical trials in patients with chronic hepatitis C (Sarrazin 2009). For a summary of the antiviral activity of nucleoside polymerase inhibitors see Figure 13.

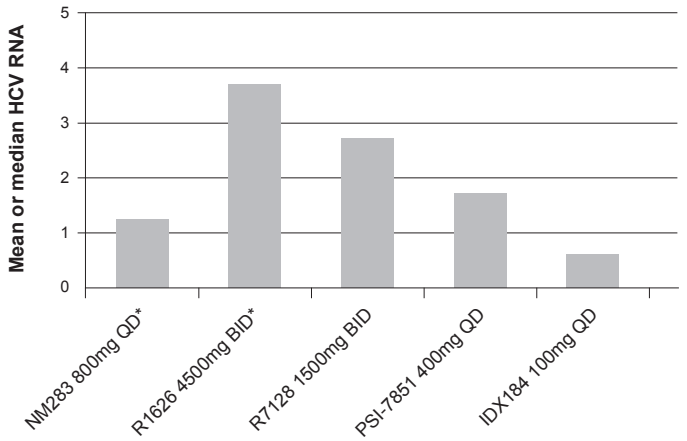


Figure 13. Antiviral activity of nucleoside analogue NS5B polymerase inhibitors.

Non-nucleoside analogs

At least 4 different allosteric binding sites have been identified for the inhibition of the NS5B polymerase by non-nucleoside inhibitors. An overview of the antiviral activities of non-nucleoside polymerase inhibitors in monotherapy studies is shown in Figure 14.

NNI site 1 inhibitors (thumb 1 / benzimidazole site)

BILB1941, BI207127 and MK-3281 are NNI site 1 inhibitors investigated in phase I clinical trials and have shown little to modest antiviral activity (Erhard 2009; Shi 2009; Sarrazin 2009). No viral breakthrough via selection of resistant variants was seen after 5 days of treatment with BILB1941 or BI207127.

NNI site 2 inhibitors (thumb 2 / thiophene site)

Filibuvir (PF-00868554) is a NNI site 2 inhibitor with modest antiviral activity in a phase I study. In a subsequent triple therapy trial with filibuvir, pegylated interferon α-2a and ribavirin for 4 weeks viral breakthrough was observed in 5/26 patients.

VCH-759, VCH-916 and VCH-222 are three other NNI site 2 inhibitors with antiviral activity in monotherapy studies (Cooper 2009; Sarrazin 2009). For VCH-759 as well as VCH-916 viral breakthroughs via selection of resistant variants were observed.

NNI site 3 inhibitors (palm 1 / benzothiadiazine site)
ANA598 is a NNI site 3 inhibitor that displayed antiviral activity during treatment of genotype 1 infected patients. Viral breakthrough was not observed during this short monotherapy trial.

NNI site 4 inhibitors (palm 2 / benzofuran site)
Monotherapy with the NNI site 4 inhibitor HCV-796 showed low antiviral activity in genotype 1 infected patients (Kneteman 2009; Villano 2007). Viral breakthrough was associated with selection of resistant variants conferring a medium to high level of phenotypic resistance. For GS-9190 low antiviral activity was observed in a clinical study and variants conferring resistance were identified in the beta-hairpin of the polymerase. ABT-333, another palm site inhibitor, demonstrated antiviral activity in patients with genotype 1 infection and from *in vitro* replicon as well as clinical studies specific variants were observed as main resistance mutations.

NS5A inhibitor

In a single ascending dose study it was shown that inhibition of the NS5A protein with BMS-790052 leads to a sharp initial decline of HCV RNA concentrations (Nettles 2008). BMS-790052 is the first NS5A inhibitor binding to domain I of the NS5A protein, which was shown to be important for regulation of HCV replication. No clinical data on resistance to this class of drugs have been presented yet and results of multiple dose studies are eagerly anticipated.

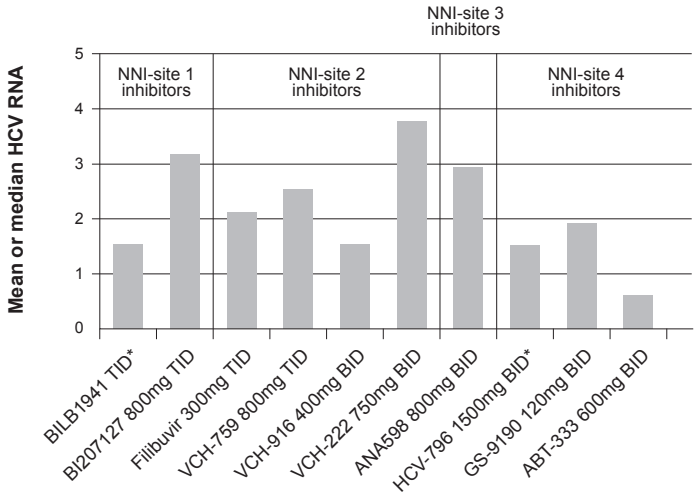


Figure 14. Antiviral activity of non-nucleoside analogue NS5B polymerase inhibitors.

Drugs targeting viral RNA

IRES inhibitors

In contrast to Cap-dependent cellular mRNA translation, HCV RNA translation is mediated by the internal ribosome entry site (IRES). The IRES assembles with several cellular and viral proteins including the 40S ribosomal subunit. The underlying molecular interactions are highly specific making IRES a suitable target for small molecule inhibitors.

VGX-410C is an orally bioavailable IRES inhibitor blocking the binding of IRES to the 40S ribosomal subunit. VGX-410C has been evaluated in one phase II clinical trial in patients with chronic hepatitis C. However, its antiviral activity was low and the development of VGX-410C was stopped. Other IRES inhibitors are in preclinical development.

Ribozymes

Ribozymes are RNA molecules that catalyze the sequence-specific cleavage of a target RNA. *In vitro* studies have identified several ribozymes specifically directed to sequences in the HCV genome. Heptazyme is an orally bioavailable, chemically modified ribozyme cleaving a sequence within the highly conserved IRES region (Foster 2004). Heptazyme inhibits HCV replication *in vitro*, but its development was halted due to animal toxicity. It is unclear whether ribozymes will move forward into clinical studies.

Antisense oligonucleotides

Antisense oligonucleotides are short, single-stranded RNA or DNA molecules with a sequence complementary to target RNA. Hybridisation with the target RNA can prevent its translation. In cell culture systems, several antisense oligonucleotides complementary to sequences in the highly conserved 5' UTR have been shown to inhibit HCV RNA replication (Guerniou 2007). The antisense oligonucleotide ISIS-14803 was evaluated in phase II clinical trials in patients with chronic hepatitis C, but its development was stopped due to insufficient antiviral efficacy (Soler 2004).

Viral assembly and release

Molecular biology of viral assembly and release

Viral assembly and release are still poorly understood. However, interactions between the HCV core protein and genomic RNA have been described, which probably initiates the formation of coated particles (Chang 1994; Suzuki 1995). Furthermore, interactions of the structural proteins E1 and E2 with endoplasmic reticulum membranes seem to be essential for viral assembly. On the cellular side, a-glucosidases, enzymes necessary in the processing of some glycoproteins, are important for the correct folding and assembly of HCV envelope proteins. These a-glucosidase inhibitors were shown to be able to interrupt viral assembly with a significant antiviral impact *in vitro* and *in vivo* (Kaita 2007).

Compounds targeting viral assembly and release

So far, drugs that target the host proteins that disturb viral assembly and release have been developed. These are summarized below.

Combination therapies of specific antivirals

It is a fundamental question whether SVR can be achieved with combination therapies of different STAT-C compounds without PEG-IFN α plus ribavirin. The INFORM-1 study was the first to evaluate the combination of a polymerase inhibitor (R7128) plus an NS3 inhibitor (R7227/ITMN191). In this proof of principle study, patients were treated with both compounds for up to 2 weeks. HCV RNA concentrations decreased by up to 5.2 $\log_{10}$ IU/ml, no viral breakthrough was observed, and HCV RNA was undetectable at the end of dosing in up to 63% of treatment-naïve patients (Gane 2009). Future clinical trials need to address whether long-term suppression of HCV replication or even SVR can be achieved with these and other antiviral combination therapies. Studies with several compounds are underway (R7128+R7227, VX-950 +VCH222, BMS790052+BMS650032, BI201335+BI207127). Intercompany collaborations might also be interesting.

Host proteins as targets in treating hepatitis C

Cyclophilin B inhibitors

Cyclophilin B is an ubiquitously-expressed cis-trans isomerase involved in the folding of various proteins. It has been shown that cyclophilin B enhances NS5B activity and thus facilitates HCV replication. The most advanced cyclophilin inhibitor is Debio-025. Debio-025 is an orally bioavailable cyclophilin B inhibitor exerting an antiviral impact on both HCV and HIV replication. In clinical trials in HIV- and HCV-coinfected patients, treatment with 1200 mg Debio-025 twice daily for two weeks led to a mean maximal $\log_{10}$ reduction of HCV RNA of 3.6 and of HIV DNA of 1.0 (Flisiak 2008). Debio-025 was well tolerated and no viral breakthrough occurred over the 14 days of treatment.

Combination therapy of Debio-025 200 mg, 600 mg or 1000 mg plus PEG-IFN α-2a was evaluated in a double-blind placebo-controlled phase II trial in treatment-naïve patients monoinfected with HCV genotypes 1, 2, 3 or 4. Treatment was administered for 29 days. Mean $\log_{10}$ reductions in HCV RNA at day 29 were 4.75 (1000 mg), 4.61 (600 mg) and 1.8 (200 mg) in the combination therapy groups compared to 2.49 (PEG-IFN α-2a alone) and 2.2 (1000 mg Debio-025 alone) in the monotherapy groups. No differences in antiviral activity were observed between individuals infected with different genotypes. Debio-025 was safe and well-tolerated but led to a reversible bilirubin increase in some patients treated daily with 1000 mg Debio-025 (Flisiak 2009). Studies determining SVR rates of combination therapy with Debio-025 and PEG-IFN α-2a are ongoing.

Glucosidase inhibitors - Celgosivir (MX-3253)

Celgosivir is an orally bioavailable prodrug of the a-glucosidase inhibitor castanospermine. In monotherapy, treatment-naive genotype 1 patients received either 200 mg or 400 mg celgosivir once daily or 200 mg twice daily. Celgosivir was generally well-tolerated. However, significant HCV RNA reductions were observed in only a minority of patients.

To assess whether celgosivir plus PEG-IFN α-2b plus ribavirin can enhance virologic response a study was done in genotype 1 patients who previously failed to respond to standard treatment. 400 mg celgosivir was administered QD over a treatment period of 12 weeks. Early virologic response rates (defined as 2 $\log_{10}$ or greater reductions in HCV RNA at week 12) were 42% after triple therapy compared to 10% in the control group treated with PEG-IFN α-2b and ribavirin alone. Mean $\log_{10}$ reductions in HCV RNA were 1.63 (triple therapy) versus 0.92 (control treatment). SVR rates, however, remain to be determined. No serious adverse events related to celgosivir were reported (Kaita 2007).

Nitazoxanide

Nitazoxanide with its active metabolite tizoxanide is a thiazolide antiprotozoal approved for the treatment of *Giardia lamblia* and *Cryptosporidium parvum* infections. *In vitro* studies have revealed an essential inhibitory impact on HCV replication by a still unknown mechanism.

Nitazoxanide monotherapy over 24 weeks led to an SVR rate of 17.4% in treatment-naïve HCV genotype 4 patients (Rossignol 2008).

Two clinical trials evaluated monotherapy as lead-in treatment, followed by the combination of nitazoxanide plus PEG-IFN α-2a. In both studies, mainly treatment-naïve genotype 4 patients were enrolled. The first study evaluated 12 weeks of nitazoxanide monotherapy 500 mg twice daily, followed by either 36 wks of nitazoxanide plus PEG-IFN a-2 and ribavirin or by 36 wks of nitazoxanide plus PEG-IFN α-2a alone. The control group was treated for 48 wks with PEG-IFN α-2a and ribavirin. SVR rates were 79% for 12 wks of nitazoxanide, followed by 36 wks of PEG-IFN α-2a and ribavirin, and 64% for 12 wks of nitazoxanide, followed by 36 wks of PEG-IFN α-2a, compared to 45% in the control group (Rossignol 2009). However, the relatively low SVR rate in the control group remains to be explained because in previous studies higher SVR rates in genotype 4 patients were reported (approximately 70%). A second study evaluating a shorter lead-in phase of 4 wks of nitazoxanide with no differences in rapid viral, early viral and end-of-treatment responses compared to the 12 weeks of lead-in treatment (Rossignol 2008). SVR rates were up to 80% with best results in those groups with a 4 week lead-in phase. In general, nitazoxanide was well tolerated and no serious adverse events were reported.

Currently, a phase II clinical trial is looking at the benefit of nitazoxanide in addition to PEG-IFN α plus ribavirin in HCV genotype 1 non-responders. Preliminary data are encouraging with an overall EVR rate of 50% after 4 weeks of nitazoxanide followed by 48 weeks of the triple combination (Bacon 2009).

Inosine monophosphate dehydrogenase inhibitors (VX-497)

The inosine monophosphate dehydrogenase (IMPDH) is an ubiquitously-expressed cellular enzyme catalysing the production of guanosine nucleotides. *In vitro* studies have revealed a significant antiviral impact of IMPDH inhibition on the replication of many RNA or DNA viruses.

VX-497 is an orally bioavailable, reversible IMPDH inhibitor. A phase II study in genotype 1 non-responders evaluated VX-497 200mg and 400mg monotherapy for 28 days. VX-497 was well-tolerated and led to a significant decrease of serum aminotransferases. HCV RNA viral load, however, was not altered.

Amantadine

Amantadine is approved for the treatment of Parkinson's Disease. In addition, antiviral activity of amantadine against a number of RNA viruses including influenza A has been shown. *In vitro* studies have observed that amantadine may be able to interfere with the function of p7, which has been shown to form an ion channel. However, this was not confirmed by a recent study in the HCV replicon (Steinmann 2007). Furthermore, no specific mutations or mutational patterns have been identified in the HCV p7 protein in association with sensitivity or resistance to amantadine (Mihm 2006).

Clinical trials evaluating amantadine in patients with chronic hepatitis C have yielded inconsistent results. Amantadine monotherapy at a dose of 100 mg twice daily for six months had no significant antiviral effect in patients with chronic hepatitis C (Andant 2000). In a randomized, double-blind, placebo-controlled clinical trial, the combination of IFN α with amantadine did not increase SVR rates compared to IFN a alone (Zeuzem 2000). The combination of amantadine with PEG-IFN α plus ribavirin showed slightly increased SVR rates in genotype 1 patients who previously were non-responders but showed no significant benefit in treatment-naïve patients or relapsers in comparison to standard treatment (Deltenre 2004; Ferenci 2006; Maynard 2006). Most recently, a large double-blind, randomized controlled trial was conducted in more than 700 treatment-naive HCV genotype 1 infected patients in Germany. In this study, no significant difference of triple therapy including amantadine 200 mg twice daily in comparison to the standard treatment of PEG-IFN α-2a plus ribavirin was observed (von Wagner 2008).

Novel interferons

Both current forms of PEG-IFN α are injected once weekly. For some patients less frequent injections are likely to be associated with fewer side effects, better tolerance, and therefore, better adherence. Novel interferons are in development designed with longer half-life and sustained plasma concentrations, resulting in the opportunity of reducing injection frequency. In addition, several companies aim to optimize the immunomodulatory and antiviral effects of interferon a to increase virologic response rates especially in patients nonresponsive to the current interferon α-2a/b based regimens. Another approach is the generation of chemically modified interferons to achieve oral bioavailability. The most promising developments in this field are summarized below.

Albinterferon

Albinterferon is a novel recombinant interferon comprising IFN α-2b genetically fused to human albumin. Human albumin has a half-life of approximately 20 days in human blood. In early clinical trials, albinterferon was shown to be safe, and constant plasma concentrations were achieved by injection every other week.

A subsequent phase II study involving 458 treatment-naïve genotype 1 patients investigated antiviral efficacy of albinterferon plus ribavirin compared to standard treatment. SVR rates were 58.5% and 55.5% in patients receiving 900 mg or 1200 mg albinterferon every other week, 50.9% in patients receiving 1200 mg albuferon once every 4 weeks and 57.9% in the control group (Zeuzem 2008).

Another study including 115 genotype 1 non-responders to standard treatment evaluated the combination of ribavirin plus 900 mg to 1200 mg albinterferon every second week for 48 or 72 weeks. The overall SVR rate was 17.4%, which is higher than SVR rates published for retreatment for 48 weeks and comparable to SVR rates after 72 weeks of re-treatment with pegylated interferons and ribavirin (Balan 2006).

In general, albinterferon was well tolerated. However, in some patients receiving 1200 mg either every second or every fourth week, serious pulmonary adverse events (pneumonitis) were observed, leading to dose reductions to 900 mg in the phase III approval studies (ACHIEVE trials). Generally, pneumonitis has also been observed upon administration of interferons and pegylated interferons, representing a class effect of interferon a.

The results of phase III approval studies have been published recently. SVR rates in both HCV genotype 1 patients (ACHIEVE 1) and HCV genotype 2/3 patients (ACHIEVE 2) were equivalent after treatment with 900 mg albuferon every second week plus ribavirin compared to the standard of care (Sulkowski 2009; Benhamou 2009).

IFN α-2bXL

IFN α-2bXL is a recombinant IFN α-2b that provides a sustained release of active IFN α with reduced peak serum concentrations. The pharmacokinetics may result in a reduced rate of side effects and may lead to increased tolerability. In a phase Ib clinical trial, genotype 1 patients (treatment-naïve, non-responders and relapsers) were treated with weekly injections of either IFN α-2bXL or PEG-IFN α-2b for 14 days. IFN α-2bXL showed an almost significant superior antiviral activity at the end of the dosing period. Importantly, IFN α-2bXL led less frequently to treatment-related adverse events (Trepo 2007). Extended clinical trials evaluating IFN α-2bXL plus ribavirin are ongoing.

Controlled-release recombinant interferon alpha-2b (Locteron)

Locteron is a novel recombinant IFN α-2b formulation that provides sustained IFN-a plasma levels when injected every other week. The SELECT-1 trial (phase II) evaluated locteron for twelve weeks at doses from 160 µg to 640 µg every other week plus ribavirin in treatment-naïve genotype 1 patients. Antiviral activity was dose-dependent and led, at higher doses, to comparable RVR and early virologic response (EVR) rates with standard interferons. Side effects were equivalent to standard interferons but administration was more convenient for patients (Herrmann 2008). A phase IIb study has recently started.

Interferon lambda

Interferon l is a type 3 interferon that binds to a unique receptor predominantly expressed in the liver. IL-28B is another type 3 interferon, which signals through the same receptor as interferon l. This might be important because recent studies have shown that distinct polymorphisms near the IL-28B gene are strongly associated with SVR and spontaneous clearance in patients with HCV genotype 1 infection (Thomas 2009; Ge 2009). Recently, a phase I clinical trial evaluating pegylated interferon l plus ribavirin was completed (Muir 2009). Interferon l was well tolerated and the majority of patients achieved a greater than 2 $\log_{10}$ decline of HCV RNA within 4 weeks.

Novel ribavirin derivates

Taribavirin

Taribavirin is an orally bioavailable prodrug of ribavirin, which was designed to avoid events of severe hemolytic anemia frequently necessitating dose reduction or treatment termination of ribavirin. Taribavirin is rapidly processed to its active metabolite ribavirin within the hepatocyte, which results in rather low ribavirin plasma concentrations. Taribavirin was shown to be safe in patients with HCV-caused, compensated liver disease.

A phase III clinical trial (VISER 1) evaluated the combination of 600 mg taribavirin together with PEG-IFN α-2b compared to weight-adjusted ribavirin. Rates of anemia were strongly reduced in the taribavirin group (5% versus 24%, defined as Hb <10g/dl) (Benhamou 2009). However, SVR rates were significantly lower in the taribavirin arm. A second trial (VISER 2) evaluated the combination of taribavirin 600 mg twice daily with PEG-IFN α-2a in an equivalent study design (Poordad 2008). Rates of anemia and SVR were comparable to those seen in VISER 1. Because a retrospective study analysis of VISER 1 and 2 indicate that higher plasma levels of taribavirin are associated with higher SVR rates, a subsequent trial evaluating body weight-adjusted taribavirin (20, 25, 30 mg/kg body weight) doses was performed. RVR, EVR and SVR rates as well as safety and tolerability between taribavirin 25 mg/kg and ribavirin were comparable in this trial with the exception of a significantly lower rate of anemia (16% versus 33%) in the taribavirin arm (Poordad 2008). Phase III studies have been initiated and taribavirin may be a good partner in STAT-C regimens.

Outlook

There is an urgent need for improvement in the treatment of hepatitis C as only around 40-50% of all patients infected with HCV can currently be cured. Moreover, interferon α-based treatment regimes require long durations and frequently cause severe side effects, challenging patient adherence. It can be anticipated that the tremendous progress in developing new antivirals will enrich future treatment regimens, especially in difficult-to-treat populations like genotype 1-infected individuals or patients suffering from reinfection after liver transplantation. Various STAT-C compounds have been shown to substantially inhibit HCV replication *in vitro* and in clinical trials. However, monotherapy results in the selection of resistant quasispecies causing viral breakthrough and loss of antiviral activity. This applies especially to the HCV protease inhibitors and to non-nucleoside inhibitors of the HCV RNA polymerase. Thus, monotherapy with small molecule inhibitors does not appear to be valuable in treating hepatitis C. However, the addition of pegylated interferons plus ribavirin has been shown to be capable of a successful reduction in the development of resistance. Based on phase II study results, these triple therapy regimes are sufficient to improve SVR rates and to shorten treatment duration. Importantly, the resistance profile of many STAT-C compounds differs and cross-resistance between compounds targeting different viral and cellular proteins is unlikely. Analogous to HIV therapy, combining drugs with different modes of action is expected to be sufficient to inhibit viral resistance. *In vitro* experiments have demonstrated that many novel antivirals with complementary mechanisms of action

have a more than additive antiviral impact when applied in combination and reduce the frequency of resistance. *In vivo*, combination therapies of these newer antivirals remain to be evaluated. In particular, it needs to be determined whether interferon-free combinations of newer antivirals are feasible.

References

Ali S, Leveque V, Le Pogam S, Ma H, et al. (2008). Selected replicon variants with low level in vitro resistance to the hepatitis C virus NS5B polymerase inhibitor PSI-6130 lack cross-resistance with R1479. Antimicrob Agents Chemother 52:4356-4369.

Andant C, Lamoril J, Deybach JC, Jouet P, et al. (2000). "Amantadine for chronic hepatitis C: pilot study in 14 patients." Eur J Gastroenterol Hepatol 12(12): 1319-22.

Appel N, Zayas M, Miller S, Krijnse-Locker J, et al. (2008). "Essential role of domain III of nonstructural protein 5A for hepatitis C virus infectious particle assembly." PLoS Pathog 4(3): e1000035.

Bacon B, Shiffman M, Lim J, Berman A, et al. (2009). Phase 2 Randomized, Double-Blind, Placebo-Controlled Study of Nitazoxanide Plus Peginterferon and Ribavirin in HCV Genotype 1 Patients: Interim Analysis Shows Increase in EVR. J. Hepatol. 50(Suppl.1): 381.

Balan V, Nelson D, Sulkowski M, Everson G, et al. (2006). "A Phase I/II study evaluating escalating doses of recombinant human albumin-interferon-alpha fusion protein in chronic hepatitis C patients who have failed previous interferon-alpha based therapy." Antivir. Ther. 4(11): 35-45.

Bartels DJ, Zhou Y, Zhang EZ, Marcial M, et al. (2008). Natural prevalence of hepatitis C virus variants with decreased sensitivity to NS3.4A protease inhibitors in treatment-naive subjects. J Infect Dis 198:800-807.

Bartenschlager R, Frese MPietschmann T (2004). "Novel insights into hepatitis C virus replication and persistence." Adv Virus Res 63: 71-180.

Barth H, Liang TJBaumert TF (2006). "Hepatitis C virus entry: molecular biology and clinical implications." Hepatology 44(3): 527-35.

Bartosch B, Cosset FL (2006). "Cell entry of hepatitis C virus." Virology 348(1): 1-12.

Bartosch B, Dubuisson JCosset FL (2003). "Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes." J Exp Med 197(5): 633-42.

Bartosch B, Vitelli A, Granier C, Goujon C, et al. (2003). "Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor." J Biol Chem 278(43): 41624-30.

Beaulieu PL (2007). "Non-nucleoside inhibitors of the HCV NS5B polymerase: progress in the discovery and development of novel agents for the treatment of HCV infections." Curr Opin Investig Drugs 8(8): 614-34.

Benhamou Y, Moussalli J, Ratziu V, Lebray P, et al. (2009). Results of a prove of concept study (C210) of telaprevir monotherapy and in combination with peginterferon alfa-2a and ribavirin in treatment-naive genotype 4 HCV patients. J Hepatol;50(Suppl.1): 6.

Benhamou Y, Nelson D, Chuang WL, Lawitz E, et al. (2009). Efficacy and safety results of albinterferon-alfa-2b and ribavirin and treatment-naïve subjects with chronic hepatitis C genotype 2 or 3. Hepatology 50(Suppl.4): 1024.

Benhamou Y, Afdhal NH, Nelson DR, Shiffman ML, et al. (2009). A phase III study of safety and efficacy of viramidine versus ribavirin in treatment-naïve patients with chronic hepatitis C: VISER1 results. Hepatology 50(3): 717-26.

Carrere-Kremer S, Montpellier-Pala C, Cocquerel L, Wychowski C, et al. (2002). "Subcellular localization and topology of the p7 polypeptide of hepatitis C virus." J Virol 76(8): 3720-30.

Chandra P, Raible D, Harper D, Speth J, et al. (2006). "Antiviral activity of the non-nucleoside polymerase inhibitor, HCV-796, in patients with chronic hepatitis C virus: preliminary results from a randomized, double-blind, placebo-controlled, ascending multiple dose study." Gastroenterology 130(Suppl. 2): 748A.

Chang SC, Yen JH, Kang HY, Jang MH, et al. (1994). "Nuclear localization signals in the core protein of hepatitis C virus." Biochem Biophys Res Commun 205(2): 1284-90.

Chen CM, He Y, Lu L, Lim HB, et al. (2007). "Activity of a potent hepatitis C virus polymerase inhibitor in the chimpanzee model." Antimicrob Agents Chemother 51(12): 4290-6.

Clarke D, Griffin S, Beales L, Gelais CS, et al. (2006). "Evidence for the formation of a heptameric ion channel complex by the hepatitis C virus p7 protein in vitro." J Biol Chem 281(48): 37057-68.

Coelmont L, Paeshuyse J, Windisch MP, De Clercq E, et al. (2006). “Ribavirin antagonizes the in vitro anti-hepatitis C virus activity of 2’-C-methylcytidine, the active component of valopicitabine.” Antimicrob Agents Chemother 50(10): 3444-6.

Collier AJ, Gallego J, Klinck R, Cole PT, et al. (2002). “A conserved RNA structure within the HCV IRES eIF3-binding site.” Nat Struct Biol 9(5): 375-80.

Cooper C, Lawitz EJ, Ghali P, Rodriguez-Torres M, et al. (2009). Evaluation of VCH-759 monotherapy in hepatitis C infection. J. Hepatol. 51:39-46.

Courcambeck J, Bouzidi M, Roche G, Pepe G, et al. (2008). “Hepatitis C Virus Drug Resistance: The case of NS3-4A macrocyclic protease inhibitor ITMN-191.” J Hepatol 48(Suppl. 2): 286.

Davis GL, Nelson DR, Terrault N, Pruett TL, et al. (2005). “A randomized, open-label study to evaluate the safety and pharmacokinetics of human hepatitis C immune globulin (Civacir) in liver transplant recipients.” Liver Transpl 11(8): 941-9.

Deltenre P, Henrion J, Canva V, Dharancy S, et al. (2004). “Evaluation of amantadine in chronic hepatitis C: a meta-analysis.” J Hepatol 41(3): 462-73.

Diedrich G (2006). “How does hepatitis C virus enter cells?” Febs J 273(17): 3871-85.

Elazar M, Liu P, Rice CMGlenn JS (2004). “An N-terminal amphipathic helix in hepatitis C virus (HCV) NS4B mediates membrane association, correct localization of replication complex proteins, and HCV RNA replication.” J Virol 78(20): 11393-400.

Erhardt A, Deterding K, Benhamou Y, Reiser M, et al. (2009). Safety, pharmacokinetics and antiviral effect of BILB 1941, a novel hepatitis C virus RNA polymerase inhibitor, after 5 days oral treatment. Antivir Ther 14:23-32.

Evans MJ, von Hahn T, Tscherne DM, Syder AJ, et al. (2007). “Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry.” Nature 446(7137): 801-5.

Ferenci P, Formann E, Laferl H, Gschwantler M, et al. (2006). “Randomized, double-blind, placebo-controlled study of peginterferon alfa-2a (40KD) plus ribavirin with or without amantadine in treatment-naive patients with chronic hepatitis C genotype 1 infection.” J Hepatol 44(2): 275-82.

Flisiak R, Dumont JMCrabbe R (2007). “Cyclophilin inhibitors in hepatitis C viral infection.” Expert Opin Investig Drugs 16(9): 1345-54.

Flisiak R, Horban A, Gallay P, Bobardt M, et al. (2008). The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology 47: 817-826.

Flisiak R, Feinman SV, Jablkowski M, Horban A, Kryczka W, Pawlowska M, Heathcote JE, Mazzella G, Vandelli C, Nicolas-Metral V, Grosgurin P, Liz JS, Scalfaro P, Porchet H, Crabbe R. The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naive hepatitis C patients. Hepatology 2009;49:1460-1468..

Flisiak R, Horban A, Gallay P, Bobardt M, et al. (2008). “The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus.” Hepatology 47(3): 817-26.

Forestier N, Reesink HW, Weegink CJ, McNair L, et al. (2007). “Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C.” Hepatology 46(3): 640-8.

Forestier N, Larrey DG, Guyader D, et al. (2008). Treatment of Chronic Hepatitis C Virus (HCV) Genotype 1 Patients with the NS3/4A Protease Inhibitor ITMN-191 Leads to Rapid Reductions in Plasma HCV RNA: Results of a Phase 1b Multiple Ascending Dose (MAD) Study. Hepatology. 48(Suppl 4): 1132A.

Forestier N, Susser S, Welker MW, Karey U, et al. (2008). Long term follow-up of patients previously treated with telaprevir. Hepatology;48(suppl.4):760A.

Forestier N, Larrey D, Marcellin P, Zeuzem S, et al. (2009). Antiviral Activity and Safety of ITMN-191 in Combination with Peginterferon Alfa-2a and Ribavirin in Patients with Chronic Hepatitis C Virus (HCV). J. Hepatol. 50(Suppl. 1): 85.

Foster G (2004). “Past, present, and future hepatitis C treatments.” Semin Liver Dis 24(Suppl. 2): 97-104.

Foster GR, Hezode C, Bronowicki JP, Carosi G, et al. (2009). Activity of telaprevir alone or in combination with peginterferon alfa-2a and ribavirin in treatment-naive genotype 2 and 3 hepatitis-C patients: interim results of study C209. J Hepatol 50(Suppl.1): 22.

Fried MW (2002). “Side effects of therapy of hepatitis C and their management.” Hepatology 36(5 Suppl 1): S237-44.

Gallego JVarani G (2002). “The hepatitis C virus internal ribosome-entry site: a new target for antiviral research.” Biochem Soc Trans 30(2): 140-5.

Gane EJ, Rodriguez-Torres M, Nelson DR, et al. (2008) Antiviral Activity of the HCV Nucleoside Polymerase Inhibitor R7128 In HCV Genotype 2 and 3 Prior Non-Responders: Interim Results of R7128 1500mg BID with PEG-IFN and Ribavirin For 28 Days. Hepatology. 48(Suppl 4):1024A..

Gane EJ, Roberts SK, Stedman C, Angus PW, et al. (2009. Combination therapy with a nucleoside polymerase (R7128) and protease (R7227/ITMN-191) inhibitor in HCV: safety, pharmacokinetics, and virologic results from INFORM-1. Hepatology 50(Suppl.4): 394.

Gaudieri S, Rauch A, Pfafferott K, Barnes E, et al. (2009). Hepatitis C virus drug resistance and immune-driven adaptations: relevance to new antiviral therapy. Hepatology 49:1069-1082.

Ge D, Fellay J, Thompson AJ, Simon JS, et al. (2009). Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461(7262):399-401.

Gish RG, Arora S, Rajender Reddy K, Nelson DR, et al. (2007). “Virological response and safety outcomes in therapy-naive patients treated for chronic hepatitis C with taribavirin or ribavirin in combination with pegylated interferon alfa-2a: a randomized, phase 2 study.” J Hepatol 47(1): 51-9.

Guerniou V, Gillet R, Berree F, Carboni B, et al. (2007). “Targeted inhibition of the hepatitis C internal ribosomal entry site genomic RNA with oligonucleotide conjugates.” Nucleic Acids Res 35(20): 6778-87.

Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, et al. (2004). “Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose.” Ann Intern Med 140(5): 346-55.

Herrmann E, Zeuzem S, Dzyublyk I, Moroz L, et al. (2008). “Viral kinetics during treatment with a controlled-release recombinant interferon alfa 2b in genotype 1 chronic hepatitis C patients.” J Hepatol 48(Suppl. 2): 318.

Hezode C, Forestier N, Dusheiko G, Ferenci P, et al. (2009). Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 360:1839-1850.

Hinrichsen H, Benhamou Y, Wedemeyer H, Reiser M, et al. (2004). “Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients.” Gastroenterology 127(5): 1347-55.

Howe A, Cheng H, Johann S, Mullen S, et al. (2006). “Molecular mechanism of hepatitis C virus replicon variants with reduced susceptibility to a benzofuran inhibitor.” Antimicrob. Agents Chemothe. 50(12): 103-13.

Kaita K, Yoshida E, Kunimoto D, Anderson F, et al. (2007). “Phase II proof of concept study of celgosivir in combination with peginterferon alfa-2b and ribavirin in chronic hepatitis C genotype 1 non-responder patients.” J. Hepatol. 46(Suppl.1): 56--57

Kaukinen P, Sillanpaa M, Kotenko S, Lin R, et al. (2006). “Hepatitis C virus NS2 and NS3/4A proteins are potent inhibitors of host cell cytokine/chemokine gene expression.” Virol J 3: 66.

Kim JL, Morgenstern KA, Griffith JP, Dwyer MD, et al. (1998). “Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding.” Structure 6(1): 89-100.

Kim JL, Morgenstern KA, Lin C, Fox T, et al. (1996). “Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide.” Cell 87(2): 343-55.

Klumpp K, Leveque V, Le Pogam S, Ma H, et al. (2006). “The novel nucleoside analog R1479 (4’-azidocytidine) is a potent inhibitor of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture.” J Biol Chem 281(7): 3793-9.

Kneteman NM, Howe AY, Gao T, Lewis J, et al. (2009). HCV796: A selective nonstructural protein 5B polymerase inhibitor with potent anti-hepatitis C virus activity in vitro, in mice with chimeric human livers, and in humans infected with hepatitis C virus. Hepatology 49:745-752.

Koch U, Narjes F (2006). “Allosteric inhibition of the hepatitis C virus NS5B RNA dependent RNA polymerase.” Infect Disord Drug Targets 6(1): 31-41.

Koch U, Narjes F (2007). “Recent progress in the development of inhibitors of the hepatitis C virus RNA-dependent RNA polymerase.” Curr Top Med Chem 7(13): 1302-29.

Koev G, Kati W (2008). “The emerging field of HCV drug resistance.” Expert Opin Investig Drugs 17(3): 303-19.

Kuntzen T, Timm J, Berical A, Lennon N, et al. (2008). Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. Hepatology 48:1769-1778.

Kwo P, Lawitz E, McCone J, Schiff E, et al. (2009). HCV SPRINT-1 final results: SVR 24 from a phase 2 study of boceprevir plus peginterferon alfa-2b/ribavirin in treatment-naive subjects with genotype-1 chronic hepatitis C. J Hepatol 50(Suppl.1): 4.

Lalezari J, Gane J, Rodriguez-Torres M, DeJesus E, et al. (2008). "Potent antiviral activity of the HCV nucleoside polymerase inhibitor R7128 with peg-ifn and ribavirin: interim results of R7128 500mg bid for 28 days." J Hepatol 48(Suppl. 2): 29.

Lamarre D, Anderson PC, Bailey M, Beaulieu P, et al. (2003). "An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus." Nature 426(6963): 186-9.

Lawitz E, Rodriguez-Torres M, Muir AJ, Kieffer TL, et al. (2008). "Antiviral effects and safety of telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in hepatitis C patients." J Hepatol49: 163-69..

Le Pogam S, Jiang WR, Leveque V, Rajyaguru S, et al. (2006). "In vitro selected Con1 subgenomic replicons resistant to 2'-C-methyl-cytidine or to R1479 show lack of cross resistance." Virology 351(2): 349-59.

Le Pogam S, Seshaadri A, Kang H, Kosaka A, et al. (2008). "Low level of resistance, low viral fitness and absence of resistance mutations at baseline quasispecies may contribute to high barrier to R1626 resistance in vivo." J Hepatol 48(Suppl. 2): 10A.

Le Pogam S, Seshaadri A, Kosaka A, Chiu S, et al. (2008). Existence of hepatitis C virus NS5B variants naturally resistant to non-nucleoside, but not to nucleoside, polymerase inhibitors among untreated patients. J Antimicrob Chemother 61:1205-1216.

Lesburg CA, Cable MB, Ferrari E, Hong Z, et al. (1999). "Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site." Nat Struct Biol 6(10): 937-43.

Lin C, Gates CA, Rao BG, Brennan DL, et al. (2005). "In vitro studies of cross-resistance mutations against two hepatitis C virus serine protease inhibitors, VX-950 and BILN 2061." J Biol Chem 280(44): 36784-91.

Lin C. et al (2007). "Telaprevir (VX-950) is a Potent Inhibitor of HCV-NS3 Proteases Derived from Genotype Non-1 HCV-Infected Patients." J. Hepatol. 46(Suppl. 1): 8.

Lin K, Kwong AD, Lin C (2004). "Combination of a hepatitis C virus NS3-NS4A protease inhibitor and alpha interferon synergistically inhibits viral RNA replication and facilitates viral RNA clearance in replicon cells." Antimicrob Agents Chemother 48(12): 4784-92.

Lin K, Perni RB, Kwong AD, Lin C (2006). "VX-950, a novel hepatitis C virus (HCV) NS3-4A protease inhibitor, exhibits potent antiviral activities in HCv replicon cells." Antimicrob Agents Chemother 50(5): 1813-22.

Lindenbach BD, Evans MJ, Syder AJ, Wolk B, et al. (2005). "Complete replication of hepatitis C virus in cell culture." Science 309(5734): 623-6.

Lohmann V, Korner F, Koch J, Herian U, et al. (1999). "Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line." Science 285(5424): 110-3.

Lorenz IC, Marcotrigiano J, Dentzer TGRice CM (2006). "Structure of the catalytic domain of the hepatitis C virus NS2-3 protease." Nature 442(7104): 831-5.

Lozach PY, Amara A, Bartosch B, Virelizier JL, et al. (2004). "C-type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype particles." J Biol Chem 279(31): 32035-45.

Lu L, Pilot-Matias TJ, Stewart KD, Randolph JT, et al. (2004). "Mutations conferring resistance to a potent hepatitis C virus serine protease inhibitor in vitro." Antimicrob Agents Chemother 48(6): 2260-6.

Ludmerer SW, Graham DJ, Boots E, Murray EM, et al. (2005). "Replication fitness and NS5B drug sensitivity of diverse hepatitis C virus isolates characterized by using a transient replication assay." Antimicrob Agents Chemother 49(5): 2059-69.

Malcolm BA, Liu R, Lahser F, Agrawal S, et al. (2006). "SCH 503034, a mechanism-based inhibitor of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral activity of alpha interferon in replicon cells." Antimicrob Agents Chemother 50(3): 1013-20.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, et al. (2001). "Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial." Lancet 358(9286): 958-65.

Manns MP, Reesink HW, Moreno C, Berg T, et al. (2008). Safety and antiviral activity of TMC435350 in treatment-naïve genotype 1 HCV-infected patients. Hepatology. 48(Suppl 4): 1023A

Maynard M, Pradat P, Bailly F, Rozier F, et al. (2006). "Amantadine triple therapy for non-responder hepatitis C patients. Clues for controversies (ANRS HC 03 BITRI)." J Hepatol 44(3): 484-90.

McCown MF, Rajyaguru S, Kular S, Cammack N, Najera I. (2009). GT-1a or GT-1b subtype-specific resistance profiles for hepatitis C virus inhibitors telaprevir and HCV-796. Antimicrob Agents Chemother 53:2129-2132.

McHutchison JG, Everson GT, Gordon SC, Jacobson IM, et al. (2009). Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 360:1827-1838.

McHutchison JG, Manns M, Muir A, Terrault N, et al. (2009). PROVE3 final results and 1 year durability of SVR with telaprevir-based regimen in hepatitis C genotype 1-infected patients with prior non-response, relapse or viral breakthrough to peginterferon-alfa-2a/b and ribavirin therapy. Hepatology. 50(Suppl 4): 334A.

McHutchison JG (2004). "Understanding hepatitis C." Am J Manag Care 10(2 Suppl): S21-9.

McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, et al. (1998). "Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group." N Engl J Med 339(21): 1485-92.

Meylan E, Curran J, Hofmann K, Moradpour D, et al. (2005). "Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus." Nature 437(7062): 1167-72.

Mihm U, Grigorian N, Welsch C, Herrmann E, et al. (2006). "Amino acid variations in hepatitis C virus p7 and sensitivity to antiviral combination therapy with amantadine in chronic hepatitis C." Antivir Ther 11(4): 507-19.

Mo H, Lu L, Pilot-Matias T, Pithawalla R, et al. (2005). "Mutations conferring resistance to a hepatitis C virus (HCV) RNA-dependent RNA polymerase inhibitor alone or in combination with an HCV serine protease inhibitor in vitro." Antimicrob Agents Chemother 49(10): 4305-14.

Moradpour D, Penin FRice CM (2007). "Replication of hepatitis C virus." Nat Rev Microbiol 5(6): 453-63.

Muir A, Shiffman ML, Zaman A, et al. (2009). A phase 1b dose-ranging study of 4 weeks of peg-interfon lambda in combination with ribavirin in patients with chronic hepatitis C virus infection. Hepatology 50(Suppl. 4): 1591.

Nelson D, Pockros P, Godofsky E, Rodriguez-Torres M, et al. (2008). "High end-of-treatment response (84%) after 4 weeks of R1626, peginterferon alfa-2a (40kd) and ribavirin followed by a further 44 weeks of peginterferon alfa-2a and ribavirin." J Hepatol 48(Suppl. 2): 371.

Nettles R, Chien C, Chung E, et al. (2008). BMS-790052 is a first-in-class potent hepatitis C virus (HCV) NS5A inhibitor for patients with chronic HCV infection: results from a proof-of-concept study. Hepatology. 48(2): 1025A.

Pascu M, Martus P, Hohne M, Wiedenmann B, et al. (2004). "Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences." Gut 53(9): 1345-51.

Pawlotsky JM, Chevaliez SMcHutchison JG (2007). "The hepatitis C virus life cycle as a target for new antiviral therapies." Gastroenterology 132(5): 1979-98.

Pierra C, Benzaria S, Amador A, Moussa A, et al. (2005). "Nm 283, an efficient prodrug of the potent anti-HCV agent 2'-C-methylcytidine." Nucleosides Nucleotides Nucleic Acids 24(5-7): 767-70.

Pileri P, Uematsu Y, Campagnoli S, Galli G, et al. (1998). "Binding of hepatitis C virus to CD81." Science 282(5390): 938-41.

Pockros PJ, Nelson D, Godofsky E, Rodriguez-Torres M, et al. (2008). "R1626 plus peginterferon Alfa-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin." Hepatology 48(2):385-97.

Poordad F, Lawitz E, Hassanein T, Shiffman ML, et al. (2009). Sustained virologic reponse (SVR) results for weight-based taribavirin versus weight-based ribavirin in naïve chronic hepatitis C, genotype 1 patients. Hepatology 50(Suppl. 4): 334.

Pottage J, Lawitz E, Mazur D, Wyles D, et al. (2007). "Short-term antiviral activity and safety of ACH-806, an NS4A antagonist, in HCV genotype 1 infected individuals." J. Hepatol. 46(Suppl. 1: 294-5).

Reesink HW, Fanning GC, Abou Farha K, Weegink C, et al. (2009). Rapid HCV-RNA decline with once-daily TMC435 : A phase I study in healthy volunteers and hepatitis C patients. Gastroenterology. epub.

Reesink HW, Zeuzem S, Weegink CJ, Forestier N, et al. (2006). "Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study." Gastroenterology 131(4): 997-1002.

Reiser M, Hinrichsen H, Benhamou Y, Reesink HW, et al. (2005). "Antiviral efficacy of NS3-serine protease inhibitor BILN-2061 in patients with chronic genotype 2 and 3 hepatitis C." Hepatology 41(4): 832-5.

Roberts SK, Cooksley G, Dore GJ, Robson R, et al. (2008). Robust antiviral activity of R1626, a novel nucleoside analog: a randomized, placebo-controlled study in patients with chronic hepatitis C. Hepatology 48:398-406.

Rodriguez-Frias F, Cubero M, Esteban JI, Buti M, et al. (2009). Detection of Clinically Relevant Minor HCV and HBV Antiviral-Resistant Mutants in Treatment-Naive Patients Using Ultra-Deep Pyrosequencing. J. Hepatol. 50(Suppl.1): 130.

Rossignol J, Elfert A, Keeffe EB, et al. (2008). Evaluation of a 4 Week Lead-In Phase with Nitazoxanide (NTZ) Prior to Peginterferon (PEG-IFN) Plus NTZ for Treatment of Chronic Hepatitis C: Final Report. Hepatology. 48(Suppl 4): 344A.

Rossignol JF, Kabil SM, El-Gohary Y, Elfert A, Keeffe EB. (2008). Clinical trial: randomized, double-blind, placebo-controlled study of nitazoxanide monotherapy for the treatment of patients with chronic hepatitis C genotype 4. Aliment Pharmacol Ther 28:574-580.

Rossignol JF, Elfert A, El-Gohary Y, Keeffe EB. (2009). Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. Gastroenterology 136:856-862.

Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, et al. (2007). "Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir." Gastroenterology 132(5): 1767-77.

Sarrazin C, Rouzier R, Wagner F, Forestier N, et al. (2007). "SCH 503034, a novel hepatitis C virus protease inhibitor, plus pegylated interferon alpha-2b for genotype 1 nonresponders." Gastroenterology 132(4): 1270-8.

Sarrazin C, Lim S, Qin X, Susser S, et al. (2009). Kinetic analyisis of viral rebound and drug resistant viral variant dynamics in patients treated with ITMN-191 (R7227) monotherapy suggests a high barrier of viral escape. Hepatology 50(Suppl. 4):953.

Sarrazin C, Zeuzem S. (2009). Resistance to direct antiviral agents in patients with hepatitis C virus infection. Gastroenterology, epub.

Schiano TD, Charlton M, Younossi Z, Galun E, et al. (2006). "Monoclonal antibody HCV-AbXTL68 in patients undergoing liver transplantation for HCV: results of a phase 2 randomized study." Liver Transpl 12(9): 1381-9.

Shi ST, Herlihy KJ, Graham JP, Nonomiya J, et al. (2009). Preclinical characterization of PF-00868554, a potent nonnucleoside inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. Antimicrob Agents Chemother 53:2544-2552.

Schiff E, Poordad F, Jacobson I, et al. (2008). Boceprevir (B) combination therapy in null responders (NR): response dependent on interferon responsiveness. J Hepatol 48(Suppl 2): 104.

Soler M, McHutchison JG, Kwoh TJ, Dorr FA, et al. (2004). "Virological effects of ISIS 14803, an antisense oligonucleotide inhibitor of hepatitis C virus (HCV) internal ribosome entry site (IRES), on HCV IRES in chronic hepatitis C patients and examination of the potential role of primary and secondary HCV resistance in the outcome of treatment." Antivir Ther 9(6): 953-68.

Steinmann E, Penin F, Kallis S, Patel AH, et al. (2007). "Hepatitis C virus p7 protein is crucial for assembly and release of infectious virions." PLoS Pathog 3(7): e103.

Sulkowski M, Zeuzem S, Lawitz E, Grigorescu M, et al. (2009).Efficacy and safety of albinterferon alfa-2b in combination with ribavirin in treatment-naive patients with HCV genotpye 1. Hepatology 50(Suppl.4): 333.

Susser S, Welsch C, Wang Y, Zettler M, et al. (2009). Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. Hepatology 50(6) : 1709-1718.

Susser S, Forestier N, Welker MW, Vermehren J, et al. (2009). Detection of Resistant Variants in the Hepatitis C Virus NS3 Protease Gene by Clonal Sequencing at Long-Term Follow-Up in Patients Treated with Boceprevir. J. Hepatol. 50(Suppl.1) : 7.

Suzuki R, Matsuura Y, Suzuki T, Ando A, et al. (1995). "Nuclear localization of the truncated hepatitis C virus core protein with its hydrophobic C terminus deleted." J Gen Virol 76 (Pt 1): 53-61.

Tellinghuisen TL, Marcotrigiano J, Rice CM. (2005). Structure of the zinc-binding domain of an essential component of the hepatitis C virus replicase. Nature 435:374-379.

Thomas DL, Thio CL, Martin MP, Qi Y, et al. (2009) Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461(7265):798-801.

Tong X, Chase R, Skelton A, Chen T, et al. (2006). "Identification and analysis of fitness of resistance mutations against the HCV protease inhibitor SCH 503034." Antiviral Res 70(2): 28-38.

Trepo C, Guest M, Meyrueix R, Rouzier R, et al. (2007). "Evaluation of antiviral activity and tolerance of a novel sustained release interferon-alpha-2b (IFN-alpha-2BXL) compared to pegylated interferon-alpha-2b (PEG-IFN-alpha-2B): a phase 1b trial in HCV patients." Hepatology 46(Suppl. 4): 238.

VanCompernolle SE, Wiznycia AV, Rush JR, Dhanasekaran M, et al. (2003). “Small molecule inhibition of hepatitis C virus E2 binding to CD81.” Virology 314(1): 371-80.

Vermehren J, Susser S, Karey U, Forestier N, et al. (2009). Clonal analysis of mutations selected in the NS3 protease domain of genotype 1 non-responders sequentially treated with boceprevir (SCH503034) and/or pegylated interferon-alfa-2b (PEG-IFN _-2b). Hepatology 50(Suppl. 4): 1040.

Villano S, Raible D, Harper D, Speth J, et al. (2007). “Antiviral activity of the non-nucleoside polymerase inhibitor, HCV-796, in combination with pegylated interferon alfa-2b in treatment-naive patients with chronic HCV.” J. Hepatol. 46(Suppl. 1): 24.

von Wagner M, Hofmann W, Teuber G, Berg T, et al. (2008). “Placebo-controlled trial of 400 mg amantadine with peginterferon alfa-2a and ribavirin for 48 weeks in chronic HCV-1-infection.” Hepatology 48: 1404-11.

Wakita T, Pietschmann T, Kato T, Date T, et al. (2005). “Production of infectious hepatitis C virus in tissue culture from a cloned viral genome.” Nat Med 11(7): 791-6.

Weiner AJ, Christopherson C, Hall JE, Bonino F, et al. (1991). “Sequence variation in hepatitis C viral isolates.” J Hepatol 13 Suppl 4: S6-14.

Welsch C, Domingues FS, Susser S, Antes I, et al. (2008). “Molecular basis of telaprevir resistance due to V36 and T54 mutations in the NS3-4A protease of the hepatitis C virus.” Genome Biol 9(1): R16.

Wohnsland A, Hofmann WPSarrazin C (2007). “Viral determinants of resistance to treatment in patients with hepatitis C.” Clin Microbiol Rev 20(1): 23-38.

Wyles D, Kaihara KSchooley R (2008). “Synergy of a Hepatitis C Virus (HCV) NS4A Antagonist in Combination with HCV Protease and Polymerase Inhibitors.” Antimicrob Agents Chemother 52(5): 1862-4.

Yang W, Zhao Y, Fabrycki J, Hou X, et al. (2008). “Selection of Replicon Variants Resistant to ACH-806, a Novel Hepatitis C Virus Inhibitor with No Cross-Resistance to NS3 Protease and NS5B Polymerase Inhibitors.” Antimicrob Agents Chemother epup.

Zeuzem S, Sarrazin C, Wagner F et al. (2005). “Antiviral activity of SCH 503034, a HCV protease inhibitor, admisitered as monotherapy in hepatitis C genotype 1 (HCV-1) patients refractory to pegylated interferon (PEG-IFN-alpha).” Hepatology 42(Suppl. 1): 276A.

Zeuzem S, Teuber G, Naumann U, Berg T, et al. (2000). “Randomized, double-blind, placebo-controlled trial of interferon alfa2a with and without amantadine as initial treatment for chronic hepatitis C.” Hepatology 32(4 Pt 1): 835-41.

Zeuzem S, Yoshida E, Benhamou Y, Pianko S, et al. (2008). “Sustained virologic response rates with albinterferon alfa-2b plus ribavirin treatment in IFN-naive, chronic hepatitis C genotype I patients.” Hepatology 48: 407-17.

Zhou Y, Bartels DJ, Hanzelka BL, Muh U, et al. (2008). “Phenotypic characterization of resistant Val36 variants of hepatitis C virus NS3-4A serine protease.” Antimicrob Agents Chemother 52(1): 110-20.

Chapter 15: Management of adverse events and drug interactions of interferon-based therapy for chronic hepatitis C

Martin Schäfer, Stefan Mauss

Introduction

Good adherence is a key factor for success in the treatment of hepatitis C. However, almost all patients on treatment with interferon plus ribavirin will experience side effects that can threaten good adherence. Therefore, proactive management of adverse events is crucial to avoid suboptimal therapy (missing doses, etc) and treatment discontinuations.

The most common clinical adverse events in patients on treatment with pegylated interferon plus ribavirin are flu-like symptoms, myalgia, sleep disturbances, asthenia, gastrointestinal disorders and depressive mood changes (Table 1).

Psychiatric side effects	Incidence
Fatigue	70-80%
Sleep disturbances	46-65%
Irritability	60-85%
Cognitive disturbances with impairments of contentration and memory	45-60%
Depressive episodes	50-60%
- mild depressive episode	20-40%
- moderate derpressive episode	15-30%
- severe depresive episode	1-5%
Delirium, psychosis	1-6%
Suicidal syndrome	<1%

**data from outpatient department, Essen*

Table 1. Incidence of the most reported IFN α induced psychiatric side effects*.

For most adverse events, clinical trials looking at dose moderation have not been done, and because of this, recommendations in this review for management are necessarily partially based on clinical experience.

Flu-like symptoms, fever, arthralgia and myalgia

Flu-like symptoms, fever, arthralgia and myalgia appear a few hours after the injection of pegylated interferon and may last for up to three days. One common approach is the use of paracetamol or other NSAIDs immediately before or after the injection of interferon. Flu-like symptoms usually diminish spontaneously during the first weeks of treatment (Figure 1).

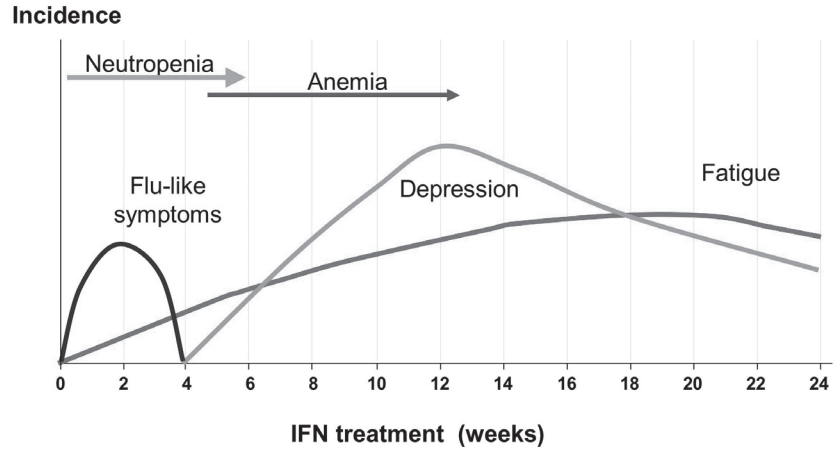


Figure 1. Time course of interferon-associated adverse events.

Low platelets are a contraindication for the use of acetylsalicylic acid, diclofenac or ibuprofen because of the inhibition of platelet aggregation. High doses of paracetamol may result in liver toxicity. Doses exceeding 2 g/day of paracetamol are not recommended.

Gastrointestinal disorders

Nausea can be mitigated by using prokinetic agents such as metoclopramide or domperidone before administering ribavirin. This may also help with the frequently observed loss of appetite.

Dry mouth has been reported as a result of inhibition of saliva production, a frequent complication of ribavirin and may continue post-therapy.

Weight loss

The average weight loss in interferon-based controlled studies is around 6-10% for a treatment period of 48 weeks (Seyam 2004). This may be predominantly due to loss of appetite and reduction in calorie intake. The weight loss is rapidly reversible upon discontinuation of therapy.

Asthenia and fatigue

Asthenia and fatigue are frequent complaints that usually increase slowly in intensity over the first couple weeks of therapy (Figure 1). In patients with marked anaemia these symptoms can be improved by raising low haemoglobin with the use of erythropoietin, a reduction of the ribavirin dosage or red blood cell transfusion (Pockros 2004). Asthenia is also reported by patients without marked anaemia. In these patients hypothyroidism may be the explanation. Symptomatic treatment of asthenia and fatigue in patients without an underlying complication such as anaemia, depression or hypothyroidism is difficult.

Chronic fatigue has been successfully treated in individual cases with antidepressants or tryptophan (Sammut 2002; Schaefer 2008). A first prospective randomised controlled trial showed superior effects of the 5-HT3 receptor antagonist ondansetron compared to placebo (Piche 2005). However, currently available data does not point to specific treatment recommendations.

Cough and dyspnoea

Cough while on therapy is frequently reported and is most probably due to oedema of the mucosa of the respiratory system. Therefore, advanced, not well-controlled asthma bronchiale may be a contraindication for hepatitis C therapy. Dyspnoea is another frequent complaint with a more complex aetiology involving mucosal swelling, anaemia and asthenia.

Disorders of the thyroid

Hypothyroidism while on interferon-based therapy is reported with an incidence of 3-10% (Bini 2004; Tran 2005). Hyperthyroidism is less frequently observed with an incidence of 1-3% (Bini 2004; Tran 2005). Interferon-induced thyroiditis or the induction of thyroid antibodies is reported as an underlying mechanism. Hypothyroidism is treated via substitution of thyroid hormone whereas clinically symptomatic hyperthyroidism may be treated with ß-blockers or carbimazole. Premature termination of interferon-based therapy is usually not necessary. Most cases of hypothyroidism are reversible upon discontinuation of interferon-based therapy, although some cases may need prolonged periods of thyroid hormone replacement therapy.

Psychiatric adverse events

Incidence and profile of psychiatric adverse events

The most commonly emerging IFN α induced psychiatric adverse events are outlined in Table 1. However, data on the frequency of psychiatric side effects differs depending on the design of the trial. Most hepatologic trials are only monitored for "major depression" without using depression scales, leading to an underreporting of mild to moderate depressive episodes. Psychiatric trials use self-rating scales (e.g., SDS-scale, BDI-Scale) or monitor patients via structured interviews utilising the Hamilton Depression Scale (HAMDS) or the Montgomery Asperg Depression Scale (MADRS), rating depressive symptoms and any changes in scores not fulfilling DSM-IV criteria of major depression. With this more sensitive psychiatric rating, over 50% of patients suffer from sleep disorders, chronic fatigue, irritability or cognitive disturbances (Schaefer 2007; Schaefer 2002; Dieperink 2000; Renault 1987). Anxiety occurs in 30-45% especially during the first 2 months of treatment. Mild depression with symptoms like reduced self-esteem, anhedonia, loss of interest, rumination, a diminished libido and spontaneous crying can be observed in 30-60% of the patients. 20-30% of treated patients develop moderate to severe depressive episodes (Bonnaccorso 2002; Dieperink 2000; Renault 1987; Schaefer 2002; Malaguarnera 2002). Suicidal ideation is seen in 5-6% of patients, while suicide attempts have been reported in individual cases (Janssen 1994). Mania has

been reported as a sporadically appearing side effect. Contrary to hitherto existing assumptions, patients with pre-existing psychiatric disturbances do not appear to have a greater risk for development of depression or attempting suicide (Schaefer 2007; Schaefer 2003; Pariante 2002). However, patients with intravenous drug histories seem more likely to discontinue treatment in the first three months compared to controls (Schaefer 2003; Mauss 2004; Schaefer 2007).

Antidepressants frequently used in the hepatitis C study population in recent trials in cases of interferon-associated depression are selective serotonin reuptake inhibitors (SSRIs) such as citaprolamin, paroxetin or tricyclic antidepressants such as doxepine. The introduction of SSRIs and newer antidepressants has markedly reduced the adverse events profile of antidepressants. Therefore, depending on the major symptoms, current sedating or activating antidepressants, especially SSRIs, are treatment of choice for interferon-induced depressive mood disorders. In patients with predominantly agitation and aggression, other strategies, e.g., modern antipsychotics, may be added.

The efficacy of antidepressants for treatment of interferon α induced depression has been shown in several cohorts (Farah 2002; Gleason 2002; Kraus 2001; Schramm 2000; Hauser 2002; Gleason 2005). Recently, early prospective controlled data shows a significantly better improvement of depressive symptoms after treatment of IFN-associated depression with citalopram (Kraus 2008). SSRIs seem to be the best-suited substances for the treatment of interferon α associated depressive symptoms. However, antidepressants with different receptor profiles (i.e., mirtazapine) and classical antidepressants (i.e., nortriptyline) are also effective (Kraus 2001; Valentine 1995). Nevertheless, tricyclic antidepressants should be used as second choice because of pharmacological interactions and anticholinergic side effects possibly leading to a higher risk of developing delirium, to affect the heart or liver or to interact with other medications. To reduce adverse events and to increase adherence, treatment with antidepressants can be started at a relatively low dose, increasing depending on the effect and tolerability. In general, a therapeutically-relevant antidepressive effect cannot be expected before 8-14 days of treatment. In case of non-response, the dose can be escalated. Treatment adherence should be assessed by monitoring serum levels before patients are switched to a different antidepressant.

Benzodiazepines can be given for a short period in case of severe sleep disturbances, irritability or depression. However, benzodiazepines should be avoided in patients with a history of IV drug or alcohol abuse because of their potential to induce addiction (Schaefer and Mauss 2008).

In case of psychotic symptoms, antipsychotics (e.g., risperidone, olanzapine) can be used at low doses, but patients should be monitored carefully by a psychiatrist. One important risk factor for the development of psychotic symptoms is a history of drug abuse.

Although history of major depression or suicide attempts is considered a contraindication for interferon-based therapy, treatment of patients with pre-existing psychiatric disorders can be initiated in close collaboration with an experienced psychiatrist in a well-controlled setting (Schaefer 2004; Schaefer 2007).

Preemptive therapy with antidepressants

One double-blind randomised study including patients with a malign melanoma demonstrated that 14 days of pre-treatment with 20 mg paroxetine per day reduced the incidence of depression during interferon therapy significantly (Musselmann 2001). Pretreatment with paroxetine also had a positive effect on the development of fears, cognitive impairments and pain during interferon treatment, but not on symptoms such as fatigue, sleep disturbances, anhedonia and irritability (Capuron 2002). A recent prospective controlled trial with HCV-positive people demonstrated that pretreatment with citalopram significantly reduced depression during the first 6 months of antiviral therapy in patients with psychiatric illness compared to controls (Schaefer 2005). Furthermore, prophylactic treatment with SSRIs was also shown to reduce the severity of depressive symptoms in patients who had suffered from severe depression during previous treatment of hepatitis C with interferon α (Kraus 2005). Finally, a recent trial confirmed a protective effect of preemptive initiation of treatment with antidepressants in cases of elevated depression scores before interferon-based therapy is started (Raison 2007). In summary, current data supports the view that all patients with pre-existing depressive symptoms should receive a prophylactic treatment with antidepressants. However, evidence from larger prospective controlled studies are needed in order to answer the question if antidepressants should be given before antiviral plus interferon-based therapy, independent of pre-existing psychiatric disorders.

Sleep disturbances

Patients who have difficulties in falling asleep can be treated with zopiclone or trimipramine. Zolpidem may be used for patients with interrupted or shortened sleep patterns. Although the risk of addiction is markedly reduced compared with other benzodiazepines, only small amounts of zoplicon or zolpidem should be prescribed at a time and therapy should be limited to the period of interferon-based therapy. As sleeping disorders can be a symptom of depression, it is also important to identify existing depressive symptoms and add antidepressants with sedative effects, such as mirtazapine, as needed.

Haematologic and immunologic effects

Interferon-based therapy is accompanied by a marked drop in white blood cells in general, neutrophils and absolute, although not relative, CD4+ cell count. This change in the cellular immune system does not result in an increased number of serious infections even in HIV-coinfected patients (Fried 2002; Manns 2001; Torriani 2004). In general the incidence of serious infections is low (<5%) in patients on interferon-based therapy.

Despite reassuring clinical data, G-CSF is not often used to correct neutropenia. G-CSF has not been proven efficacious in clinical trials for this purpose and its use is off-label.

Haemolytic anaemia induced by ribavirin is further aggravated by the myelosuppressive effect of interferon that inhibits compensatory reticulocytosis (De Franceschi 2000). As a consequence, anaemia (<10 g/dl) is reported in up to 20% of patients (Hadziyannis 2004). In severe cases of anaemia dose reduction of ribavirin is required. In rare cases, red blood cell transfusion may be necessary. Erythropoetin can be successfully used to correct ribavirin-induced anaemia at least partially and to

avoid ribavirin dose reduction or red blood cell transfusions. However, prospective controlled trials have not shown an improved efficacy of hepatits C therapy in patients who take erythropoietin (Afdahl 2004; Pockros 2004; Shiffman 2007). Erythropoietin is not approved for correction of ribavirin-induced anaemia in hepatitis C therapy.

Mild to moderate thrombocytopenia is frequently observed in patients with advanced liver fibrosis and may complicate interferon-based therapy. Reduction of interferon dosing may be indicated to reverse severe thrombocytopenia. Eltrombopag has been used successfully in studies to increase platelet count in patients with hepatitis C-associated thrombocytopenia (McHutchinson 2007).

Skin disorders and hair loss

Skin disorders such as lichen ruber planus, necrotising vasculitis or porphyrea cutanea tarda are associated with hepatitis C infection. The effects of hepatitis C therapy are often not well-studied and based only on cohort data (Berk 2007).

Interferon plus ribavirin may have an effect on the skin itself including dry skin, itching, eczema and new or exacerbated psoriasis. Ointments with rehydrating components, urea or steroids can be used depending on the nature of the skin disorders. In severe cases a dermatologist should be involved. In particular, eczema and psoriasis may last substantially longer than the treatment period with interferon-based therapy.

Local skin reactions to the injection of pegylated interferon are common and usually present as red indurations lasting days to weeks. Repeated injections at the same site may cause ulcers and should be avoided. Hypersensitivity reactions to pegylated interferons are reported anecdotally.

Hair loss is frequent, usually appearing after the first months of therapy and continuing for some weeks after the cessation of therapy. Alopecia is very rare and hair loss is usually fully reversible, although the structure of the hair may be different after therapy.

Adherence

Adherence data from retrospective analyses suggest that at least 80% of the cumulative dosing of ribavirin and interferon should be taken by patients as a prerequisite for treatment success. Cumulative doses of less than 80% were associated with a steep drop in sustained virologic response (Camma 2005). Another surrogate of adherence is the premature treatment discontinuation rate, which usually ranges from 10–15% with pegylated interferon plus ribavirin (Fried 2002; Manns 2001).

Conclusion

In summary, the toxicity of interferon-based therapy in combination with ribavirin is considerable and requires a deep-seated knowledge and active management, in particular involving psychiatric adverse events.

The first generation of HCV protease and polymerase inhibitors will be combined with interferon and ribavirin as triple combination therapy to improve efficacy of therapy, in particular in HCV genotype 1 patients. Current studies indicate that most agents will have a substantial adverse event profile increasing haematological or dermatological problems while on therapy. Early assessment and therapy of adverse events may prevent premature treatment discontinuation, thereby improving the efficacy of hepatitis C therapy.

References

Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Proactive Study Group. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. Gastroenterology. 2004; 126(5):1302-11.

Berk DR, Mallory SB, Keeffe EB, Ahmed A. Dermatologic disorders associated with chronic hepatitis C: effect of interferon therapy. Clin Gastroenterol Hepatol. 2007; 5(2):142-51.

Bini EJ, Mehandru S. Incidence of thyroid dysfunction during interferon alfa-2b and ribavirin therapy in men with chronic hepatitis C: a prospective cohort study. Arch Intern Med. 2004; 164(21):2371-6.

Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J Clin Psychopharmacol 2002; 22[1]:86-90.

Cammà C, Licata A, Cabibbo G, Latteri F, Craxì A. Treatment of hepatitis C: critical appraisal of the evidence. Expert Opin Pharmacother. 2005;6(3):399-408.

Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Mol Psychiatry 2002; 7[5]:468-473.

De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, Stanzial AM, Solero P, Corrocher R. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology 2000; 31(4): 997-1004.

Dieperink E, Willenbring M, Ho SB. Neuropsychiatric symptoms associated with hepatitis C and interferon alpha: A review. Am J Psychiatry 2000; 157[6]:867-876.

Farah A. Interferon-induced depression treated with citalopram. J Clin Psychiatry 2002; 63(2):166-7.

Fried M, Shiffman M, Reddy R, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975-982.

Gleason OC, Yates WR, Isbell MD, Philipsen MA. An open-label trial of citalopram for major depression in patients with hepatitis C. J Clin Psychiatry, 2002; 63(3):194-8.

Gleason OC, Yates WR, Philipsen MA. Major depressive disorder in hepatitis C: an open-label trial of escitalopram. Prim Care Companion J Clin Psychiatry 2005; 7(5):225-230.

Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin in combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004; 140: 346-55.

Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J et al. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry 2002; 7(9):942-947.

Janssen HL, Brouwer JT, van der Mast RC, Schalm SW. Suicide associated with alfa-interferon therapy for chronic viral hepatitis. J Hepatol 1994; 21[2]:241-243.

Kraus MR, Schafer A, Scheurlen M. Paroxetine for the prevention of depression induced by interferon alfa. N Engl J Med 2001; 345(5):375-6.

Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M. Paroxetine for the treatment of interferon-alpha-induced depression in chronic hepatitis C. Aliment Pharmacol Ther 2002; 16(6):1091-9.

Kraus MR, Schafer A, Al-Taie O, Scheurlen M. Prophylactic SSRI during interferon alpha re-therapy in patients with chronic hepatitis C and a history of interferon-induced depression. J Viral Hepat 2005;12(1):96-100.

Kraus MR, Schafer A, Schottker K, Keicher C, Weissbrich B, Hofbauer I et al. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: A randomized, double-blind, placebo-controlled study. Gut. 2008; 57(4):531-6

Malaguarnera M, Laurino A, Di F, I, Pistone G, Castorina M, Guccione N et al. Neuropsychiatric effects and type of IFN-alpha in chronic hepatitis C. J Interferon Cytokine Res 2001; 21[5]:273-278.

Manns M, McHutchison J, Gordon S, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958-965.

McHutchison JG, Dusheiko G, Shiffman ML, Rodriguez-Torres M, Sigal S, Bourliere M, et al. Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. N Engl J Med. 2007; 357(22):2227-36.

Mauss S, Berger F, Goelz J, Jacob B, Schmutz G. A prospective controlled study of interferon-based therapy of chronic hepatitis C in patients on methadone maintenance. Hepatology. 2004;40(1):120-4.

Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001; 344[13]:961-966.

Pariante CM, Landau S, Carpiniello B. Interferon alfa-induced adverse effects in patients with a psychiatric diagnosis. N Engl J Med 2002; 347[2]:148-149.

Pockros PJ, Shiffman ML, Schiff ER, Sulkowski MS, Younossi Z, Dieterich DT, et al. Epoetin alfa improves quality of life in anemic HCV-infected patients receiving combination therapy. Hepatology 2004; 40(6):1450-8.

Piche T, Vanbiervliet G, Cherikh F, Antoun Z, Huet PM, Gelsi E et al. Effect of ondansetron, a 5-HT3 receptor antagonist, on fatigue in chronic hepatitis C: a randomised, double blind, placebo controlled study. Gut 2005; 54(8):1169-1173.

Raison CL, Woolwine BJ, Demetrashvili MF, Borisov AS, Weinreib R, Staab JP et al. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. Aliment Pharmacol Ther 2007; 25(10):1163-1174

Renault PF, Hoofnagle JH, Park Y, Mullen KD, Peters M, Jones DB et al. Psychiatric complications of long-term interferon alfa therapy. Arch Intern Med 1987; 147[9]:1577-1580.

Sammut S, Bethus I, Goodall G, Muscat R. Antidepressant reversal of interferon-alpha-induced anhedonia. Physiol Behav 2002; 75(5):765-772.

Schaefer M, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, Schmidt F et al. Interferon alpha [IFNalpha] and psychiatric syndromes: a review. Prog Neuropsychopharmacol Biol Psychiatry 2002; 26[4]:731-746.

Schaefer M, Mauss S. Clinical management of Interferon-alpha-associated psychiatric side effects during hepatitis C treatment in patients with drug addiction. Current Drug Abuse Reviews 2008; 1:177-187.

Schaefer M, Schmidt F, Folwaczny C, Lorenz R, Martin G, Schindlbeck N et al. Adherence and mental side effects during hepatitis C treatment with interferon alfa and ribavirin in psychiatric risk groups. Hepatology 2003; 37[2]:443-451.

Schaefer M, Heinz A, Backmund M. Treatment of chronic hepatitis C in patients with drug dependence: time to change the rules? Addiction 2004; 99(9):1167-1175.

Schafer A, Wittchen HU, Seufert J, Kraus MR. Methodological approaches in the assessment of interferon-alfa-induced depression in patients with chronic hepatitis C - a critical review. Int J Methods Psychiatr Res 2007; 16(4):186-201.

Schaefer M, Hinzpeter A, Mohmand A, Janssen G, Pich M, Schwaiger M et al. Hepatitis C treatment in “difficult-to-treat” psychiatric patients with pegylated interferon-alpha and ribavirin: Response and psychiatric side effects. Hepatology 2007; 46(4):991-998.

Schaefer M, Schwaiger M, Garkisch AS, Pich M, Hinzpeter A, Uebelhack R, Heinz A, van Boemmel F, Berg T. Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. J Hepatol. 2005; 42(6):793-8.

Schaefer M, Winterer J, Sarkar R, Uebelhack R, Franke L, Heinz A et al. Three cases of successful tryptophan add-on or monotherapy of Hepatitis C and IFN-alpha associated mood disorders . Psychosomatics 2008; 2008; 49(5):442-6.

Schramm TM, Lawford BR, Macdonald GA, Cooksley WG. Sertraline treatment of interferon-alfa-induced depressive disorder. Med J Aust, 2000; 173(7):359-61.

Seyam MS, Freshwater DA, O’Donnell K, Mutimer DJ. Weight loss during pegylated interferon and ribavirin treatment of chronic hepatitis C. J Viral Hepat. 2005; 12(5):531-5.

Shiffman ML, Salvatore J, Hubbard S, Price A, Sterling RK, Stravitz RT, Luketic VA, Sanyal AJ. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. Hepatology. 2007;46(2):371-9.

Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, Carosi G, Sasadeusz J, Katlama C, Montaner J, Sette H Jr, Passe S, De Pamphilis J, Duff F, Schrenk UM, Dieterich DT; APRICOT Study Group. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. N Engl J Med. 2004; 351(5):438-50.

Tran HA, Jones TL, Batey RG.The spectrum of thyroid dysfunction in an Australian hepatitis C population treated with combination Interferon-alpha 2-beta and Ribavirin. BMC Endocr Disord. 2005; 2:5.

Valentine AD, Meyers CA. Successful treatment of interferon-alpha-induced mood disorder with nortriptyline. Psychosomatics, 1995; 36(4):418-9.

Chapter 16: Extrahepatic manifestations

Karl-Philipp Puchner, Thomas Berg

Introduction

Patients with chronic hepatitis C virus (HCV) infection are at risk of a great number of extrahepatic manifestations (EHMs) (Table 1) – up to 40-76% of patients infected with HCV develop at least one EHM during the course of the disease (Cacoub 2000; Cacoub 1999). EHMs are often the first and only clinical sign of chronic hepatitis C infection. Evidence of HCV infection should always be sought out in cases of non-specific chronic fatigue and/or rheumatic, haematological, endocrine or dermatological disorders. The pathogenesis of EHM is still not fully understood, although most studies suggest that the presence of mixed cryoglobulinaemia, particular lymphotropism of the virus, molecular mimicry and non-cryoglobulinaemic autoimmune phenomena constitute the major pathogenic factors (Ferri 2007). Nevertheless, pathogenesis and epidemiology of many EHMs requires further investigation (Figure 1). Our aim is to give a brief insight into the epidemiology, pathogenesis, clinical relevance and therapeutic management of HCV-associated EHM (Zignego 2007a).

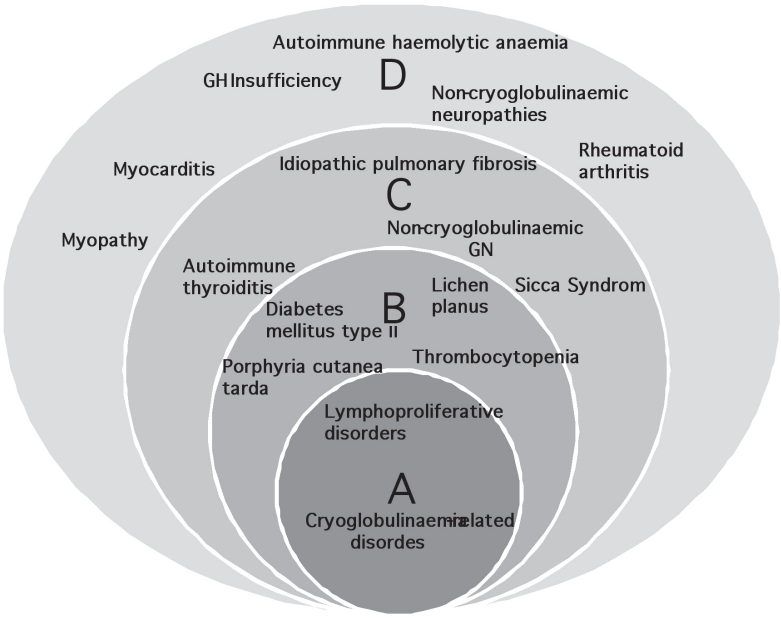


Figure 1. Schematic representation of EHM categories (modified after Zignego 2007a). A) Associations that rest upon strong epidemiological evidence and clear pathogenetic mechanisms; B) Associations that rest upon high prevalence, but still unclear pathogenetic mechanisms; C) Associations for which the high prevalence in HCV collectives could be due to HCV infection and/or confounding factors; D) Anecdotal observations.

Organ/System	Manifestation
Endocrine disorders	
	Autoimmune thyroidopathies (in particular, Hashimoto thyreoiditis)
	Insulin resistance/diabetes mellitus*
	GH-insufficiency
Rheumatic disorders	
	Mixed cryoglobilinaemia*
	Cryoglobulinaemic vasculitis*
	Peripheral neuropathy*
	Membrano-proliferative glomerulonephritis (GN)*
	Membranous GN*
	Rheumatoid arthralgias/oligo-polyarthritis
	Rheumatoid factor positivity*
	Sicca syndrome
Haematologic disorders	
	Lymphoproliferative disorders/Non-Hodgkin Lymphomas*
	Immune thrombocytopaenic purpura (ITP)
	Monoclonal gammopathies*
	Autoimmune haemolytic anaemia
Dermatologic disorders	
	Palpable purpura
	Porphyria cutanea tarda (PCT)
	Lichen planus
	Pruritus
Miscellaneous	
	Chronic fatigue*, subclinical cognitive impairment, psychomotoric deceleration, symptoms of depression*
	Myopathy
	Cardiomyopathy/Myocarditis
	Idiopatic pulmonal fibrosis

* *Associations that rest upon strong epidemiological prevalence and/or clear pathogenetic mechanisms*

Table 1. HCV-related extrahepatic manifestations.

Lymphoproliferative disorders

Mixed cryoglobulinaemia (MC)

Cryoglobulinaemia refers to the presence of abnormal immunoglobulins in the serum, which have the unusual property of precipitating at temperatures below 37°C and redissolving at higher temperatures. The phenomenon of cryoprecipitation was first described in 1933 (Wintrobe 1933). Cryoglobulins (CGs) are nowadays classified, on the basis of

their clonality, into three types [Table 2]. Type II CG and type III CG, consisting of monoclonal and/or polyclonal immunoglobulins, are prevalent in patients with a chronic HCV infection, while type I CGs, consisting exclusively of monoclonal components, are mostly found in patients with lymphoproliferative disorders (multiple myeloma, B cell lymphoma, Waldenström macroglobulinaemia). Type II or type III mixed cryoglobulinaemia is found in 19%-50% of patients with chronic HCV, but leads to clinical manifestations, through vascular precipitation of immunocomplexes, in only 30% of them (Lunel 1994; Womg 1996). Asymptomatic mixed cryoglobulinaemia, during the course of chronic HCV infection, may evolve into symptomatic disease. Patients with symptomatic mixed cryoglobulinaemia exhibit higher cryoglobulin concentrations (cyocrit >3%) (Weiner 1998) and lower concentrations of complement factors C3 and C4. Thus CG-triggered complement activation may constitute a key incidence in cryoglobulinaemia-derived pathogenesis.

Factors that seem to favour the development of MC are female sex, age, alcohol intake (>50g/d), advanced liver fibrosis and steatosis (Lunel 1994; Wong 1996; Saadoun 2006).

Clonality	
Type I	Monoclonal immunoglobulins (IgG or IgM)
Type II	Polyclonal immunoglobulins (mainly IgG) and monoclonal IgM with rheumatoid factor activity (RF)
Type III	Polyclonal IgG and IgM

Table 2. Classification of cryoglobulinaemia types.

Diagnosis. Detection of CG is carried out by keeping patient serum at 4° for up to 7 days. After cryoprecipitate is visible, CG can be purified and characterized using immunfixation electrophoresis. In case of evidence of mixed cryoglobulinaemia in HCV-positive patients, the presence of cryoglobulinaemic-syndrome must be sought out. Vigilant monitoring is required, as asymptomatic mixed cryoglobulinaemia patients may develop MC-related disorders in the course of the disease. The diagnosis of the MC syndrome is based on serologic, pathologic and clinical criteria (Table 3).

Serologic	**Histopathologic**	**Clinical**
C4 reduction	Leukocytoclastic vasculitis	Purpura
Positive rheumatoid factor (RF)	Infiltrates of monoclonal B-cells	Fatigue
CGs type II or III		Arthralgien
HCV antibodies		Membranoproliferative GN
		Peripheral neuropathy

Table 3. Diagnostic criteria of cryoglobulinaemic syndrome.

In the presence of mixed CG, low C4 counts, leucocytoclastic vasculitis and purpura, a definite symptomatic MC can be diagnosed. Rheumatoid factor (RF) determination constitutes a reliable surrogate parameter for detection of CG. Finally, presence of CG may impair HCV RNA determination as viral RNA can accumulate in precipitated cryocrit (Colantoni 1997).

Clinical features of mixed cryoglobulinaemia. HCV-related MC proceeds mostly asymptomatically and has no significant influence on the course of chronic liver inflammation. On the other hand, symptomatic mixed cryoglobulinaemia is associated with higher mortality (Ferri 2004). Clinical manifestations of symptomatic mixed cryoglobulinaemia are listed below:

Systemic vasculitis: HCV-related vasculitis relies on a deposition of immunocomplexes, containing CGs, complement and large amounts of HCV antigens in the small- and medium-sized blood vessels. HCV accumulates in the CG-immunoglobulins. Pathohistological findings reveal a leucocytoclastic vasculitis (Agnello 1997). The most common symptoms of mixed cryoglobulinaemic vasculitis are weakness, arthralgia and purpura (the Meltzer and Franklin triad). Mixed cryoglobulinaemic vasculitis may also lead to Raynaud's Syndrome and Sicca Syndrome, glomerulonephritis and peripheral neuropathy.

Renal impairment: The predominant renal impairment associated with mixed cryoglobulinaemia is the membranous proliferative glomerulonephritis (MPGN), characterized in most cases by proteinuria, mild haematuria and mild renal insufficiency. The presence of kidney impairment is considered to be a negative prognostic factor in the course of the disease (Ferri 2004). In 15% of patients, MC-related nephropathy may progress towards terminal chronic renal failure requiring dialysis (Tarantino 1995).

Peripheral neuropathy: Peripheral neuropathy, on the basis of endoneural microangiopathy, constitutes a further typical complication of mixed cryoglobulinaemia. MC-related neuropathy, presenting clinically as mononeuropathy or polyneuropathy, is mostly sensory and is characterized by numbness, burning skin crawling and pruritus, predominantly in the hands and feet (Tembl 1999; Lidove 2001). Epidemiological data from Italy suggest that peripheral neuropathy is the second most common symptom after the Meltzer and Franklin triad in patients with symptomatic HCV-associated mixed cryoglobulinaemia (Ferri 2004).

Cirrhosis: The causal association between CG and progression of liver fibrosis, suggested by numerous authors has not been confirmed in a recently published 10-year prospective study. The 10-year rates of progression to cirrhosis were similar in cryoglobulinaemic and non-cryoglobulinaemic HCV-infected patients (Vigano 2007). With respect to this recent data, it is unlikely that mixed cryoglobulinaemia constitutes an independent risk factor for the progression of liver fibrosis.

Malignant lymphoproliferative disorders/NHL

The association between infectious agents and potentially reversible "antigen driven" lymphoproliferative disorders, such as Helicobacter pylori-related gastric marginal zone B cell lymphoma has been known for many decades. Recent data suggest a causative association between HCV and Non-Hodgkin Lymphoma (NHL) (Mele 2003; Duberg 2005; Giordano 2007). HCV infection leads per se to a twofold higher risk

of developing NHL (Mele 2003; Duberg 2005). The most prevalent HCV-associated lymphoproliferative disorders according to the REAL/WHO classification are: follicular lymphoma, B cell chronic lymphocytic leukaemia/small lymphocyte lymphoma, diffuse large B cell lymphoma and marginal zone lymphoma, including the mucosa-associated lymphoid tissue lymphoma. Overall, marginal zone lymphoma appears to be the most frequently encountered low grade B cell lymphoma in HCV patients.

HCV-associated lymphoproliferative disorders (LPDs) are observed over the course of MC. 8%-10% of mixed cryoglobulinaemia type II evolve into B cell NHL after long-lasting infection. However, a remarkably high prevalence of B cell NHL was also found in HCV patients without mixed cryoglobulinaemia (Silvestri 1997). Genetic predisposition and factors seem to have a major impact on the development of LPDs in HCV-positive patients (Matsuo 2004).

Aetiology and pathogenesis of LPDs in patients with HCV infection. In the development of LPDs direct and indirect pathogenic HCV-associated factors (Figure 2) are seen. Sustained B cell activation and proliferation, noticed during chronic HCV infection, is an indirect pathogenic mechanism.

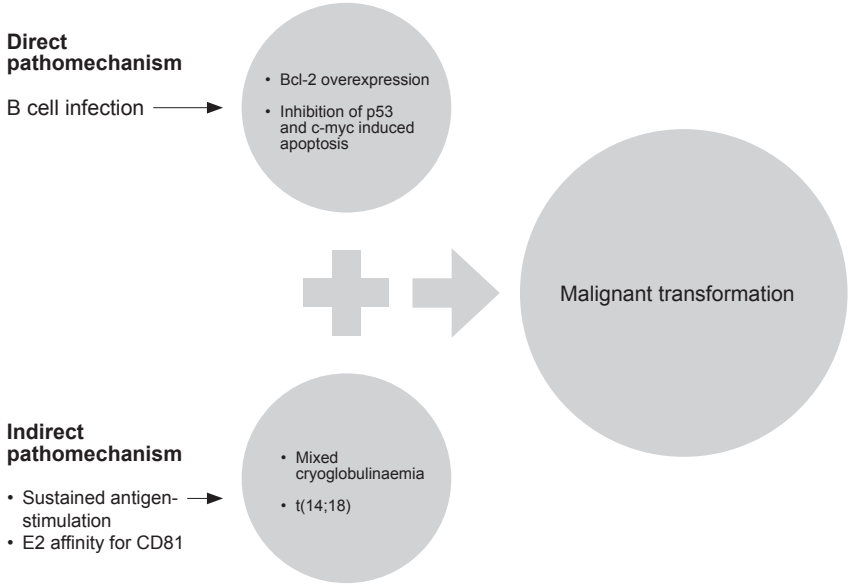


Fig 2. Pathomechanisms involved in the development of malignant lymphoproliferative disorders in patients with chronic HCV infection. Indirect pathomechanism: Sustained antigen stimulation, as binding of viral envelope protein to CD81 receptor, leads to excessive B cell proliferation, which in turn favors development of mixed cryoglobulinaemia and/or genetic aberrations, such as t(14;18) translocation. Direct pathomechanism: Viral infection of B cells, as viral replication in them may result in activation of proto-oncogenes (i.e., Bcl-2) and/or inhibition of apoptotic factors (i.e., p53, c-myc).One of the factors favoring this polyclonal B cell activation and proliferation is probably the HCV E2 protein, which binds specifically to CD81, a potent B cell activator (Cormier 2004).

Direct pathogenic mechanism are based on lymphotropic properties of HCV, hence on the very invasion of HCV into the B cells. HCV RNA sequences were first detected in mononuclear peripheral blood cells (Zignego 1992). Especially CD19+ cells seem to be permissive for certain HCV quasispecies (Roque Afonso 1999). Active replication of the HCV genome in B cells is associated with activation of anti-apoptotic gene bcl-2 and inhibition of p53 or c-Myc-induced apoptosis (Sakamuro 1995; Ray 1996). In this light, direct involvement of HCV in the immortalisation of B cells can be envisioned (Zignego 2000; Machida 2004).

Treatment of lymphoproliferative disorders

Mixed cryoglobulinaemia: While asymptomatic MC per se does not constitute an indication for treatment, symptomatic mixed cryoglobulinaemia should be always treated. Because asymptomatic cryoglobulinaemia may evolve into symptomatic in the course of disease, vigilant monitoring is required and introduction of antiviral therapy in terms of prophylaxis should be considered.

A casual correlation between HCV infection and mixed cryoglobulinaemia has been established, the therapeutic approach of symptomatic mixed cryoglobulinaemia should primarily concentrate on the eradication of the virus. Indeed, clinical improvement of MC is reported in 50 to 70% of patients receiving antiviral therapy with interferon a (IFN a) and ribavirin and mostly correlates with a drastic reduction of HCV RNA concentrations (Calleja 1999). However, cryoglobulinaemic vasculitis following successful antiviral treatment persists in a small collective (Levine 2005). On the other hand, IFN α is a promising therapeutic tool irrespective of virologic response. Due to its antiproliferative properties on IgM-RF producing B cells and stimulation of macrophage-mediated clearance of immunocomplexes, IFN a may lead to clinical amelioration even in virological nonresponders. Therefore, therapeutic success should be primarily evaluated on the basis of clinical response irrespective of virologic response. In case of treatment failure of antiviral therapy and/or fulminant manifestations, contraindications or severe side effects, alternative therapeutic strategies, such as cytostatic immunosuppresive therapy and/or plasmapheresis should be taken into consideration (Craxi 2008) (Figure 3; Table 4).

In cases of severe systemic vasculitis, initial therapy with rituximab, a monoclonal chimeric antibody against CD20 B cell specific antigen, is suggested. Its efficacy and safety have also been demonstrated in patients with symptomatic MC resistant to IFN a therapy, although HCV RNA increased approximately twice the baseline levels in responders (Sansonno 2003). In this light, combined application of rituximab with PEG-IFN α plus ribavirin in cases of severe mixed cryoglobulinaemia-related vasculitis resistant to antiviral therapy seems to be the optimal therapeutic strategy, achieving amelioration of MC-related symptoms and a complete eradication of HCV in responders (Saadoun 2008). In severe rituximab-refractory mixed cryoglobulinaemia-related vasculitis or acute manifestations, cycles of plasma exchange plus corticosteroids and eventually cyclophosphamide are indicated.

Effectiveness of antiviral therapy on cryoglobulinaemic-induced peripheral neuropathy is still being discussed. While HCV-related peripheral neuropathy responsive to antiviral

therapy with IFN a and ribavirin in 4 patients with chronic HCV was reported (Koskinas 2007), several authors report on aggravation of cryoglobulinaemic neuropathy or even *de novo* occurance of demyelinating polyneuropathy during IFN α and PEG-IFN α treatment (Boonyapist 2002; Khiani 2008). Therefore, application of IFN α in presence of HCV-related neuropathy requires a cautious risk-benefit assessment.

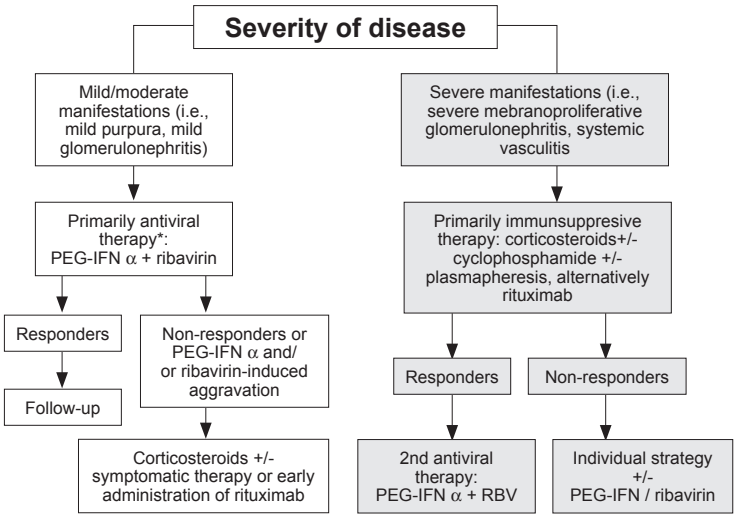


Fig. 3. Therapy algorithm for symptomatic HCV-related mixed cryoglobulinaemia (modified after Craxi 2008). Antiviral therapy, on the basis of PEG-IFN α and ribavirin, is regarded as first line therapy in cases of mild/moderate manifestations. In case of contraindications, patients should be treated primarily with corticosteroids. Non-response to antiviral therapy or drug-induced aggravation make application of corticosteroids essential. Long-term therapy with corticosteroids may result in elevation of viral load and progression of hepatic disease. In this light, rituximab represents an attractive alternative, for in this case, drug-induced viral load elevation is of minor extent. In patients with severe manifestations, treatment should focus on immunosupression (± plasmapheresis). Due to its excellent immunosuppressive properties and relatively mild side effect profile, use of rituximab should be favored in this constellation. In case of good clinical response, consecutive antiviral treatment in terms of PEG-IFN α and ribavirin may serve as a maintenance therapy. Therapy refractory cases require individual treatment according to the particular center experience. Supplementation of therapeutic strategy by antiviral therapy should be taken into consideration.

As eradication of Helicobacter pylori may lead to complete remission of MALT lymphoma, so antiviral therapy can lead to regression of low-grade NHL in patients with HCV-related malignant lymphoproliferative disorders. PEG-IFN α plus ribavirin should be regarded in such cases as first line therapy (Giannelli 2003; Vallisa 2005). Thus, remission of the haematologic disorders is closely associated with virologic response or rather achievement of sustained virologic response. Effectiveness of IFN α in this context should be ascribed primarily to its antiviral and less to its anti-proliferative drug properties.

Author	Patients	Therapy	Notes
Zuckerman	N=9 Virological non-responders with symptomatic cryoglobulinaemia after IFN α monotherapy	Standard IFN α plus ribavirin 15mg/kg/d	CGs undetectable within 6 weeks in 7/9 patients; Clinical improvement in 9/9 within 10 week
Sansonno	N=20 Clinical non-responders with vasculitis and peripheral neuropathy after IFN α montherapy	Rituximab 375 mg/m^2/ week for 4 weeks	16 patients with complete clinical response; 12 with sustained response throughout follow-up. Viraemia increase in responders
Saadoun	N=16 Cryoglobulinaemic-vasculitis in relapsers or non-responders to IFN α/PEG-IFN α + ribavirin	Rituximab 375 mg/m^2/wk for 4 weeks combined with PEG-INF α 1.5 ug/kg/wk plus RBV (600 mg - 1200 mg/d) for 12 months	10/16 complete clinical response; CGs and RNA HCV undetectable in responders
Bruchfeld	N=7 HCV-related renal manifestations (2/7 cryoglobulinaemia-related)	IFN α plus low-dose RBV (200-600mg) or PEG-INFα plus low-dose ribavirin	Improvement of GRF and proteinuria in 4/7 patients and sustained viral response in 5/7.
Roccatello	N=6 Cryoglubulinaemic-systematic manifestations; predominantly renal (5/6)	Rituximab 375 mg/m^2/wk for 4weeks plus rituximab 375mg/m^2 1 month and 2 months later	Decrease of cryocrit and proteinuria at month 2, 6, 12.
Koskinas	N=4 Cryoglobulinaemic-patients with severe sensory-motor polyneuropathy	INF α-2b 1,5ug/kg/week + ribavirin 10.6 mg/kg/d for 48 weeks	Significant improvement of neurological parameters in 4/4; Undetectable HCV RNA and lower CG-levels in 3/4 at the end of therapy.

Table 4. Treatment of cryoglobulinaemia-related disorders in patients with chronic HCV infection.

Treatment of HCV infected patients with high-grade NHL should be based on cytostatic chemotherapy. HCV infection does not constitute a contraindication for cytostatic chemotherapy. Unlike HBV infection, antiviral prophylaxis before chemotherapy introduction is not obligatory. Chemotherapy may lead to a substantial increase in viraemia. Consecutive exacerbation of the infection, making discontinuation of chemotherapy mandatory, is unlikely to occur. However, treatment- related liver toxicity is more frequent in HCV-positive NHL and is often associated with severe hepatic manifestations (Besson 2006; Arcaini 2009). Current data suggest that antiviral treatment may serve as maintenance therapy for achieving sustained remission of NHL after chemotherapy completion (Gianelli 2003).

Further haematological manifestations

HCV-associated thrombocytopenia

Thrombocytopenic conditions (platelet counts below 150 x 103/uL) are often observed in patients with chronic hepatitis C and result mainly from advanced liver fibrosis and manifest cirrhosis (Wang 2004). Lack of hepatic-derived thrombopoetin can inter alia be recognized as an important causal factor (Afdhal 2008). As HCV RNA can be abundant in platelets (Takehara 1994) and megakariocytes of thrombocytopenic patients, direct cytopathic involvement of HCV can be hypothesized (Bordin 1995; De Almeida 2004). Furthermore, it has been suggested that exposure to HCV may be a causative factor for the production of platelet-associated immunoglobulins, inducing thrombocytopenia through a similar immunological mechanism to that operating in immune thrombocytopenic purpura (ITP) (Aref 2009). There is a high HCV prevalence in patients with ITP (García-Suaréz 2000), and these patients exhibit diverse characteristics to HCV-negative patients with ITP, which supports the hypothesis of direct viral involvement in the development of thrombocytopenia (Rajan 2005).

There is no consensus regarding the optimum treatment of HCV-related ITP. Along with classical therapeutic approaches such as corticosteroids, intravenous immunoglobulins and splenectomy, antiviral therapy constitutes another option. Substantial increase of platelets after application of antiviral therapy is registered in a significant percentage of patients with HCV-related ITP (Iga 2005), although evidence from further studies is required to confirm this hypothesis. However, caution is recommended in thrombocytopenic patients treated with PEG-IFN α plus ribavirin as significant aggravation of HCV-related ITP may occur under this regimen (Fattovich 1996). On the other hand, long-term use of steroids and immunosuppressive drugs respectively is limited by an increased risk of fibrosis progression and a substantial elevation of virus. A new orally active thrombopoetin-receptor agonist, elthrombopag, may be used in thrombocytopenic HCV patients in the future. Its efficacy was recently documented in patients with HCV-related ITP (Bussel 2007) as well as in HCV-positive patients suffering from thrombocytopenia due to cirrhosis (McHutchison 2007). In case of refractory disease or aggravation during the course of antiviral therapy, rituximab should be considered (Weitz 2005).

HCV-related autoimmune haemolytic anaemia

Interpretation of autoimmune haemolytic anaemia (AHA) as a possible EHM is based mainly on a few well-documented case reports (Chao 2001; Fernandéz 2006; Srinivasan 2001). AHA has been frequently observed in HCV patients treated with IFN a with and without ribavirin and consequently recognized as a possible side effect of antiviral treatment (De la Serna-Higuera 1999; Nomura 2004). Recently, a large-scale epidemiological study confirmed a high incidence of AHA in HCV patients undergoing antiviral treatment. However, the incidence rate of AHA in treatment-naïve HCV patients was statistically insignificant (Chiao 2009). In this light, there is, for the time being, little evidence for regarding AHA as a possible EHM of chronic HCV infection.

HCV-related glomerulonephritis

Glomerulonephritis (GN) constitutes a rare extrahepatic complication of chronic HCV. Predominant manifestations are cryoglobulinaemic or non-cryoglobulinaemic membranous proliferative GN and mesangioproliferative GN. Far less common is membranous nephropathy (Arase 1998). Other forms of GN do not correlate significantly with HCV infection (Daghestani 1999). Microhaematuria and proteinuria are among the most frequent medical findings in patients with membranous proliferative GN. Approximately 50% of patients exhibit a mild renal insufficiency. 20-25% may present an acute nephritic syndrome (haematuria, hypertension and proteinuria) as in 25% of patients nephrotic syndrome represents the initial manifestation. In contrast, >80% of patients with HCV-related membranous nephropathy suffer primarily a nephrotic syndrome (Doutrelepont 1993; Rollino 1991). The mesangioproliferative form proceeds mostly asymptomatically, where typical findings such as haematuria and proteinuria are often missing (McGuire 2006).

The pathomechanism of renal impairment is yet not fully understood. It can be hypothesized that glomerular injury is primarily caused by a deposition of circulating immunocomplexes containing anti-HCV antibodies, HCV antigens and complement factors. Formation and deposition of such immunocomplexes occurs also in the absence of CGs. HCV-proteins in glomerular and tubolointerstitial structures are immunohistologically detectable in approximately 70% of the patients with chronic HCV (Sansonno 1997). Further possible pathomechanisms of glomerular injury encompass formation of glomerular autoantibodies, glomerular impairment due to chronic hepatic injury, or IgM overproduction with consecutive glomerular IgM deposition as result of HCV-triggered cryoglobulinaemia type II. GN prevalence in HCV patients is estimated at 1.4% and is comparably high to its prevalence among blood donors (Paydas 1996).

HCV induced GN has mostly a benign prognosis (Daghestani 1999). 10-15% of patients with nephritic syndrome experience spontaneous complete or partial remission. Frequently persisting mild proteinuria exhibits no tendency to progression. It is estimated that only approximately 15% of the patients with HCV-related GN develop terminal renal failure requiring dialysis (Tarantino 1995). Nevertheless, presence of kidney impairment is considered to be a negative prognostic factor for long-term survival (Ferri 2004).

Patients with HCV-related GN should be primarily treated with antivirals. Sustained viral response leads normally in cases of mild renal impairment to amelioration of proteinuria or even full remission of GN. In case of high baseline viraemia and advanced renal insufficiency application of antiviral therapy is subject to certain limitations (Sabry 2002). Despite amelioration of proteinuria achieved after antiviral therapy, significant improvement of renal function is often lacking (Alric 2004). PEG-IFN and ribavirin dosage must be cautiously adjusted to glomerular filtration rate (GFR), in order to prevent mainly ribavirin accumulation with consecutive haemolytic anaemia (Fabrizi 2008). Even in advanced renal failure, use of ribavirin is recommended, due to superior efficacy of the combination therapy vs. IFN monotherapy (Bruchfeld 2003; Baid-Agrawal 2008). In patients with GFR <30 ml/min ribavirin dosage should not exceed 600mg/week. Careful dosage augmentation may be undertaken

in the absence of side effects. Ribavirin dosages up to 100-400mg/day was done under vigilant blood level monitoring in dialysis patients. Ribavirin-induced haemolytic anaemia was efficiently treated by administration of erythropoetin and erythrocyte concentrates (van Leusen 2008). As determination of ribavirin blood levels is not an established laboratory procedure, implementation of such a therapeutic approach in clinical routine remains arduous.

Fulminant manifestations with impending acute renal failure make administration of corticosteroids, immunosuppressive drugs such as cyclophosphamid and eventually plasmapheresis necessary (Garini 2007; Margin 1994). In cases of simultaneous bone marrow B cell infiltration and/or resistance to conventional therapy, application of rituximab is indicated (Roccatello 2004). Rituximab may be used as an alternative first line therapy in severe renal manifestations (Roccatello 2008). Antiviral and immunosuppressive therapy should always be supplemented with symptomatic therapy with ACE inhibitors or AT1 receptor antagonists (Kamar 2006).

Endocrine manifestations

Thyroid disease is found more commonly in patients with chronic HCV infection than in general population. About 13% of HCV-infected patients have hypothyroidism and up to 25% have thyroid antibodies (Antonelli 2004). On the other hand, there is evidence that IFN α may induce thyroid disease or unmask preexisting silent thyroidopathies (Graves disease, Hashimoto thyroiditis) (Prummel 2003). In addition, some studies suggest that thyroid autoimmune disorders were significantly present in patients with chronic hepatitis C during but not before IFN α therapy (Marazuela 1996; Vezali 2009). In this light, the role of chronic hepatitis C infection per se in the development of thyroid disorders remains to be determined. The presence of autoantibodies against thyroid with/or without clinical manifestations increases the risk of developing an overt thyroiditis significantly during antiviral therapy. Therefore, monitoring of the thyroid function should be performed during treatment.

Association between chronic HCV infection and development of insulin resistance and diabetes mellitus has been discussed in the past (Knobler 2000; Mason 1999; Hui 2003; Mehta 2003). In the meantime, a causal association is backed up by studies demonstrating that antiviral therapy with consecutive sustained viral response correlates with improved diabetic metabolic status and resolution of insulin resistance (Kawaguchi 2007). A recently published meta-analysis of retrospective and prospective studies confirms a high risk for the development of diabetes mellitus type II in patients with chronic HCV infection (OR=1.68, 95%, CI 1.15-2.20) (White 2008). Viral induction of insulin resistance seems to be HCV-specific, as prevalence of diabetes mellitus in HBV-infected patients is significantly lower (White 2008; Imazeki 2008). The pathomechanism of HCV-induced insulin resistance is yet not fully understood. It has been suggested that the appearance of insulin resistance could correlate with certain genotypes of HCV. Furthermore, HCV-dependent upregulation of cytokine suppressor SOC-3 may be responsible for the induction of cell desensitization towards insulin. Insulin resistance in turn, represents an independent risk factor for progression of liver fibrosis in patients with chronic HCV infection (Moucari 2008; Kawaguchi 2004).

Finally, the link between HCV, growth hormone (GH) insufficiency and low insulin-like growth factor (IGF-1) has been hypothesized. Reduced GH secretion could be the result of a direct inhibitory effect of HCV infection at the level of the pituitary or hypothalamus (Plöckinger 2007).

Dermatologic and miscellaneous manifestations

A multitude of cutaneous disorders has been sporadically associated with chronic HCV infection (Hadziyannis 1998). Epidimiologic studies have confirmed the existence of a strong correlation between the sporadic form of porphyria cutanea tarda (PTC) and HCV, though the presence of HCV in PTC patients seems to be subject to strong regional factors. Indeed, HCV prevalence in PTC patients is higher than 50% in Italy, while only 8% in Germany (Fargion 1992; Stölzel 1995).

Strong evidence of a close association between HCV and lichen planus was provided by studies performed in Japan and southern Europe (Nagao 1995; Carrozzo 1996), yet these observations do not apply to all geographic regions (Ingafou 1998). HLA-DR6 has been recognized as a major predisposing factor for development of lichen planus in HCV-positive patients. One hypothesis suggests that geographical fluctuation of HLA-DR6 is responsible for the diverse prevalence among HCV patients (Gandolfo 2002).

Idiopathic pulmonary fibrosis (IPF) represents potentially an EHM, as prevalence of anti-HCV in patients with this disease is notably high (Ueda 1992). Interestingly, alveolar lavage in therapy-naïve HCV patients yielded frequently findings consistent with a chronic alveolitis. Alveolar lavage in the same patients after completion of antiviral therapy showed a remission of inflammatory activity (Yamaguchi 1997). Involvement of CGs in the genesis of IPF is also probable (Ferri 1997).

Numerous central nervous manifestations have been described in association with HCV infection. Cryoglobulinaemic or non-cryoglobulinaemic vasculitis of cerebral blood vessels may be responsible for the relatively high prevalence of both ischaemic and haemorrhagic strokes in young HCV-positive patients (Cacoub 1998). Transverse myelopathies leading to symmetrical paraparesis and sensory deficiency have been recently observed (Aktipi 2007).

Furthermore, chronic HCV infection is associated with significant impairment of quality of life. 35-68% of HCV patients suffer from chronic fatigue, subclinical cognitive impairment and psychomotor deceleration. Symptoms of depression are evident in 2-30% of HCV patients examined (Perry 2008; Forton 2003; Carta 2007). Psychometric as functional magnetic resonance spectroscopy studies suggest altered neurotransmission in HCV-positive groups (Weissenborn 2006; Forton 2001). In addition, significant tryptophan deficiency is detectable in patients with chronic HCV infection. Resulting deficiency of the tryptophan-derived serotonin is likely to favor an occurrence of depressive disorders. There is evidence to suggest that antiviral therapy can lead to elevation of tryptophan blood levels and thus contribute to amelioration of depressive symptoms in HCV patients (Zignego 2007c).

Occasionally, chronic HCV infection has been seen in association with cardiac pathologies such as chronic myocarditis and dilatative/hypertrophic cardiomyopathy. Pathogenesis seems to rely on genetic predisposition and is assumed to be immunologically triggered (Matsumori 2000).

References

Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, Esteban R. Thrombocytopenia associated with chronic liver disease. J Hepatol 2008;48: 1000-1007.

Agnello V., G. Ábel. Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. Arthritis Rheumatism 1997; 40: 2007-2015.

Aktipi KM, Ravaglia S, Ceroni M. Severe recurrent myelitis in patients with hepatitis C virus infection. Neurology2007;68: 468–469.

Alric L, Plaisier E, Thébault S, Péron JM, Rostaing L, Pourrat J, Ronco P, Piette JC, Cacoub P. Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. Am J Kidney Dis 2004;43: 617-623.

Antonelli A, Ferri C, Pampana A, et al. Thyroid disorders in chronic hepatitis C. Am J Med 2004;117: 10-13.

Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I,et al. Glomerulonephritis in autopsy cases with hepatitis C virus infection. Intern Med 1998;37:836-40.

Arcaini L, Merli M, Passamonti F, Bruno R, Brusamolino E, Sacchi P, Rattotti S, Orlandi E, Rumi E, Ferretti V, Rizzi S, Meli E, Pascutto C, Paulli M, Lazzarino M. Impact of treatment-related liver toxicity on the outcome of HCV-positive non-Hodgkin's lymphomas. 2010;85: 45-50.

Aref S, Sleem T, El Menshawy N, Ebrahiem L, Abdella D, Fouda M, Samara NA, Menessy A, Abdel-Ghaffar H, Bassam A, Abdel Wahaab M. Antiplatelet antibodies contribute to thrombocytopenia associated with chronic hepatitis C virus infection. Hematology 2009;14: 277-281.

Baid-Agrawal S, Pascual M, Moradpour D, Frei U, Tolkoff-Rubin N. Hepatitis C virus infection in haemodialysis and kidney transplant patients. 2008;18: 97-115.

Besson C, Canioni D, Lepage E, Pol S, Morel P, Lederlin P, Van Hoof A, Tilly H, et al. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. 2006;24: 953-60.

Boonyapisit K, Katirji B. Severe exacerbation of hepatitis C-associated vasculitic neuropathy following treatment with interferon alpha: a case report and literature review. Muscle Nerve 2002;25: 909-913.

Bordin G, Ballare M, Zigrossi P, Bertoncelli MC, Paccagnino L, et al. A laboratory and thrombokinetic study of HCV-associated thrombocytopenia: a direct role of HCV in bone marrow exhaustion? Clin Exp Rheumatol 1995;Suppl 13: S39-43.

Bruchfeld A, Lindahl K, Stahle L, Schvarcz R. Interferon and ribavirin treatment in patients with hepatitis C-associated renal disease and renal insufficiency. Nephrol Dial Transplant 2003;18(8):1573-80.

Bussel JB, Cheng G, Saleh MN, Psaila B, Kovaleva L, Meddeb B, Kloczko J, Hassani H, Mayer B, Stone NL, Arning M, Provan D, Jenkins JM. Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. 2007;357:2237-47.

Cacoub P, Sbai A, Hausfater P, et al. Central nervous system involvement in hepatitis C virus infection. Gastroenterol Clin Biol 1998;22: 631–633.

Cacoub P, Poynard T, Ghillani P, et al. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC group. Multidepartment Virus C. Athritis Rheum 1999;42: 2204-2212.

Cacoub P, Renou C, Rosenthal E, et al. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. Groupe d' Etude et de Recherche en Medicine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. Medicine 2000;79: 47-56.

Calleja JL, Albillos A, Moreno-Otero R, et al. Sustained response to interferon-alpha or to interferon-alpha plus ribavirin in hepatitis C virus-associated symptomatic mixed cryoglobulinaemia. Aliment Pharmacol Ther 1999;13: 1179-1186.

Carrozzo M, Gandolfo S, carbone M, Colombatto P, Broccoletti R, Garzino-Demo P, et al. Hepatitis C virus infection in Italian patients with oral lichen planus: a prospective case-control study. J Oral Pathol Med 1996;25: 527-33.

Carta MG, Hardoy MC, Garofalo A, Pisano E, Nonnoi V, Intilla G, Serra G, Balestrieri C, Chessa L, Cauli C, Lai ME, Farci P. Association of chronic hepatitis C with major depressive disorders: irrespective of interferon-alpha therapy. Clin Pract Epidemol Ment Health 2007;3: 22.

Chao TC, Chen CY, Yang YH, et al. Chronic hepatitis C virus infection associated with primary warm-type autoimmune hemolytic anemia. J Clin Gastroenterol 2001;33: 232-233.

Chiao EY, Engels EA, Kramer JR, Pietz K, Henderson L, Giordano TP, Landgren O. Risk of immune thrombocytopenic purpura and autoimmune hemolytic anemia among 120 908 US veterans with hepatitis C virus infection. Arch Intern Med 2009;169: 357-363.

Colantoni A De Maria N, Idilman R, Van Thiel DH. Polymerase chain reaction for the detection of HCV-RNA: cryoglobulinaemia as a cause for false negative results. Ital J Gastroenterol Hepatol 1997;29: 273–277.

Cormier EG, Tsamis F, Kajumo F, Durso RJ, Gardner JP, Dragic T. CD81 is an entry coreceptor for hepatitis C virus. Proc Natl Acad Sci US 2004;101: 7270-7274.

Craxì A, Laffi G, Zignego AL. Hepatitis C virus (HCV) infection: a systemic disease. Mol Aspects Med 2008;29: 85-95.

Daghestani L, Pomeroy C. Renal manifestations of hepatitis C infection. Am J Med 1999;106: 347-354.

De Almeida AJ, Campos-de-Magalhaes M, Okawa MY, Vieira de Oliveira R, et al. Hepatitis C virus-associated thrombocytopenia: a controlled prospective, virological study. Ann Hematol 2004;83: 434-440.

De la Serna- Higuera C, Bárcena-Marugán R, Sanz-de Villalobos E. Hemolytic anemia secondary to alpha-interferon treatment in a patient with chronic C hepatitis. J Clin Gastroenterol 1999;28: 358-9.

Doutrelepont J-M., M. Adler, M. Willems, P. Durez, S-H. Yap. Hepatitis C virus infection and membranoproliferative glomerulonephritis. Lancet 1993;341:317.

Duberg AS, Nordström M, Törner A, Reichard O, Strauss R, Janzon R, Bäck E, Ekdahl K. Non-Hodgkin's lymphoma and other nonhepatic malignancies in Swedish patients with hepatitis C virus infection. Hepatology 2005;41: 652-659.

Fabrizi F, Lunghi G, Messa P, Martin P. Therapy of hepatitis C virus-associated glomerulonephritis: current approaches. J Nephrol 2008;21:813-825.

Fargion S, Piperno A, Cappellini MD et al. Hepatitis C virus and porphyria cutanea tarda: evidence of a strong association. Hepatology 1992;16: 1322-26.

Fattovich G, Giustina G, Favarato S, Ruol A. A survey of adverse events in 11,241 patients with chronic viral hepatitis treated with alfa interferon. J Hepatol 1996;24:38-47.

Fernandéz A. An unusual case of autoimmune hemolytic anemia in treatment naïve hepatitis C virus infection. Hematology 2006;11: 385–387.

Ferri C, La Civita L, Fazzi P, Solfanelli S, Lombardini F, Begliomini E, et al. Intestinal lung fibrosis and rheumatic disorders in patients with hepatitis C virus infection. Br J Rheumatol 1997;36: 360-365.

Ferri C, Sebastiani M, Guiggiolo D, Cazzato M, Longombardo G, Antonelli A, et al. Mixed cryoglobulinaemia: demographic, clinical, and serologic features and survival in 231 patients. Semin Arthritis Rheum 2004;33: 355-374.

Ferri C, Antonelli A, Mascia M.T, et al. B cells and mixed cryoglobulinemia. Autoimmun Rev 2007;7: 114-20.

Forton DM, Allsop JM, Main J, et al. Evidence for a cerebral effect of the hepatitis C virus. Lancet 2001;358: 38–39.

Forton DM, Taylor-Robinson SD, Thomas HC. Cerebral dysfunction in chronic hepatitis C infection. J Viral Hepat 2003;10: 81-86.

Gandolfo S, Carrozzo M. Lichen planus and hepatitis C virus infection. Minerva Gastroenterol Dietol 2002;48: 89.

García-Suárez J, Burgaleta C, Hernanz N, Albarran F, Tobaruela P, Alvarez-Mon M. HCV-associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy. Br J Haematol 2000;110: 98-103

Garini G, Allegri L, Lannuzzella F, Vaglio A, Buzio C. HCV-related cryoglobulinemic glomerulonephritis: implications of antiviral and immunosuppressive therapies. Acta Biomed 2007;78:51-59.

Giannelli F, Moscarella S, Giannini C, Caini P, Monti M, et al. Effect of antiviral treatment in patients with chronic HCV infection and t(14;18) translocation. Blood 2003;102: 1196-201.

Giordano TP, Henderson L, Landgren O, Chiao EY, Kramer JR, El-Serag H, Engels EA. Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. JAMA 2007;297: 2010-2017.

Hadziyannis SJ. Skin diseases associated with hepatitis C virus infection. J Eur Acad Dermatol Venereol 1998;10: 12-21.

Hui JM, Sud A, Farrell GC, Bandara P, et al. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. Gastroenterology 2003;125 :1695- 1704.

Iga D, Tomimatsu M, Endo H, Ohkawa S-I, Yamada O. Improvement of thrombocytopenia with disappearance of HCV RNA in patients treated by interferon-a therapy: possible etiology of HCV-associated immune thrombocytopenia. Eur J Haematol 2005;75: 417-423.

Imazeki F, Yokosuka O, Fukai K, Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus- infected and hepatitis C virus-cleared patients. Liver international 2008;28: 355-362.

Ingafou M, Porter SR, Scully C, Teo CG. No evidence of HCV infection or liver disease in British patients with oral lichen planus. Int J Oral Maxillofac Surg 1998;27: 65-66.

Kamar N, Rostaing L, Alric L. Treatment of hepatitis C-virus-related glomerulonephritis. Kidney Int 2006;69: 436-439.

Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004;165: 1499-508.

Kawaguchi T, Ide T, Taniguchi E, et al. Clearance of HCV improves insulin resistance, ß-cell function, and hepatic expression of insulin receptor substrates 1 and 2. Am J Gastroenterol 2007;102: 1-7.

Khiani V, Kelly T, Adeel S, Jensen D, Mohanty SR. Acute inflammatory demyelinating polyneuropathy associated with pegylated interferon a 2a therapy for chronic hepatitis C virus infection. World J Gastroenterol 2008;14: 318-321.

Knobler H, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of diabetes in noncirrhotic patients with chronic hepatitis C virus infection. Mayo Clin Proc 2000;75: 355-359.

Koskinas J, Kilidireas C, Karandreas N, Kountouras D, Savvas S, et al. Severe hepatitis C virus-related cryoglobulinaemic sensory-motor polyneuropathy treated with pegylated interferon-a2b and ribavirin: clinical, laboratory and neurophysiological study. Liver Int 2007;27:414-420.

Levine JW, Gota C, Fessler BJ, et al. Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. J Rheumatol 2005;32:1164–1167.

Lidove O, Cacoub P, Maisonobe T, Servan J, Thibault V, Piette JC, et al. Hepatitis C virus infection with peripheral neuropathy is not always associated with cryoglobulinaemia. Ann Rheum Dis 2001;60: 290-292.

Lunel F, Musset L, Cacoub P, Franguel L, Cresta P, Perri M, et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology 1994;106: 1291-300.

Machida K, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, et al. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci USA 2004;101:4262-4267.

Mason AL, Lau JY, Hoang N, et al. Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 1999;29: 328-333.

Marazuela M, Garcia-Buey L, Gonzalez-Fernandez B, et al. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-alpha therapy. Clinical Endocrinology 1996;44: 635-642.

Margin S, Craxi A, Fabiano C et al. Hepatitis C viraemia in chronic liver disease: relationship to interferon alpha or corticosteroid treatment. Hepatology 1994;19: 273–279.

Matsumori A, Yutani C, Ikeda Y, Kawai S, Sasayama S. Hepatitis C virus from the hearts of patients with myocarditis and cardiomyopathy. Lab Invest 2000;80: 1137-42.

Matsuo K, Kusano A, Sugumar A, Nakamura S, Tajima K, Mueller NE. Effect of hepatitis C virus infection on the risk of non-Hodgkin's lymphoma: a meta-analysis of epidemiological studies. Cancer Sci 2004;95:745-52.

McGuire BM, Julian BA, Bynon JS Jr, Cook WJ, King SJ, Curtis JJ, Accortt NA, Eckhoff DE. Brief communication: Glomerulonephritis in patients with hepatitis C cirrhosis undergoing liver transplantation. Ann Intern Med 2006;144(10):735-41.

McHutchison JG, Dusheiko G, Shiffman ML, Rodriguez-Torres M, Sigal S, Bourliere M, Berg T, et al. Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. N Engl J Med 2007;357:2227-2236.

Mele A, Pulsoni A, Bianco E, Musto P, Szklo A, Sanpaolo MG, Iannitto E, et al. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study. Blood 2003;102: 996-9.

Mehta SH, Brancati FL, Strathdee SA, Pankow JS, et al. Hepatitis C virus infection and incident type 2 diabetes. Hepatology 2003;38: 50-56.

Moucari R, Asselah T, Cazals-Hatem D, Voitot H, et al. Insulin resistance in chronic hepatitis C: Association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology 2008;134: 416-423.

Nagao Y, Sata M, Tanikawa K, Itoh K, Kameyama T. Lichen planus and hepatitis C virus in the northern Kyushu region of Japan. Eur J Clin Invest 1995;25: 910-914.

Nomura H, Tanimoto H, Kajiwara E, Shimono J, Maruyama T, Yamashita N, et al. Factors contributing to ribavirin-induced anemia. J Gastroenterol Hepatol 2004;19(11):1312-7.

Paydas S., N. Seyrek, G. Gonlusen, Y. Sagliker. The frequencies of hepatitis B virus markers and hepatitis C virus antibody in patients with glomerulonephritis. Nephron 1996;74: 617-619.

Perry W, Hilsabeck RC, Hassanein TI. Cognitive dysfunction in chronic hepatitis C: a review. Dig Dis Sci 2008;53: 307-321.

Plöckinger U, Krüger D, Bergk A, Weich V, Wiedenmann B, Berg T. Hepatitis-C patients have a reduced growth hormone (GH) secretion which improves during long-term therapy with pegylated interferon-a. Am J Gastroenterol 2007;102: 2724-2731.

Prummel MF, Laurberg P. Interferon-alpha and autoimmune thyroid disease. Thyroid 2003;13: 547-551.

Rajan SK, Espina BM, Liebman HA. Hepatitis C virus-related thrombocytopenia: clinical and laboratory characteristics compared with chronic immune thrombocytopenic purpura. Br J Haematol 2005;129: 818-824.

Ray RB, Meyer K, Ray R. Suppression of apoptotic cell death by hepatitis C virus core protein. Virology 1996;226(2): 176-182.

Roccatello D, Baldovino S, Rossi D, Mansouri M, Naretto C et al. Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. Nephrol Dial Transplant 2004;19: 3054-61.

Roccatello D, Baldovino S, Rossi D, Giachino O, Mansouri M et al. Rituximab as a Therapeutic Tool in Severe Mixed Cryoglobulinemia. Clin Rev Allergy Immunol 2008;34: 111-117.

Rollino C., D. Roccatello, O Giachino, B. Basolo, G. Piccoli. Hepatitis C virus Infection and membranous glomerulonephritis. Nephron 1991;59: 319-320.

Roque Afonso AM, Jiang J, Penin F, Tareau C, Samuel D, Petit MA, et al. Nonrandom distribution of hepatitis C virus quasispecies in plasma and peripheral blood mononuclear cell subsets.J Virol 1999;73: 9213-21.

Saadoun D, Asselah T, Resche-Rigon M, Bedossa P, Valla D, et al. Cryoglobulinemia is associated with steatosis and fibrosis in chronic hepatitis C. Hepatology 2006;43: 1337-1345.

Saadoun D, Resche-Rigon M, Sene D, Perard L, Piette JC, Cacoub P. Rituximab combined with Peg-Interferon-Ribavirin in refractory HCV-associated cryoglobulinemia vasculitis. Ann Rheum Dis 2008;67: 1431-1436.

Sabry AA, Sobh MA, Sheaashaa HA, Kudesia G, Wild G, Fox S, et al. Effect of combination therapy (ribavirin and interferon) in HCV-related glomerulopathy. Nephrol Dial Transplant 2002;17: 1924-30.

Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. J Virol 1995;69(6):3893-3896.

Sansonno D., L. Gesualdo, C. Manno, F.P. Schena, F. Dammacco. Hepatitis C virus-related proteins in kidney tissue from hepatitis C virus-infected patients with cryoglobulinemic membranoproliferative glomerulonephritis. Hepatology 1997;25, 1237-1244.

Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. Blood 2003;101: 3818-26.

Silvestri F, Barillari G, Fanin R, Pipan C, Falasca E, Salmaso F, et al. Hepatitis C virus infection among cryoglobulinemic and non-cryoglobulinemic B-cell non-Hodgkin's lymphomas. Haematologica 1997;82: 314-7.

Srinivasan R. Autoimmune hemolytic anemia in treatment naıve chronic hepatitis C infection. J Clin Gastroenterol 2001;32: 245–247.

Stölzel U, Köstler E, Koszka C, Stöffler-Meilicke M, Schuppan D, Somasundaram R, et al. Low prevalence of hepatitis C virus infection in porphyria cutanea tarda in Germany. Hepatology 1995;21:1500-3.

Takehara K, Otsuka T, Arai T, Matsuzaki Y, et al. Detection of hepatitis C virus (HCV) in platelets of type C chronic liver diseases by polymerase chain reaction (PCR). Gastroenterology 1994;106: A995.

Tarantino A, Campise M, Banfi G, Confalonieri R, Bucci A, Montoli A, et al. Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. Kidney Int 1995;47: 618-623.

Tembl JI, Ferrer JM, Sevilla MT, Lago A, Mayordomo F, Vilchez JJ. Neurologic complications associated with hepatitis C virus infection. Neurology 1999;19: 889-95.

Ueda T, Ohta K, Suzuki N, Yamaguchi M, Hirai K, et al. Idiopathic pulmonary fibrosis and high prevalence of

serum antibodies to hepatitis C virus. Am Rev respire Dis 1992;146: 266-268.

Vallisa D, Bernuzzi P, Arcaini L, Sacchi S, Callea V, Marasca R, et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: a multicenter Italian experience. J Clin Oncol 2005;23: 468-73.

van Leusen R, Adang RP, de Vries RA, Cnossen TT, Konings CJ, Schalm SW, Tan AC. Pegylated interferon alfa-2a (40 kD) and ribavirin in haemodialysis patients with chronic hepatitis C. Nephrol Dial Transplant 2008;23: 721-725

Vezali E, Elefsiniotis I, Mihas C, Konstantinou E, Saroglou G. Thyroid dysfunction in patients with chronic hepatitis C: Virus-or therapy-related? J Gastroenterol Hepatol 2009;24: 1024-1029.

Vigano M, Lampertico P, Rumi MG, Folli C, Maggioni L, et al. Natural history and clinical impact of cryoglobulins in chronic Hepatitis C: 10-year prospective study of 343 patients. Gastroenterology 2007;133: 835-42.

Wang CS, Yao WJ, Wang ST, Chang TT, Chou P. Strong association of Hepatitis C virus (HCV) infection and thrombocytopenia: implications from a survey of a community with hyperendemic HCV infection. Clin Infect Dis 2004;39: 790-796.

Weiner SM, Berg T, Berthold C, Weber S, Peters T, Blum H, Hopf U, Peter HH. A clinical and virological study of hepatitis C virus-related cryoglobulinemia in Germany. J Hepatol 1998;29: 375-384

Weissenborn K, Ennen JC, Bokemeyer M, Ahl B, Wurster U, Tillmann H, Trebst C, Hecker H, Berding G. Monoaminergic neurotransmission is altered in hepatitis C virus infected patients with chronic fatigue and cognitive impairment. Gut. 2006;55: 1624-1630.

Weitz IC. Treatment of immune thrombocytopenia associated with interferon therapy of hepatitis C with the anti-CD20 monoclonal antibody, rituximab. Am J Hematol 2005;78:138–141.

White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: A systematic review and meta-analysis. J Hepatol 2008;49: 831-844.

Wintrobe M., M. Buell. Hyperproteinemia associated with multiple myeloma. With report of a case in which extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynoud's disease. Bull Johns Hopkins Hosp 1933;52: 156-165.

Wong VS, Egner W, Elsey T, Brown D, Alexander GJ. Incidence, character and clinical relevance of mixed cryoglobulinaemia in patients with chronic hepatitis C virus infection. Clin Exp Immunol 1996;104: 25-31.

Yamaguchi S, Kubo K, Fujimoto K, Hanaoka M, Hayasaka M, et al. Bronchoalveolar lavage fluid findings in patients with chronic hepatitis C before and after treatment with interferon alpha. Thorax 1997;52: 33-37.

Zignego AL, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, et al. Infection of peripheral mononuclear blood cells by hepatitis C virus. J Hepatol 1992;382-6.

Zignego AL, Giannelli F, Marrocchi ME, Mazzocca A, Ferri C, Giannini C, et al. T(14;18) translocation in chronic hepatitis C virus infection. Hepatology 2000;31: 474-9.

Zignego AL, Ferri C, Pileri SA, Caini P, Bianchi FB. Extrahepatic manifestations of the Hepatitis C Virus infection: a general overview and guidelines for clinical approach. Dig Liver Dis 2007a;39: 2-17

Zignego AL, Giannini C, Ferri C. Hepatitis C virus-related lymphoproliferative disorders: an overview. World J Gastroenterol 2007b;13: 2467-78.

Zignego AL, Cozzi A, Carpenedo R, Giannini C, Rosselli M, Biagioli T, et al. HCV patients, psychopathology and tryptophan metabolism: analysis of the effects of pegylated interferon plus ribavirin treatment. Dig Liver Dis 2007c;39: 107-111.

Part 4

Coinfections

Chapter 17: Management of HBV/HIV coinfection

Stefan Mauss, Jürgen Rockstroh

Introduction

The prevalence and transmission routes of HBV coinfection in the HIV population vary substantially by geographic region (Alter 2006; Konopnicki 2005). In the United States and Europe the majority of homosexual men in the HIV population have evidence of past HBV infection, and 5-10% show persistence of HBs antigen with or without replicative hepatitis B as defined by the presence of HBV DNA (Konopnicki 2005). Overall, rates of HBV/HIV coinfection are slightly lower among intravenous drug users compared to homosexual men and much lower among people infected through heterosexual contact (Núñez 2005).

In endemic regions of Africa and Asia, the majority of HBV infections are transmitted vertically at birth or before the age of 5 years through close contact within households, medical procedures and traditional scarification (Modi 2007). The prevalence in the young population in some Asian countries has substantially decreased since the introduction of vaccination on a nationwide level (Shepard 2006). In Europe vaccination of children and members of risk groups is reimbursed by health care systems in most countries.

The natural history of hepatitis B is altered by simultaneous infection with HIV. Immune control of HBV is negatively affected leading to a reduction of HBs antigen seroconversion. If HBV persists, the HBV DNA levels are generally higher in untreated patients (Bodsworth 1989; Bodsworth 1991; Hadler 1991). In addition, with progression of cellular immune deficiency, reactivation of HBV replication despite previous HBs antigen seroconversion may occur (Soriano 2005). In untreated HIV populations faster progression to liver cirrhosis is reported for HBV/HIV-coinfected patients (Puoti 2006). Moreover, hepatocellular carcinoma may develop at an earlier age and is more aggressive in this population (Puoti 2004; Brau 2007).

Being HBV-coinfected results in increased mortality for HIV-seropositive individuals, even after the introduction of highly active antiretroviral combination therapy (HAART), as demonstrated by an analysis of the EuroSIDA Study, which shows a 3.6-fold higher risk of liver-related deaths among HBsAg-positive patients compared to HBsAg-negative individuals (Konopnicki 2005; Nikolopoulos 2009) (Figure 1). In the Multicentre AIDS Cohort Study, an 8-fold increased risk of liver-related mortality was seen among HBV/HIV-coinfected compared to HIV-monoinfected individuals, particularly among subjects with low CD4 nadir counts (Thio 2002). An independent observation from a large cohort confirming this association is the reduction in mortality for HBV/HIV-coinfected patients treated with lamivudine compared to untreated patients (Puoti 2007). This result is even more remarkable because lamivudine is one of the least effective HBV polymerase inhibitors due to a rather rapid development of resistance.

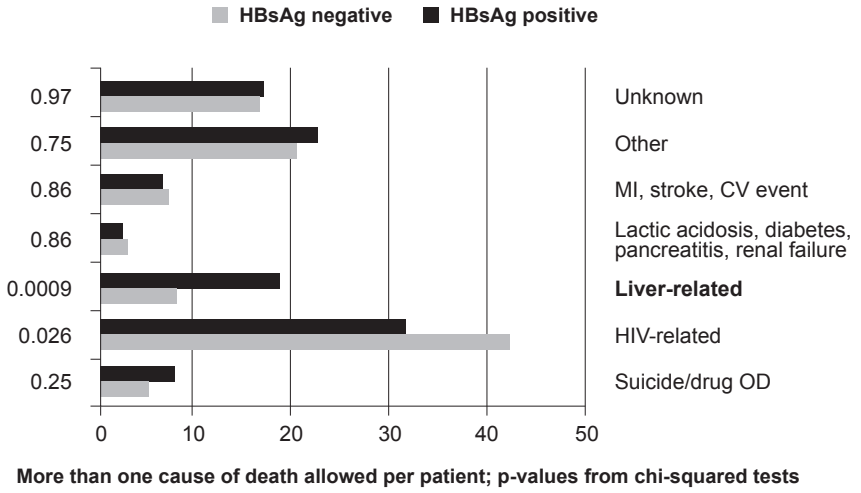


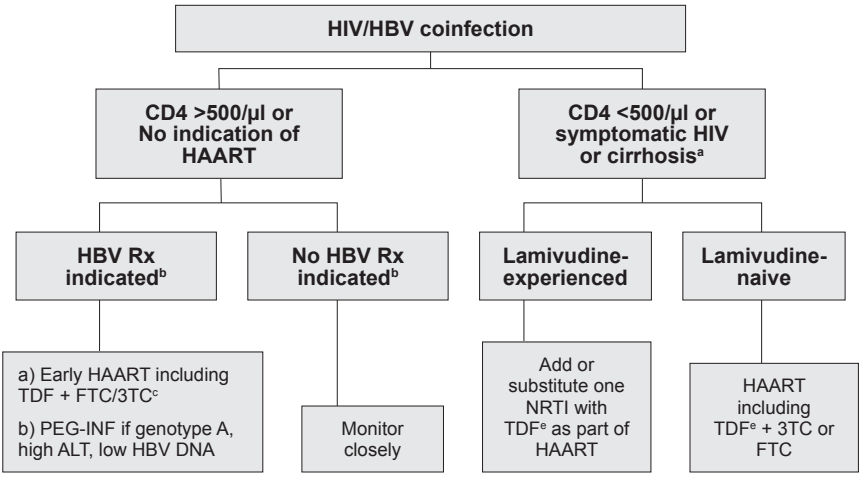
Figure 1: Association of HBV/HIV coinfection and mortality (Konopnicki 2005).

These two large cohort studies along with data from HBV monoinfection studies showing a reduction in morbidity and mortality justify treatment of hepatitis B in HBV/HIV-coinfected patients. HBV is often treated simultaneously with HIV, as some nucleoside and nucleotide reverse transcriptase inhibitors are active as HBV polymerase inhibitors as well. Therefore, antiretroviral therapy should be adjusted according to HBV status wherever possible to avoid higher pill burden and additional toxicities. A less frequent but more challenging situation is the initiation of HBV therapy in HIV-coinfected individuals who are not on antiretroviral therapy. Treatment with interferon is one possible therapeutic option in this situation. The main limitation of HBV polymerase inhibitors may be induction of HIV resistance by the anti-HBV agents as they act simultaneously as HIV reverse transcriptase inhibitors.

HBV therapy in HBV/HIV-coinfected patients without antiretroviral therapy

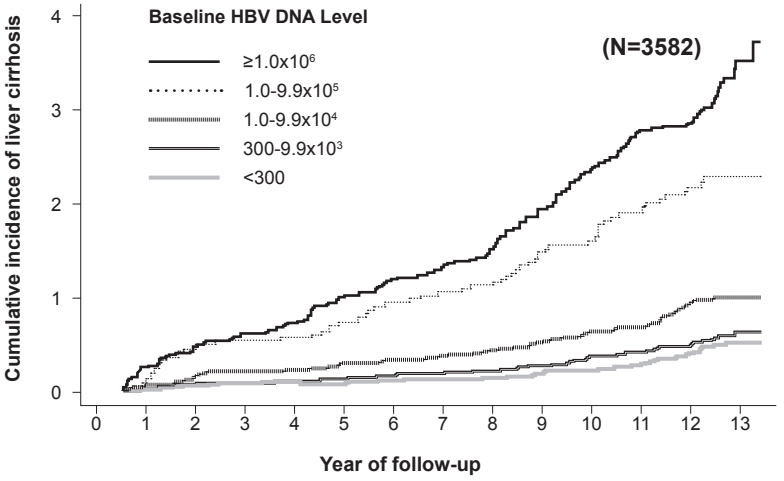
The recommendations of the updated European AIDS Clinical Society (EACS) for the treatment of chronic hepatitis B in HIV-coinfected patients without antiretroviral therapy are shown in Figure 2 (EACS 2009). Starting hepatitis B therapy depends on the degree of liver fibrosis and the HBV DNA level. Using the level of HBV replication as basis for treatment decisions is an important change of paradigm in HBV therapy. This decision is based on the results of the REVEAL study (Iloeje 2006). REVEAL followed the natural course of chronic hepatitis B without liver cirrhosis in about 3700 Taiwanese patients for more than 10 years. In these HBV-monoinfected patients an HBV DNA of >10,000 copies/ml (i.e., 2000 IU/ml) had a markedly increased risk of developing liver

cirrhosis and hepatocellular carcinoma (Figure 3). This association was even observed in patients with normal ALT levels (Chen 2006) (Figure 4). It should be mentioned that this cohort consisted of Asian patients without HIV coinfection predominantly infected at birth or in early childhood. However, the results were considered too important not to form part of the management of HIV-coinfected patients.



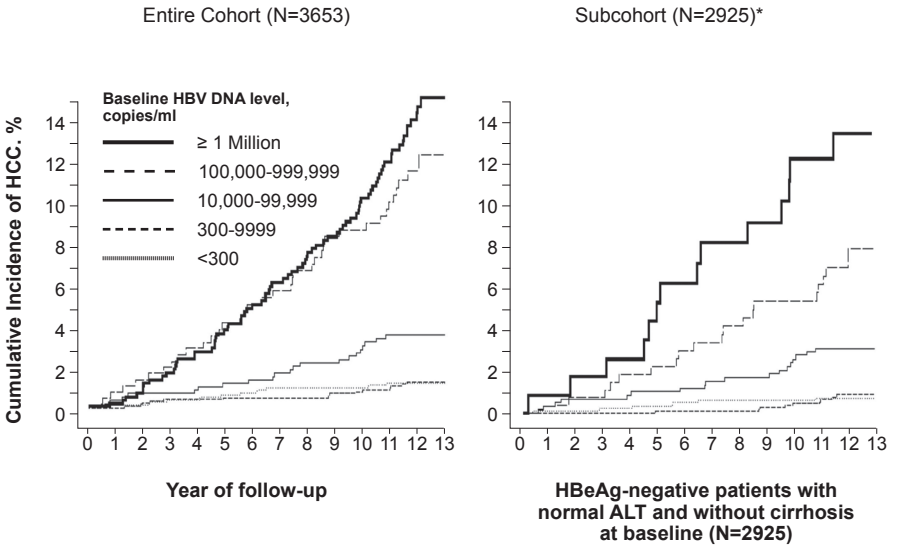
a) Cirrhotic patients should be referred for variceal assessment, have regular HCC monitoring and be referred early for transplant assessment.
b) See Figure 2 for assessment of HBV Rx indication. Some experts strongly think that any HBV-infected patient requiring HAART should receive TDF + 3TC or FTC unless history of TDF intolerance, particularly in HIV/HBV co-infected patients with advanced liver fibrosis (F3/F4).
c) If patient is unwilling to go on early HAART, adefovir and telbivudine may be used as an alternative to control HBV alone. Recently a case report suggested anti-HIV activity of telbivudine. In vitro *data using an assay able to demonstrate anti-HIV activity of entecavir failed to detect an influence of telbivudine on the replicative capacity of HIV-1. Treatment duration: in patients not requiring HAART and on treatment with telbivudine +/- adefovir, or those on HAART where nucleoside backbone needs changing, anti-HBV therapy may be stopped cautiously in HBeAg+ patients who have achieved HBe seroconversion or HBs seroconversion for at least six months or, after HBs seroconversion; for at least six months in those who are HBeAg-.*
d) Treatment length: 48 weeks for PEG-INF; on-treatment quantification of HBsAg in patients with HBeAg-negative chronic hepatitis B treated with PEG-INF may help identify those likely to be cured by this therapy and optimize treatment strategies.
e) In some cases of tenofovir intolerance (i.e., renal disease), entecavir + adefovir or tenofovir in doses adjusted to renal clearance in combination with effective HAART may be advisable. NRTI substitution should only be performed if feasible and appropriate from the perspective of maintaining HIV suppression. Caution is warranted in switching from a tenofovir-based regimen to drugs with a lower genetic barrier, e.g., FTC/3TC, in particular in lamivudine-pretreated cirrhotic patients, as viral breakthrough due to archived YMDD mutations has been observed. This has also been described in individuals with previous 3TC HBV resistance who have been switched from tenofovir to entecavir.

Figure 2: Treatment algorithm for therapy of HBV in HIV-coinfected patients (EACS 2009).



Lloeje UH et al. Gastroenterology 2006; 130:678

Figure 3: REVEAL Study: Association of HBV DNA level and liver cirrhosis (lloeje 2006).



Chen CJ et al. JAMA 2006; 295:65

Figure 4: REVEAL Study: Association of HBV DNA and the development of hepatocellular carcinoma (Chen 2006).

Usually patients with an HBV DNA of less than 2000 IU/ml have no substantial necroinflammatory activity in the liver and therefore a benign course of fibrosis progression and a low risk for the development of hepatocellular carcinoma. However, especially in patients harbouring HBV precore mutants, fluctuations in HBV DNA and ALT are not rare. Monitoring of the activity of the HBV DNA and ALT accompanied by a sonography every 6-12 months is recommended. In the case of HBV DNA <2000 IU/ml and elevated transaminases and/or signs of advanced liver fibrosis, alternative causes of hepatitis and liver toxicity should be excluded.

For patients with HBV DNA >2000 IU/ml the ALT level is the next decision criterion. Patients with normal ALT should be assessed for liver fibrosis by liver biopsy or elastometry. In case of lack of substantial liver fibrosis (METAVIR stage F0/1) monitoring of the activity of the HBV DNA and ALT accompanied by an ultrasound every 3-6 months is recommended. In the presence of liver fibrosis of METAVIR F2 or higher, hepatitis B treatment should be initiated.

For patients with HBV DNA >2000 IU/ml and increased ALT, treatment for HBV is an option particularly in the presence of relevant liver fibrosis.

In patients not taking antiretroviral therapy, pegylated interferon α-2a or -2b seems a suitable option. However, data in the literature for HIV-coinfected patients on interferon therapy for HBV infection are limited and not very encouraging (Núñez 2003). For pegylated interferons no data from larger cohorts exist and one study combining pegylated interferon with adefovir did not show encouraging results (Ingiliz 2008). Favourable factors for treatment success with interferon are low HBV DNA, increased ALT, HBV genotype A or infection wth HBV wild type.

Alternatively patients can be treated with polymerase inhibitors. However, due to their antiretroviral activity tenofovir, emtricitabine and lamivudine are contraindicated in the absence of effective HIV therapy. In contrast to *in vitro* data reported by the manufacturer, antiretroviral activity and induction of the HIV reverse transcriptase mutation M184V was recently reported for entecavir (MacMahon 2007). Currently only telbivudine and adefovir are considered reasonably safe treatment options. There is limited *in vivo* data for adefovir to support this recommendation (Delaugerre 2002; Sheldon 2005). For telbivudine *in vitro* data are available showing a specific inhibitory activity on the HBV polymerase and no effect on HIV (Avilla 2009). However, in contrast with this, two case reports have suggested antiretroviral activity of telbivudine (Low 2009; Milazzo 2009).

Because of its greater antiviral efficacy, telbivudine is preferred by most experts to adefovir (Chan 2007). Alternatively an add-on strategy of telbivudine to adefovir in the case of not fully suppressive antiviral therapy or primary combination therapy of both drugs can be considered although clinical data are not yet available for this strategy.

As both drugs have limitations in the setting of HBV-monoinfected patients due to considerable development of resistance against telbivudine and the limited antiviral efficacy of adefovir, the initiation of antiretroviral therapy using tenofovir plus lamivudine or emtricitabine should be considered, particularly in HIV-coinfected patients with advanced liver fibrosis.

The treatment duration is determined by HBe antigen seroconversion as in HBV-monoinfected patients. In case of infection with a precore mutant HBs antigen seroconversion is the biological endpoint.

Antiretroviral treatment of chronic hepatitis B in HBV/HIV-coinfected patients

For patients on antiretroviral therapy a wider choice of polymerase inhibitors is available. In principle the treatment algorithm in Figure 5 is based on the same principles as outlined above (EACS 2009).

For patients with an HBV DNA <2000 IU/ml and no relevant liver fibrosis no specific antiretroviral regimen is recommended. However when choosing an HBV polymerase inhibitor, the complete suppression of HBV DNA is important to avoid the development of HBV resistance mutations. The activity of the HBV infection in these patients should be assessed at least every six months as part of routine monitoring of the HIV infection including an ultrasound due to the slightly increased risk of hepatocellular carcinoma.

When HBV DNA is above 2000 IU/ml in naive patients a combination of tenofovir plus lamivudine/emtricitabine to treat both infections is recommended. Even for patients who harbour lamivudine-resistant HBV due to previous therapies this strategy stands. The recommendation to continue lamivudine/emtricitabine is based on the delay of resistance to adefovir seen when doing so (Lampertico 2007).

For patients with liver cirrhosis a maximally active continuous HBV polymerase inhibitor therapy is important to avoid hepatic decompensation and reduce the risk of developing hepatocellular carcinoma. Tenofovir plus lamivudine/emtricitabine is the treatment of choice. If the results are not fully suppressive, adding entecavir should be considered. At least every six months, assessement of the liver by ultrasound for early detection of hepatocellular carcinoma is necessary. In patients with advanced cirrhosis gastroscopy should be performed as screening for esophageal varices.

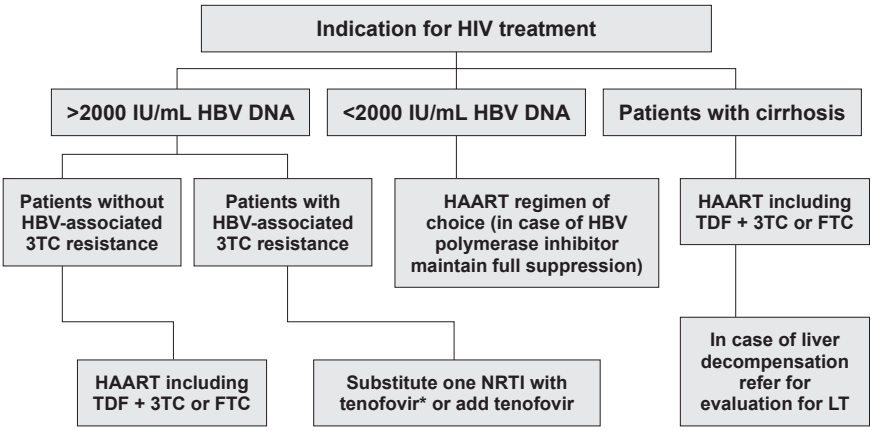


Figure 5. Treatment algorithm for HBV therapy in patients with antiretroviral therapy (EACS 2009).

For patients with hepatic decompensation and full treatment options for HBV and stable HIV infection, liver transplantation should be considered, as life expectancy seems to be the same as for HBV-monoinfected patients (Coffin 2007; Tateo 2009). Patients with hepatocellular carcinoma may be considered liver transplant candidates as well, although according to preliminary observations from small cohorts, the outcome may be worse than for HBV-monoinfected patients with hepatocellular carcinoma (Vibert 2008).

In general tenofovir can be considererd the standard of care for HBV in HIV-coinfected patients, because of its efficacy and its strong HBV polymerase activity. Tenofovir has been a long-acting and effective therapy in the vast majority of treated HBV/HIV-coinfected patients (van Bömmel 2004; Mathews 2009). No conclusive pattern of resistance mutations has been identified in studies or cohorts. But resistance is likely to occur in patients with long term therapy as with any other antiviral. In prospective controlled studies tenofovir was clearly superior to adefovir for treatment of HBe antigen positive and HBe antigen negative patients (Heathcote 2007; Marcellin 2007).

The acquistion of adefovir resistance mutations and multiple lamivudine resistance mutations may impair the activity of tenofovir (Fung 2005; Lada 2008; van Bömmel 2010), although even in these situations tenofovir retains activity against HBV (Berg 2008; Petersen 2009).

In lamivudine-resistant HBV the antiviral efficacy of entecavir in HIV-coinfected patients is reduced, as it is in HBV monoinfection (Shermann 2008). Because of this and the property of tenofovir as an approved antiretroviral, tenofovir is the preferred choice in treatment naïve HIV-coinfected patients who have an antiretroviral treatment indication.

The use of entecavir, telbivudine or adefovir as an add-on to tenofovir or other drugs in the case of not fully suppressive antiviral therapy has not been studied in HIV-coinfected patients so far. The decision to do so is made on a case-by-case basis.

It is a general belief that combination therapy with tenofovir plus lamivudine/emtricitabine is superior to monotherapy, in particular in patients with highly replicative HBV infection. However, to date no conclusive studies supporting this are available (Schmutz 2006; Mathews 2008; Mathews 2009).

In the case of development of HIV resistance to tenofovir it is important to remember its HBV activity before switching to another regimen without antiviral activity against HBV. Discontinuation of the HBV polymerase inhibitor without maintaining the antiviral pressure on HBV can lead to necroinflammatory flares which can result in acute liver decompensation in serious cases.

Management of resistance to HBV polymerase inhibitors

Issues concerning the avoidance and management of resistance to HBV polymerase inhibitors are discussed in detail in Chapter 10.

Conclusion

The number of available HBV polymerase inhibitors for chronic hepatitis B has increased substantially over the last few years. In general though, the choice is still limited to two mostly non-cross-resistant classes, the nucleotide and nucleoside com-

pounds. In HIV-coinfected patients where antiretroviral therapy is not indicated the choice is more limited with only adefovir and telbivudine as treatment options. Alternative options in these patients may be interferon therapy or the initiation of full antiretroviral therapy, which is currently preferred by most experts, although both toxicities and costs may increase.

For HBV/HIV-coinfected patients on antiretroviral therapy the treatment of choice is tenofovir in the majority of treatment-naïve or lamivudine-pretreated cases. Due to rapid development of resistance in not fully suppressive HBV therapy lamivudine or emtricitabine monotherapy should never be considered. A combination of tenofovir plus lamivudine or emtricitabine as a primary combination therapy has theoretical advantages, but studies supporting this concept have not been carried out to date.

In general, treatment of HBV as a viral disease follows the same rules as HIV therapy aiming at a full suppression of the replication of the virus to avoid the development of resistance. Successful viral suppression of hepatitis B results in inhibition of necroinflammatory activity, reversion of fibrosis and the ultimate goal of immune control of the infection.

References

Alter M. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol 2006; 44: 6-9.

Avila C, Karwowska S, Lai C, Evans T. Telbivudine has no in vitro activity against laboratory and clinical HIV-1, including 5 clades and drug-resistant clinical isolates. Proceedings of the 12th Conference on Retroviruses and Opportunistic Infections; Montréal, Canada; 2009 February 8-11; poster 813b.

Berg T, Moller B, Trinh H, Chan S, Marcellin P, Suarez E, Snow-Lampart A, Frederick D, Oldach D, Sorbel J, Borroto-Esoda K, Rousseau F.Tenofovir disoproxil fumarate (TDF) versus emtricitabine plus TDF for treatment of chronic Hepatitis B (CHB) in subjects with persistent viral replication receiving adefovir dipivoxil (ADV). Journal of Hepatology. 2008; 48(Suppl.2): S34 (abstract 76).

Bodsworth N, Donovan B, Nightingale BN. The effect of concurrent human immunodeficiency virus infection on chronic hepatitis B: a study of 150 homosexual men. J Infect Dis 1989;160(4):577-82.

Bodsworth NJ, Cooper DA, Donovan B. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. J Infect Dis 1991;163(5):1138-40.

Brau N, Fox R, Xiao P, Marks K, Naqvi Z, Taylor L, et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: A US-Canadian multicenter study. J Hepatol. 2007;47(4):527-37.

Chan HL, Heathcote EJ, Marcellin P et al. Treatment of Hepatitis B e Antigen-Positive Chronic Hepatitis with Telbivudine or Adefovir: A Randomized Trial. Ann Intern Med. 2007;147:2-11.

Chen CJ, Yang HI, Su J, Jen CL et al. REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006; 4; 295(1):65-73.

Coffin CS, Berg CL, Dove LM et al. Survival and risk of HBV recurrence in HIV-HBV coinfected liver transplant recipients: Preliminary findings from the HIV-TR study. Hepatology 2007; 46 Suppl.1:245A.

Delaugerre C, Marcelin AG, Thibault V, Peytavin G, Bombled T, Bochet MV, Katlama C, Benhamou Y, Calvez V. Human immunodeficiency virus (HIV) Type 1 reverse transcriptase resistance mutations in hepatitis B virus (HBV)-HIV-coinfected patients treated for HBV chronic infection once daily with 10 milligrams of adefovir dipivoxil combined with lamivudine. Antimicrob Agents Chemother. 2002;46(5):1586-8.

EACS guidelines 2009: http://www.europeanaidsclinicalsociety.org/guidelines.asp

Fung SK, Andreone P, Han SH et al. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. J Hepatol 2005; 43(6):937-43.

Hadler SC, Judson FN, O'Malley PM, et al. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. J Infect Dis 1991;163(3):454-9.

Heathcote EJ, Gane E, DeMan R et al. A randomized, double-blind, comparison of tenofovir DF (TDF) versus adefovir dipivoxil (ADV) for the treatment of HBeAg positive chronic hepatitis B (CHB): Study GS-US-174-0103. Hepatology 2007; 46, No. 4, (Suppl.1): 861A, Abstract LB6.

Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130 (3): 678-86.

Ingiliz P, Valantin MA, Thibault V, Duvivier C, Dominguez S, Katlama C, Poynard T, Benhamou Y. Efficacy and safety of adefovir dipivoxil plus pegylated interferon-alpha2a for the treatment of lamivudine-resistant hepatitis B virus infection in HIV-infected patients. Antivir Ther. 2008 13(7): 895-900.

Konopnicki D, Mocroft A, de Wit S et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. AIDS 2005; 19(6):593-601.

Lada O, Gervais A, Branger M, Peytavin G, Colin G, Fraqueiro G, et al. Low rate of delayed response in lamivudine experienced HIV/HBV co-infected patients treated with enofovir disoproxil fumarate (TDF). Journal of Hepatology. 2008; 48(Suppl.2): S259 (abstract 695).

Lampertico P, Viganò M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with Lamivudine: a 3-year study of 145 Lamivudine-resistant hepatitis B patients. Gastroenterology 2007; 133(5):1445-51.

Low E, Cox A, Atkins M and Nelson M. Telbivudine Has Activity against HIV. Proceedings of the 12th Conference on Retroviruses and Opportunistic Infections; Montréal, Canada; 2009 February 8-11; poster 813a

Marcellin P, Buti M, Krastev Z et al. A randomized, double-blind, comparison of tenofovir DF (TDF) versus adefovir dipivoxil (ADV) for the treatment of HBeAg-negative chronic hepatitis B (CHB): Study GS-US-174-0102. Hepatology 2007; 46, No. 4, (Suppl.1): 290A, Abstract LB2.

Matthews GV, Avihingsanon A, Lewin SR, Amin J, Rerknimitr R, Petcharapirat P, et al. A randomized trial of combination hepatitis B therapy in HIV/HBV coinfected antiretroviral naïve individuals in Thailand. Hepatology. 2008 48(4): 1062-9.

Matthews GV, Seaberg E, Dore GJ, Bowden S, Lewin SR, Sasadeusz J, et al. Combination HBV therapy is linked to greater HBV DNA suppression in a cohort of lamivudine-experienced HIV/HBV coinfected individuals. AIDS. 2009 23(13): 1707-15.

McMahon MA, Jilek BL, Brennan TP et al. The HBV drug entecavir - effects on HIV-1 replication and resistance. N Engl J Med 2007;356:2614-2621.

Milazzo L, Caramma I, Lai A, Violin M, De Maddalena C, Cesari M, Galli M, Balotta C. Telbivudine in the treatment of chronic hepatitis B: experience in HIV type-1-infected patients naive for antiretroviral therapy. Antivir Ther. 2009 14(6):869-72.

Modi A, Feld J. Viral hepatitis and HIV in Africa. AIDS Rev 2007; 9: 25-39.

Núñez M, Puoti M, Camino N, Soriano V. Treatment of chronic hepatitis B in the human immunodeficiency virus-infected patient: present and future. Clin Infect Dis 2003 15;37(12):1678-85.

Nikolopoulos GK, Paraskevis D, Hatzitheodorou E, Moschidis Z, Sypsa V, Zavitsanos X, Kalapothaki V, Hatzakis A. Impact of hepatitis B virus infection on the progression of AIDS and mortality in HIV-infected individuals: a cohort study and meta-analysis. Clin Infect Dis. 2009 48(12):1763-71.

Núñez M, Soriano V. Management of patients co-infected with hepatitis B virus and HIV. Lancet Infect Dis 2005; 5:374-382.

Petersen J, Lutgehetmann M, Zoulim F, Sterneck M, Janssen HL, Berg T, et al. Entecavir and tenofovir combination therapy in chronic hepatitis B: rescue therapy in patients with advanced fibrosis and multiple previous treatment failures. Hepatology 2009 50,4: 496A.

Puoti M, Bruno R, Soriano V, Donato F, Gaeta G, Quinzan G, et al. Hepatocellular carcinoma in HIV-infected patients: epidemiological features, clinical presentation and outcome. AIDS 2004; 18: 2285-93.

Puoti M, Cozzi-Lepri A, Arici C et al.Impact of lamivudine on the risk of liver-related death in 2,041 HBsAg- and HIV-positive individuals: results from an inter-cohort analysis. Antivir Ther. 2006;11(5):567-74.

Puoti M, Torti C, Bruno R. Natural history of chronic hepatitis B in co-infected patients. J Hepatol 2006; 44: 65-70.

Rockstroh JK, Bhagani S, Benhamou Y, Bruno R, Mauss S, Peters L, et al. European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of chronic hepatitis B and C coinfection in HIV-infected adults. HIV Med. 2008 Feb;9(2):82-8.

Schmutz G, Nelson M, Lutz T et al. Combination of tenofovir and lamivudine versus tenofovir after lamivudine failure for therapy of hepatitis B in HIV-coinfection. AIDS. 2006;20(15):1951-4.

Sheldon JA, Corral A, Rodés B, Mauss S, Rockstroh J, Berger F, Schwarze-Zander C, Soriano V. Risk of selecting K65R in antiretroviral-naive HIV-infected individuals with chronic hepatitis B treated with adefovir. AIDS 2005;19(17):2036-8.

Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev. 2006;28:112-25.

Sherman M, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, et al. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. Hepatology. 2008 ;48(1):99-108

Soriano V, Puoti M, Bonacini M. Care of patients with chronic hepatitis B and HIV co-infection: recommendations from an HIV-HBV international panel. AIDS 2005; 19: 221-240.

Soriano V, Mocroft A, Peters L, Rockstroh J, Antunes F, Kirkby N, et al. Predictors of hepatitis B virus genotype and viraemia in HIV-infected patients with chronic hepatitis B in Europe´.J Antimicrob Chemother 2010

Tateo M, Roque-Afonso AM, Antonini TM, Medja F, Lomes A, Jardel C, et al. Long-term follow-up of liver transplanted HIV/hepatitis B virus coinfected patients: perfect control of hepatitis B virus replication and absence of mitochondrial toxicity. AIDS. 2009 23(9):1069-76.

Thio C, Seaberg E, Skolasky R. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter AIDS Cohort Study (MACS). Lancet 2002; 360: 1921-1926.

Van Bömmel F, Wünsche T, Mauss S et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004 40(6):1421-5.

Van Bömmel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology. 2010 51(1):73-80.

Chapter 18: Management of HCV/HIV coinfection

Christoph Boesecke, Stefan Mauss, Jürgen Kurt Rockstroh

Epidemiology of HIV and HCV coinfection

HIV and HCV share transmission pathways, which explains the high rate of coinfection with both viruses. Of the 33.4 million HIV-infected persons worldwide in 2008 it is estimated that at least 5 million of them have concomitant hepatitis C virus infection. Whereas both viruses are transmitted with high efficacy via blood-to-blood contact, HCV is less easily transmitted sexually. Thus, the prevalence of hepatitis C coinfection within different countries, regions and populations is closely related to the prevalence of blood-borne transmission (mainly intravenous drug use) of HIV. Among HIV-infected patients in Europe, Australia and the US, at least one out of four is coinfected with hepatitis C (Rockstroh 2004). Hepatitis C coinfection rates as high as 70% can be seen in Eastern European countries like Belorus and the Ukraine where intravenous drug use (IVDU) is the main route of HIV transmission. On the other hand, in Central European countries such as Belgium, Austria or Germany, where sexual intercourse dominates as mode of HIV transmission, hepatitis C coinfection rates are rather low, between 10 and 15% (Rockstroh 2005). Similar rates can be found in HIV-positive patients in Australia (Jin 2009) and the UK (Turner 2009). Interestingly, recent data from the US indicate that 25% to 35% of patients with HIV are coinfected with HCV (Singal 2009) reflecting the contribution of at-risk populations such as prison inmates to the overall numbers. 65-70% of HIV-positive prisoners in the US are coinfected with hepatitis C, in contrast to 18-25% within the general US HIV-positive population (Weinbaum 2005). In Asia, coinfection rates of up to 85% have been observed among Chinese plasma donors whereas in countries with predominantly heterosexual HIV transmission like Thailand coinfection rates are around 10% (Qian 2006). In sub-Saharan Africa, where again the primary route of transmission of HIV is sexual, HCV coinfection rates so far have been reported to be relatively low.

Although the traditional route of HCV transmission is blood-borne and includes IVDU, snorting drugs, sharing toothbrushes/razors, and tattooing (Bollepalli 2007), recent epidemic outbreaks among HIV-positive men who have sex with men (MSM) from several major European cities such as London, Paris, Amsterdam, and Berlin as well as more recent reports from the US and Canada, document that HCV may well be sexually transmitted and should therefore also be taken into account at regular STD screenings (Gotz 2005; Danta 2007; Vogel 2009; Vogel 2010).

HCV is detected in 4-8% of infants born to HCV-infected mothers (Bevilacqua 2009). Dual HCV/HIV infection increases the risk for transmission of both viruses and high levels of HCV viremia in the mother increases the risk of perinatal HCV transmission (Zanetti 1995). However, in HIV/HCV-coinfected mothers receiving HAART and undergoing cesarean section the risk of HCV transmission is strikingly reduced to less than 1%.

In summary, the prevalence of hepatitis C within the HIV-infected population is far higher than in the general population where the global burden of hepatitis C is estimated to be roughly 2%. This highlights the importance of preventing further spread of hepatitis C infection as one of the major co-morbidities in HIV-infected individuals. The average estimated risk of transmission for hepatitis C in HIV is depicted in Table 1. Although they share common routes of infection, the viruses are transmitted with varying efficacy depending upon the mode of transmission.

Mode of transmission	HIV	HCV	HCV / HIV coinfection
Perinatal	7-50%	1-7%	1-20%
Sexual contact*	1-3%	<1%	<4%
Needle stick injury	0.3%	<1%	Unknown

* With sexual contact the risk refers to cumulative exposure.

Table 1. Average estimated risk of transmission for HIV, HCV and HCV/HIV coinfection.

Specifics regarding the diagnosis of HCV in HIV coinfection

The presence of HCV can be confirmed serologically by the detection of antibodies to the virus via ELISA testing. Loss of HCV antibodies observed in rare cases in very advanced immune deficiency in HIV/HCV coinfection does not necessarily indicate viral clearance (Cribier 1999). Therefore, a single negative HCV antibody ELISA does not necessarily exclude HCV infection in HIV-positive patients, especially in severe immune deficiency. Additionally, a rise of liver transaminases has been proven to be more sensitive in the detection of acute HCV infection in HIV-positive patients than repeated testing for the presence of antibodies against HCV (Thomson 2009). However, in more than 80% of HIV-positive individuals with positive HCV antibodies, HCV RNA is detected in the blood. Higher concentrations of HCV RNA are found in HIV-positive individuals than in HIV-negative patients with hepatitis C (Perez-Olmeda 2002). Interestingly, recent data from a cross-trial comparison showed that HIV-positive patients were less likely to present with elevated serum ALT and clinical signs or symptoms of hepatitis than HIV-negative patients (Vogel 2009). In observations from hemophiliac patients the mean concentrations of HCV RNA increase by 1 log over the first two years after HIV seroconversion (Eyster 1994). The levels of HCV viremia increase eight times faster in HIV-positive individuals than in patients with hepatitis C who are not infected with HIV. The highest concentrations for HCV viremia have been reported in patients who subsequently develop liver failure.

Interestingly, spontaneous clearance of HCV RNA has been observed in some HIV/HCV-coinfected patients experiencing significant immune reconstitution following HAART initiation (Fialaire 1999; Thomson 2009). In contrast, there are also patients with positive HCV antibodies and negative HCV RNA, where after initiation of

HAART, HCV RNA was noted to reemerge frequently in combination with a flare of liver transaminases. Therefore, regular monitoring of HCV RNA levels is warranted in HIV/HCV-coinfected patients.

The distribution of HCV genotypes in HIV-positive patients reflects the route of transmission. Genotype 1b accounts for 2/3 of post-transfusion HCV infections and is the predominant genotype in hemophiliacs. In contrast, genotypes 1a and 3a are more common in intravenous drug users (Pol 1994).

The natural history of hepatitis C in HIV-positive patients

Various studies have demonstrated that underlying HIV infection weakens the immune response to hepatitis C, thereby diminishing the chance of spontaneous viral clearance of HCV infection. Interestingly, data from the European epidemic of sexually transmitted acute hepatitis C infection in HIV-positive individuals suggest that despite underlying HIV infection spontaneous resolution of HCV may occur in up to 20-30% of newly infected patients (Vogel 2010).

Numerous large cohort studies have demonstrated that once chronic hepatitis C is established the presence of HIV leads to a faster HCV clinical progression due to the lack of critical CD4-positive T cell responses against HCV (Danta 2008). In the American multicenter Hemophiliac Cohort Study liver failure occurred in 9% of multi-transfused HCV/HIV-coinfected adult hemophiliacs without an AIDS-defining opportunistic infection or malignancy (Eyster 1993). In the same time period, no case of liver failure was observed in HCV-positive HIV-negative hemophiliacs. Subsequently, several studies have confirmed the unfavorable course of hepatitis C in HIV-coinfected hemophiliacs, particularly in the setting of progressive immunodeficiency and lower CD4 counts (Rockstroh 1996; Puoti 2001).

In addition, the time interval between HCV exposition and development of cirrhosis was found to be shortened in coinfected subjects. Indeed, within 10-15 years of initial HCV infection, 15-25% of HIV-coinfected patients develop cirrhosis compared with 2-6% of HIV-negative patients (Soto 1997). Importantly, mortality due to advanced liver disease happens ten years earlier in coinfected hemophiliacs than in HIV-negative hemophiliacs with hepatitis C (Darby 1997). The incidence of hepatocellular carcinoma is also higher in coinfected patients (Giordano 2004).

Effect of hepatitis C on HIV infection

As clear as HIV's influence on the accelerated disease progression for HCV-associated liver disease is, HCV's influence on the course of HIV disease is conflicting. The Swiss Cohort first revealed a blunted CD4 cell response associated with a faster progression to AIDS after initiation of HAART in HIV/HCV-coinfected patients (Greub 2000). Interestingly, four-year follow-up data from the same cohort study did not see significant differences with regard to CD4 cell count recovery between HCV-positive and HCV-negative HIV-positive patients (Kaufmann 2003). Subsequent studies have indeed found that after adjusting for use of HAART, no difference in CD4 cell count recovery can be observed (Sulkowski 2002). Updated information from an analysis of

the large EuroSIDA cohort, after taking into account ongoing chronic (persistent HCV replication) and resolved (positive HCV antibodies but negative HCV RNA) hepatitis C infection, confirm that no difference in CD4 cell count recovery is observed in patients with chronic hepatitis C infection and detectable HCV RNA in comparison to HIV-monoinfected patients (Rockstroh 2007). In addition, recent data from the same cohort revealed that CD4-positive T cell recovery in HIV-positive patients with maximal suppression of HIV replication is not influenced by HCV serostatus in general or HCV genotype or level of HCV in particular (Peters 2009).

Effect of HAART on hepatitis C

In HIV/HCV-coinfected patients starting antiretroviral therapy a transient increase in HCV RNA levels may occur at week 4 but thereafter no significant changes in concentrations of HCV RNA happen over the first six months of treatment (Rockstroh 1998). However, a 1 log decrease of HCV RNA has been reported in HIV/HCV-coinfected individuals receiving more than 12 months of HAART and having significant immune reconstitution. Other investigators, however, have not observed this decrease in HCV RNA. Moreover, eradication of HCV has been reported in individual patients receiving HAART following CD4 count recovery.

There is increasing evidence that HAART-induced immune reconstitution might reverse the unfavorable accelerated course for hepatitis C in patients with severe HIV-associated immune deficiency (Verma 2006; Vogel 2009). Taking into account that liver disease progresses especially in patients whose CD4 count drops below 200/µl it is appealing to think that CD4 increases on HAART may impact the further course of liver disease. In an early study of 162 individuals with HIV/HCV coinfection who underwent liver biopsy, the use of protease inhibitors as part of their HAART regimen was associated with significantly lower rates of progression of liver fibrosis that could not be explained by other cofactors (Benhamou 2000). These findings were then reinforced by several cohort analyses which showed that HIV/HCV-coinfected individuals on HAART had significantly lower liver-related mortality than patients receiving either suboptimal (one or two nucleoside reverse transcriptase inhibitors) or no antiretroviral therapy (Qurishi 2003).

One paper also addressed the amount of immune reconstitution achieved on HAART and the subsequent risk for developing hepatic decompensation in HIV/HCV-coinfected individuals commencing HAART (Pineda 2007). Those patients who experienced the highest CD4 cell count gain on HAART were the least likely to develop further complications of liver disease, again highlighting a favorable impact of HAART-induced immune reconstitution on the course of liver disease. As a consequence, the recently updated antiretroviral treatment guidelines of the European AIDS Clinical Society recommend earlier initiation of antiretroviral therapy in HIV patients with HCV coinfection (CD4 T cell count between 350-500/µl in asymptomatic patients).

Short-term and long-term virologic success rates of HAART in HIV/HCV coinfection are, however, limited by an increased risk of hepatotoxicity. Various studies have shown that the presence of HCV was independently associated with an increased risk of rises in serum aminotransferases (Lichterfeld 2004) highlighting the need for close monitoring.

Therapy

The most important reason to treat hepatitis C in HIV-coinfected individuals is the unfavorable course of hepatitis C in the setting of HIV coinfection particularly with the increased life expectancy gained by successful HAART. An increased risk of hepatotoxicity after HAART initiation in HIV/HCV-coinfected patients, possibly limiting the long-term benefit of HAART in this particular patient group, further underlines the need for successful treatment of hepatitis C (Sulkowski 2000). Several studies have been able to demonstrate that successful treatment of hepatitis C dramatically reduces subsequent complications of preexisting liver disease. This implies that once viral clearance is achieved with hepatitis C combination therapy the prognosis of liver disease dramatically improves (even in the presence of already developed liver cirrhosis) and once HCV infection is eradicated further liver complications are very unlikely.

The goal of hepatitis C treatment is to achieve persistently negative HCV RNA levels. This is generally referred to as a sustained virologic response (SVR). It is defined as negative HCV RNA six months after completion of HCV therapy. Negative HCV RNA at the end of the treatment period is described as an end-of-treatment response (EOT). Negative HCV RNA after four weeks of HCV treatment initiation is referred to as rapid treatment response. Failure to respond to treatment is referred to as non-response.

The combination of pegylated interferon and ribavirin is regarded as standard therapy in coinfected patients. Table 2 summarizes the main results from randomized clinical trials investigating the efficacy of pegylated interferon and ribavirin in HIV/HCV-coinfected individuals. Recently published data from the GESIDA study show similar efficacy and safety for both pegylated IFN α-2b and pegylated IFN α-2a in the treatment of chronic HCV infection in HIV-infected patients (Berenguer 2009).

	ACTG5071	APRICOT	RIBAVIC	Laguno	PRESCO
Number of Patients	66	289	194	52	389
PEG-INF α	2a	2a	2b	2b	2a
IV drug use	-	62%	80%	75%	90%
Liver cirrhosis	11%	15%	39%		
(F3-F4)	19%	28%			
(F3-F4)					
Genotype 1,4	77%	67%	61%	63%	61%
Normal ALT	34%	0%	16%	0%	0%
Mean CD4+	495	520	477	570	546
HAART	85%	83%	83%	94%	74%
Discontinuation rate due to AE*	12%	25%	17%	17%	9%
Discontinuation rate due to other reasons	-	31%	39%	23%	7%
EOT (ITT)**	41%	49%	35%	52%	67%
SVR (ITT)***	27%	40%	27%	44%	50%

adverse events, **end-of-treatment response, intent-to-treat analyses, * sustained virological response, intent-to-treat analyses*

Table 2: Results from randomized clinical trials investigating the efficacy of pegylated interferon and ribavirin in HIV/HCV-coinfected individuals.

Overall, SVR rates of up to 50% can be achieved (Torriani 2004; Nunez 2007). The difference in rates of SVR in various studies can be explained mainly by differences in ribavirin dosages used. In the initial HCV treatment trials in HIV-coinfected individuals, due to the fear of interactions between ribavirin and commonly used NRTIs for HIV treatment, an 800 mg daily dose of ribavirin was chosen for most patients independent of the prevailing genotype. This led to suboptimal SVR rates. However, in the PRESCO trial, where weight-adjusted daily ribavirin dosages of 1000-1200 mg were used independent of genotype, SVR rates almost doubled in comparison to some of the earlier studies such as APRICOT, most likely due to the higher ribavirin levels. In spite of this, very recently presented data from the PARADIGM trial, a double-blind, multicenter study comparing 800 vs 1000/1200 mg of ribavirin plus PEG-IFN in HCV/HIV coinfected patients showed no significant differences in the rates of SVR (Rodriguez-Torres 2009).

In the current guidelines, daily administration of ribavirin 1000 mg (<75 kg body weight) and 1200 mg (>75 kg body weight) split into 2 doses (BID) is recommended for HCV therapy in HIV coinfection for all genotypes in combination with pegylated interferon.

The standard dosage for PEG-IFN α-2a is 180 mg/kg body weight once weekly and for PEG-IFN α-2b it is 1.5 mg/kg body weight once weekly. Duration of therapy is individualized taking into account factors for HCV treatment response such as genotype, baseline viral load and time to reach HCV undetectability (see Figure 1). Results from the PRESCO trial indicate that at least some patients may benefit from a longer duration of HCV combination therapy, of up to 72 weeks (see Figure 1). This mainly refers to patients infected with HCV genotypes 1 and 4 (Núñez 2007) for whom poorer response rates have been extensively shown when compared with genotypes 2 and 3.

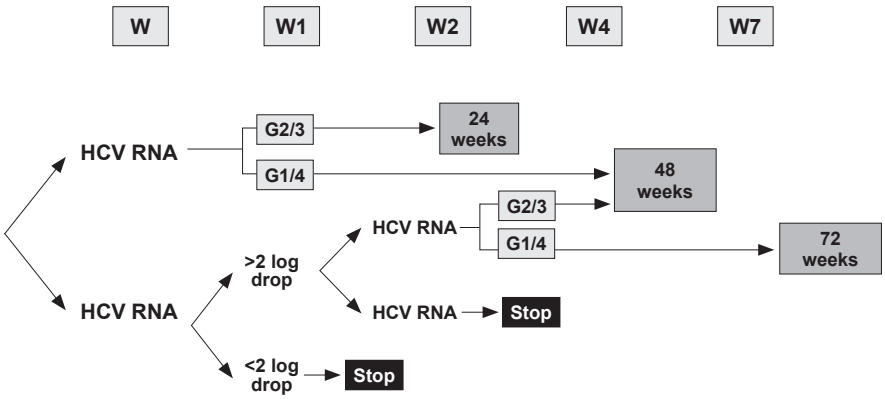


Figure 1: Algorithm for management of hepatitis C in HIV coinfection.
Proposed optimal duration of hepatitis C virus (HCV) therapy in HIV/HCV-coinfected patients (w: week; G: genotype) (modified according to Rockstroh 2009). *In patients with low baseline viral load (<400,000 IU/l) and minimal liver fibrosis.

Unlike HAART, HCV treatment offers the possibility of eradicating HCV within defined treatment periods and this clearly appears potentially advantageous for the subsequent management of the patient's HIV infection. Every patient should be considered for HCV treatment when the benefits of therapy outweigh the risks. Benefits of therapy also need to be seen in the context of rapid liver fibrosis progression in HIV/HCV coinfection and improved HCV treatment outcome under optimized management in these patients. Information on liver fibrosis staging is important for making treatment decisions in coinfected patients. However, a liver biopsy is not mandatory for decisions on treatment of chronic HCV infection. Recently introduced noninvasive markers such as blood tests or transient elastography constitute new and exciting means of assessing liver disease in HIV and hepatitis-coinfected individuals (Rockstroh 2009). When liver biopsy or non-invasive tests for assessing hepatic fibrosis (e.g., elastometry by Fibroscan®, Echosense, France) demonstrate lower grades of liver fibrosis (F0-F1) regardless of HCV genotype, treatment can be deferred. Assessment of fibrosis should be repeated frequently to monitor progression in these cases. It is especially important to perform a liver disease stage assessment in patients with a low likelihood of achieving SVR.

Diagnosis of hepatitis C
HCV Ab (positive 1-5 months after infection, may rarely be lost with immunosuppression) HCV RNA level* (while not prognostic for progression, it is for response to treatment)
Status of liver damage
Grading of fibrosis (e.g., Fibroscan®, liver biopsy, serum fibromarkers**) Hepatic synthetic function (e.g., coagulation, protein, albumin, CHE) Ultrasound and AFP every 6 months in cirrhotics (gastroscopy upon diagnosis of cirrhosis and every 1-2 years thereafter)
Before HCV treatment
HCV genotype and serum HCV RNA Auto-antibodies (ANA, SMA, ANCA and LKM1***) TSH, thyroid autoantibodies if applicable
Monitoring of HCV treatment
Differential blood count and liver enzymes every 2-4 weeks HCV RNA at week 4 (to evaluate rapid virological response), week 12, 24, 48, (72 if applicable) and 24 weeks after stopping HCV therapy CD4 count every 12 weeks TSH every 12 weeks

**Low viral load defined as less than 400,000 IU/l when using PEG-IFN+RBV; there is no standard conversion formula for converting the amount of HCV RNA reported in copies/ml to the amount reported in IU. The conversion factor ranges from about one to five HCV RNA copies per IU.*
***Serum fibromarkers include APRI, FIB-4, Hyaluronic acid, Fibrometer, Fibrotest, Forns, Hepascore and other indices; recently more complex tests such as Fibrometer, Fibrotest and Hepascore have shown more accuracy in predicting liver fibrosis than simple biochemical tests such as APRI, FIB-4 or Forns.*
****Patients with positive anti-LKM or -ANA with homogeneous pattern should be evaluated for concurrent autoimmune hepatitis especially in the presence of ALT elevation during treatment.*

Table 3. Diagnostic procedures for hepatitis C in HIV coinfection (adapted from Rockstroh 2008).

In addition to this, insulin resistance (which can be determined using the homeostasis model assessment of insulin resistance (HOMA-IR) score) has been reported as a negative predictor of achieving SVR and therefore may also be considered during evaluation.

Current therapy is particularly recommended in all those patients with a high likelihood of achieving an SVR, i.e., patients infected with genotype 2 or 3 and those infected with genotype 1 if the viral load is low (<400,000-500,000 IU/ml) (Rockstroh 2008 and 2009). If chronic hepatitis C is detected early in the course of HIV infection (before the initiation of HAART) treatment for chronic HCV is advised. However, if a coinfected patient has severe immune deficiency (CD4 count <200 cells/ml), the CD4 count should be improved using HAART before beginning HCV treatment. Patients with a CD4 relative percentage of >25% are more likely to achieve SVR than those with lower CD4 percentages (Opravil 2007). If an early virological response of at least 2 $\log_{10}$ reduction in HCV RNA compared with baseline is not achieved by week 12, treatment should be discontinued as an SVR is unlikely. The current European recommendations for treatment initiation of PEG-INF and ribavirin for HIV/HCV coinfected patients are shown in Figure 1. The procedures for diagnosis of hepatitis C, assessment of liver disease stage and control examinations before and during HCV therapy are summarized in Table 3.

The choice of antiretrovirals while on HCV therapy

The choice of the best-tolerated HIV drugs appears crucial for completing the planned treatment duration of hepatitis C therapy of 24-72 weeks. ddI use has been independently associated with increased adverse event rates including lactic acidosis and hepatic decompensation in patients who have liver cirrhosis prior to commencement of PEG-IFN/RBV therapy (Mauss 2006). Apparently, ribavirin enhances the phosphorylation of ddI and thereby leads to an increased risk of pancreatitis and mitochondrial toxicity in subjects receiving concomitant ribavirin and ddI therapy (Moreno 2004). ddI use is therefore contraindicated in combination with ribavirin, especially in patients who have already developed liver cirrhosis. The use of HIV antiretrovirals such as AZT and d4T are also discouraged whenever possible, as increased toxicity can be expected. RBV + AZT is associated with enhanced anemia (Alvarez 2006) while RBV + d4T is associated with increased mitochondrial toxicity and weight loss and a high potential to worsen pre-existing lipoatrophy. Patients on atazanavir-containing HAART may develop jaundice due to an increase in total serum bilirubin levels following initiation of ribavirin (Rodriguez-Novoa 2008). The role of abacavir is uncertain at this point but cohort data suggest lower SVR results in patients on abacavir-containing HAART (Bani-Sadr 2007). As abacavir and ribavirin are both guanosine analogues it is speculated that there may be interference or competition in the phosphorylation pathway. Interestingly, in the presence of therapeutic ribavirin levels no difference was observed between abacavir and other nucleosides in achieving SVR in HIV/HCV-coinfected patients receiving PEG-IFN/ribavirin therapy and concomitant HAART (Laufer 2008).

Treatment of HCV for relapsers or non-responders

Patients with a history of previous HCV therapy who were either non-responders or who relapsed while on previous HCV therapy need to be reassessed with regard to a new HVC treatment optimizing the dose and duration as well as the best supportive therapy. Recent results from the SLAM-C trial (ACTG 5178) have attenuated hopes that maintenance therapy with PEG-INF might be beneficial for non-responders. In the meantime, data from the ENDURE trial comparing half-doses of PEG-IFN α-2b vs. placebo in coinfected, pre-treated patients with compensated liver cirrhosis are eagerly awaited. Table 4 summarizes possible interventions for HCV/HIV-coinfected non-responders and relapsers to previous interferon-based therapies (Rockstroh 2008 and 2009).

Category	Subgroup	Recommended intervention
Suboptimal treatment	Suboptimal schedule • Interferon monotherapy • Low doses of ribavirin • Short length of therapy Limiting toxicities & poor adherence	Re-treatment using combination therapy of PEG-IFN plus weight-based dose of ribavirin
		Optimal support (SSRI, paracetamol/NSAID*, adherence support, use of hematopoietic growth factors**)
Optimal treatment with virologic failure	Relapse (HCV RNA negative at the end of treatment)	Re-treatment using combination therapy of PEG-IFN plus weight-based ribavirin dosing (consider longer treatment duration)
	Non-response (no HCV RNA negativization during treatment)	Wait until new antivirals become available either through clinical trials or upon licensure

**NSAID, non-steroidal anti-inflammatory drugs; PEG, polyethylene glycol; SSRI, selective serotonin reuptake inhibitors.*
***Data on the use of hematopoietic growth factors in HIV/HCV co-infection so far is limited to an improvement in quality of life but not antiviral efficacy; treatment with growth factors is currently mostly off-label in Europe.*

Table 4: Classification of and interventions for HCV/HIV-coinfected patients who are non-responders/relapsers to prior IFN-based therapies.

Treatment of acute HCV in HIV

In patients with acute HCV infection HCV therapy is recommended if the HCV RNA is confirmed positive (1 week apart) by week 12 post-HCV transmission, as SVR rates following treatment of acute HCV infection are higher than for treatment of chronic HCV. Uncontrolled pilot studies of treatment of acute HCV infection in HIV-coinfected patients demonstrate SVR rates above 60% mostly with combination therapy of PEG-IFN/RBV for 24-48 weeks. Unfortunately, clear guidance is difficult at this point due to the lack of controlled data. HCV RNA levels at weeks 4 and 12 may help to guide treatment duration.

Liver transplantation in HIV/HCV coinfected patients

In general, HIV/HCV-coinfected individuals develop more rapid HCV-related hepatic injuries such as liver fibrosis and cirrhosis. Additionally, HIV/HCV coinfection is associated with an increased rate of hepatocellular carcinoma (HCC). Typically HCC occurs in HIV/HCV-coinfected patients at an earlier age and the course is more aggressive with a shorter survival compared to HCV-monoinfected individuals. Therefore, the presence of esophageal varices using upper-gastrointestinal endoscopy should be monitored in patients with liver cirrhosis every 1-2 years, and an ultrasound of the liver and a serum alpha-fetoprotein determination should be performed at least every 6 months in patients with F3/F4 fibrosis according to the recommendations of the European Consensus Guidelines (Alberti 2005).

Liver transplant should be considered in patients with decompensated liver cirrhosis, as this is a contraindication for HCV treatment. To fulfill the selection criteria for a liver transplant in HIV/HCV-coinfected individuals the CD4+ count has to be at least 100 cells/µl. Additionally, the patient has to have either undetectable HIV viremia (<400 copies/ml) or at least rational treatment options to control HIV infection successfully after liver transplantation. Further contraindications for transplantation are opportunistic diseases, ongoing alcohol or drug abuse, HCC metastasis in other organs, a second malignant disease, cardiopulmonary disease or older age with an elevated risk of mortality related to the operation. Recent data from a large US cohort sheds light on survival rates after liver transplantation (Mindikoglu 2008). The estimated 2-year survival rate was found to be somewhat lower in HIV-positive patients (70%) compared with HIV-negative patients (81%). This was mostly attributable to HBV or HCV coinfection. Other studies have shown good outcome results in the setting of HBV/HIV coinfection when compared to HIV mono-infection (Vogel 2005). This highlights the major problem in HCV/HIV-coinfected transplant recipients: HCV re-infection of the transplanted organ. Re-infection with HBV can be prevented with anti-HBs immunoglobulin and HBV antivirals.

In the context of post-transplant immunosuppression, it is important to point out that there are crucial pharmacokinetic drug-drug interactions on the level of the cytochrome P450 metabolism and p-glycoprotein induction between the key immunosuppressive drugs tacrolimus or cyclosporine A and the antiretroviral agents used for HIV therapy. Determinations of the plasma levels of the antiretroviral drugs are necessary. Furthermore, the doses of cyclosporine A or tacrolimus usually need to be reduced when the patient is treated concomitantly with a protease inhibitor, especially if boost-

ed with ritonavir (Vogel 2004). By contrast, NNRTIs can lower the concentrations of immunosuppressive drugs.

Recurrence of chronic hepatitis C in the liver graft is frequently observed in HIV-positive patients and a more rapid progression to graft cirrhosis and liver disease related mortality compared to HCV-monoinfected patients has been reported. Therefore, combination therapy with pegylated interferon plus ribavirin seems to be the best management option 1-3 months after liver transplantation and after re-infection with hepatitis C virus is detected.

Conclusion

HIV has been shown to accelerate the progression of hepatitis C and to result in higher liver disease-related mortality and morbidity in HIV/HCV-coinfected patients compared to HCV- or HIV-monoinfected individuals. Enhanced hepatotoxicity of HAART as well as drug-drug interactions between HAART and ribavirin clearly underline the need for specific treatment strategies in these patients. A number of important clinical studies have established PEG-IFN plus ribavirin combination therapy as the current gold standard allowing sustained virologic response rates of almost 50% in HIV/HCV-coinfected individuals under optimized management conditions (weight-based ribavirin 1000-1200 mg daily and individualized treatment duration).

Nevertheless, the proportion of patients not treatable or those who relapse, especially in patients with genotype 1 infection, remains high. In addition, only one treatment modality is currently available. Luckily, analogous to antiretroviral therapy in HIV patients, new HCV polymerase and protease inhibitors are being developed and investigated in clinical trials, and results are impatiently awaited.

References

Alberti A, Clumeck N, Collins S, et al. ECC Jury. Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. J Hepatol. 2005 May;42:615-24.

Alvarez D, Dieterich DT, Brau N, Moorehead L, Ball L, Sulkowski MS. Zidovudine use but not weight-based ribavirin dosing impacts anaemia during HCV treatment in HIV-infected persons. J Viral Hepat. 2006 Oct;13(10):683-9.

Bani-Sadr F, Denoeud L, Morand P, et al. Agence Nationale pour la Recherche contre le SIDA et les Hépatites Virales HC02-Ribavic Study Team. Early virologic failure in HIV-coinfected hepatitis C patients treated with the peginterferon-ribavirin combination: does abacavir play a role? J Acquir Immune Defic Syndr. 2007;45:123-5.

Benhamou Y, Demartinio V, Boche T et al: Factors effecting liver fibrosis in human immunodeficiency virus and hepatitis C virus coinfected patients. Impact of protease inhibitor therapy. Hepatology 2001; 34: 283-287.

Berenguer J, González-García J, López-Aldeguer J, Von-Wichmann MA, Quereda C, Hernando A, et al. Pegylated interferon {alpha}2a plus ribavirin versus pegylated interferon {alpha}2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. J Antimicrob Chemother. 2009 Jun;63(6):1256-63.

Bevilacqua E, Fabris A, Floreano P, Pembrey L, Newell ML, Tovo PA, Amoroso A; EPHN collaborators. Genetic factors in mother-to-child transmission of HCV infection. Virology. 2009 Jul 20;390(1):64-70.

Bollepalli S, Mathieson K, Bay C, Hillier A, Post J, Van Thiel DH, Nadir A. Prevalence of risk factors for hepatitis C virus in HIV-infected and HIV/hepatitis C virus-coinfected patients. Sex Transm Dis. 2007 Jun;34(6):367-70.

Cribier B, Rey D, Schmitt C et al: High hepatitis C viremia and impaired antibody response in patients coinfected with HIV. AIDS 1995; 9: 1131-1136.

Danta M, Brown D, Bhagani S, et al. and Acute HCV (HAAC) group. Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours. AIDS. 2007 May 11;21:983-91.

Danta M, Semmo N, Fabris P, Brown D, Pybus OG, Sabin CA, et al. Impact of HIV on host-virus interactions during early hepatitis C virus infection. J Infect Dis. 2008 Jun 1;197(11):1558-66.

Darby SC, Ewart DW, Giangrande PL, et al. Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. Lancet 1997; 350(9089): 1425-1431.

Eyster ME, Diamondstone LS, Lien JM et al: Natural history of hepatitis C virus infection in multitransfused hemophiliacs: effect of coinfection with human immune deficiency virus – a multicenter hemophiliac cohort study. J Acquir Immune Def Syndr 1993; 6: 602-610.

Eyster ME, Fried MW, Di Bisceglie AM, Goeddert JJ: Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. Blood 1994; 84: 1020-1023.

Fialaire P, Payan C, Vitour D et al: Sustained disappearance of hepatitis C viremia in patients receiving protease inhibitor therapy for human immunodeficiency virus infection. J Infect Dis 1999; 180: 574-575.

Giordano TP, Kramer JR, Souchek J, Richardson P, El-Serag HB. Cirrhosis and hepatocellular carcinoma in HIV-infected veterans with and without the hepatitis C virus: a cohort study, 1992-2001. Arch Intern Med. 2004 Nov 22;164(21):2349-54.

Gotz HM, van Doormun G, Niesters HG et al: A cluster with acute hepatitis C virus infection among men who have sex with men – results from contact tracing and public health implications. AIDS 2005; 19: 969-974.

Greub G, Ledergerber B, Battegay M et al: Clinical progression, survival and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection. Lancet 2003; 356: 1800-1805.

Jin F, Prestage GP, Matthews GV, Zablotska I, Rawstorne P, Kippax SC, Kaldor J, Grulich AE. Prevalence, incidence and risk factors for hepatitis C in homosexual men:Data from two cohorts of HIV negative and HIV positive men in Sydney, Australia. Sex Transm Infect. 2009 Oct 19.

Kaufmann GR, Perrin L, Pantaleo G, et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. Arch Intern Med 2003; 163(18): 2187-2195.

Laufer N, Laguno M, Perez I, Cifuentes C, Murillas J, Vidal F, Bonet L, Veloso S, Gatell JM, Mallolas J. Abacavir does not influence the rate of virological response in HIV-HCV-coinfected patients treated with pegylated interferon and weight-adjusted ribavirin. Antivir Ther. 2008;13(7):953-7.

Mauss S, Valenti W, DePamphilis J, et al. Risk factors for hepatic decompensation in patients with HIV/HCV coinfection and liver cirrhosis during interferon-based therapy. AIDS. 2004;18:F21-25.

Mindikoglu AL, Regev A, Magder LS. Impact of human immunodeficiency virus on survival after liver transplantation: analysis of United Network for Organ Sharing database. Transplantation. 2008 Feb 15;85(3):359-68.

Moreno A, Quereda C, Moreno L, Perez-Elías MJ, Muriel A, Casado JL, et al. High rate of didanosine-related mitochondrial toxicity in HIV/HCV-coinfected patients receiving ribavirin. Antivir Ther. 2004 Feb;9(1):133-8.

Nunez M, Miralles C, Berdun MA et al for the PRESCO study group: Role of weight-based ribavirin dosing and extended duration of therapy in chronic hepatitis C in HIV-infected patients: The PRESCO trial. AIDS Res Hum Retrovir 2007, 23: 972-982.

Opravil M, Sasadeusz J, Cooper DA et al: Effect of baseline CD4 cell count on the efficacy and safety of peginterferon-a 2a (40 kd) + ribavirin in patients with HIV-HCV coinfection. J Acquir Immune Def Syndr 2008; 47: 36-49.

Perez-Olmeda M, Rios P, Nunez M, Garcia-Samaniego J, Romero M, Soriano V. Virological characteristics of hepatitis C virus infection in HIV-infected individuals with chronic hepatitis C: implications for treatment. AIDS 2002; 16(3): 493-495.

Peters L, Mocroft A, Soriano V, Rockstroh JK, Losso M, Valerio L, et al. Hepatitis C virus coinfection does not influence the CD4 cell recovery in HIV-1-infected patients with maximum virologic suppression. J Acquir Immune Defic Syndr. 2009 Apr 15;50(5):457-63.

Pineda JA, García-García JA, Aguilar-Guisado M, et al. Grupo para el Estudio de las Hepatitis Víricas de la Sociedad Andaluza de Enfermedades Infecciosas (SAEI).Clinical progression of hepatitis C virus-related chronic liver disease in human immunodeficiency virus-infected patients undergoing highly active antiretroviral therapy. Hepatology. 2007;46:622-30.

Pol S, Thiers V, Nousbaum J, et a. Changing distribution of HCV genotypes in Europe in the last decades. J Hepatol 1994; 21: S13.

Puoti M, Bonacini M, Spinetti A, Putzolu V, Govindarajan S, Zaltron S, et al. Liver fibrosis progression is related to CD4 cell depletion in patients coinfected with hepatitis C virus and human immunodeficiency virus. J Infect Dis. 2001 Jan 1;183(1):134-7. Epub 2000 Nov 16.

Qian HZ, Vermund SH, Kaslow RA, et al. Co-infection with HIV and hepatitis C virus in former plasma/blood donors: challenge for patient care in rural China. AIDS 2006; 20(10): 1429-1435.

Qurishi N, Kreuzberg C, Lüchters G et al: Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C coinfection. Lancet 2003; 362: 1708-1713.

Ragni MV, Belle SH, Im K, et al. Survival of human immunodeficiency virus-infected liver transplant recipients. J Infect Dis. 2003;188:1412-20.

Rockstroh JK, Spengler U, Sudhop T et al: Immunosuppression may lead to progression of hepatitis C virus associated liver disease in hemophiliacs coinfected with HIV. Am J Gastroenterol 1996; 91: 2563-2568.

Rockstroh JK, Theisen A, Kaiser R et al: Antiretroviral triple therapy decreases the HIV viral load and does not alter hepatitis C virus serum levels in HIV/HCV-coinfected hemophiliacs. AIDS 1998; 12: 829-830.

Rockstroh JK, Spengler U: HIV and HCV coinfection. Lancet Infect Dis 2004; 4: 437-444.

Rockstroh JK, Mocroft A, Soriano V et al: Influence of hepatitis C virus infection on HIV-1 disease progression and response to highly antiretroviral therapy. J Infect Dis 2005; 192: 992-1002.

Rockstroh JK, Bhagani S, Benhamou Y et al: European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of chronic hepatitis B and C coinfection in HIV-infected adults. HIV Med 2008; 9: 82-88.

Rockstroh JK, Bhagani S, Benhamou Y et al: European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of chronic hepatitis B and C co-infection in HIV-infected adults. Version 5, November 2009. Presented at the EACS Conference 2009, Cologne, Germany.

Rockstroh JK. Hot topics in HIV and hepatitis coinfection: noninvasive diagnosis of liver disease, liver transplantation, and new drugs for treatment of hepatitis coinfection. HIV Clin Trials. 2009 Mar-Apr;10(2):110-5.

Rodriguez-Torres M, Slim J, Bhatti L, Sterling RK, Sulkowski MS, Hassanein T, et al. Standard versus high dose ribavirin in combination with peginterferon alfa-2a (40KD) in genotype 1 (G1) HCV patients coinfected with HIV: Final results of the PARADIGM study. AASLD 2009, Abstract 1561.

Rodríguez-Nóvoa S, Morello J, González M, Vispo E, Barreiro P, González-Pardo G, et al. Increase in serum bilirubin in HIV/hepatitis-C virus-coinfected patients on atazanavir therapy following initiation of pegylated-interferon and ribavirin. AIDS. 2008 Nov 30;22(18):2535-7.

Singal AK, Anand BS. Management of hepatitis C virus infection in HIV/HCV co-infected patients: clinical review. World J Gastroenterol. 2009 Aug 14;15(30):3713-24.

Soto B, Sanchez-Quijano A, Rodrigo L, et al. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol 1997; 26(1): 1-5.

Sulkowski NS, Thomas DL, Chaisson RE, Moore D: Hepatotoxicity associated with antiretroviral in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA 2000; 283: 74-80.

Sulkowski MS, Moore RD, Mehta SH, et al. Hepatitis C and progression of HIV disease. JAMA. 2002;288:199-206.

Thomson EC, Nastouli E, Main J, Karayiannis P, Eliahoo J, Muir D, McClure MO. Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV. AIDS. 2009 Jan 2;23(1):89-93.

Torriani FJ, Rodriguez-Torres M, Rockstroh JK et al: peg-interferon-a 2a + ribavirin for chronic hepatitis C virus infection in HIV-infected patients. New Engl J Med 2004; 358: 438-450.

Turner J, Bansi L, Gilson R, Gazzard B, Walsh J, Pillay D, et al. The prevalence of hepatitis C virus (HCV) infection in HIV-positive individuals in the UK - trends in HCV testing and the impact of HCV on HIV treatment outcomes. J Viral Hepat. 2009 Oct 13.

Verma S, Wang CH, Govindarajan S, Kanel G, Squires K, Bonacini M. Do type and duration of antiretroviral therapy attenuate liver fibrosis in HIV-hepatitis C virus-coinfected patients? Clin Infect Dis. 2006 Jan 15;42(2):262-70.

Vogel M, Voigt E, Michaelis HC, et al.: Management of drug-to-drug interactions between cyclosporin A and the protease inhibitor saquinavir/ritonavir in liver transplanted HIV-infected patients. Liver Transplant 2004;10: 939-944.

Vogel M, Voigt E, Schäfer N, Goldmann G, Schwarz N, Kalff JC, et al. Orthotopic liver transplantation in human immunodeficiency virus (HIV)-positive patients: outcome of 7 patients from the Bonn cohort. Liver Transpl. 2005 Dec;11(12):1515-21.

Vogel M, Deterding K, Wiegand J, Grüner NH, Baumgarten A, Jung MC, et al. Hep-Net. Initial presentation of acute hepatitis C virus (HCV) infection among HIV-negative and HIV-positive individuals-experience from 2 large German networks on the study of acute HCV infection. Clin Infect Dis. 2009 Jul 15;49(2):317-9.

Vogel M, Rockstroh JK. Liver disease: the effects of HIV and antiretroviral therapy and the implications for early antiretroviral therapy initiation. Curr Opin HIV AIDS. 2009 May;4(3):171-5.

Vogel M, Rockstroh JK. Treatment of acute hepatitis C in HIV infection. J Antimicrob Chemother. 2010 Jan;65(1):4-9.

Weinbaum CM, Sabin KM, Santibanez SS. Hepatitis B, hepatitis C, and HIV in correctional populations: a review of epidemiology and prevention. AIDS 2005; 19 Suppl 3: S41-46.

Zanetti AR, Tanzi E, Paccagnini S et al: Mother-to-infant transmission of hepatitis C virus. Lombard study group on vertical HCV transmission. Lancet 1995; 345: 289-291.

Chapter 19: Management of HBV/HCV coinfection

Carolynne Schwarze-Zander, Jürgen Kurt Rockstroh

Epidemiology of HBV/HCV coinfection

Hepatitis B (HBV) and hepatitis C (HCV) viruses are the most common causes of chronic liver disease world-wide. Due to shared routes of transmission, coinfection with HBV and HCV is not uncommon among individuals in HBV endemic areas who also have a high risk of parenteral infections, such as injection drug users (Pallas 1999), patients on hemodialysis (Reddy 2005), patients undergoing organ transplantation (Aroldi 2005) and HIV-positive individuals (Zhou 2007). Due to a lack of large-scale population-based studies the exact number of HBV/HCV coinfected patients is unknown. Dual infection with HBV and HCV in the same host ranges from 9% to 30%, depending on the geographic region (Zarski 1998; Liaw 1995). These numbers may underestimate the true number of people with HBV/HCV coinfection as there is a well-known entity of occult HBV infection (i.e., patients with negative hepatitis B surface antigen (HBsAg) but detectable serum HBV DNA) in patients with chronic hepatitis C (Cacciola 1999).

Screening for HBV/HCV coinfection

Persons with a first episode of acute hepatitis should be screened for all viral causes including HBV and HCV (see Chapter 8 on diagnostic tests for hepatitis B and Chapter 12 for hepatitis C). Some patients may be inoculated with both viruses simultaneously and will present with acute hepatitis due to both viruses. In addition, HBV superinfection in patients with chronic hepatitis C, and HCV superinfection in patients with chronic hepatitis B have both been reported (Liaw 2004; Liaw 2000; Liaw 2002). Therefore, episodes of acute hepatitis in patients with known chronic HBV or HCV infection, especially those with ongoing risk behaviour for infection with the other virus such as injection drug users, should prompt screening for superinfection. In addition, in patients with chronic hepatitis C, ruling out occult HBV infection beyond HBsAg testing, i.e., by polymerase chain reaction (PCR), should be done when clinically indicated.

Viral interactions between HBV and HCV

Patients with both HBV and HCV infections may show a large spectrum of virologic profiles. HCV infection can suppress HBV replication and it has been shown that HBV/HCV-coinfected patients have lower HBV DNA levels, decreased activity of HBV DNA polymerase, and decreased expression of HBsAg and hepatitis B core antigen in the liver (Chu 1998). Moreover, patients with chronic HBV infection who become superinfected with HCV can undergo seroconversion of HBsAg (Liaw 1994; Liaw 1991). Several authors have reported that HBV can reciprocally inhibit HCV replication as well (Sato 1994). Specifically, HBV DNA replication has been shown

to correlate with decreased HCV RNA levels in coinfected patients (Zarski 1998). Furthermore, coinfected patients have been shown to have lower levels of both HBV DNA and HCV RNA than corresponding monoinfected controls, indicating that simultaneous suppression of both viruses by the other can also occur (Jardi 2001). Thus, HBV or HCV can play the dominant role, HBV and HCV can inhibit each other simultaneously and they can alternate their dominance (Liaw 1995). Both viruses have the ability to induce seroconversion of the other. The chronology of infection may have a role in determining the dominant virus. However, the overall effect appears to be HCV suppression of HBV (Liaw 2001).

Clinical scenarios of HBV and HCV infection

Different scenarios of infection have been described with HBV/HCV coinfection including acute hepatitis with HBV and HCV (Alberti 1995), occult HBV coinfection of chronic hepatitis C (Sagnelli 2001), and superinfection by either virus in patients with pre-existing chronic hepatitis due to the other virus (Figure 1).

Acute hepatitis by simultaneous infection of HBV and HCV

Simultaneous coinfection with HBV and HCV is rarely seen, but the interaction of HBV and HCV appears to be similar to chronic infection. In acute infection with HBV and HCV, patients show delayed HBsAg appearance and a shorter hepatitis B surface antigenemia compared to those with acute HBV alone (Mimms 1993). Biphasic alanine aminotransferase (ALT) elevation was found in some patients (Alberti 1995).

HCV superinfection

HCV superinfection is frequent in endemic areas of HBV infection, such as in Asian countries (Liaw 2002; Liaw 2004), which can result in the suppression of HBV replication and termination of HBsAg carriage. However, long-term follow-up analyses have described a higher rate of liver cirrhosis and hepatocellular carcinoma. Fulminant hepatic failure was significantly higher among patients with underlying HBV infection than those without (23% vs. 3%) (Chu 1999; Chu 1994).

HBV superinfection

HBV superinfection is less common in HCV-infected patients and very limited data is available. In one report a patient became seronegative for HCV RNA after HBV superinfection, indicating that superinfection of HBV may lead to suppression of HCV (Liaw 2000;Wietzke 1999). Other reports have shown that HBV superinfection may be associated with acute deterioration of liver function among patients with chronic HCV infection, and the risk of fulminant hepatitis may be increased (Sagnelli 2002).

Occult HBV infection in patients with HCV infection

Occult HBV infection has been identified in up to 50% of patients with chronic HCV. Importantly, a relation to HCV treatment outcomes has been described (Zignego 1997; Fukuda 2001; Sagnelli 2001). HCV infection with occult HBV infection has been associated with higher ALT levels, greater histological activity index and liver disease more often progressing to liver cirrhosis (Fukuda 1999; Cacciola1999; Sagnelli 2001).

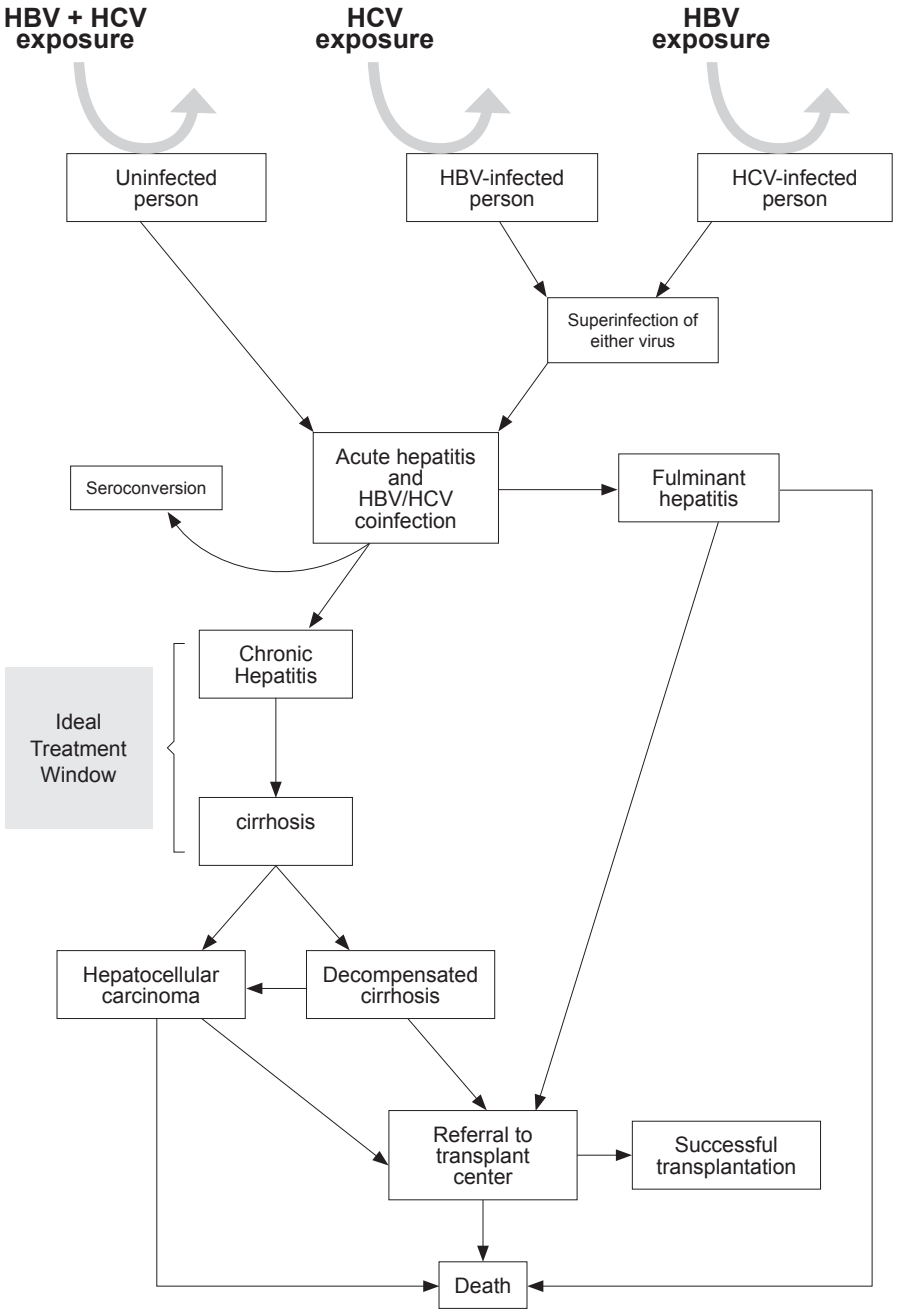


Figure 1. Clinical scenarios of HBV/HCV coinfection (modified after Crockett & Keeffe 2005).

Chronic hepatitis in HBV/HCV coinfection

Various immune profiles are found in patients with chronic HBV/HCV hepatitis (Table 1). Patients with detectable serum HBV DNA and HCV RNA are at highest risk of progression to cirrhosis and liver decompensation and therefore should be considered for treatment. Active HCV infection (HCV RNA+) in the setting of inactive HBsAg (HBsAg+/HBV DNA-) behaves similarly to patients with HCV monoinfection. Another possibility is active HBV infection in patients with inactive or prior HCV infection (HBV DNA +/HCV RNA-/anti-HCV+). This immune profile is less common, and may indicate HBV suppression of HCV.

	HBV and HCV active	Occult HBV in chronic active HCV	HCV active in HBs Ag carrier
HBsAg	+	-	+
HBV DNA	+	+	-
Anti-HCV	+	+	+
HCV RNA	+	+	+

Table 1. Immune profiles in HBV/HCV coinfected patients with chronic hepatitis.

Cirrhosis

Higher rates of cirrhosis have been demonstrated in HBV/HCV-coinfected patients. In comparison to patients with HBV monoinfection higher rates of cirrhosis (44% vs. 21%) and decompensated liver disease (24% vs. 6%) were demonstrated in coinfected patients (Fong 1991). Compared to HCV monoinfected patients a higher rate of cirrhosis (95% vs. 49%) and more decompensated liver disease (Child-Pugh class C 37% vs. 0%) were found in HBV/HCV-coinfected patients (Mohamed Ael 1997).

Hepatocellular carcinoma

In many studies coinfection with HBV and HCV has been shown to be associated with an increased risk of HCC development (Kaklamani 1991; Mohamed Ael 1997).

In one longitudinal study incidence of HCC was 6.4 per person years in HCV/HBV-coinfected patients compared to 2.0 in HBV and 3.7 in patients with HCV monoinfection. The cumulative risk of developing HCC after 10 years was 45% in HBV/HCV-coinfected patients compared with 16% in HBV and 28% in HCV monoinfected patients (Chiaramonte 1999). HBV/HCV-coinfected patients should undergo a screening routine for HCC with liver ultrasound and alpha-fetoprotein levels in serum at least every 6 months.

Treatment of HBV and HCV coinfection

Currently there are no well-established treatment guidelines for HBV/HCV-coinfected patients. Generally, treatment guidelines for monoinfected patients should be applied to coinfected patients. As with HBV and HCV monoinfection, treatment of coinfected

patients should be started in patients with active chronic hepatitis or cirrhosis before liver decompensation. Due to the variety of virological profiles in HBV/HCV coinfection it is important to assess the dominant virus prior to initiating therapy. Treatment studies for HBV/HCV coinfection are reviewed in (Crockett 2005) and (Chu 2008). In patients with HBV/HCV coinfection treatment should be initiated when inclusion criteria for standard treatment guidelines of HBV or HCV monoinfection are met (see Chapter 9 for HBV Therapy and Chapter 13 for HCV Therapy).

In coinfected patients with dominance of HCV infection, IFN plus ribavirin has been well-studied and proven efficient. However, pegylated IFN is the standard of care for HCV monoinfected patients and future studies in HBV/HCV-coinfected patients will be carried out using pegylated IFN. A recent prospective multicenter study including 19 coinfected patients found the combination of PEG-IFN α-2b plus ribavirin to be effective to induce a sustained HCV RNA response in 93% (88% in HCV genotype 1 and 100% in genotype 2 and 3) of coinfected patients (Potthoff 2008).

In patients with dominance of HBV disease IFN +/- HBV polymerase inhibitor is a possible option. Until now most data available are for lamivudine. There is very little experience with the newer anti-HBV agents. Future studies are needed to assess the safety and effectiveness of antiviral therapy with pegylated interferon, ribavirin and a combination of the newer nucleos(t)ide analogues. Due to loss of viral suppression from the successfully treated dominant virus, deterioration of liver disease has been reported (Yalcin 2003), thus caution must be exercised upon initiation of therapy.

Conclusion

Coinfection with HBV and HCV is not uncommon, especially within areas of high hepatitis B prevalence. HBV/HCV coinfection is a challenge for clinicians due to the complex interaction of HBV and HCV, and the propensity for developing severe liver disease. No treatment standard has been established for HBV/HCV-coinfected patients. Treatment decisions must be made based upon identification of the dominant virus. Standard IFN, ribavirin and lamivudine are the best-studied treatment agents. However, larger randomized, controlled trials are needed to establish the role of PEG-IFN in combination with ribavirin and nucleos(t)ide analogues for treatment of HBV/HCV coinfection. Finally, caution must be exercised in treating coinfected patients, as flares of the untreated virus may occur.

References

Alberti, A., Pontisso, P., Chemello, L., Fattovich, G., Benvegnu, L., Belussi, F. & De Mitri, M.S. (1995). J Hepatol, 22, 38-41.

Aroldi, A., Lampertico, P., Montagnino, G., Passerini, P., Villa, M., Campise, M.R., Lunghi, G., Tarantino, A., Cesana, B.M., Messa, P. & Ponticelli, C. (2005). Transplantation, 79, 1132-6.

Cacciola, I., Pollicino, T., Squadrito, G., Cerenzia, G., Orlando, M.E. & Raimondo, G. (1999). N Engl J Med, 341, 22-6.

Chiaramonte, M., Stroffolini, T., Vian, A., Stazi, M.A., Floreani, A., Lorenzoni, U., et al. (1999). Cancer, 85, 2132-7.

Chu, C.J. & Lee, S.D. (2008). J Gastroenterol Hepatol, 23, 512-20.

Chu, C.M., Sheen, I.S. & Liaw, Y.F. (1994). Gastroenterology, 107, 189-95.

Chu, C.M., Yeh, C.T. & Liaw, Y.F. (1998). J Clin Microbiol, 36, 2084-6.

Chu, C.M., Yeh, C.T. & Liaw, Y.F. (1999). Gut, 45, 613-7.

Crockett, S.D. & Keeffe, E.B. (2005). Ann Clin Microbiol Antimicrob, 4, 13.

Fong, T.L., Di Bisceglie, A.M., Waggoner, J.G., Banks, S.M. & Hoofnagle, J.H. (1991). Hepatology, 14, 64-7.

Fukuda, R., Ishimura, N., Hamamoto, S., Moritani, M., Uchida, Y., Ishihara, S., et al. (2001). J Med Virol, 63, 220-7.

Fukuda, R., Ishimura, N., Niigaki, M., Hamamoto, S., Satoh, S., Tanaka, S., et al. (1999). J Med Virol, 58, 201-7.

Jardi, R., Rodriguez, F., Buti, M., Costa, X., Cotrina, M., Galimany, R., Esteban, R. & Guardia, J. (2001). Hepatology, 34, 404-10.

Kaklamani, E., Trichopoulos, D., Tzonou, A., Zavitsanos, X., Koumantaki, Y., Hatzakis, A., et al. (1991). Jama, 265, 1974-6.

Liaw, Y.F. (1995). Hepatology, 22, 1101-8.

Liaw, Y.F. (2001). J Gastroenterol Hepatol, 16, 597-8.

Liaw, Y.F. (2002). J Gastroenterol, 37 Suppl 13, 65-8.

Liaw, Y.F., Chen, Y.C., Sheen, I.S., Chien, R.N., Yeh, C.T. & Chu, C.M. (2004). Gastroenterology, 126, 1024-9.

Liaw, Y.F., Lin, S.M., Sheen, I.S. & Chu, C.M. (1991). Infection, 19, 250-1.

Liaw, Y.F., Tsai, S.L., Chang, J.J., Sheen, I.S., Chien, R.N., Lin, D.Y. & Chu, C.M. (1994). Gastroenterology, 106, 1048-53.

Liaw, Y.F., Yeh, C.T. & Tsai, S.L. (2000). Am J Gastroenterol, 95, 2978-80.

Mimms, L.T., Mosley, J.W., Hollinger, F.B., Aach, R.D., Stevens, C.E., Cunningham, M., et al. (1993). BMJ, 307, 1095-7.

Mohamed Ael, S., al Karawi, M.A. & Mesa, G.A. (1997). Hepatogastroenterology, 44, 1404-6.

Pallas, J.R., Farinas-Alvarez, C., Prieto, D. & Delgado-Rodriguez, M. (1999). Eur J Epidemiol, 15, 699-704.

Potthoff, A., Wedemeyer, H., Boecher, W.O., Berg, T., Zeuzem, S., Arnold, J., et al. (2008). J Hepatol.

Reddy, G.A., Dakshinamurthy, K.V., Neelaprasad, P., Gangadhar, T. & Lakshmi, V. (2005). Indian J Med Microbiol, 23, 41-3.

Sagnelli, E., Coppola, N., Messina, V., Di Caprio, D., Marrocco, C., Marotta, A., et al. (2002). Hepatology, 36, 1285-91.

Sagnelli, E., Coppola, N., Scolastico, C., Mogavero, A.R., Filippini, P. & Piccinino, F. (2001). J Med Virol, 64, 350-5.

Sato, S., Fujiyama, S., Tanaka, M., Yamasaki, K., Kuramoto, I., Kawano, S., et al. (1994). J Hepatol, 21, 159-66.

Wietzke, P., Schott, P., Braun, F., Mihm, S. & Ramadori, G. (1999). Liver, 19, 348-53.

Yalcin, K., Degertekin, H., Yildiz, F. & Kilinc, N. (2003). J Gastroenterol, 38, 796-800.

Zarski, J.P., Bohn, B., Bastie, A., Pawlotsky, J.M., Baud, M., Bost-Bezeaux, F., et al. (1998). J Hepatol, 28, 27-33.

Zhou, J., Dore, G.J., Zhang, F., Lim, P.L. & Chen, Y.M. (2007). J Gastroenterol Hepatol, 22, 1510-8.

Zignego, A.L., Fontana, R., Puliti, S., Barbagli, S., Monti, M., Careccia, G., et al. (1997). J Med Virol, 51, 313-8.

Part 5

Liver fibrosis

Chapter 20: Assessment of hepatic fibrosis in chronic viral hepatitis

Frank Grünhage, Frank Lammert

Introduction

Non-invasive methods for the assessment of liver fibrosis versus invasive liver biopsy are increasingly being used thanks to patient acceptance and the low but ever-present morbidity of biopsies. Yet, despite recent advances in the use of surrogate markers and the development of new technical developments such as elastography, liver histology remains the gold standard for fibrosis staging (Goodman 2007). Most experts agree that non-invasive techniques will not replace liver biopsies completely but will help reduce the number of biopsies required (Leroy 2007; Pinzani 2005; Sebastiani 2006).

Non-invasive tests should be able to discriminate between non-significant (stages F0-F1) and significant (stages ≥F2) fibrosis, to help either delay or initiate antiviral treatment. Non-invasive markers should be able to reliably predict liver cirrhosis, and dose adjustments or monitoring can occur while on antiviral treatment.

Mechanisms of liver fibrosis in chronic viral hepatitis

Liver fibrosis is characterised by the loss of hepatocytes, destruction of hepatic (micro) architecture, proliferation of hepatic (myo)fibroblasts, and excess deposition of extracellular matrix components (Friedman 2008). Endstage liver fibrosis (cirrhosis) may include insufficient detoxification, hepatocellular carcinoma, portal hypertension, renal and pulmonary failure, and is associated with excess mortality. In chronic viral hepatitis, fibrosis develops as a consequence of the host immunological response. This immunological response activates antiviral defence mechanisms that aim to clear infected hepatocytes. The mechanisms underlying fibrogenesis in HBV or HCV are complex (Friedman 2007).

A key feature of hepatic fibrosis is the activation and proliferation of hepatic stellate cells. Quiescent hepatic stellate cells store vitamin A and reside in the subendothelial space of Disse. Chronic liver injury leads to activation of these cells, which become contractile, produce extracellular matrix components and secrete pro-inflammatory cytokines and chemokines such as transforming growth factor ß. The activation of these cells is believed to represent the key event in hepatic fibrogenesis (Friedman 2008). Hepatic stellate cell activation depends on signalling by Kupffer cells, endothelial cells, hepatocytes, and platelets. The deposition of the extracellular matrix is constantly opposed by degradation of these proteins. In progressive liver fibrosis, this balance is skewed in favour of excess extracellular matrix deposition. Matrix metalloproteinases and their regulators (tissue inhibitors of metalloproteinases, TIMPs) control matrix deposition and degradation. In liver fibrogenesis, TIMP-1 is also produced by activated hepatic stellate cells.

Liver histology, by helping visualise the fibrosis, is regarded as the gold standard for the assessment and progression of fibrosis. However, the disadvantages of this method have motivated researchers and clinicians to test non-invasive strategies. These strategies are based either on single serum surrogate markers, compositional scores derived from combinations of different surrogate markers, or modifications of imaging techniques.

Liver biopsy – the gold standard for staging of liver fibrosis

In the majority of liver centres worldwide, liver biopsy is performed as a "blind" or ultrasound-guided puncture, as either an out- or in-patient procedure. Liver punctures are considered to be relatively safe procedures with complication rates ranging from 0.75% up to 13.6% (Myers 2008; Piccinino 1986; van der Poorten 2006). The most frequent complications are minor bleeding or pain. After efficient substitution with clotting factors, percutaneous liver biopsy is also possible in patients with inherited bleeding disorders with no obvious increase of complication rates (DiMichele 2003; Schwarz 2008). Procedure-related mortality rates are reported to range from 0.001 to 0.003% (Piccinino 1986). Of note, excess rates with severe bleedings and biopsy related deaths have been reported after percutaneous biopsy in populations with advanced fibrosis, cirrhosis, or hepatic tumors (Terjung 2003). Thus, liver biopsies in these patients should always be performed as in-patient procedures, as >90% of complications are detected within the first 24 hours (Piccinino 1986).

Transjugular puncture of the liver via cannulation of an hepatic vein is an alternative, that can be performed in patients with severe coagulation deficiencies. It is resource-intensive and carries a risk of intrahepatic haemorrhage or capsule perforation with intra-abdominal bleeding. Complication rates are lower as compared to percutaneous biopsies and range from 2.5% (Mammen 2008) to 6.5% with a reported mortality rate of up to 0.09% in high-risk groups (Kalambokis 2007). However, the quality of specimens from transjugular biopsies may be lower because of the higher fragmentation of specimens and the lower numbers of portal fields in transjugular biopsies (Cholongitas 2006).

Laparoscopy and mini-laparoscopy are even more invasive procedures for obtaining liver biopsies. A recent randomized trial showed a higher detection rate of liver cirrhosis as compared to percutaneous biopsies with lower complication rates for laparoscopy (Denzer 2007). No data is available for detection in lower fibrosis stages. Thus, we recommend this procedure only in selected cases if the results might have an impact on the clinical management of the patient (Helmreich-Becker 2003).

The quality and reliability of fibrosis staging via histopathological assessment of liver biopsy specimens depends largely on the size of the specimen and the number of portal fields. The biopsy should be 20-25 mm long and more than 11 portal tracts should be visible (Cholongitas 2006; Rousselet 2005; Bedossa 2003). However, in daily practice these requirements may not be easy to achieve; and even if a large enough biopsy is acquired, the specimen only reflects about 1/50,000 of the whole liver. Thus, liver biopsies are particularly prone to sampling errors and may – like non-invasive markers – have difficulties in discriminating between adjacent stages of fibrosis (i.e., F1 vs. F2 or F2 vs. F3). Recent studies reported up to one stage difference between specimens from the right and the left lobe in up to 38% of biopsies (Regev 2002; Siddique 2003). Discrepancies of more than one stage are rare (Regev 2002; Siddique 2003; Skripenova 2007). Intra- and inter-observer variability may be unaffected by specimen sizes but can lead to discrepancies in up to 20% of cases, even if one stage difference between estimates is accepted (Gronbaek 2002; Petz 2003). Standardized automatic staging via image analysis may improve inter-observer variability (Hui 2003).

All staging systems for liver fibrosis are based on the definition of categorical stages of liver fibrosis that describe the increase of deposition of collagen and the progressive destruction of liver architecture ranging from no fibrosis to cirrhosis with a variable number of intermediate stages (Table 1). The use of categories decreases interobserver variation, but also results in a loss of information that may be covered by more detailed scoring systems (Standish 2006).

Whereas the METAVIR score is considered best in HCV fibrosis, there is a wide variability in the use of other staging systems in patients with chronic viral hepatitis. In Germany, current guidelines recommend the staging system defined by Desmet & Scheuer (Table 1) (The French METAVIR Cooperative Study Group 1994; Knodell 1981; Ishak 1995; Desmet 1994; Schirmacher 2004).

Staging System	Fibrosis stages	Remark	
METAVIR Score	F0, F1, F2, F3, F4	Best evaluated in HCV fibrosis	(The French METAVIR Cooperative Study Group 1994)
Knodell Score	F0, F1, F3, F4	No intermediate stage	(Knodell 1981)
Desmet & Scheuer	Analogous to METAVIR	Recommended by German guidelines for the assessment of liver fibrosis	(Desmet 1994; Schirmacher 2004)
Batts & Ludwig	Similar to METAVIR		(Batts 1995)
Ishak Score	F0, F1, F2, F3, F4, F5, F6		(Ishak 1995)

Table 1. Commonly used liver fibrosis staging scores.

Surrogate markers of liver fibrosis

Liver fibrosis develops as a continuous process rather than in a stepwise manner. Thus, so-called surrogate markers, which are also continuous variables, may provide a more precise grading system. Surrogate makers can be subdivided into direct and indirect markers. Direct markers reflect changes in the content of extracellular matrix proteins (such as collagen) in the liver. In contrast, indirect markers reflect alterations in hepatic function, increase in portal hypertension with subsequent splenic enlargement, and/or grade of hepatic inflammation that may correlate with liver fibrosis stage (Table 2). Direct and indirect markers may be used alone or - more commonly - in combination ("composite scores"). The calculation of such scores can be simple or based on complicated formulas (e.g., Fibrotest / Fibrosure) (Table 2). Most studies of non-invasive markers were performed in HCV patients, while studies in HBV or co-infected cohorts are sparse (Pinzani 2008). Primary endpoints of the studies that evaluated surrogate markers vary from discrimination of no fibrosis and cirrhosis to the determination of fibrosis stages. However, for the clinical management of patients with chronic viral hepatitis both are needed: whereas the former is needed to identify patients in need of urgent treatment, the latter may separate those patients with an indication for antiviral treatment due to significant fibrosis from those with no or minor fibrosis in whom treatment may be postponed.

Index	Markers	Calculation	Interpretation	PPV/NPV (%)
Direct surrogate markers				
MP3	PIIINP, MMP-1	0.5901(logPIINP[ng/ml]) -0.1749(logMMP-1[ng/ml])	<0.3≈F0-2 >0.4≈F3-4 <0.3≈F0-1 >0.4≈F2-4	NPV=95 PPV=66 NPV=75 PPV=91
ELF	PIIINP, HA	Proprietary	>0.102 Scheuer 3–4 <0.102 Scheuer 0–2	PPV = 35 NPV = 92
Indirect surrogate markers				
Forns	Age, plt, γGT, cholesterol	7.811 - 3.131 xln(plt) + 0.781 xln(γGT) + 3.467 x ln(age) - 0.014 (cholesterol)	>6.9 ≈Scheuer 2–4 <4.2 ≈Scheuer 0–1	PPV = 66 NPV = 96
APRI	AST, plt	([AST/ULN]/plt [x109/l]) x100	>1.5 ≈Ishak 3–6 ≤0.5 ≈Ishak 0–2	PPV = 91 NPV = 90
Fibrotest **Fibrosure**	Haptoglobin, a2-MC, apo-A1, γGT, bilirubin, γ–globulin	Proprietary	0.75–1.00 ≈F4 0.73–0.74 ≈F3–F4 0.59–0.72 ≈F3 0.49–0.58 ≈F2 0.32–0.48 ≈F1–F2 0.28–0.31 ≈F1 0.22–0.27 ≈F0–F1 0.00–0.21 ≈F0	PPV = 78 PPV = 76 PPV = 76 PPV = 67 PPV/NPV = 61/85 NPV = 91 NPV = 92 NPV = 94
Fibroindex	Plt, AST, γ GT	1.738 - 0.064 (plt [¥104/mm3]) + 0.005 (AST [IU/L]) + 0.463 x (γGT(g/dl)	≤ 1.25 ≈F0–F1 ≥ 2.25 ≈F2–F3	NPV = 61.7 PPV = 90
Testa	Plt, spleen diameter	Plt count/spleen diameter	>1750 ≈Ishak ≤2 ≤1750 ≈Ishak >2	NPV = 79 PPV = 78.9c
Fibrosis probability index	AST, cholesterol, past alcohol intake, HOMA, age	E*/1 + e*, where * = -10.929 + (1.827 xln[AST]) + (0.081xage) + (0.768 x [past alcohol use graded as 0–2]) + (0.385 x HOMA)	<0.2 ≈F0–F1 ≥0.8 ≈F2–F4	NPV = 77.4 PPV = 87
FIB-4	Plt, AST, ALT, age	(Age x AST)/(plt count x ALT1/2)	<1.45 ≈Ishak <4–6 >3.25 ≈Ishak ≥4–6	NPV = 90 PPV = 65
Bonancini	ALT, AST, INR, plt	Sum (range 0–11) of (plt score) + (ALT/AST score) + (INR score) plt (x109/l): >340 = 0; 280–339 = 1; 220 –279 = 2; 160–219 = 3; 100–159 = 4; 40–99 = 5; <40 = 6 ALT/AST ratio: >1.7 = 0; 1.2–1.7 = 1; 0.6–1.19 = 2; <0.6 = 3 INR: \1.4 = 2	>8 ≈Knodell 3–4	PPV = 92.9
Pohl	AST, ALT, plt	Positive if: AST/ALT ≥1 and platelet count <150 x109/l	Positive ≈F3-F4	PPV = 93
Shet **Park**	AST, ALT AST, ALT	AST/ALT AST/ALT	≥1 ≈ Scheuer 4 ≥1≈ Scheuer 4	PPV = 100 PPV = 73.7
Age-Platelet	Plt, age	Age score + plt score (0–10 possible score) age: <30 = 0; 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; ≥70 = 5. Plt (x109/l): ≥225 = 0; 200–224 = 1; 175–199 = 2; 150–174 = 3; 125–149 = 4; <125 = 5	≥6 ≈F2–F4	PPV = 96
Combined direct and indirect surrogate markers				
SHASTA	HA, AST, albumin	-3.84 + 1.70 (1 if HA 41–85 ng/ml, 0 otherwise) + 3.28 (1 if HA >85 ng/ml, 0 otherwise) + 1.58 (1 if albumin <3.5 g/dl, 0 otherwise) + 1.78 (1 if AST>60 IU/l, 0 otherwise)	>0.8 ≈Ishak ≥3 <0.3 ≈Ishak ≤2	PPV = 100 NPV = 94
FM	plt, PI, AST, HA, α2-MC, gender, age	- 0.007 plt (G/L) - 0.049 PI (%) + 0.012 AST (IU/l) + 0.005 α2-MC (mg/dl) + 0.021 HA (g/l) - 0.270 urea (mmol/l) + 0.027 age (years)+3.718	≥F2	PPV = 86.3/96.6
Hepascore	HA, α2-MC, γGT, age, gender	y/1 + y, where y = exp [- 4.185818 - (0.0249 x age) + 0.7464 x sex) + (1.0039 x α2-MC) + (0.0302 x HA) + (0.0691 x bilirubin) - (0.0012 x γGT)]	≥0.5 ≈F2–F4 <0.5 ≈F0–F1	PPV = 88 NPV = 98
FSII	HA, α2-MC, TIMP-1	Proprietary	≥42 ≈F2–F4 <40 ≈F0–F1	PPV = 77.4 NPV = 78

Table 2. Summary of surrogate markers of liver fibrosis modified according to Pinzani.

From the whole range of surrogate markers only a few are in clinical use. The simple APRI score has been widely studied in HCV and HBV as well as in coinfected patients (Cacoub 2008; Vallet-Pichard 2008; Wai 2006; Lebensztejn 2005). A recent comprehensive meta-analysis of the performance of the APRI test showed that its major strength is the exclusion of significant fibrosis, defined as F2-F4, or cirrhosis with cut-offs of 0.5 and 1.0. However, the authors conclude that using this marker alone, only about one third of all biopsies can be avoided. Importantly, the test performance varied with the quantity of advanced fibrosis in the different patient groups (Shaheen 2007a; Shaheen 2007b). Fibrotest has also achieved some clinical significance. However, this test may not be available for all patients. Recent meta-analyses of the predictive performance of Fibrotest summarize that the reliability for the detection of advanced fibrosis or cirrhosis is adequate for clinical practice and a cut-off of 0.6 is suggested (Shaheen 2007b; Poynard 2007). Of note, the reliability for the detection of earlier fibrosis stages appears to be relatively low (Shaheen 2007b; Poynard 2007) and the most positive conclusions concerning the Fibrotest come from authors who are directly involved in the commercial distribution of this test (Shaheen 2007b; Poynard 2007).

In summary, surrogate markers may support the clinical decision making process, but a single surrogate marker or score can not replace the liver biopsy. On the other hand, attempts have been made to combine different surrogate markers and biopsy in clinical decision algorithms that aim to reduce the need for liver punctures (Table 2).

Transient elastography

Transient elastography (TE) is a non-invasive technique to assess liver fibrosis that was first described in the medical literature in 1999 (Sandrin 1999). TE allows the assessment of liver fibrosis by calculating the velocity of a low-frequency transient shear wave produced by a mechanical probe that is placed directly on the skin of the patient. The velocity of the wave that penetrates the liver tissue depends on the actual stiffness of the liver, which in turn correlates with the extent of liver fibrosis. In practice, a probe is placed in an intercostal space at a position that is comparable to the position for standard liver biopsy. 10 successful measurements are usually necessary for the assessment of liver stiffness. This can be done in less than 5 minutes. At present TE machines are exclusively available by echosense (FibroScan®). Liver stiffness is expressed in kilo Pascal (kPa). The method is easy to learn, quick, results are available immediately, and a technical assistant may perform the procedure. TE displays robust intra- and inter-observer variability (Fraquelli 2007) and may be applied in children and adults (de Ledinghen 2007).

Evaluation of liver stiffness in subjects without apparent liver disease shows that liver stiffness is influenced by gender and body mass index (BMI). In general, liver stiffness is higher in men than in women (5.81±1.54 vs. 5.23±1.59 kPa) (Roulot 2008). It is important to note that the applicability of TE is limited to relatively lean patients (BMI $\leq$28 kg/m^2), patients without ascites, and "cooperative" patients. In addition, TE is hampered in those with acute liver injury such as acute viral or alcoholic hepatitis, or chronic viral hepatitis flares, that may lead to an overestimation of liver fibrosis (Arena 2008; Coco 2007; Sagir 2008). Unlike liver histology, no published data is available on the variability ("sampling error") of TE results. TE correlates well with other surrogate markers of liver fibrosis such as APRI and FIB-4 (Vidovic unpublished data).

In patients with chronic liver disease eligible for TE, liver stiffness values correlate well with the stage of fibrosis, irrespective of the underlying disease aetiology. TE has been evaluated in patients with chronic viral hepatitis, PBC, PSC, and NASH. Due to high acceptance by patients, it can easily be used to monitor progression or regression of fibrosis in patients under observation or on therapy (Yoneda 2007). TE has been evaluated for the detection of liver fibrosis in patients with acute and chronic viral hepatitis and has also been positively evaluated for HIV/HCV-coinfected patients and in patients with HCV reoccurrence post-transplantation (de Ledinghen 2006; Maida 2007; de Ledinghen 2006; Carrion 2006). In chronic viral hepatitis, it is unknown whether there is a difference in TE results between patients with chronic HBV, HCV and/or HIV/HCV-coinfected patients.

In some clinical situations, e.g., older patients or patients with risk factors for therapy, a positive decision for treatment of chronic hepatitis B and C is guided by the diagnosis of significant fibrosis. The presence of F2 fibrosis indicates significant liver fibrosis, which justifies treatment according to treatment guidelines for chronic hepatitis B, C and coinfected patients (e.g., German Guidelines for the Management of Patients with Chronic Hepatitis C Viral Infection 2009).

Study	Population	Cut-off (kPa)				
Castera 2006	HCV N = 183	F = 0	F≥1	F≥2	F≥3	F = 4
		n.d	n.d	7.1 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	9.5 Se: 0.73 Sp: 0.91 PPV: .87 NPV: .81	12.5 Se: 0.87 Sp: 0.91 PPV: .77 NPV: .97
Ziol 2005	HCV N = 327	n.d	n.d	8.8	9.6	14.6
		n.d	n.d	Se: 0.56 Sp: 0.91 PPV: .88 NPV: .56	Se: 0.86 Sp: 0.85 PPV: .71 NPV: .93	Se: 0.86 Sp: 0.96 PPV: .78 NPV: .97
Foucher 2006	HCV / HBV N = 711	n.d	n.d	7.2 Se: 0.64 Sp: 0.85 PPV: .90 NPV: .52	12.5 Se: 0.65 Sp: 0.95 PPV: .90 NPV: .80	17.6 Se: 0.77 Sp: 0.97 PPV: .91 NPV: .92
Ogawa 2007	HCV / HBV N = 229	3.5	6.4	9.5 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	11.4 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	15.4 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48
		6.3	6.7	9.1 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	13.7 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	26.4 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48
Arena 2008	HCV N = 150			7.8 Se: 0.83 Sp: 0.82 PPV: .83 NPV: .79	10.8 Se: 0.91 Sp: 0.94 PPV: .89 NPV: .95	14.8 Se: 0.94 Sp: 0.92 PPV: .73 NPV: .98
de Ledinghen 2006	HIV/HCV N = 72	n.d	n.d	4.5 Se: 0.93 Sp: 0.18 PPV: n.d. NPV: n.d.	n.d	11.8 Se: 1.0 Sp: 0.93 PPV: n.d. NPV: n.d.

Table 3. Cut-off values for transtient elastography in different study populations.

Recent studies comparing TE with liver biopsy demonstrate both high sensitivity and specificity for the detection of advanced fibrosis and cirrhosis. However, TE performance is less reliable for the detection of fibrosis stages ≥2 compared to more advanced stages of liver fibrosis (sensitivity 56-67%), resulting in moderate negative predictive values. Thus, assessment of liver fibrosis by TE alone may result in the underestimation of liver fibrosis in some patients. Vice versa, if TE predicts significant fibrosis a biopsy may not be necessary. One drawback in clinical practice is that the different TE studies suggest slightly different cut-off values (Table 3). A recent meta-analysis that evaluated the predictive performance of TE in patients with chronic liver disease suggested that the optimal cut-off value for the diagnosis of significant fibrosis is 7.65 kPa (Friedrich-Rust 2008). This cut-off proved to be robust especially in patients with chronic HCV infection.

In addition to the assessment of liver fibrosis stages, TE may also be used to predict the presence of portal hypertension and thus the need to evaluate the patient for the presence of oesophageal varices (Rockey 2008). Whether TE is reliable enough to predict the stage of cirrhosis is still debatable and needs further study (Foucher 2006).

Other imaging techniques

A number of different imaging techniques such as conventional ultrasound, real-time elastography, NMR imaging and CT have been applied for the assessment of liver fibrosis. None of these methods has yet achieved an overall clinical acceptance regarding the detection of early forms of liver fibrosis, either due to low sensitivity and/or specificity, or high costs.

Clinical decision algorithms

Until now, no non-invasive marker for staging of liver fibrosis has been able to replace the liver histology as the gold standard. This is largely due to the fact that outcome studies with clear endpoints like mortality have not been performed. These will probably not be available in the near future. The advantages of these non-invasive tests in comparison to liver biopsy are striking. In order to overcome test limitations and to benefit from their specific advantages, a frequent strategy is to combine different non-invasive tests, thereby using liver biopsy only in case of doubt. However, current algorithms vary greatly. Whereas some authors have calculated a reduction of liver biopsies of 30%, others have estimated reductions of up to 80% (Leroy 2007; Sebastiani 2007; Sebastiani 2004). Interestingly, the performance of such algorithms and their components depend on underlying diseases (HCV, HBV or coinfections). Thus to date, no widely applicable algorithm is available. However, Figure 1 shows a concept used in our daily practice.

Summary

Non-invasive tests will not replace liver biopsies, but smart combinations of both options may save many patients from the more invasive procedure. Whatever the current standard of care, the patient should be informed about non-invasive tests, their applicability and their limitations. The decision to biopsy should ultimately be made together with the informed patient.

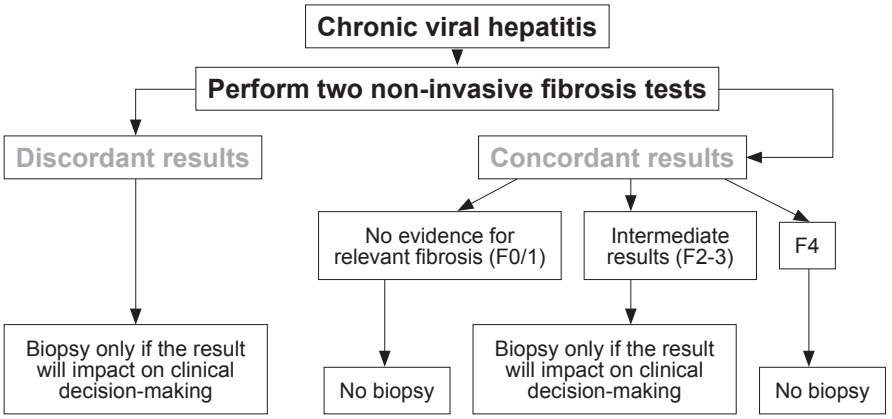


Figure 1. Potential clinical decision algorithm for safer liver biopsies in patients with chronic viral hepatitis.

References

Arena U, Vizzutti F, Abraldes J, Corti G, Stasi C, Moscarella S, Milani S, Lorefice E, Petrarca A, Laffi G, Romanelli RG, Bosch J, Marra F, Pinzani M. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. Gut 2008.

Arena U, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, Moscarella S, Boddi V, Petrarca A, Laffi G, Marra F, Pinzani M. Acute viral hepatitis increases liver stiffness values measured by transient elastography. Hepatology 2008, 47(2):380-4.

Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. Am J Surg Pathol 1995, 19(12):1409-17.

Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003, 38(6):1449-57.

Cacoub P, Carrat F, Bedossa P, Lambert J, Penaranda G, Perronne C, Pol S, Halfon P. Comparison of non-invasive liver fibrosis biomarkers in HIV/HCV co-infected patients: The fibrovic study - ANRS HC02. J Hepatol 2008.

Carrion JA, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. Liver Transpl 2006, 12(12):1791-8.

Castera L. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005, 128:343.Foucher J. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 2006, 55:403.

Cholongitas E, Quaglia A, Samonakis D, Senzolo M, Triantos C, Patch D, et al. Transjugular liver biopsy: how good is it for accurate histological interpretation? Gut 2006, 55(12):1789-94.

Cholongitas E, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. Am J Clin Pathol 2006, 125(5):710-21.

Coco B. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. J Viral Hepat 2007, 14:360.

de Ledinghen V, Le Bail B, Rebouissoux L, Fournier C, Foucher J, Miette V, et al. Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. J Pediatr Gastroenterol Nutr 2007, 45(4):443-50.

de Ledinghen V, Douvin C, Kettaneh A, Ziol M, Roulot D, Marcellin P, et al. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. J Acquir Immune Defic Syndr 2006, 41(2):175-9.

de Ledinghen V. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus co-infected patients. J Acquir Immune Defic Syndr 2006, 41:175.

Denzer U, Arnoldy A, Kanzler S, et al. Prospective randomized comparison of minilaparoscopy and percutaneous liver biopsy: diagnosis of cirrhosis and complications. J Clin Gastroenterol 2007, 41(1):103-10.

Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994, 19(6):1513-20.

DiMichele DM, Mirani G, Wilfredo Canchis P, Trost DW, Talal AH. Transjugular liver biopsy is safe and diagnostic for patients with congenital bleeding disorders and hepatitis C infection. Haemophilia 2003, 9(5):613-8.

Foucher J, Chanteloup E, Vergniol J, Castera L, Le Bail B, Adhoute X, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 2006, 55(3):403-8.

Fraquelli M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. Gut 2007, 56:968.

Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008, 88(1):125-72.

Friedman SL, Rockey DC, Bissell DM. Hepatic fibrosis 2006: report of the Third AASLD Single Topic Conference. Hepatology 2007, 45(1):242-9.

Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008, 134(4):960-74.

Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol 2007, 47(4):598-607.

Gronbaek K, Christensen PB, Hamilton-Dutoit S, et al. Interobserver variation in interpretation of serial liver biopsies from patients with chronic hepatitis C. J Viral Hepat 2002, 9(6):443-9.

Helmreich-Becker I, Schirmacher P, Denzer U, Hensel A, Meyer zum Buschenfelde KH, Lohse AW. Minilaparoscopy in the diagnosis of cirrhosis: superiority in patients with Child-Pugh A and macronodular disease. Endoscopy 2003, 35(1):55-60.

Hui AY, Liew CT, Go MY, Chim AM, Chan HL, Leung NW, Sung JJ. Quantitative assessment of fibrosis in liver biopsies from patients with chronic hepatitis B. Liver Int 2004, 24(6):611-8.

Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994, 20(1 Pt 1):15-20.

Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995, 22(6):696-9.

Kalambokis G, Manousou P, Vibhakorn S, Marelli L, Cholongitas E, Senzolo M, Patch D, Burroughs AK. Transjugular liver biopsy--indications, adequacy, quality of specimens, and complications--a systematic review. J Hepatol 2007, 47(2):284-94.

Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981, 1(5):431-5.

Lebensztejn DM, Skiba E, Sobaniec-Lotowska M, Kaczmarski M. A simple noninvasive index (APRI) predicts advanced liver fibrosis in children with chronic hepatitis B. Hepatology 2005, 41(6):1434-5.

Leroy V. Prospective comparison of six non-invasive scores for the diagnosis of liver fibrosis in chronic hepatitis C. J Hepatol 2007, 46:775.

Maida I, Soriano V, Barreiro P, Rivas P, Labarga P, Nunez M. Liver fibrosis stage and HCV genotype distribution in HIV-HCV coinfected patients with persistently normal transaminases. AIDS Res Hum Retroviruses 2007, 23(6):801-4.

Mammen T, Keshava SN, Eapen CE, Raghuram L, Moses V, Gopi K, Babu NS, Ramachandran J, Kurien G. Transjugular liver biopsy: a retrospective analysis of 601 cases. J Vasc Interv Radiol 2008, 19(3):351-8.

Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. Liver Int 2008, 28(5):705-12.

Ogawa E, Furusyo N, Toyoda K, Takeoka H, Otaguro S, Hamada M, et al. Transient elastography for patients with chronic hepatitis B and C virus infection: Non-invasive, quantitative assessment of liver fibrosis. Hepatol Res 2007, 37(12):1002-10.

Petz D, Klauck S, Rohl FW, Malfertheiner P, Roessner A, Rocken C. Feasibility of histological grading and staging of chronic viral hepatitis using specimens obtained by thin-needle biopsy. Virchows Arch 2003, 442(3):238-44.

Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. J Hepatol 1986, 2(2):165-73.

Pinzani M, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. Nat Clin Pract Gastroenterol Hepatol 2008, 5(2):95-106.

Pinzani M. Fibrosis in chronic liver diseases: diagnosis and management. J Hepatol 2005, 42(Suppl 1):S22.

Poynard T, Morra R, Halfon P, Castera L, Ratziu V, Imbert-Bismut F, et al. Meta-analyses of FibroTest diagnostic value in chronic liver disease. BMC Gastroenterol 2007, 7:40.

Regev A. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002, 97:2614.

Rockey DC. Noninvasive assessment of liver fibrosis and portal hypertension with transient elastography. Gastroenterology 2008, 134(1):8-14.

Roulot D, Czernichow S, Le Clesiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: Influence of gender and metabolic syndrome. J Hepatol 2008.

Rousselet MC, Michalak S, Dupre F, Croue A, Bedossa P, Saint-Andre JP, Cales P. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology 2005, 41(2):257-64.

Sagir A, Erhardt A, Schmitt M, Haussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatology 2008, 47(2):592-5.

Sandrin L, Catheline S, Tanter M, Hennequin X, Fink M. Time-resolved pulsed elastography with ultrafast ultrasonic imaging. Ultrason Imaging 1999, 21(4):259-72.

Schirmacher P, Fleig WE, Dienes HP. (Biopsy diagnosis of chronic hepatitis). Z Gastroenterol 2004, 42(2):175-85.

Schwarz KB, Zellos A, Stamato L, Boitnott J, Perlman E, Chong S, Casella JF. Percutaneous liver biopsy in hemophiliac children with chronic hepatitis C virus infection. J Pediatr Gastroenterol Nutr 2008, 46(4):423-8.

Sebastiani G, Vario A, Guido M, Noventa F, Plebani M, Pistis R, Ferrari A, Alberti A. Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. J Hepatol 2006, 44(4):686-93.

Sebastiani G. Sequential algorithms combining non-invasive markers and biopsy for the assessment of liver fibrosis in chronic hepatitis B. World J Gastroenterol 2007, 13:525.

Sebastiani G. Stepwise combination of algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. J Hepatol 2004, 44:686.

Siddique I, El-Naga HA, Madda JP, Memon A, Hasan F. Sampling variability on percutaneous liver biopsy in patients with chronic hepatitis C virus infection. Scand J Gastroenterol 2003, 38(4):427-32.

Shaheen AA, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. Hepatology 2007, 46(3):912-21.

Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. Am J Gastroenterol 2007, 102(11):2589-600.

Skripenova S, Trainer TD, Krawitt EL, Blaszyk H. Variability of grade and stage in simultaneous paired liver biopsies in patients with hepatitis C. J Clin Pathol 2007, 60(3):321-4.

Standish RA, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. Gut 2006, 55(4):569-78.

Terjung B, Lemnitzer I, Dumoulin FL, Effenberger W, Brackmann HH, Sauerbruch T, Spengler U. Bleeding complications after percutaneous liver biopsy. An analysis of risk factors. Digestion 2003, 67(3):138-45.

Vallet-Pichard A, Mallet V, Pol S. Predictive value of FIB-4 versus fibrotest, APRI, FIBROINDEX and FORNS to noninvasively estimate fibrosis in hepatitis C. Hepatology 2008, 47(2):763.

van der Poorten D, Kwok A, Lam T, Ridley L, Jones DB, Ngu MC, Lee AU. Twenty-year audit of percutaneous liver biopsy in a major Australian teaching hospital. Intern Med J 2006, 36(11):692-9.

Wai CT, Cheng CL, Wee A, Dan YY, Chan E, Chua W, Mak B, Oo AM, Lim SG. Non-invasive models for predicting histology in patients with chronic hepatitis B. Liver Int 2006, 26(6):666-72.

Yoneda M. Transient elastography in patients with nonalcoholic fatty liver disease (NAFLD). Gut 2007, 56:1330.

Ziol M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology 2005, 41:48.

Part 6

Hepatocellular carcinoma

Chapter 21: Diagnosis, Prognosis & Therapy

Ulrich Spengler

Classification of HCC

Tumours are classified in order to stratify patients with respect to their prognosis for survival, to select and offer optimised therapeutic options at any tumour stage. In HCC the Barcelona Clinic Liver Cancer (BCLC) Classification has been adopted as the international standard, which is recommended by both the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) (Table 1). The BCLC classification takes into account several aspects of the disease: the patient's general state of health, the severity of the liver disease as well as the extent of tumour spread (Llovet 1999). Patients in stages BCLC 0 and A have a considerably better prognosis than patients in advanced stages of liver cancer (Mazzaferro 1996). Nevertheless approximately only 25% of patients with liver cancer are diagnosed at an early stage. Both EASL and AASLD guidelines also provide recommendations regarding which therapy is best-suited to treat patients at each stage of the BCLC classification. Unlike classification schemes in other types of malignancy the BCLC classification is particularly helpful because it is entirely based on clinical parameters - molecular characteristics are not yet able to reliably assess the individual prognosis of patients with HCC.

Tumor stage	General state of health	Tumour characteristics	Child stage
0 Very early	good	single nodule <2 cm	A & B
A Early	good	single nodule <5 cm, 3 nodules <3 cm	A & B
B Intermediate	good	large, multiple nodules	A & B
C Advanced	reduced	vascular invasion, extrahepatic secondaries	A & B
D Terminal	severely reduced	any form	C

Sala et al. Sem Liever Dis 2005

Table 1. Barcelona Clinic Liver Cancer (BCLC) Classification.

Epidemiology

HCC has an annual incidence of more than 600,000 newly diagnosed patients. Thus, HCC constitutes the sixth most frequent form of cancer worldwide, and it holds third place concerning malignancy-related mortality (Parkin 2005). Incidence rates of HCC are steadily rising both in Europe and the US.

Chronic hepatitis B is the major risk factor for developing HCC in Africa and Asia, while in the US, Europe and Japan chronic hepatitis C is the leading cause of HCC. Eighty

percent of liver cancers are found in cirrhotic livers, which in themselves carry a high risk for HCC. Chronic carriers of hepatitis B virus (HBV) have a 100-fold increased risk as compared to a non-infected healthy reference population. Reports from Taiwan indicate a direct link between HBV viral loads and the risk of developing liver cancer within 10 years (Chen 2006; Iloeje 2006). The risk of HCC is significantly increased once HBV viral loads exceed 10,000 copies/ml irrespective of the degree of hepatic inflammation. In developing world countries exposure to aflatoxins further increases this risk of HCC.

Approximately 170 million people are infected with the hepatitis C virus worldwide, 20 to 30% of whom will develop liver cirrhosis, which carries a 3-5% annual risk of ultimately progressing to liver cancer. In practical terms this means that approximately one third of cirrhotic patients with hepatitis C will go on to develop HCC. Unlike hepatitis B a close relationship between HCV viral loads and the risk of developing HCC apparently does not exist (Bralet 2000). As a general rule patients will not develop liver cancer in chronic hepatitis C before their disease has progressed to the stage of cirrhosis. Consumption of alcohol or tobacco enhances the risk of HCC (Donato 2002; Gelatti 2005). Beyond that, obesity (Calle 2003) and diabetes mellitus (Davila 2005) must be considered neglected but nevertheless pivotal factors that can multiply the risk of liver cancer in western countries resulting in 4 to 40-fold increased HCC rates among patients with chronic viral hepatitis.

Surveillance of patients at high risk and early HCC diagnosis

Surveillance using ultrasound at 6-month intervals is generally recommended for all patients with liver cirrhosis or other risk factors of HCC. Significantly more patients with early hepatocellular carcinoma were detected in a single large randomised study in China, when patients were in a regular HCC screening programme, irrespective of the presence of cirrhosis (Zhang 2004). When 3- versus 6-month surveillance intervals were compared in a randomized study involving 1200 patients, there was no evidence that the shorter interval improved rates of early diagnosis and therapeutic outcomes (Trinchet 2007). If patients with cirrhosis harbour nodular lesions, however, the 3-month control interval is preferred due to the high potential of malignancy and growth characteristics of such lesions (Yao 2006). Alfa-foetoprotein (AFP) is no longer recommended as a tool for HCC surveillance, because repeated AFP measurements have proven only marginally beneficial for HCC outcomes. Novel biomarkers such Des-Gamma-Carboxyprothrombin (DCP) or the Lectin 3-Fraction of AFP (AFP-L3) have also not been established as reliable tools to detect early HCC. Nevertheless the consistent use of ultrasound for patients with early carcinoma enable us to make an early diagnosis in 30% of patients who then have a reasonable chance of curative therapy via the improved treatment options available.

Diagnosis

The diagnosis of HCC can either be made by detecting malignantly transformed hepatocytes in a liver biopsy or by demonstrating characteristic radiological features in a hepatic lesion after application of contrast media, which confirm arterial hyperperfusion of the tumour. Thus, these novel guidelines enable the diagnosis of HCC in

a cirrhotic liver without histopathology or reference to elevated tumour markers.

The distinction between a dysplastic nodule and early HCC poses a particularly challenging and as yet unsolved task for the pathologist, because markers showing the unequivocal differentiation between these two entities in difficult-to-assess histological specimens have yet to be identified. Glypican-A or a combination of three markers (glypican-A, LYVE-1 and survivin) may become tools for the pathologist enabling a correct histological diagnosis in up to 85-95% of patients. Other markers like serin/threonin kinase 15, phospholipase A2 or telomerase reverse transcriptase (TERT) are currently under evaluation. At the present time, clearly dysplastic nodules should be submitted to radiological surveillance quickly, since such lesions retain a high potential for malignant transformation resulting in transition to HCC in approximately one-third of cases.

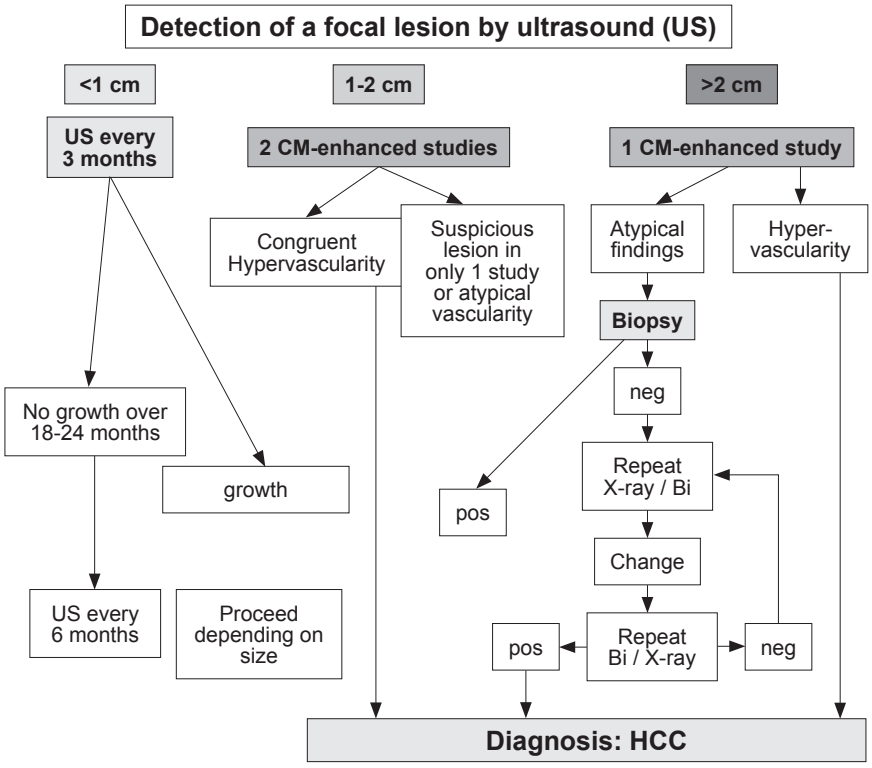


Figure 1. Diagnostic algorithm for the diagnosis of hepatocellular carcinoma depending on tumour size.

Radiological diagnosis of HCC uses detection of hyper-vascularized nodular lesions. Contrast-enhanced ultrasound, computed tomography (CT) or nuclear magnetic spin resonance tomography (MRT) are all considered to be equivalent diagnostic tools, and novel international consensus guidelines accept a diagnosis of HCC without

histopathology, if the patient has a nodular lesion in the cirrhotic liver that exhibits obvious hyper-vascularisation after application of contrast medium. Hyper-vascularisation is characterised by contrast enhancement in the early arterial phase, which rapidly disappears in the late venous phase (the so-called wash-out phenomenon) of the contrast study. Contrast-enhanced ultrasound, spiral CT and MRT in combination with gadolinium-enhancement exhibit similarly excellent diagnostic sensitivity and specificity in lesions larger than 2 cm. For this very reason detection of signs of hyper-vascularisation with any one of these three radiological techniques is sufficient to make a confident diagnosis of HCC in tumours >2 cm. Diagnostic precision is considerably less in lesions of 1-2 cm diameter. To account for that loss of precision, a diagnosis of HCC in these smaller tumours must be based on the congruent detection of a hyper-vascularised lesion in at least two independent radiological procedures. In equivocal situations the diagnosis must be clarified by biopsy in small nodules, which may have to be repeated within a short period of time. A diagnostic algo-rithm recommended by EASL and AASLD is shown in Figure 1. Small tumours should be either monitored in short-term intervals (every 3 months) or directly investigated by a liver biopsy to clarify their significance.

Stage-adapted therapy for liver cancer

A. Potentially curative therapy in stages BCLC 0-A

Patients with early HCC have excellent chances for curative cancer treatment. They can achieve 5-year survival rates of 50-70% by surgical resection, liver transplant or percutaneous, ablative procedures.

Surgical resection constitutes the backbone of curative treatment in patients with early HCC. It is the treatment of choice in patients with localised tumour spread and small-size cancers and tumours in a non-cirrhotic liver (evidence grade IIIA). Prognosis after surgical resection is excellent, if the tumour is not larger than 2 cm in diameter (5-year survival rates 70-90% with rates of tumour recurrence below 10%). Excluding patients with poor liver function keeps peri-operative mortality below 5%. Favourable criteria for surgical resection comprise single nodules less than 5 cm in size or a maximum of 3 nodules in a single liver lobe in patients with only moderately impaired liver function (cirrhosis stage Child A) without portal hypertension (hepato-portal-venous pressure gradient >10 mm Hg or presence of oesophageal varices or splenomegaly together with reduced platelet counts <100,000/µl) and serum bilirubin in the normal range. However, it is noteworthy that even the most modern CT and MRT scanner still underestimate the extent of vascular invasion in 30% of patients with early HCC.

Liver transplantation is an alternative therapeutic option, if the liver cancer cannot be cured by local resection due to anatomical reasons, if residual liver function after resection is anticipated to be poor or there is multi-nodular tumour spread into both liver lobes (evidence grade IIIA). Commonly patients with HCC are selected for liver transplant according to the so-called Milan criteria, i.e., the patient has a single nodule

of less than 5 cm in diameter or at most has 3 nodules, none of which exceeds 3 cm in diameter (Mazzaferro 1996). Milan criteria patients usually achieve survival rates of 80% and 70% one and five years after liver transplantation. However, it has been demonstrated that patients with more extensive stages of liver cancer can be transplanted with reasonable long-term outcomes (Yao 2001). Selection of patients according to the so-called San Francisco criteria comprises solitary large nodules up to 6.5 cm as well as multi-nodular HCC with a maximum of 3 nodules, each of which must be smaller than 4.5 cm with a total sum of all nodule diameters below 8 cm. Patients who remain within these extended selection criteria can still reach 70-80% five-year survival rates after liver transplantation.

A central issue in liver transplantation is the process of fair organ allocation. Shortage of donor organs is particularly critical in patients with liver cancer, because the tumour will continue to expand while the patient is on the waiting list, and can ultimately reach a stage that makes liver transplantation a futile option. It has been estimated that after one year on the waiting list approximately 40% of patients can no longer be cured by liver transplant (Poon 2007). In the Eurotransplant registry donor livers are allocated to patients according to their MELD scores, which take into account kidney function, serum bilirubin and the degree of coagulopathy. As a rule, patients with early HCC, who are eligible for liver transplantation, still have rather low MELD scores, which would give them only low priority for organ allocation. To circumvent this shortcoming of the MELD-based allocation system and to ensure a fairer organ allocation, Eurotransplant accepts the diagnosis of HCC within the Milan criteria as a so-called standard exemption, and the patient receives additional points on top of his so-called lab-MELD score. More points are added after each 3-month waiting period to adjust the patient's total MELD score to the steadily increasing risk of tumour spread and to accelerate organ allocation.

Most transplant centres have adopted the supplementary strategy of treating liver cancers locally while the patient is on the waiting list. It is recommended to immediately treat patients by transarterial chemoembolisation once the patient has been accepted onto the waiting list. This strategy probably also improves selection of patients for liver transplantation, because those with stable disease after chemoembolisation achieve a greater than 90% five-year survival rate after liver transplant, while only 35% of patients in the group with progressive tumour expansion survive five years after liver transplantation (Otto 2006).

Patients with HCC that is limited to a distinct region of the liver but who are older or have significant co-morbidity for other reasons are candidates for local-ablative procedures. Percutaneous ethanol injection or radiofrequency ablation, at least midterm, achieves equal outcomes to resection and liver transplantation. Five-year survival rates are estimated at 70-80% for nodules less than 3 cm in diameter and at 50% for tumours between 3 and 5 cm in size (Lopez 2006). Radio frequency ablation seems to do a little better than ethanol injection owing to the more favourable rates of local tumour recurrence of 2-18% after 2 years (evidence grade ID). Best outcomes are achieved in patients with Child A liver cirrhosis and tumours <2 cm in size (Sala 2004). A direct head-to-head comparison of the different local-ablative procedures within the same study is still pending.

Adjuvant therapy, in the context of resection, liver transplantation or local-ablative procedures, does seem to offer additional benefits. Thus far, antiviral treatment of hepatitis B with nucleos(t)ide analogues remains the single approved treatment after removal or local destruction of HCC that has been proven an effective therapeutic adjuvant to reduce the risk of tumour recurrence.

B. Palliative therapy in stages BCLC B and C

Palliative treatment remains the only therapeutic option for patients with advanced stages of liver cancer that cannot be controlled by local therapy.

Arterial chemoembolisation is the most frequent intervention offered to patients whose HCC cannot be resected. Usually lipiodol combined with an embolising agent such as gelatine or microspheres is mixed with cytostatic drug and applied to the liver via an intra-arterial catheter. Suitable cytotoxic agents are doxorubicin, mitomycin and cis-platinum, but the optimal combination of drugs and treatment schedules has not been established. In randomised studies demonstrating a benefit of chemoembolisation doxorubicin or cis-platinum were given in 3-4 angiographic sessions per year. Chemoembolisation carries the risk of ischemic damage to the liver, potentially leading to fulminant liver failure. To minimize this risk chemoembolisation should be offered only to patients with good residual hepatic function, who h ave asymptomatic multi-nodular liver cancer without vascular invasion or extrahepatic tumour spread. Vice-versa patients with decompensated liver disease (liver cirrhosis, Child B or C) or imminent hepatic failure should not undergo chemoembolisation.

Taken together, chemoembolisation is currently the only palliative treatment demonstrated to significantly improve patient survival in controlled studies (Llovet 2002). It has been shown to achieve partial responses in 15-55% of patients in tumour progression as well as vascular invasion (evidence grade 1D).

Radiotherapy applying ^{90m}Yttrium-loaded microspheres has recently been developed as a novel alternative palliative treatment of liver cancer with unexpectedly impressive anti-tumoural activity in selected individual cases (Sangro 2006; Jacobs 2007; Salem 2006; Liu 2004). Of note, unlike chemoembolisation, some types of microspheres do not occlude the blood vessels and can be applied irrespective of the presence of portal vein thrombosis. However, the therapeutic potential of ^{90m}Yttrium-loaded microspheres cannot currently be assessed with certainty because these novel procedures have not yet been evaluated in randomized, prospective controlled studies.

Systemic chemotherapy on the other hand has been proven repeatedly not to offer survival benefits, irrespective of whether it is given as a single agent or as part of combination chemotherapy (Llovet 2003). Likewise, anti-hormonal therapy with tamoxifen or octreotide has not provided any improved patient survival when studied under controlled conditions (Gallo 2006; Yuen 2002).

Molecular-targeted therapeutic strategies, based on improved knowledge of intracellular signal transmission and regulation of apoptosis, offer new hope for effective pallia-

tive therapy in liver cancer. Such strategies are targeted at inhibition of growth factors or interruption of signalling pathways that are essential for tumour growth and expansion such as angiogenesis or activation of telomerases. Sorafenib (Nexavar®) is a novel orally available multi-kinase-inhibitor acting on several distinct tyrosine kinases (VEGF-R2, PDGF-R, c-Kit receptor) as well on serine/threonine kinases (b-Raf. P38). Thus, by inhibiting angiogenesis and cellular proliferation, sorafenib can block two of the major signalling pathways pivotally involved in the pathogenesis of HCC development. In a phase III study involving 602 patients, sorafenib (400 mg BID) was well tolerated and associated with improved survival in 44% of patients resulting in 3 months extended survival in treated patients (10.7 months in the sorafenib arm versus 7.9 months in the control arm). Diarrhea, weight loss, hand-foot-syndrome and hypophosphatemia were the most important side effects that occurred significantly more frequently in patients on sorafenib. Thus, sorafenib has become the first systemically acting substance demonstrated to prolong life at the expense of moderate side effects in patients on palliative treatment of liver cancer. Further antagonists which probably block VEGF-R, EGF-R, ErbB2, Akt/mTor or Wnt/b-catenin signal transmission pathways are still awaited and are currently under evaluation in phase II studies. Figure 2 gives a summary and succinct overview of stage-adapted therapy for hepatocellular carcinoma.

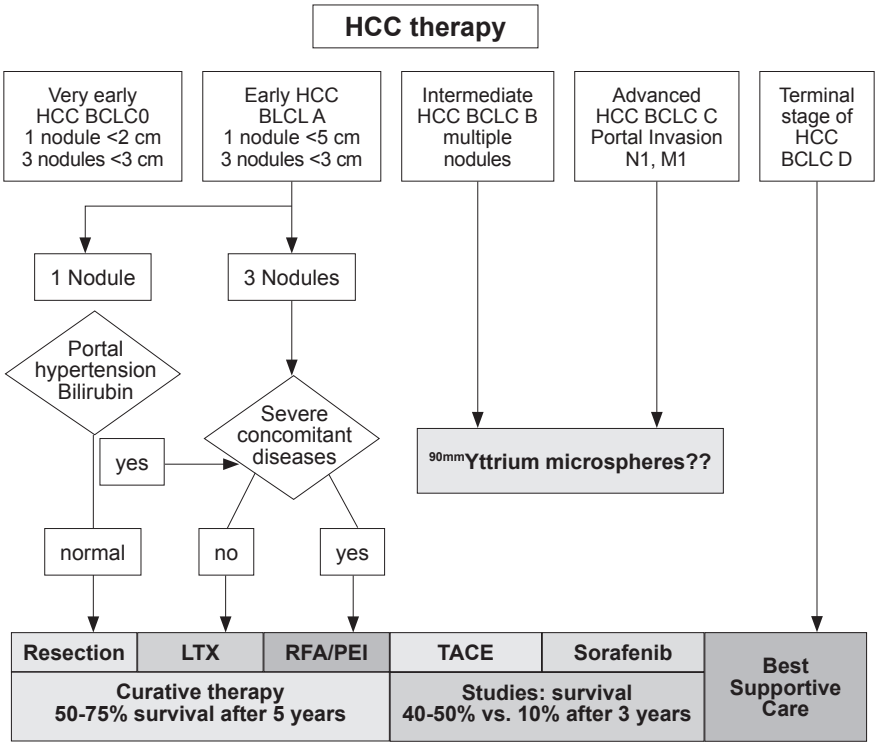


Figure 2. Overview of stage-adapted liver cancer therapy in relationship to BLCL criteria.

Prophylaxis of liver cancer

Despite conspicuous progress concerning liver cancer diagnosis and therapy, prognosis of HCC has not improved very much over time. Thus, prophylactic measures are of pivotal importance. Vaccination against HBV, as is now recommended by many national vaccination councils, has been proven in Taiwan to markedly reduce HBV infection rates along with the incidence of HCC as a complication of chronic hepatitis B in later life (Lok 2004).

Patients with chronic HBV and patients with chronic hepatitis C should be offered antiviral therapy as effective secondary prophylaxis of HCC. Both HBe-antigen positive (van Zonneveld 2004) and HBe-antigen negative patients with chronic hepatitis B show reduced incidence rates of HCC (Papatheoridis 2001; Brunetto 2002; Lampertico 2003) when successfully treated with interferon. Likewise, antiviral therapy with nucleo(t)side analogues has been demonstrated to reduce the risk of HCC in patients with chronic hepatitis B (Liaw 2005) and several meta-analyses confirm that successful interferon therapy leads to a reduced risk of HCC in chronic hepatitis (Camma 2001; Paptheoridis 2001a; Veldt 2004). Nevertheless, patients who have reached the stage of cirrhosis prior to starting antiviral therapy should be maintained on close HCC surveillance programmes, since the risk of developing liver cancer remains high in this subgroup of patients even after sustained virologic elimination is achieved (Yu 2006). Therapeutic management of additional risk factors such as obesity and poorly controlled diabetes mellitus provide additional chances for prophylactic measures to reduce the risk of HCC development. Finally, consumption of two or more cups of coffee per day seems to reduce the risk of liver cancer by 40-50% in patients with chronic viral hepatitis (Gelatti 2005; Bravi 2007; Larsson 2007; Wakai 2007).

References

Bralet MP, Regimbeau JM, Pineau P, Dubois S, Loas G, Degos F, et al. Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases. Hepatology 2000; 32: 200-4.

Bravi F, Bosetti C, Tavani A, Bagnardi V, Gallus S, Negri E, Franceschi S, La Vecchia C. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. Hepatology 2007; 46: 430-5.

Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Gorin JM, Bonino F. Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study. J Hepatol 2002; 36: 263-70.

Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol 2001; 34: 593-602.

Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003; 348: 1625-38.

Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295: 65-73.

Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 2005; 54: 533-9.

Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002; 155: 323-31.

Gallo C, De Maio E, Di Maio M, Signoriello G, Daniele B, Pignata S, et al. Tamoxifen is not effective in good prognosis patients with hepatocellular carcinoma. BMC Cancer 2006 24;6:196.

Gelatti U, Covolo L, Talamini R, Tagger A, Barbone F, Martelli C, et al. N-Acetyltransferase-2, glutathione S-transferase M1 and T1 genetic polymorphisms, cigarette smoking and hepatocellular carcinoma: a case-control study. Int J Cancer 2005; 115: 301-6.

Gelatti U, Covolo L, Franceschini M, Pirali F, Tagger A, Ribero ML, et al. Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: a case-control study. J Hepatol. 2005; 42:528-34.

Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006; 130: 678-86.

Jakobs TF, Hoffmann RT, Poepperl G, Schmitz A, Lutz J, Koch W, et al. Mid-term results in otherwise treatment refractory primary or secondary liver confined tumours treated with selective internal radiation therapy (SIRT) using (90)Yttrium resin-microspheres. Eur Radiol 2007; 17: 1320-30.

Lampertico P, Del Ninno E, Vigano M, Romeo R, Donato MF, Sablon E, et al. Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003; 37: 756-63.

Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. Gastroenterology 2007; 132: 1740-5.

Liaw YF. Prevention and surveillance of hepatitis B virus-related hepatocellular carcinoma. Semin Liver Dis 2005; 25 Suppl 1: 40-7.

Liu MD, Uaje MB, Al-Ghazi MS, Fields D, Herman J, Kuo JV, et al. Use of Yttrium-90 TheraSphere for the treatment of unresectable hepatocellular carcinoma. Am Surg 2004; 70: 947-53.

Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology 2003; 37: 429-42.

Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Bru C, Rodes J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. Hepatology 1999; 29: 62-7.

Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, et al. Arterial embolisation or chemo-embolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002; 359: 1734-9.

Lok AS. Prevention of hepatitis B virus-related hepatocellular carcinoma. Gastroenterology. 2004; 127(Suppl 1):S303-9.

Lopez P, Villanueva A, Llovet JM, Updated systematic review of randomized controlled trials in hepatocellular carcinoma. 2002-2005. Aliment Pharmacol Ther 2006; 23: 1535-1547.

Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996; 334: 693-9.

Otto G, Herber S, Heise M, Lohse AW, Monch C, Bittinger F, et al. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. Liver Transpl 2006;12:1260-7.

Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. J Hepatol. 2001; 34: 306-13.

Papatheodoridis GV, Papadimitropoulos VC, Hadziyannis SJ. Effect of interferon therapy on the development of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a meta-analysis. Aliment Pharmacol Ther 2001; 15: 689-98.

Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Difference in tumor invasiveness in cirrhotic patients with hepatocellular carcinoma fulfilling the Milan criteria treated by resection and transplantation: impact on long-term survival. Ann Surg 2007;245: 51-8.

Sala M, LLovet JM, Vilana R, Bianchi L, Solé M, Ayusa C, et al. Initial response to percutaneous ablation predicts serviva in patients with hepatocellular carcinoma. Hepatology 2004; 40: 1352-1360.

Salem R, Hunter RD. Yttrium-90 microspheres for the treatment of hepatocellular carcinoma: a review. Int J Radiat Oncol Biol Phys 2006; 66(2 Suppl): S83-8.

Sangro B, Bilbao JI, Boan J, Martinez-Cuesta A, Benito A, Rodriguez J, Panizo A, Gil B, Inarrairaegui M, Herrero I, Quiroga J, Prieto J. Radioembolization using 90Y-resin micro-spheres for patients with advanced hepatocellular carcinoma. Int J Radiat Oncol Biol Phys. 2006; 66: 792-800.

Trinchet J, Beaugrand M from GRETCH. A randomized trial comparing 3-month vs. 6 month screening for HCC by ultrasonography Abstrac tbook International Liver Cancer Association 2007.

van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004; 39: 804-10.

Veldt BJ, Saracco G, Boyer N, Camma C, Bellobuono A, Hopf U, et al. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. Gut 2004; 53: 1504-8.

Wakai K, Kurozawa Y, Shibata A, Fujita Y, Kotani K, Ogimoto I, et al. Liver cancer risk, coffee, and hepatitis C virus infection: a nested case-control study in Japan. Br J Cancer 2007; 97: 426-8.

Yao FY. Should surveillance for hepatocellular carcinoma be increased in patients with cirrhosis and small liver nodules? Nature Clin Pract Gastroenterol Hepatol 2006; 3: 544-545.

Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology 2001; 33:1394-403.

Yu ML, Lin SM, Lee CM, Dai CY, Chang WY, Chen SC, et al. A simple noninvasive index for predicting long-term outcome of chronic hepatitis C after interferon-based therapy. Hepatology 2006;4 4: 1086-97.

Yuen MF, Poon RT, Lai CL, Fan ST, Lo CM, Wong KW, et al. A randomized placebo-controlled study of long-acting octreotide for the treatment of advanced hepatocellular carcinoma. Hepatology 2002; 36: 687-91.

Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol 2004; 130: 417-422.

Part 7

Liver transplantation

Chapter 22: Current concepts in the management of patients before and after liver transplantation

Susanne Beckebaum, Guido Gerken, Vito R Cicinnati

Introduction

The first attempt at heterotopic grafting of a liver in a dog was reported more than 50 years ago (Welch 1955). The first known experimental orthotopic liver transplantation (LT) was reported in 1956 at the University of California (Cannon 1956). In the early sixties, Starzl performed a human-to-human LT in a 3-year-old child with congenital biliary atresia who died intraoperatively (Starzl 1963). The following 2 transplant recipients lived for 22 days and 1 week, respectively (Starzl 1963). Starzl finally transplanted several patients with success in 1967 (Starzl 1968).

With the advances in immunosuppression, surgical techniques, organ preservation and improvements in patient management, LT has become the gold standard in the treatment of advanced chronic liver disease and fulminant hepatic failure and has achieved 1-year and 5-year survival rates of 80-90% and 60-80%, respectively (Seaberg 1999).

This chapter focuses on important issues in the field of transplant hepatology and may provide helpful information to physicians involved in the care of liver transplant recipients. It includes indications for LT, current organ allocation policy, pretransplant evaluation, management while on the waiting list, living donor liver transplantation (LDLT), and management of early and long term complications after LT. Furthermore, it deals with immunosuppressive agents and regimens that have been evaluated in clinical practice and studies.

Timing and indications for liver transplantation

Appropriate selection of candidates and timing of LT is crucial to reduce mortality and improve outcome in LT recipients. A patient is considered too healthy to undergo LT if the expected survival is greater without LT. Therefore, criteria are needed in order to select patients who can most benefit from transplantation and allocate donor organs to the sickest patients first. In 2002, the Organ Procurement and Transplantation Network, along with the United Network of Organ Sharing, developed a new system based on the model for end-stage liver disease (MELD) to prioritize patients on the waiting list. This new system leaves no room for subjective criteria or favoritism, as it is based on a mathematical equation (Table 1). In the Eurotransplant countries, Child Pugh Turcotte score was replaced by the MELD score in December 2006.

In a large study (Merion 2005) investigating the survival benefit of liver transplantation, candidates transplanted with a MELD score <15 had a significantly higher mortality risk as compared to those remaining on the waiting list. Patients with a MELD score of 18 or higher have a significant transplant benefit. The implementation of the new allocation system resulted in reduced registrations and improved transplantation rates without increased mortality rates (Freeman 2008; Freeman 2004).

MELD Score = 0.957 x $\log_e$ (creatinine mg/dL)
0.378 x $\log_e$ (bilirubin mg/dL)
1.120 x $\log_e$ (INR**)
+ 0.643

*Model of End-stage Liver Disease, **International Normalized Ratio

Table 1. Calculation of the MELD* Score.

However, the MELD score does not accurately predict mortality in approximately 15-20% of patients. A potential modification of the MELD allocation system that is currently under investigation is to allocate organs by not only taking into account pretransplant mortality but also posttransplant mortality and donor-related factors. Furthermore, standardization of laboratory assays and incorporation of sodium might improve the MELD score's predictive abilities (Choi 2008).

Candidates for LT must have irreversible acute or chronic end stage liver disease. Hepatitis C virus (HCV)- or alcohol-induced liver disease account for the most common disease indications in adults with liver cirrhosis (http://www.eltr.org) (Figure 1). Other indications include cholestatic liver disorders [primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC)], hepatitis B virus (HBV) infection, autoimmune hepatitis (AIH), inherited metabolic diseases (cystic fibrosis, Wilson's disease, hemochromatosis, alpha-1-antitrypsin deficiency), nonalcoholic steatohepatitis, nonmetastatic hepatocellular carcinoma (HCC), and acute virally-, toxin-, or drug-induced hepatic failure. In children, biliary atresia and metabolic liver diseases are the most common indications.

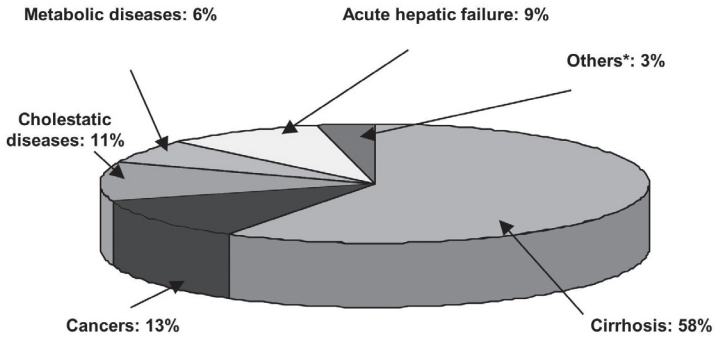


Figure 1. Indications for liver transplantation.
Primary diseases leading to liver transplantion in Europe, 01/1988 - 06/2007 (Data kindly provided from European Liver Transplant Registry, http://www.eltr.org).

Contraindications for LT include active alcohol and drug abuse, extrahepatic malignancies, sepsis, uncontrolled pulmonary hypertension, and coexistent medical disorders such as severe cardiopulmonary condition, technical or anatomical barriers such as thrombosis of the entire portal and superior mesenteric venous system. A history of a previous malignancy must be carefully considered and likelihood of recurrence must be estimated.

A major issue in patients transplanted for alcoholic liver disease is the likelihood of relapse (Neuberger 2007). Stated in the policy is that patients with alcoholic liver disease must be abstinent for at least 6 months before liver transplantation. The Department of Psychosomatic Medicine and Psychotherapy at our university hospital established a group psychotherapy program with the aim of establishing alcohol abstinence and compliance of healthy behavior. The therapy consists of a 6-month program with 18 hours of group sessions. Alcohol concentration in the breath and alcohol metabolites in the urine are measured at every group session. Results suggest that structured cognitive-behavioral group therapy has a beneficial effect on the health behavior of these patients (Erim 2007).

Patient evaluation

Evaluation of a potential transplant candidate is a complex and time-consuming process that requires a multidisciplinary approach. This process must identify extrahepatic diseases that may exclude the patient from transplantation or require treatment before surgical intervention. The protocol for evaluation of potential transplant candidates at our transplant center is shown in Table 2.

- Physical examination
- Diagnostic tests (baseline laboratory testing; serologic, tumor/virologic, and microbiologic screening; autoantibodies; thyroid function tests)
- Ultrasonography with Doppler
- Abdominal MRI or CT scan
- Chest X-rays
- Electrocardiogram (ECG), stress ECG, 2-dimensional echocardiography (further cardiological screening if abnormal or risk factors are present)
- Upper and lower endoscopy
- Pulmonary function testing
- Mamography (females >35 years)
- Physician consultations (anaesthesiologist, gynecologist, urologist, cardiologist, neurologist, dentist, ENT physician)
- A meticulous psychosocial case review (psychiatrist or psychologist consultation)

Table 2. Evaluation protocol for potential transplant candidates.

Pre-transplantation management issues

In cases of recurrent variceal hemorrhage despite prior interventional endoscopic therapy (and non-selective beta-blockade) or refractory ascites, transjugular intrahepatic portosystemic shunts (TIPS) have been used as an approach to lower portal pressure and as bridging therapy for transplant candidates. The identification of predisposing factors and the application of lactulose, nonabsorbed antibiotics and protein-restricted diets remain essential for prophylaxis and management of hepatic encephalopathy (HE).

Hepatorenal syndrome (HRS) represents a complication of end-stage liver disease and is classified into type 1 HRS characterized by a rapid impairment of renal function with a poor prognosis; type 2 HRS is a moderate steady renal impairment (Wong 2008). Vasoconstrictors including commonly used terlipressin in combination with volume expansion, have been shown to be effective and serve as bridging therapy to LT with effective restoration of arterial blood flow in up to 75% of patients. TIPS has also demonstrated success in up to 50% of patients. Extracorporeal liver support systems based on exchange or detoxification of albumin have been successfully employed in a number of patients. After wait-listing, laboratory values must be updated according to the recertification schedule shown in Table 3, otherwise transplant candidates will automatically revert to the previous lower MELD score.

Special attention regarding specific, disease-related therapy prior to surgery should be given to transplant candidates undergoing LT for HCC or virally-related liver diseases.

Score	Recertification	Lab values
≥25	every 7 days	≤48 hours old
24-19	every 30 days	≤7 days old
18-11	every 90 days	≤14 days old
≤10	every year	≤30 days old

Table 3. Recertification schedule of MELD data.

Waiting list monitoring of hepatitis B liver transplant candidates

The goal of antiviral therapy in HBV patients on the waiting list is to achieve viral suppression to undetectable HBV DNA levels prior to transplantation (Figure 2) (Cornberg 2008; Cornberg 2007). Several studies have demonstrated clinical benefits under viral suppression in patients with decompensated cirrhosis as reflected by a decrease in CPT score, improvement of liver values and resolution of clinical complications (Kapoor 2000; Schiff 2007; Villeneuve 2000; Yao 2001; Nikolaidis 2005).

A major concern of long-term lamivudine (LAM) therapy is the emergence of mutations in the YMDD motif of the DNA polymerase (Tillmann 2007; Beckebaum 2008a; Ono 2001; Mutimer 2000; Seehofer 2001). LAM has been proposed to be downgraded from first-line to second-line therapy (Tan 2007). The use of a more potent nucleos(t)ide analogue (entecavir or tenofovir disoproxil fumarate) should be favored.

An open-label study was conducted in HBV patients with LAM resistance who were wait-listed (n=226) or post-LT (n=241) (Schiff 2007). Add-on therapy with ADV was performed at some time in almost all patients; however, the exact time period of combined treatment was not clearly documented. They found that serum HBV DNA became undetectable in wait-listed patients in only two-thirds of patients at week 96, indicating the need for a more potent antiviral therapy in non-responders.

In patients who have LAM resistance, TDF may be a better option than ADV as rescue therapy in LAM resistance (van Bommel 2004; Neff 2004; Del Poggio 2007). ETV has a high resistance barrier as multiple mutations are necessary for development of resistance and, for nucleos(t)ide analogue-naïve patients, it is highly efficacious in therapy-naïve pre-transplant candidates (Colonno 2006; Chang 2006).

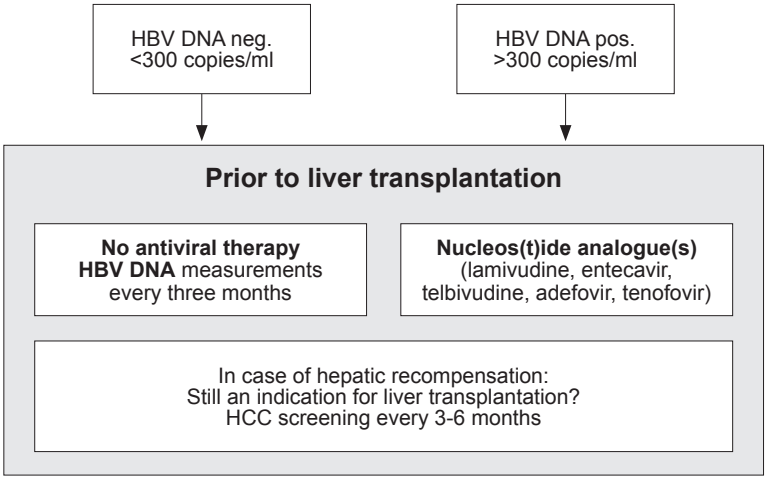


Figure 2. Management of HBV patients prior to liver transplantation.
In all viremic (>300 copies/ml) patients awaiting liver transplantation for HBV-related liver damage, efficient antiviral therapy is required. Suppression of HBV DNA may lead to clinical stabilization resulting in removal from the waiting list or in a delay in the need for liver transplantation. The German Guidelines propose monitoring for HBV DNA at least every three months (Cornberg 2007). Neg., negative, pos., positive, HCC, hepatocellular carcinoma

Waiting list monitoring and treatment of hepatitis C liver transplant candidates

The number of studies investigating the tolerability and efficacy of antiviral therapy in HCV patients before LT is limited (Crippin 2002; Iacobellis 2007; Everson 2005; Triantos 2005). Wait-listed patients who have viral response under antiviral therapy have a lower reinfection rate and better outcome after LT (Thomas 2003; Picciotto 2007). Thus, there is an indication for therapy with pegylated interferon (PEG-IFN) plus ribavirin in patients with compensated HCV cirrhosis on the waiting list. Antiviral therapy in dec-

ompensated cirrhosis and with MELD score ≥18 should be restricted to exceptional cases and monitored by a transplant center. Although pretransplantation IFN-alpha therapy has shown to suppress HCV in some patients, adverse events associated with therapy are frequent (Crippin 2002). These side-effects and the need for dose reduction or withdrawal often prevent efficient eradication of the virus in HCV patients awaiting LT.

Adjunctive treatment and staging of hepatocellular carcinoma transplant candidates

Although LT has been recognized as the most effective means of treating HCC patients, the success has been limited by long waiting times for transplantation, with disease progression or death while on the waiting list. Waiting-list drop-out rates may be reduced by the application of bridging therapies such as transarterial chemoembolization or radiofrequency ablation (Roayaie 2007).

Under MELD allocation, patients must meet the Milan criteria (one tumor £5 cm in diameter or up to three tumors, none >3 cm) to qualify for exceptional HCC waiting list consideration. Diagnosis of HCC is confirmed if the following criteria are given according to the German guidelines for organ transplantation (Bundesärztekammer 2008): (1) liver biopsy-proven or (2) AFP >400 ng/ml and hypervascular liver lesion detectable in one imaging technique (magnetic resonance imaging (MRI), spiral computed tomography (CT), angiography) or (3) hypervascular liver lesion detectable in 2 different imaging techniques. Patients may be registered at a MELD score equivalent to a 15% probability of pre-transplant death within 3 months. Patients will receive additional MELD points equivalent to a 10% increase in pre-transplant mortality to be assigned every 3 months until these patients receive a transplant or become unsuitable for LT due to progression of their HCC. The listing center must enter an updated MELD score exception application in order to receive additional MELD points.

Pre-listing the patient should undergo a thorough assessment to rule out extrahepatic spread and/or vascular invasion. The assessment should include CT scan or MRI of the abdomen and chest and a bone scan. Trimonthly routine follow-up examinations (MRI or CT scan) of wait-listed HCC patients for early detection of disease progression are required.

Accurate discrimination of HCC patients with good and poor prognosis by appropriate criteria (genomic or molecular strategies) is highly warranted and still in the exploratory phases (Marsh 2003, Finkelstein 2003). In patients with alcohol-related liver disease and HCC, a multidisciplinary approach and thorough work-up of both the alcoholic and oncologic problem is mandatory (Sotiropoulos 2008a).

Living donor liver transplantation: indications, donor evaluation, and outcome

LDLT was introduced in 1989 with the first successful series of pediatric patients (Broelsch 1991). Adult-to-adult living donor liver transplantation (ALDLT) was first performed in Asian countries where cadaveric organ donation is rarely practiced (Sugawara 1999; Kawasaki 1998). LDLT peaked in the US in 2001 (Qiu 2005). While the number of LDLT is the US has declined the number in Asia has continued to increase.

The evaluation of donors is a cost-effective although time-consuming process. Clinical examinations, imaging studies, special examinations, biochemical parameters, and psychosocial evaluation prior to donation varies from center to center and has been described elsewhere (Valentin-Gamazo 2004). Using Germany as an example, the expenses for evaluation, hospital admission, surgical procedure, and follow-up examinations of donors are paid by the recipient's insurance. Due to the increasing number of potential candidates and the more stringent selection criteria, rejection of potential donors has been reported in about 69-86% of cases (Valentin-Gamazo 2004; Pascher 2002). The advantages of LDLT include the feasibilty of performing the operation when medically indicated and the short duration of cold ischemia time.

The surgical procedures for LDLT are more technically challenging than those for cadaver liver transplantation. In the recipient operation, bile duct reconstruction has proven to be the most challenging part of the procedure with biliary complications ranging from 15% to 60% (Sugawara 2005).

Regarding the donor outcome, morbidity rates vary considerably in literature (Patel 2007; Beavers 2002). Possible complications include wound infection, pulmonary problems, vascular thrombosis with biliary leaks, strictures, and incisional hernia. Biliary complications are the most common postoperative complication in LDLT and occur in up to 7% of donors (Perkins 2008; Sugawara 2005). Liver regeneration can be documented with imaging studies and confirmed by normalization of bilirubin, liver enzymes, and synthesis parameters. LDLT should be performed only by established transplant centers with appropriate medical expertise.

Perioperative complications

Despite advances in organ preservation and technical procedures, postoperative complications due to preservation/reperfusion injury have not markedly decreased over the past several years. Perioperative ischemic injuries include hepatocellular damage during cold ischemia time from prolonged preservation and warm ischemia during implantation of the allograft. Typical histological features of preservation and reperfusion injury include centrilobular pallor and ballooning degeneration of hepatocytes. Bile duct cells are more sensitive to reperfusion injury than hepatocytes (Washington 2005) resulting in increased levels of bilirubin, gamma-glutamyl transpeptidase (gGT) and alkaline phosphatase (AP). Vascular complications such as hepatic artery thrombosis (HAT) occur in 1.6-4% of patients. Thus, Doppler exams of the hepatic artery and portal vein are frequently performed in the early postoperative setting. HAT in the early postoperative period can be managed with thrombectomy. Late HAT is managed by interventional endoscopic retrograde cholangiography (ERC) in those cases of bile duct strictures but requires retransplantation in the majority of patients due to large bile duct injuries. Early portal vein thrombosis is rare (<1%) but may lead to graft loss if not revascularized.

Primary non-functioning graft (PNFG) may be clinically obvious immediately after revascularization of the allograft. Early signs of liver dysfunction include prolonged coagulation times, elevated liver enzymes (transaminases, cholestasis parameter) without a downward trend, rising lactate, and hypoglycemic episodes. PNFG is a critical situation and requires immediate retransplantation.

In the first month after LT, mostly bacterial as well as fungal infections are seen. Correct differentiation between colonization and true infection is important. Common bacterial pathogens include gram-negative organisms (enterobacteriaceae, pseudomonas aeruginosa) and gram-positive organisms (staphylococcus aureus, enterococcus fecalis, enterococcus faecium). Of fungal infections, greater than 90% are nosocomial candidal infections of wounds, intra-abdominal organs, or intravascular catheters (Singh 2003). Aspergillus accounts for approximately 15% of all fungal infections. Sporadic fungal infections may be caused by cryptococcus, mucor, trichosporon or fusarium (Walsh 1999). Reported risk factors for infections include advanced age, accompanying renal insufficiency, malnutrition, and a high number of perioperative blood product transfusions (Patel 1996).

The clinical symptoms of acute cellular rejection are unspecific, may not be apparent or may manifest as fever, right upper quadrant pain, and malaise. A liver biopsy is indispensable for confirming the diagnosis of acute rejection. High dose corticosteroids (3 days of 500-1000 mg methylprednisolone) are the first-line treatment for acute rejection.

Long-term complications after liver transplantation

Due to excellent results in the short-term outcome after LT, attention has shifted to reducing long-term complications. Management issues for the long-term include opportunistic infections, chronic ductopenic rejection, side effects due to immunosuppression including cardiovascular complications, *de novo* malignancies, biliary complications, osteoporosis and disease recurrence.

Opportunistic Infections

Opportunistic infections in the medium- and long-term after LT are primarily viral and fungal in origin. Opportunistic bacterial infections are uncommon after 6 months in patients receiving stable and reduced maintenance doses of immunosuppression with good graft function. Cytomegalovirus (CMV) is a frequent cause of infection in the posttransplant setting (Figure 3). Diagnostic assays, such as CMV pp65 Ag, and quantitative PCR have demonstrated similar efficacy for the diagnosis and monitoring of CMV infection in liver transplant recipients (Martin-Davila 2005). Valganciclovir is an oral prodrug of ganciclovir and has various advantages over the original formulation (10 times higher bioavailability, lower application frequency, lower occurrence of resistance) (Lake 2003). A recent controlled clinical trial demonstrated that valganciclovir is as effective and safe as intravenouos (IV) ganciclovir for the prophylaxis of CMV disease in solid organ (including liver) transplant recipients (Paya 2004). Time-to-onset of CMV disease and to viremia were delayed with valganciclovir; rates of acute allograft rejection were lower in the valganciclovir-treated group.

A high Epstein-Barr virus (EBV) infection viral load and a high level of immunosuppression are reported as risk factors for posttransplant lymphoproliferative disease (PTLD, Smets 2002). The clinical presentation varies and may manifest as an impaired general condition with fatigue, weight loss, tonsilitis, lymph node enlargement, and gastrointestinal symptoms. PTLD is more frequent in children after LT, but still represents 15% of tumors in adults. Lowering immunosuppression is the current method to prevent PTLD in patients with a high viral load. Treatment with anti-CD20 monoclonal antibodies (rituximab) has been found to effectively suppress viral replication

in recipients of hematopoietic stem cell transplantation (Opelz 2007). A recently published study in LT children with EBV infection showed that long-term valganciclovir therapy achieved undetectable EBV DNA in 47.6 % of patients (Hierro 2008).

The clinical manifestation of infection with human herpes virus (HHV)-6 may vary between asymptomatic infection to severe symptoms (Lautenschlager 1998). Furthermore, there is a potential role of HHV-6 and HHV-7 as copathogens in the direct and indirect illnesses caused by CMV. Oral reactivation of herpes simplex virus after liver transplantation is common. Development of varicella-zoster virus after LT is often related to intense immunosuppressive therapy and its therapy does not differ from the non-transplant setting.

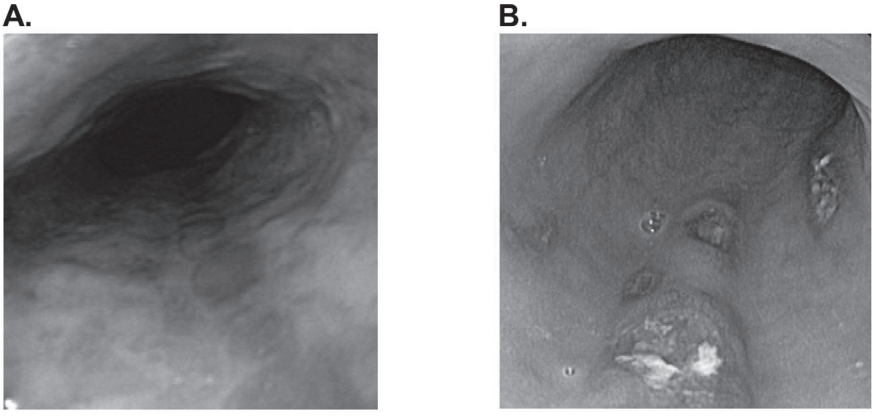


Figure 3. Cytomegalovirus infection of the upper gastrointestinal tract.
[A.] Patient complaining of dysphagia and epigastric discomfort with multiple longitudinal esophageal ulcers seen at upper endoscopy. [B.] Endoscopic findings of deep esophageal ulcerations with fibrinoid necrosis in another immunocompromised patient. In both cases, lesions were caused by cytomegalovirus infection. Diagnosis depends on a positive mucosal biopsy which should include specimens from the ulcer margins and ulcer base. Haematoxylin and Eosin staining typically reveals “owl’s-eye” cytoplasmic and intranuclear inclusion bodies.

Chronic ductopenic rejection

Advances in immunosuppressive regimens have greatly reduced the incidence of chronic ductopenic rejection and allograft failure. Chronic rejection begins within weeks to months or years after LT and affects about 4% to 8% of patients (Neuberger 1999). Risk factors for chronic rejection include alloimmune immunologic injury and nonimmunologic factors such as older donor age, prolonged cold ischemia time, and donor atherosclerosis. The most widely recognized manifestation of chronic rejection is obliterative arteriopathy (Demetris 1997). Chronic rejection may appear indolently and might only become apparent as liver test injury abnomalities (gGT, AP, bilirubin, transaminases). The diagnosis needs to be confirmed by histopathologic examination. Switching the baseline immunosuppression from CSA to TAC and initiating myco-

phenolate mofetil (MMF) rescue therapy represent a treatment option in these patients (Daly 2002). A recent study investigating the efficacy and safety of anti-interleukin (IL)-2 receptor antibodies (daclizumab and basiliximab) for steroid-resistant rejection revealed a poor histologic response in chronic rejection but successful resolution (75%) in patients with acute cellular rejection (Orr 2005).

CNI-induced nephrotoxicity and alternative immunosuppressive protocols

Despite the introduction of new immunosuppressive agents (Table 4), calcineurin inhibitors (CNI) remain the key drugs of most immunosuppressive regimens. Both cyclosporine A (CSA) and tacrolimus (TAC) inhibit the calcineurin-calmodulin complex and therefore IL-2 production. Renal failure, mainly due to CNI nephrotoxicity, is the most common complication following orthotopic LT. The incidence of chronic renal dysfunction has been reported in up to 70% of patients (Afonso 2008; Ziolkowski 2003). End stage renal disease has been described to occur in 18% of patients during a follow-up of 13 years after LT (Gonwa 2001).

Immunosuppressant Class	Immunosuppressive Agent
Corticosteroids	Prednisone, prednisolone, methylprednisolone
Calcineurin inhibitors	Cyclosporin A, tacrolimus
Antimetabolites	Mycophenolate mofetil, azathioprine
mTOR Inhibitors	Sirolimus, everolimus
Polyclonal antibodies	Antithymocyte globulin (ATG)
Monoclonal anti-CD3 antibodies	Muromonab-CD3 (OKT3)
Chimeric monoclonal antibodies	Anti-IL-2 inhibitors (basiliximab)
Monoclonal anti-CD52 antibodies	Alemtuzumab (campath-1H)

Table 4. Clinically used immunosuppressive agents in liver transplantation.

In LT patients with CNI-induced nephrotoxicity, a complete replacement of CNI with conversion to MMF has shown conflicting results with respect to occurrence of rejection ranging between 0% and 60% (Creput 2007; Moreno 2003; Stewart 2001; Moreno 2004; Schlitt 2001). MMF inhibits inosine monophosphate dehydrogenase, a critical enzyme in the de novo pathway of purine synthesis. Results from previous studies with immunosuppressive regimens including MMF and minimal CNI treatment suggest a significant improvement in renal function in this patient group (Cicinnati 2007a; Beckebaum 2004a; Raimondo 2003; Cantarovich 2003; Garcia 2003).

Sirolimus (SRL) and everolimus (EVL) constitute a new class of compounds called the mammalian target of rapamycin (mTOR) inhibitors, which exhibit immunosuppressive and antiproliferative effects.

Sirolimus (SRL) is a macrolide isolated from streptomyces hygroscopius. It binds to a highly conserved cellular protein, FKBP12, and to the rapamycin/FKBP12 complex targets, and it inactivates mTOR, which is considered a master switch for cell cycle progression (Luan 2003). Reported side-effects of SRL include increased incidence of wound infection and dehiscence, HAT, hyperlipidemia, thrombocytopenia, leucopenia, and anemia. The antifibrotic effect of SRL may provide an explanation for impaired wound healing (Watson 1999). SRL is currently being investigated in clinical studies as an alternative or complementary agent to CNI (Sanchez 2005; Neff 2003; Trotter 2003). There are conflicting results with respect to renal improvement upon switching to mTOR inhibitor therapy with concomitant reduction/elimination of CNI. Further trials will determine whether earlier conversion to mTOR inhibitors enables prevention of CNI-related renal dysfunction.

Individual studies have demonstrated a benefit of SRL/MMF combination therapy (Kniepeiss 2003; Maheshwari 2006). Recently, a satisfactory outcome and potential survival benefit was reported in HCC patients with SRL-based immunosuppression (Kneteman 2004; Zimmerman 2008; Toso 2007). However, only results from randomized controlled studies in the future will show whether SRL can improve recurrence-free survival in this patient group.

A second mTOR inhibitor, EVL, may exhibit improved bioavailability as compared to SRL. The half-life of SRL and EVL is 62h and approximately 30h, respectively, in stable kidney recipients treated with CSA. The correlation between AUC and dose is excellent for both drugs, but the pharmacokinetics of EVL are less influenced by CSA. A recently published study demonstrated that everolimus in combination with oral CSA had an acceptable safety and tolerability profile (Levy 2006). However, the side effects were more frequent in the ERL as compared to the ERL-free control group. Of note, there was no difference in the incidence of thrombocytopenia or leukopenia between the groups.

Other side effects of CNI

Beside potential nephrotoxicity, CNI therapy is associated with side-effects which include cardiovascular complications, tremor, headache, electrolyte abnormalities, hyperuricemia, hepatotoxicity, and gastrointestinal symptoms. Neurotoxicity, including tremor, paresthesia, muscle weakness, and seizures, more often occurs in TAC-treated patients; gingival hyperplasia and hirsutism are associated with CSA treatment.

Cardiovascular side-effects due to CNI and steroids include hyperlipidemia, arterial hypertension, and diabetes (Beckebaum 2004b). Treatment of hyperlipidemia with reductase inhibitors (statins) is safe and well tolerated.

There is ongoing discussion of steroid avoidance due to dyslipidemia, osteoporosis, development of cataracts, weight gain, hypertension, and a deleterious impact on glucose control. The Ochsner Clinic investigated the efficacy of polyclonal rabbit anti-thymocyte globulin (RATG) induction followed by TAC monotherapy in a randomized, prospective trial (Eason 2003). Compared to the control group with steroids, the RATG plus TAC group had a lower incidence of posttransplant diabetes, CMV infection, and steroid-requiring rejections. Other research groups have reported encouraging findings with steroid-free protocols including basiliximab induction therapy (Filipponi 2004; Neuhaus 2002).

The prevalence of new-onset diabetes mellitus after LT has been reported to occur in 9-21% of patients (John 2002; Konrad 2000). The prevalence of posttransplant diabetes is even higher if cofactors such as hepatitis C are present. In various studies, the diabetogenic potential has been reported to be higher in patients receiving TAC than in those receiving CSA. In contrast, CSA has a more pronounced effect on lipid levels. CSA can act by modulating the activity of the LDL receptor or by inhibiting the bile acid 26-hydroxylase that induces bile acid synthesis from cholesterol.

De novo malignancies

Malignancies occur in 4-16% of transplant patients depending on the length of follow-up, characteristics of the transplant population, choice of immunosuppression and era in which LT was performed (Fung 2001). The highest risks in the transplant setting are nonmelanoma skin cancers, mainly as squamous cell carcinoma and basal cell carcinoma (Figure 4), which range from 6-70% of the tumors observed followed by PTLD (4.3-30%) (Yao 2006; Vallejo 2005).

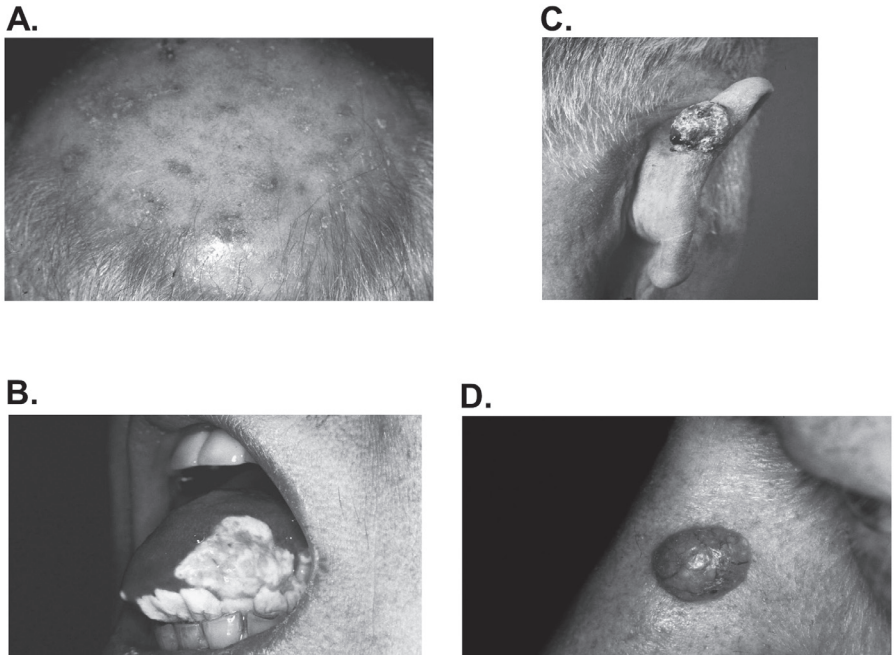


Figure 4. Nonmelanoma skin scancers and liver transplantation.

Organ transplant recipients have an increased risk of development of nonmelanoma skin cancers as compared to the non-transplant setting. Premalignant lesions such as actinic keratoses [A.] are predominantly located on sun-exposed areas. Squamous cell carcinoma [B.,C.] is the most frequent skin cancer after liver transplantion followed by basal

cell carcinoma [D.] (Photographs kindly provided by Dr. Hillen, Transplant Dermatology Outpatient Unit, Department of Dermatology, University Hospital Essen, Germany).

Premaligant lesions such as actinic keratoses are mostly located on sun-exposed areas. Squamous cell carcinoma and basal cell carcinoma are increased by factors of ~100 and 10, respectively, in organ transplant recipients as compared to the immunocompetent population (Ulrich 2008). An annual routine dermatological follow-up exam, limitation of sun exposure and sun protective measures including sunscreens are highly recommended for transplant patients.

A trend has been recently reported toward an increased incidence of advanced colon polyps and colon carcinoma in immunosuppressed patients after LT. However, larger studies are needed to determine whether posttransplant colon cancer surveillance should be performed more frequently than in the non-transplant setting (Rudraraju 2008). Recent studies reported a significantly higher incidence of aerodigestive cancer including lung cancer among patients who underwent LT for alcohol-related liver disease (Vallejo 2005; Jimenez 2005). SRL exerts antiangiogenic activities that are linked to a decrease in production of vascular endothelial growth factor (VEGF) and to a markedly inhibited response of vascular endothelial cells to stimulation by VEGF (Guba 2002). Furthermore, the ability of SRL to increase the expression of E-cadherin suggests a candidate mechanism for its ability to block regional tumor growth and for inhibiting metastatic progression. Therefore, not only patients transplanted for HCC but also those with de novo malignancies after LT should be given special consideration for SRL-based immunosuppressive regimens.

Biliary complications

Biliary strictures are one of the most common complications after LT, with a reported incidence of 5.8-34% (Graziadei 2006). Risk factors contributing to biliary strictures include ischemia/reperfusion injuries, prolonged warm and cold ischemia times, bacterial and viral infections, especially CMV, age cross match, acute and chronic rejection, a small-for-size graft, HAT, ABO blood incompatibility, hepatotoxic drugs, and recurrent viral or cholestatic disease. The spectrum of biliary complications has evolved over recent years, due to the introduction of reduced size, split liver and LDLT.

Endoscopic retrograde cholangiography (ERC) or percutaneous transhepatic cholangiography (PTC) have often been used as the primary approach, leaving surgical intervention for those who are nonresponsive to endoscopic interventions or who have diffuse intrahepatic bile duct dammage. Novel radiological methods such as magnetic resonance cholangiopancreaticography (MRCP) have been introduced as an additional diagnostic tool for biliary complications.

Biliary leaks generally occur as an early posttransplant complication while strictures may develop postoperatively over months and years. The long-term efficacy and safety of endoscopic techniques have been evaluated in various transplant centers (Qin 2006; Zoepf 2006; Pascher 2005). Nonanastomotic strictures are commonly associated with a less favorable response to interventional endoscopic therapy, in comparison to anastomosis stenosis (Figure 5). An Austrian group found anastomotic strictures in 12.6% of patients transplanted between October 1992 and December 2003 and nonanastomic strictures in 3.7% during a mean follow-up of 53.7 months after

LT (Graziadei 2006). Interventional endoscopic procedures were effective in 77% of patients with anastomosis stenosis; whereas treatment of nonanastomotic strictures showed long-term effectiveness in 63% of patients. A surgical approach was required in 7.4% of transplant recipients.

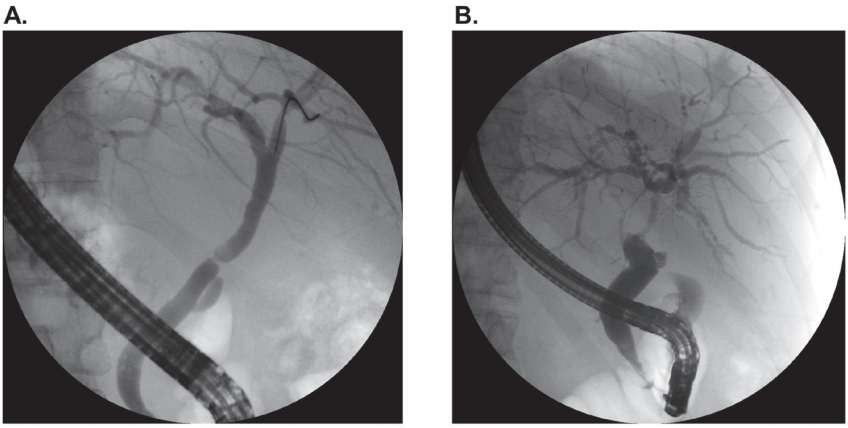


Figure 5. Biliary tract complications after liver transplantation.
[A.] Endoscopic retrograde cholangiography (ERC) showing posttransplant short filiform anastomotic biliary stricture in a 46-year-old patient transplanted for HCV and alcohol-related cirrhosis 6 months ago. Therapy sessions include dilatation and an increasing number of bile duct endoprostheses at short intervals of every 2-3 months. Prior to endoscopic therapy an endoscopic spinkterotyoms is performed. [B.] ERC of a 41-year-old patient transplanted for HCV diagnosed with ischemic-type biliary lesions (type 3) with long nonanastomotic stricture extending proximally from the site of the anastomosis and strictures throughout the entire liver.

At our center, results from 75 transplanted patients undergoing ERC for suspected anastomic strictures were retrospectively analyzed (Zoepf 2006). Balloon dilatation alone and combined dilatation and endoprotheses placement was efficacious in 89% and 87% of cases respectively, but recurrence occurred in 62% and 31% of cases respectively. We therefore use dilatation plus stenting with endoscopic reassessment in anstomotic strictures. Repeated ERC sessions are performed with increasing endoprothesis diameter in trimonthly time intervals and double or triple parallel stenting in selected cases. Up to 75% of patients were stent-free after 18 months of endoscopic intervention (Tung 1999).

Medical treatment for bile duct strictures consists of UDCA and additional antibiotic treatment in stricture-induced cholangitis. Complications related to bilioenteric anastomosis require PTC or surgical intervention. Ampullary and sphincter of Oddi dysfunction occur in up to 5% of transplanted patients with typical signs of biliopancreatic reflux of contrast medium during ERC. Various centers have reported on endoscopic sphincterotomy or transpapillary stenting as endoscopic treatment (Clavien 1995; Douzdjian 1994). In patients with biliary stones, endoscopic sphincterotomy and stone extraction have been reported to be successful in nearly all patients (Tung 1999).

Metabolic bone disease

Metabolic bone disease is a common cause of morbidity after LT. Liver cirrhosis and therapy with corticosteroids are risk factors for the development of osteoporosis. Screening with bone densitometry should therefore begin prior to LT. Patients with reduced bone mineral density should be administered calcium and vitamin D. In addition, bisphosphonates are currently the most promising approach for the management of transplantation osteoporosis (Ebeling 2007).

Recurrent diseases after liver transplantation

Disease recurrence may occur in patients transplanted for viral hepatitis, tumor disease, autoimmune or cholestatic or alcohol-related liver diseases. With universal recurrence of HCV in all replicative patients, hepatitis C continues to pose one of the greatest challenges for preventing disease progression in the allograft.

Recurrence of hepatitis B in the allograft

Combined use of hepatitis B immune globulin (HBIG) and nucleos(t)ide analogues has emerged as the treatment of choice in transplant HBV recipients (Figure 6) (Han 2003; Yan 2006; Ferretti 2004; Beckebaum 2004c; Rosenau 2001; Yao 1999; Marzano 2005; McCaughan 1999) and its efficacy has been investigated extensively.

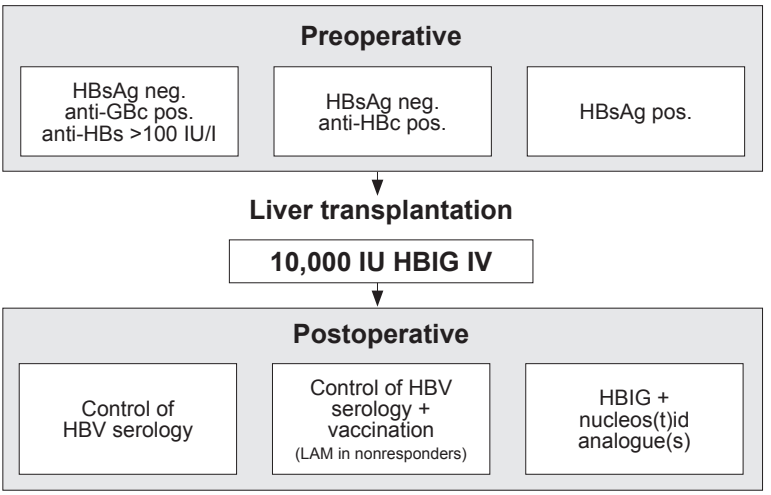


Figure 6. Prophylaxis of HBV recurrence after liver transplantation.
Combined use of nucleos(t)ide analogue(s) and hepatitis B immunoglobulin (HBIG) in patients who are hepatitis B surface antigen (HBsAg)-positive prior to liver transplantation is the current gold standard for prophylaxis of HBV reinfection after liver transplantation. Those who are anti-hepatitis B core (anti-HBc)-positive and without detectable anti-hepatitis B surface (anti-HBs) titers or anti-HBs titers <100 IU/L should be vaccinated according to the German Guidelines (Cornberg 2007). In cases of no or little response (anti-HBs <100 IU/L) to vaccination, lamivudine (LAM) monotherapy can be initiated. In patients who have protective anti-HBs titers of >100 IU/L, antiviral therapy is not necessary but long term monitoring of HBV serology including anti-HBs titer is required. Neg., negative; pos., positive.

Recurrence rates differ considerably among various studies using combined prophylactic therapy as most of these studies are small, with varying proportions of included patients with active viral replication at LT and varying follow-up periods after LT. Furthermore there is a high variability (dose, duration and method of HBIG administration) in the prophylactic protocols. HBIG prophylaxis is in the range of 1 euro per unit. Long-term HBIG prophylaxis at our LT center consists of 2000 international units (IU)/d (IV) for 5 consecutive days in individual frequencies (mostly every 2-3 months) to maintain trough anti-HBs levels at or above 100 IU/L.Subcutaneous (sc) HBIG application has various advantages over intramuscular (im) and IV administration (Beckebaum 2008b). It is better tolerated and patients can perform injections in a home setting, thus reducing time-consuming physician consultations. Results are pending from a current ongoing open, prospective, randomised parallel study conducted by the Berlin Transplant Group and our transplant center investigating the efficacy and safety of sc-administered human HBIG (BT088) in HBV-transplanted recipients.

Economic issues have led to a controversial discussion of whether indefinite passive immunization is necessary and if nucleos(t)ide analogue therapy is sufficient for antiviral prophylaxis (Naoumov 2001; Buti 2007; Gane 2007; Angus 2007; Neff 2007; Lo 2005; Wong 2007; Nath 2006; Yoshida 2007). Studies have described unacceptable 2-3 year resistance rates of about 30-40% under LAM monotherapy and with no initial phase of HBIG therapy (Table 5) (Marzano 2001; Jiao 2007; Zheng 2006) - this monoprophylactic regimen is not sufficient except in patients who are seropositive only for anti-HBc.

A small cohort of non-HBV replicating patients who were converted from HBIG plus LAM (150 mg/d) to ADV (5 mg/d)/LAM (150 mg/d) therapy after a mean post-LT period of 6.5 months was retrospectively investigated (Neff 2007) (Table 5). The mean length of follow-up since therapy conversion is 21 months. They found that none of the patients showed an increase in transaminases while on dual nucleos(t)ide analogue therapy. Although the authors mentioned that HBV serologic testing was performed, there were no results given after the therapy switch.

A prospective, open-label, multicenter study on the safety and efficacy of combined LAM/ADV therapy in HBsAg-positive LT recipients was conducted (Gane 2007) (Table 5). Patients with clinical and virologic LAM resistance were excluded. Combined nucleos(t)ide analogue treatment started upon wait-listing. The median duration of antiviral therapy prior to LT was 3.6 months. HBIG (800 IU im) was administered for only one week post-transplant. During the study 19 patients were transplanted, and of those, none had recurrent HBV during a median follow-up of 11.7 months. The same group conducted a study comparing patients who switched from HBIG/LAM to LAM/ADV versus those who maintained the HBIG/LAM therapy (Table 5) (Angus 2007). One patient in the switching group became HBsAg-positive, but remained HBV DNA-negative after 5 months; all others remained HBsAg- and HBV DNA-negative at a median of 17.2 months from randomization.

Other promising results have been obtained (Nath 2006) (Table 5). Studies to date are limited, small and with short follow-up (Table 5). Thus, larger, prospective studies are needed to show if combination of a nucleoside and a nucleotide analogue will be sufficient as a prophylactic strategy against recurrent hepatitis B infection.

Authors	Study type	Patients, (n)	Antiviral therapy before LT, n (%)	HBV DNA pos. at LT, n (%)	Antiviral therapy after LT	Follow-up period after LT*	HBV recurrence [HBV DNA pos.], n (%)
Naoumov 2001	Prospective, randomized	12	NA	0 (0%)	HBIG for alt least 6 months, LAM thereafter	at week 52	1 (8%)
		12	NA	0 (0%)	HBIG		2 (17%)
Buti 2007	Prospective, randomized	14	8 (57%)	0 (0%)	HBIG+LAM for 1 month, LAM thereafter	mean 83 weeks	3 (15%)
		15	9 (60%)	0 (0%)	HBIG+LAM, n=6 switched to LAM monotherapy (at month 18)		1 (11%)**
Gane 2007	Prospective, non-randomized	26	26 (100%)	13 (68%) (LT in 19)	HBIG+ADV+LAM for 7 days, ADV+LAM thereafter	12 months	0 (0%)
Marzano 2001	Prosepective, non-randomized	12 (historic controls)	NA	9 (75%)	LAM	mean 42 months	6 (50%)
		33	33 (100%)	7 (21%)	HBIG+LAM	mean 30 months	1***(4%)
Augus 2007	Prospective, randomized	16	NA	NA	HBIG+LAM for at least 12 months thereafter ADV/LAM	17 months	0 (0%)
		18	NA	NA	HBIG+LAM		0 (0%)

Table 5. Prophylaxis of hepatitis B reinfection without HBIG maintenance therapy.

16 patients with LAM resistance who had treatment at LT with LAM plus ADV therapy were looked at (Lo 2005). One-half of the patients were administered HBIG for a median of 24 months. None of them had detectable HBV DNA, 13 were HBsAg-negative, and 2 without combined HBIG therapy became HBsAg-positive.

The final long-term results of ADV treatment in LAM-resistant post-transplant patients were recently published (Schiff 2007). At weeks 48 and 96, mean serum HBV DNA levels decreased by 4.0±1.6 and 4.5±1.5 $\log_{10}$ copies/ml, respectively, and serum HBV DNA became undetectable in 40% and 65%, respectively. After 48 weeks, ALT, bilirubin, albumin, and prothrombin time normalized in 51%, 76%, 81%, and 76%, respectively. Kaplan-Meier estimates of survival in these post-transplant patients at weeks 48, 96, and 144 were 91%, 88%, and 87%, respectively. Incomplete collection of data does not allow comparison of resistance rates in patients with combined LAM/ADV therapy with those receiving ADV monotherapy.

The choice of the antiviral therapy in patients with HBV recurrence depends on the current antiviral medication, on the viral load, and the resistance profile. There is no rationale for continuing HBIG therapy in case of viral breakthrough with detectable HBV DNA. Antiviral drug resistance can easily be established by genotypic assays that identify specific mutations known to be associated with decreased susceptibility to particular drugs.

The successful use of TDF in eight transplant patients who developed resistance to LAM at a median postoperative follow-up period of 26 months and were switched to TDF 1-66 months post-LT has been reported (Neff 2007). All experienced HBV DNA suppression, and seven of eight patients achieved an undetectable viral load.

Due to the lower antiviral activity of ADV and the increased potential for nephrotoxicity, accumulating data indicate that TDF seems to be more favourable (van Bommel 2008). In contrast to ADV, switching to TDF instead of adding on to LAM when there is resistance seems to be justified (Cornberg 2008; Cornberg 2007). Results from studies in LT patients treated with combinations, including TDF + LAM, TDF + ETV, or TDF + emtricitabine are urgently needed.

Experience with ETV in LT is very limited (Shakil 2002). Antiviral efficacy, safety, and the pharmacokinetic profile have been evaluated in a multicenter open-label study (AI463015) in nine LT patients (Shakil 2002). At week 48, all patients had a viral reduction ≥2 $\log_{10}$ copies/ml and four had normalization of their ALT. One patient had seroconversion and a sustained virological response.

Recurrence of hepatitis C in the allograft

The influence of HCV infection on allograft histology is highly variable. The liver injury can vary from absent or mild disease despite high viral burden to cirrhosis in the allograft (approximately 25% of recipients within 5-10 years of follow-up) (Berenguer 2003a). There are also patients who achieve viral response under therapy but still have progression of liver fibrosis (Cicinnati 2007b). It has been reported that patient and graft survival in HCV-infected transplant recipients is worse compared to those with other indications (Berenguer 2007; Forman 2002; Testa 2000). After the diagnosis of cirrhosis, the decompensation risk appears to be accelerated (17% and 42% at 6 and 12 months, respectively) (Berenguer 2000) and patient survival is significantly decreased (66% and 30% at 1 and 5 years, respectively) (Saab 2005).

Several factors have been suggested that may accelerate HCV reinfection of the allograft (Belli 2007; Berenguer 2003b; Iacob 2007; Saab 2005). Significance of variables depicted in Table 6 has been discussed widely except for listed donor factors, high dose corticosteroids and OKT3 therapy.

Donor factors	Donor age Liver steatosis
Recipient factors	Surgical factors (cold/warm ischemia time) Recipient age Recipient gender Non-caucasian race High viral load pre-transplant/early post-transplant Genotype 1b Muromonab-CD3 (OKT3) Bolus corticosteroids Rapid tapering of corticosteroids

Table 6. Factors that may accelerate histological progression in HCV patients after liver transplantation.

In particular, there are insufficient and somewhat controversial data regarding the relationship between immunosuppressive agents and clinical expression of HCV recurrence (Table 7).

Immunosuppressive agent	Viral load	Severity of HCV recurrance
Calcineurin inhibitors	No difference between cyclosporine A and tacrolimus	No difference between cyclosporine A and tacrolimus
Bolus corticosteroids	↑	↑
Azathioprine	↓ (in the replicon system)	Controversial discussion
Mycophenolate mofetil	Controversial discussion	Controversial discussion
T-lymphocyte depleting agents	Not known	Controversial discussion
Sirolimus	Not known	Not known

Table 7. Factors that may accelerate histological progression in HCV patients after liver transplantation.

TAC and CSA do not seem to be significantly different (Berenguer 2006a; Lake 2003; Martin 2004; Hilgard 2006) with respect to their impact on the course of hepatitis C recurrence. However, results are conflicting (Iacob 2007; Cescon 2009;

Firpi 2009; Watashi 2003). Data so far have shown that CSA has a strong suppressive effect on HCV replication using the HCV replicon cell culture system (Watashi 2003) and is associated with a higher sustained viral response in interferon-treated HCV recipients (Cescon 2009; Firpi 2009). However, large, randomized controlled studies are warranted to determine if one CNI is better than the other in patients transplanted due to hepatitis C.

Both CNI can increase TGF-β gene transcription and thus contribute to the development of chronic and progressive disease. However, the accelerated fibrosis observed in LT patients with hepatitis C recurrence does not seem to be related to a greater amount of activated hepatic stellate cells and TGFβ-1 expression in the grafts of these patients as compared to non-LT patients with chronic hepatitis C. In LT patients, the amount of activated hepatic stellate cells and TGFβ-1 expression correlated with the fibrosis stage and progression without any apparent influence of the type of CNI administered (Cisneros 2007).

Various studies have demonstrated that slowly tapering off corticosteroids over time may prevent progression to severe forms of recurrent disease (Brillanti 2002; Berenguer 2002; McCaughan 2003).

Iinduction with MMF is reported to be associated with more severe recurrence of HCV (Berenguer 2003). Other investigators have found that MMF has no impact on patient survival, rejection, or rate of HCV recurrence in HCV-infected transplant recipients based on biochemical changes and histological findings (Jain 2002). A recent study showed significantly better patient survival and graft survival for HCV-infected patients treated with MMF, TAC, and steroids than for patients treated only with TAC and steroids, with 4-year patient survival rates of 79.5% vs. 73.8% and 4 year graft survival rates of 74.9% vs. 69.5% (Wiesner 2005). Another study has shown a positive effect of MMF in combination with CNI taper for 24 months on fibrosis progression, graft inflammation, and alaninaminotransferase levels (Bahra 2005). It can be speculated that MMF may prevent fibrosis progression through an antiproliferative effect on myofibroblast-like cells as well as to the inhibition of adhesion molecules involved in the migration of immune cells towards the allograft, reduced nitric oxide production and subsequent suppressed allograft injury.

With respect to SRL, there are few case reports and no published data from randomised controlled studies available in HCV patients (Samonakis 2005; Schacherer 2007). Data are also insufficient regarding the impact of IL-2 receptor antibodies on the course of HCV reinfection (Nelson 2001; Calmus 2002). Results from a randomised controlled, multicenter study revealed that IL-2 induction therapy was associated with a significantly lower mortality and rate of allograft loss 6 and 12 months after LT (Calmus 2002).

HCV RNA concentrations in the medium term and long term after LT do not correlate with the severity of inflammation in the liver. Thus, regular histological evaluation of posttransplant chronic hepatitis C in 1-year (or maximum 2-year) intervals is recommendable to determine the grade of inflammation and fibrosis stage. In particular, the biopsy result is important for therapy decision, to exclude signs of rejection prior to antiviral therapy and to determine the efficacy of antiviral therapy.

There are only a few published studies evaluating the predictability of fibrosis using FibroScan in the LT setting. The diagnostic value of single laboratory tests, combinations of routinely available laboratory values with or without clinical parameters, direct biochemical markers of hepatic extracellular matrix turnover, and more complex assays based on a statistical approach has been assessed in immunocompetent patients. The diagnostic use of many of these non-invasive tests, however, remains to be determined in LT patients. To date, there is no model available for transplant recipients to be used irrespective of the indication for LT.

There is increasing evidence that IFN α and ribavirin therapy may prevent the development of cirrhosis, even in the absence of sustained viral response in a subset of patients (Cicinnati 2007b). This treatment is however associated with more side-effects and is far less effective than in the non-transplant setting. The most applicable treatment strategy is treatment of established HCV recurrence with PEG-IFN α and ribavirin, which results in a sustained viral response of 20-30%. Preemptive antiviral therapy (Shergill 2005; Sugawara 2004; Sheiner 1998; Singh 1998; Chalasani 2005) has not shown superior effects as compared to established HCV therapy (Berenguer 2008; Chalasani 2005; Abdelmalek 2004; Giostra 2004; Beckebaum 2004d; Beckebaum 2003; Bizollon 2005; Castells 2005; Toniutto 2005; Neff 2004; Dumortier 2004) and should only be considered in cases of rapid progression of HCV infection in the early posttransplant period. Most published studies in the transplant setting are not controlled, monocentric and/or comprise a small patient cohort (Shergill 2005; Sugawara 2004; Gane 1998; Kizilisik 1997; Ghalib 2000).

Optimal onset, dose and duration of therapy are not known yet. Positive predictive factors for sustained viral response include use of erythropoietin, patient compliance, treatment with pegylated interferon (versus standard interferon) and an early histological response (Berenguer 2006b).

The proportion of patients who need a dose reduction of their antiviral therapy due to anemia or leucopenia may be reduced by the use of erythropoietin or granulocyte macrophage colony-stimulating factor (not approved for this indication). Reported risk of rejection is low if close monitoring of antiviral therapy is provided (Gane 1998; Kizilisik 1997). Therapy needs to be withdrawn in case of histologically-proven rejection.

Recurrence of cholestatic liver diseases and autoimmune hepatitis

Data about the frequency of recurrent cholestatic and AIH-related liver disease vary in the literature depending on the follow-up period and criteria chosen for definition of disease recurrence.

Presently, PBC represents the sixth leading indication for LT in the US. The prognosis after LT is excellent, with an approximately 80% 5-year survival reported by most large centres. A recently published study reported recurrent PBC in one-third of patients at 11-13 years postransplant (Charatcharoenwitthaya 2007). Various other studies support this (Jakob 2006; Sylvestre 2003; Liermann-Garcia 2001; Dmitrewski 1996).

Diagnosis of PBC in the transplanted liver is usually more challenging than diagnosis in the native liver. Immunoglobulin M and anti-mitochondrial antibodies (AMA) often persist, and elevated cholestatic enzymes may be due to other causes of bile duct damage such as ischemic cholangiopathy or chronic ductopenic rejection. Recurrent PBC is a histologic diagnosis and if a liver biopsy is carried out only when clinical features are apparent, the frequency of recurrence will be considerably underestimated.

Some investigators have found that CSA-based immunosuppressive therapy is associated with lower recurrence rates as compared to TAC-based immunosuppression (Dmitrewski 1996; Wong 1993). The impact of ursodeoxycolic acid (UDCA) on the natural history of recurrent disease remains unknown. In the Mayo Clinic transplant series, 50% of recurrent PBC patients receiving UDCA showed normalization of serum alkaline phosphatase and alanine aminotransferase levels over a 36-month period compared with 22% of untreated patients (Charatcharoenwitthaya 2007). Although no significant differences in the rate of histological progression could be detected between the treated and untreated subgroups, the proportion of individuals with histological progression was significantly lower in those that showed improvement of biochemical parameters regardless of treatment.

It has been reported that HLA-A, -B, and -DR mismatches between the donor and the recipient decreases the risk of disease recurrence in PBC patients (Morioka 2007; Hashimoto 2001). However, the association among LT outcomes, and recurrent PBC after LT should be further investigated both in the LDLT and DDLT setting.

The reported recurrence rate for PSC after LT ranges between 9% and 37% (Cholongitas 2008; Yamagiwa 2007; Vera 2002; Graziadei 1999; Goss 1997). Recurrent PSC is diagnosed by histology and/or imaging of the biliary tree and exclusion of other causes of nonanastomotic biliary strictures. Histopathological findings in PSC include fibrous cholangitis, fibro-obliterative lesions, ductopenia, and biliary fibrosis. In a recent study at the Mayo clinic, recurrence of PSC was defined by strict cholangiographic and histological criteria in patients with PSC, in whom other causes of bile duct strictures were absent (Graziadei 2002). However, due to the lack of a histological gold standard, the diagnosis of PSC recurrence is based primarily on cholangiographic features.

Seddon et al. investigated the clinical course of ulcerative colitis in recipients transplanted for PSC (Ho 2005). Interestingly, despite immunosuppression, significantly higher relapse rates and a significantly higher corticosteroids requirement were detected, with 20% of patients becoming corticosteroid dependent after LT (Ho 2005). A recent study reported that maintenance steroids (>3 months) for ulcerative colitis post-LT was the only risk factor significantly associated with recurrent PSC (Cholongitas 2008).

Results from various studies have not revealed any differences in the overall patient survival or graft survival in patients with or without recurrent PSC disease (Ben-Ari 2003; Graziadei 2002).

AIH recurrence has been reported in about one-third of patients within a follow-up period of ≥5 years (Duclos-Vallee 2003; Campsen 2008a; Vogel 2004). Incidence increases over time as immunosuppression is reduced (Prados 1998). A long-term follow-up study (>10 years) by a French group found AIH recurrence in 41% of the patients. The authors recommended regular liver biopsies, because histological signs precede abnormal biochemical liver values in about one-fourth of patients (Duclos-

Vallee 2003). The diagnosis of recurrent AIH may include histological features, the presence of autoantibodies, and increased gamma-globulins. The majority of published studies did not confirm a posttransplant prognostic role of antibodies in patients undergoing LT for AIH. Conflicting data exist regarding the presence of specific HLA antigens that predispose patients to AIH recurrence after LT (Gonzalez-Koch 2001; Molmenti 2002). Histological signs of recurrence include interface hepatitis, lymphoplasmocytic infiltration, and/or lobular involvement. In an analysis of data from 28 patients with AIH between 1987 and 1999, a 5-year survival rate of 78.2% was seen, which was not significantly different from controls with genetic liver diseases (Vogel 2004). Patients had more episodes of acute rejection though, in comparison to the control group.

Patients with AIH typically receive low-dose steroid therapy after LT. The transplant center in Colorado attempted to minimize or stop steroid therapy in patients who had undergone a transplant due to AIH (Campsen 2008a). They found that an increased dose of immunosuppressive therapy and presence of inflammatory bowel disease were negatively associated with steroid withdrawal.

Outcome in patients transplanted for hepatic malignancies

The results of early studies of LT for HCC were disappointing. More than 60% of patients developed tumor recurrence within the first two transplant years (Ringe 1989) and reported 1-year and 5-year survival rates were 42-71% and 20-45%, respectively (Busuttil 1996; Yokoyama 1990). Currently, there are 1-year survival rates up to 80%, 5-year survival rates up to 70%, and recurrence rates of 10-15% in patients fulfilling the Milan criteria (Yoo 2003; Zavaglia 2005). In an analysis of predictors of survival and tumor-free survival in a cohort of 155 OLT recipients, histological grade of differentiation and macroscopic vascular invasion were identified as independent predictors of survival and tumor recurrence in patients transplanted for HCC (Zavaglia 2005).

Expansion beyond the Milan criteria to University of California San Francisco (UCSF) criteria (single tumor <6.5 cm, two to three tumors: none >4.5 cm or total diameter <8 cm, no vascular invasion) or even more liberal criteria (no portal invasion, no extrahepatic disease) has been discussed widely (Sotiropoulos 2007; Kaihara 2003; Malago 2006; Lo 2004). However, centers such as the San Francisco Transplant Group as well as the UCLA Transplant Group have demonstrated acceptable 5-year survival rates of 50-80% after LT for tumors greater than the Milan criteria but within UCSF criteria (Duffy 2007; Yao 2007).

Expansion of criteria in the LDLT setting is even more challenging due to the donor risk and the risk of selection of tumors with unfavorable biology following the concept of fast-tracking (Hiatt 2005). According to a multicenter study from Korea, the 3-year survival rate was 91% in LDLT patients and 88% in DDLT patients meeting the UCSF criteria (Hwang 2005). Another Korean study (Lee 2008) reported comparable survival rates in LDLT patients meeting Milan criteria, UCSF criteria or expanded criteria (largest tumor size <5 cm, HCC number <6, and no gross vascular invasion) (76%, 75.9% and 76.3%, respectively). Moreover, use of expanded criteria yielded a higher discriminatory power than the Milan and UCSF criteria. Recently, our transplant group was able to widen the age range (>60 years); MELD score >22 and AFP

>400 ng/ml were negatively associated with survival in HCC patients undergoing LDLT (Sotiropoulos 2008b). Novel molecular biology techniques, such as genotyping for HCC, may be relevant for determining recurrence-free survival and improving organ allocation.

Neoadjuvant chemoradiation and subsequent LT has shown promising results for patients with localized, unresectable hilar cholangiocellular carcinoma (CCC) (Rea 2005; Shimoda 2001). Challenges of LT attributable to neoadjuvant therapy include tissue injury from radiation therapy and vascular complications that include hepatic artery thrombosis. Predictors of response to the neoadjuvant protocol prior to LT need to be determined (Heimbach 2008). Increasing age, high pretransplant tumor marker, residual tumor size in the explant >2 cm, tumor grade, previous cholecystectomy and perineural invasion were identified as predictors of recurrence following LT (Knight 2007).

Metastatic lesions originating from neuroendocrine tumors (NET) may be hormone-producing (peptide hormones or amines) or may present as nonfunctional tumors (Frilling 2006; Coppa 2001; Lehnert 1998). They are characterized by slow growth and frequent metastasis to the liver, and their spread may be limited to the liver for protracted periods of time. The currently available data in patients transplanted for NET are limited and ususally restricted to small numbers of patients, which suggests that so far LT should be considered only in highly selected cases. Long-term results from prospective studies are needed to further define selection criteria for patients with NET for LT, to identify predictors for disease recurrence, and to determine the influence of the primary tumor site on patient posttransplant survival.

Recurrent alcohol abuse after liver transplantation for alcoholic liver disease

Alcoholic liver disease has become a leading indication of LT and represents the second most frequent transplant indication in Europe and the United States. Patient and graft survival is excellent in those maintaining alcohol abstinence after LT. Studies evaluating recurrent alcohol abuse have reported a mean incidence of relapse in one third of patients ranging from 10% to 50% in up to 5 years of follow-up (Burra 2005). The role of the length of pretransplant abstinence as a predictor of posttransplant abstinence has been widely discussed. Many studies have assessed possible risk factors for alcoholic relapse after LT. Perney et al. recently identified the following factors as risks for recurrent alcohol abuse: a shorter length of abstinence before LT, more than one pretransplant alcohol withdrawal, alcohol abuse in first relatives, younger age, and alcohol dependence (Perney 2005). Accordingly, the results from the Pittsburgh Transplant Center revealed that the prognosis regarding continued abstinence post-transplant is much more favorable for individuals with a diagnosis of abuse than for those who meet criteria for alcohol dependence (DiMartini 2008).

A recently published study reported that poorer social support, family alcohol history, and pretransplant abstinence of ≤6 months showed significant associations with relapse (Dew 2008). In addition, an Australian study identified the presence of psychiatric comorbidities, or a score higher than 3 on the High-Risk Alcoholism Relapse (HRAR) scale as factors predictive of relapse into harmful drinking

(Haber 2007). Information on previous alcohol consumption (dependence, number of withdrawals, family history) has been described to predict severe relapse after LT in patients with alcohol-related liver disease (Perney 2005). Severe chronic alcohol consumption after LT significantly decreases the medium and long-term survival (Pfitzmann 2007; Bellamy 2001).

Experiences with liver transplantation in inherited metabolic liver diseases

LT is regarded as an effective treatment strategy for patients with Wilson's Disease that presents as deterioration of cirrhosis not responsive to treatment, as acute on chronic disease or fulminant hepatic failure. LT reverses the abnormalities of copper metabolism by converting the copper kinetics from a homozygous to a heterozygote phenotype, thus providing an adequate increase of ceruloplasmin levels and a decrease of urinary copper excretion posttransplant. The Kings College Hospital reported excellent long-term results after LT with no deaths or graft loss in patients who have undergone LT for Wilson's Disease since 1994 with 5-year patient and graft survival rates of 87.5% (Sutcliffe 2003). There are several reports in the literature indicating a reversal of neurological symptoms after LT (Martin 2008; Sevmis 2008). However, the course of neurological symptoms remains unpredictable and it is still a matter of debate if LT should be considered in patients with severe neurological impairment (Pabón 2008).

Alpha-1 antitrypsin deficiency is one of the most common genetic causes of liver disease in the world. It is a common genetic reason for pediatric LT, but a rare indication in adults. It has been suggested that a subgoup of PiZZ individuals are predisposed to liver damage, due to an insufficient degradation of mutant alpha-1 antitrypsin Z within the endoplasmatic reticulum (Perlmutter 1998). A 1-year survival rate of 73% for adults has been reported in the literature (Vennarecci 1996).

In hemochromatosis, the metabolic defect resides in the small intestine, while LT cures the metabolic defect of the liver. Iron depletion therapy prior to LT may be associated with a better outcome after LT and is therefore strongly recommended (Weiss 2007). It has been shown that the survival of patients who undergo LT for hereditary hemochromatosis is markedly lower in comparison to other indications (Brandhagen 2001). Data on 56 patients with hemochromatosis compared to 5180 liver transplant recipients with other indications revealed 1-year survival rates of 69% vs 79% and 5-year survival rates of 43% vs 54% for those transplanted between 1982 and 1991 (Kilpe 1993). Findings derived from the UNOS database revealed 1-year and 5-year survival rates of 75% and 64% in patients with iron overload, as compared to 83% and 70% in those without iron overload (Brandhagen 2001). Reduced posttransplant survival in patients with hemochromatosis has been attributed to cardiac problems and increased infectious complications.

Conflicting and very limited data are available about recurrent iron deposition in the liver. Nonetheless, there is a need for careful monitoring of patients with hereditary hemochromatosis in order to determine whether iron reaccumulates in the allograft.

Outcome after liver transplantation for acute hepatic failure

Acute hepatic failure (AHF) accounts for 9% of liver transplant activity (Figure 1). The most common causes of AHF include acetaminophen overdose, idiosyncratic drugs (paracetamol, isoniazid/rifampicin, cumarins, ectasy, tricyclic antidepressants, etc.) and hepatitis B infection (Khashab 2007). In addition, Budd Chiari syndrome, Wilson's Disease, hepatitis A infection and in rare cases autoimmune disease may also present as AHF.

Available data document that survival in patients with AHF is inferior to that of recipients with nonacute indications for LT within the first year but comparable in the long-term (Wigg 2005). Early postoperative complications in patients transplanted for AHF include sepsis, multisystem organ failure, and primary graft failure. Serum creatinine concentrations above 200 μmol/l pretransplant, non-white race of the recipient, donor body mass index >35 kg/m^2 and recipient age >50 years have been suggested as risk factors for posttransplant mortality (Wigg 2005). A study correlating the causes of AHF and the transplant outcome has suggested that the best outcome is found in patients transplanted for Wilson's Disease and the worst outcome in those transplanted for idiosyncratic drug reactions (O'Grady 2005). The results in patients transplanted for AHF have improved within the last decade, due to the establishment of prognostic models and the option for LDLT which has a limited role in the US and Europe but plays a major role in Asia (Lo 2008). AHF was the indication for LDLT in more than 10% of the series reported by the Kyoto group and by the Hong Kong group (Morioka 2007; Lo 2004). Recently, Campsen et al. reported the outcome of patients with AHF who were evaluated for LDLT and included in the Adult to Adult Living Donor Liver Transplantation (A2AAL) Cohort Study (Campsen 2008b). Of all evaluated patients, only 1% were diagnosed with AHF. However, the authors concluded that LDLT is associated with acceptable patient survival (70%) and donor outcome in this small patient group.

Conclusion

Liver transplantation is challenged by a shortage of organs and a prolonged waiting-list time. The large disparity between the number of available cadaver donor organs and recipients awaiting LT has created an ongoing debate regarding the appropriate selection criteria. The rationale of allocation systems utilizing the MELD score is to prioritize patients with severe liver dysfunction ("the sickest first"). Novel surgical techniques, including split cadaveric livers, LDLT, and broadening the donor criteria towards acceptance of marginal donors have been used as strategies in order to expand the donor pool.

HCV has become the leading indication for cadaveric transplantation and LDLT in the United States, accounting for approximately 50% of all cases. Moreover, the number of patients with HCV cirrhosis continues to increase. There is ongoing research aiming to define host or viral factors that predict recurrence, the impact of immunosuppressive regimens, and the appropriate timepoint and dose for antiviral treatment.

Due to the availability of antiviral drugs, the survival of patients undergoing LT for HBV infection has dramatically improved and has become comparable to or even better than the survival of patients with non-virus-related liver diseases. HBIG-free

therapeutic regimens with new promising nucleos(t)ide analogue combinations are currently being investigated for their efficacy and safety as first-line therapy in clinical studies. The ultimate goal is to prevent antiviral drug resistance and to identify predictors involved in response to treatment and treatment failure or relapse.

Data about the frequency of disease recurrence in cholestatic and autoimmune liver diseases vary in the literature. Diagnosis of disease relapse in cholestatic and autoimmune liver disease is more challenging than in the non-transplant setting. Patients have excellent medium-term and long-term results despite limited therapeutic options for recurrent liver disease.

Abstinence of ≥6 months pretransplant is widely considered the prerequisite time for listing for LT. There are few reliable predictors of relapse in alcoholic patients after LT. Survival rates in patients with alcohol-related liver disease are similar or even better as compared to the outcomes of patients who undergo transplantation for other types of chronic liver disease. In contrast, survival is worse in patients with heavy alcohol consumption after LT.

LT in HCC patients provides excellent outcomes and low recurrence rates following the Milan criteria. Expansion of transplantation criteria beyond the Milan criteria has been discussed at length. Recent developments in genomic and proteomic approaches may allow the identification of new biomarkers for prediction of HCC recurrence.

The management of cardiovascular, renal, coagulopathic, cerebral and infectious complications in patients with AHF is clinically challenging. Prognostic models are helpful but not entirely accurate in predicting those who will require LT. Due to advances in intensive care medicine and surgical techniques outcomes for patients with AHF have progressively improved over the last decades.

Due to excellent short-term outcomes after LT, attention has shifted to reducing CNI-associated long-term complications. Cardiovascular comorbidities due to metabolic complications, such as diabetes mellitus, dyslipidemia, obesity, and arterial hypertension, account for 30-70% of long-term morbidity. Numerous ongoing studies aim to determine the most effective immunosuppressive protocols while minimizing drug-related side effects. These protocols often combine several drugs with different mechanisms of action and toxicities allowing dose adjustment. Current trends of immunosuppressive strategies include CNI-sparing protocols, mTOR inhibitor based-protocols and corticosteroid-avoidance protocols. There is a trend towards tailored immunosuppressive regimens according to the etiology of the liver disease and comorbidities such as renal dysfunction and cardiovascular disease.

References

Afonso RC, Hidalgo R, Zurstrassen MP, et al. Impact of renal failure on liver transplantation survival. Transplant Proc 2008;40(3):808-10.

Angus PW, Strasser SI, Patterson S, et al. A randomized study to assess the safety and efficacy of adefovir dipivoxil substitution for hepatitis B immune globulin in liver transplantation patients receiving long-term low dose IM HBIG and lamivudine prophylaxis. Hepatology 2007;46:238A.

Abdelmalek MF, Firpi RJ, Soldevila-Pico C, et al. Sustained viral response to interferon and ribavirin in liver transplant recipients with recurrent hepatitis C. Liver Transpl 2004;10:199-207.

Bahra M, Neumann UI, Jacob D, et al. MMF and calcineurin taper in recurrent hepatitis C after liver transplantation: impact on histological course. Am J Transplant 2005;5:406-11.

Beavers KL, Sandler RS, Shrestha R. Donor morbidity associated with right lobectomy for living donor liver transplantation to adult recipients. Liver Transpl 2002:8:110-7.

Beckebaum S (a), Sotiropoulos G, Gerken G, Cicinnati V. Hepatitis B and liver transplantation: 2008 update. Rev Med Virol 2008;18. Epub ahead of print.

Beckebaum S (a), Cicinnati V, Brokalaki E, Frilling A, Gerken G, Broelsch CE. CNI-sparing regimens within the liver transplant setting: experiences of a single center. Clin Transpl 2004;215-20.

Beckebaum S (b), Cicinnati VR, Broelsch CE. Future directions in immunosuppression. Transplant Proc 2004; 36(2Suppl):574S-6S.

Beckebaum S (b), Cicinnati V, Gerken G. Current concepts for prophylaxis and treatment of hepatitis B reinfection after liver transplantation. Med Klin (Munich) 2008; 103(4):190-7.

Beckebaum S (d), Cicinnati VR, Zhang X, et al. Combination therapy with peginterferon alpha-2B and ribavirin in liver transplant recipients with recurrent HCV infection: preliminary results of an open prospective study. Transplant Proc 2004;36(5): 1489-91.

Beckebaum S, Cicinnati VR, Karliova M, et al. Daily interferon alpha-2B and ribavirin combination therapy for liver transplant patients with chronic hepatitis C infection. Transplant Proc 2003; 35(6):2080-1.

Beckebaum S (c), Cicinnati VR, Gerken G, et al. Management of chronic hepatitis B in the liver transplant setting. Transplantat Rev 2004;18:3-12.

Bellamy CO, DiMartini AM, Ruppert K, et al. Liver transplantation for alcoholic cirrhosis: long term follow-up and impact of disease recurrence. Transplantation 2001;72:619-26.

Belli LS, Burroughs AK, Burra P, et al. Liver Transplantation for HCV cirrhosis: improved survival in recent years and increased severity of recurrent disease in female recipients: results of a long term retrospective study. Liver Transpl 2007;13:737-40.

Ben-Ari Z, Pappo O, Mor E. Intrahepatic cholestasis after liver transplantation. Liver Transpl 2003;9:1005-18.

Berenguer M (b), Crippin J, Gish R, et al. A model to predict severe HCV-related disease following liver transplantation. Hepatology 2003;38:34-41.

Berenguer M (a). Host and donor risk factors before and after liver transplantation that impact HCV recurrence. Liver Transpl 2003;9:S44-7.

Berenguer M. Recurrent hepatitis C: worse outcomes established, interventions still inadequate. Liver Transplantation 2007;13:641-3.

Berenguer M, Prieto M, Rayon JM, et al. Natural history of clinically compensated HCV-related graft cirrhosis after liver transplantation. Hepatology 2000;32(4 PT 1):852-8.

Berenguer (a) M, Aguilera V, Prieto M, et al. Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected liver transplant recipients. Liver Transpl 2006;12:762-7.

Berenguer M (b), Palau A, Fernandez A, et al. Efficacy, predictors of response, and potential risks associated with antiviral therapy in liver transplant recipients with recurrent hepatitis C. Liver Transpl 2006;12:1067-76.

Berenguer M. Outcome of posttransplantation hepatitis C virus disease - is it the host, the virus, or how we modify the host and/or the virus? Liver Transpl 2002;8:889-91.

Berenguer M, Palau A, Aquilera V, Rayón JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. Am J Transplant 2008;8:679-87.

Bizollon T, Ahmed SN, Radenne S, et al. Long term histological improvement and clearance of intrahepatic hepatitis C virus RNA following sustained viral response to interferon-ribavirin combination therapy in liver transplant patients with hepatitis C virus recurrence. Gut 2005;52:283-7.

Brandhagen DJ. Liver transplantation for hereditary hemochromatosis. Liver Transpl 2001;7:663-7.

Brillanti S, Vivarelli M, De Ruvo N, et al. Slowly tapering off steroids protects the graft against hepatitis C recurrence after liver transplantation. Liver Transpl 2002;8(10):884-8.

Broelsch CE, Whitington PF, Emond JC, et al. Liver transplantation in children from living related donors. Surgical techniques and results. Ann Surg 1991;214 (4):428-37.

Bundesärztekammer. Richtlinien zur Organtransplantation gem. §16 TPG. Deutsches Ärzteblatt 2008;105(26):A1461-4.

Burra P, Lucey MR. Liver transplantation in alcoholic patients. Transpl Int 2005;18:491-8.

Busuttil RW, Farmer DG. The surgical treatment of primary hepatobiliary malignancy. Liver Transpl Surg 1996; 2(Suppl 1):114-30.

Buti M, Mas A, Prieto M, et al. Adherence to lamivudine after an early withdrawal of hepatitis B immune globulin plays an important role in the long-term prevention of hepatitis B virus recurrence. Transplantation 2007;84:650-4.

Calmus Y, Scheele JR, Gonzalez-Pinto I, et al. Immunoprophylaxis with basiliximab, a chimeric anti-interleukin-2 receptor monoclonal antibody, in combination with azathioprine-containing triple therapy in liver transplant recipients. Liver Transpl 2002;8:123-31.

Campsen J (b), Blei AT, Emond JC, et al. Outcomes of living donor liver transplantation for acute liver failure: the adult-to-adult living donor liver transplantation cohort study. Liver Transpl 2008;14(9):1273-80.

Campsen J (a), Zimmerman MA, Trotter JF, et al. Liver transplantation for autoimmune hepatitis and the success of aggressive corticosteroid withdrawal. Liver Transpl 2008;14(9):1281-6.

Cannon JA. Organs. Transplant Bull 1956;3-7.

Cantarovich M, Tzimas GN, Barkun J, Deschênes M, Alpert E, Tchervenkov J. Efficacy of mycophenolate mofetil combined with very low-dose cyclosporine microemulsion in long-term liver-transplant patients with renal dysfunction. Transplantation 2003;76:98-102.

Castells L, Vargas V, Aleende H, et al. Combined treatment with pegylated interferon (alpha-2b) and ribavirin in the acute phase of hepatitis C virus recurrence after liver transplantation. J Hepatol 2005;43:53-9.

Cescon M, Grazi GL, Cucchetti A, Vetrone G, Ravaioli M, Ercolani G, et al. Predictors of sustained virological response after antiviral treatment for hepatitis C recurrence following liver transplantation. Liver Transpl 2009; 15:782-9.

Cicinnati VR (a), Yu Z, Klein CG, et al. Clinical trial: switch to combined mycophenolate mofetil and minimal dose calcineurin inhibitor in stable liver transplant patients-assessment of renal and allograft function, cardiovascular risk factors and immune monitoring. Aliment Pharmacol Ther 2007;26(9):1195-208.

Cicinnati V (b), Iacob S, Klein C, et al. Ribavirin with either standard or pegylated interferon to treat recurrent hepatitis C after liver transplantation. Aliment Pharmacol Ther 2007;26:291-303.

Chalasani N, Manzarbeitia C, Ferenci P, et al. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. Hepatology 2005;41:289-98.

Chang TT, Gish RG, de Man R, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006; 354: 1001-10.

Charatcharoenwitthaya P, Pimentel S, Talwalkar, et al. Long-term survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. Liver Transpl 2007;13(9):1236-45.

Choi PC, Kim HJ, Choi WH, et al. Model for end stage liver disease, model for end-stage liver disease-sodium and Child-Turcotte-Pugh scores over time for the prediction of complications of liver cirrhosis. Liver International 2008; Epub ahead of print.

Clavien PA, Camargo CA, Baillie L, Fitz JG. Sphincter of Oddi dysfunction after liver transplantation. Dig Dis Sci 1995;40:73-4.

Colonno RJ, Rose R, Baldick CJ, et al. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology 2006;44:1656-65.

Coppa J, Pulvirenti A, Schiavo M, et al. Resection versus transplantation for liver metastases from neuroendocrine tumors. Transplant Proc 2001;33:1537-9.

Cornberg M, Protzer U, Dollinger MM, et al. [Prophylaxis, Diagnosis and Therapy of Hepatitis-B-Virus-(HBV-) Infection: upgrade of the guideline, AWMF-Register 021/011]. Z Gastroenterol 2007;45:525-74.

Cornberg M, Protzer U, Dollinger MM, et al. The German guideline for the management of hepatitis B virus infection: short version. J Viral Hepatitis 2008;15:1-20.

Créput C, Blandin F, Deroure B, et al. Long-term effects of calcineurin inhibitor conversion to mycophenolate mofetil on renal function after liver transplantation. Liver Transpl 2007;13(7):1004-10.

Crippin JS, McCashland T, Terrault N, Sheiner P, Charlton MR. A pilot study of the tolerability and efficacy of antiviral therapy in hepatitis C virus-infected patients awaiting liver transplantation. Liver Transpl 2002;8:350-5.

Daly I, Jain A, Reyes J, Fung J. Mycophenolate mofetil for treatment of chronic rejection in liver allograft under tacrolimus. Transplant Proc 2002;34:1503.

Del Poggio P, Zaccanelli M, Oggionni M, et al. Low-dose tenofovir is more potent than adefovir and is effective in controlling HBV viremia in chronic HBeAg-negative hepatitis B. World J Gastroenterol 2007;13:4096-9.

Demetris AJ, Murase N, Lee RG et al. Chronic rejection. A general overview of histopathology and pathophysiology with emphasis on liver, heart and intestinal allografts. Ann Transplant 1997;2:27-44.

Dew MA, DiMartini AF, Steel J, et al. Meta-analysis of risk for relapse to substance use after transplantation of the liver or other solid organs. Liver Transpl 2008;14(2):159-72.

DiMartini A, Dew MA, Fitzgerald MG, Fontes P. Clusters of alcohol use disorders diagnostic criteria and predictors of alcohol use after liver transplantation for alcoholic liver disease. Psychosomatics 2008;49(4):332-40.

Dmitrewski J, Hubscher SG, Mayer AD, Neuberger JM. Recurrence of primary biliary cirrhosis in the liver allograft: the effect of immunosuppression. J Hepatol 1996;24:253-7.

Douzdjian V, Abecassis MM, Johlin FC. Sphincter of Oddi dysfunction following liver transplantation. Screening by bedside manometry and definitive manometric evaluation. Dig Dis Sci 1994;39:253-6.

Duclos-Vallee JC, Sebagh M, Rifai K, et al. A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence. Gut 2003;52:893-97.

Duffy JP, Vardanian A, Benjamin E, et al. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. Ann Surg 2007;246(3):502-9.

Dumortier J, Scoazek JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. J Hepatol 2004;40:669-74.

Eason JD, Nair S, Cohen AJ, Blazek JL, Loss GE. Steroid-free liver transplantation using rabbit antithymocyte globulin and early tacrolimus monotherapy. Transplantation 2003;75:1396-9.

Ebeling PR. Transplantation osteoporosis. Curr Osteoporos Rep 2007;5(1):29-37.

Erim Y, Böttcher M, Dahmen U, Beck O, Broelsch CE, Helander A. Urinary ethyl glucuronide testing detects alcohol consumption in alcoholic liver disease patients awaiting liver transplantation. Liver Transpl 2007;13(5):757-61.

Everson GT, Trotter J, Forman L, et al. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. Hepatology 2005;42(2):255-62.

Cholongitas E, Shusang V, Papatheodoridis GV, et al. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl 2008;14(2):138-43.

Ferretti G, Merli M, Ginanni CS, et al. Low-dose intramuscular hepatitis B immune globulin and lamivudine for long-term prophylaxis of hepatitis B recurrence after liver transplantation. Transplant Proc 2004;36:535-8.

Filipponi F, Callea F, Salizzoni M, et al. Double-blind comparison of hepatitis C histological recurrence rate in HCV+ liver transplant recipients given basiliximab + steroids or basiliximab + placebo, in addition to cyclosporine and azathioprine. Transplantation 2004;78:1488-95.

Finkelstein SD, Marsh W, Demetris AJ, et al. Microdissection-based allelo-typing discriminates de novo tumor from intrahepatic spread in hepatocellular carcinoma. Hepatology 2003;37:871-9.

Firpi R, Soldevila-Pico C, Morelli G, Cabrera R, Levy C, Clark VC, et al. The use of cyclosporine for recurrent hepatitis C after liver transplant: a randomized pilot study. Dig Dis Sci 2009 Oct 2 [Epub ahead of print].

Forman LM, Lewis DJ, Berlin JA, et al. The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002;122:889-96.

Freeman RB. Model for end stage liver disease (MELD) for liver allocation: A 5-year score card. Hepatology 2008;47(3):1052-7.

Freeman RB. Overview of the MELD/PELD system of liver allocation indications for liver transplantation in the MELD era: evidence-based patient selection. Liver Transpl 2004;10(10 Suppl 2):S2-3.

Frilling A, Malago M, Weber F, et al. Liver transplantation for patients with metastatic endocrine tumors. Liver Transpl 2006; 12(7):1089-96.

Fung JJ, Jain A, Kwak EJ, Kusne S, Dvorchik I, Eghtesad B. De novo malignancies after liver transplantation: a major cause of late death. Liver Transpl 2001;7(11 Suppl 1):S109-18.

Gane EJ, Lo SK, Riordan SM, et al. A randomized study comparing ribavirin and interferon alfa monotherapy for hepatitis C recurrence after liver transplantation. Hepatology 1998;27:1403-7.

Gane E, Strasser SI, Patterson S, et al. A prospective study on the safety and efficacy of lamivudine and adefovir dipivoxil prophylaxis in HBsAg positive liver transplantation candidates. Hepatology 2007;46:479A.

Garcia CE, Ribeiro HB, Garcia RL, et al. Mycophenolate mofetil in stable liver transplant patients with calcineurin inhibitor-induced renal impairment: single-center experience. Transplant Proc 2003;35:1131-2.

Ghalib R, Pandula R, Kadhim T, et al. Treatment of recurrent hepatitis C after liver transplantation with alpha-2b interferon plus ribavirin. Hepatology 2000;32:291A.

Giostra E, Kullak-Ublick GA, Keller W, et al. Ribavirin/interferon-alpha sequential treatment of recurrent hepatitis C after liver transplantation. Transpl Int 2004;17:169-76.

Gonwa TA, Mai ML, Melton LB, Hays SR, et al. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. Transplantation 2001;72(12):1934-9.

Gonzalez-Koch A, Czaja AJ, Carpenter HA, et al. Recurrent autoimmune hepatitis after orthotopic liver transplantation. Liver Transpl 2001;7:302-10.

Goss JA, Shackleton CR, Farmer DG, et al. Orthotopic liver transplantation for primary sclerosing cholangitis. A 12-year single center experience. Ann Surg 1997;225:472-81.

Graziadei IW. Recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl 2002;8:575-81.

Graziadei IW, Wiesner RH, Marotta PJ, et al. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. Hepatology 1999;30:1121-7.

Graziadei IW, Schwaighofer H, Koch R, et al. Long-term outcome of endoscopic treatment of biliary strictures after liver transplantation. Liver Transpl 2006;12(5):718-25.

Guba M, von Breitenbuch P, Steinbauer M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 2002;8:128-35.

Haber PS, McCaughan GW. “I’ll never touch it again, doctor!”-harmful drinking after liver transplantation. Hepatology 2007;46(4):1302-4.

Han SH, Martin P, Edelstein M, et al. Conversion from intravenous to intramuscular hepatitis B immune globulin in combination with lamivudine is safe and cost-effective in patients receiving long-term prophylaxis to prevent hepatitis B recurrence after liver transplantation. Liver Transpl 2003;9:182-7.

Hashimoto E, Shimada M, Noguchi S, et al. Disease recurrence after living liver transplantation for primary biliary cirrhosis: a clinical and histological follow-up study. Liver Transpl 2001;7:588-95.

Heimbach JK. Successful liver transplantation for hilar cholangiocarcinoma. Curr Opin Gastroenterol. 2008;24(3):384-8.

Hiatt JR, Carmody IC, Busuttil RW. Should we expand the criteria for hepatocellular carcinoma with living-donor liver transplantation?-no, never. J Hepatol 2005;43:573-7.

Hierro L, Diez-Dorado R, Diaz C, et al. Efficacy and safety of valganciclovir in liver-transplanted children infected with Epstein-Barr virus. Liver Transpl 2008;14:1185-93.

Hilgard P, Kahraman A, Lehmann N, et al. Cyclosporine versus tacrolimus as immunosuppression after liver transplantation in patients with chronic hepatitis C: effects on virus replication and recurrent hepatitis. World J Gastroenterol 2006:12:1258-62.

Ho GT, Seddon AJ, Therapondos G, Satsangi J,Hayes PC. The clinical course of ulcerative colitis after orthotopic liver transplantation for primary sclerosing cholangitis: further appraisal of immunosuppression post transplantation. Eur J Gastroenterol Hepatol 2005;17:1379-85.

Hwang S, Lee SG, Joh JW, Suh KS, Kim DG. Liver transplantation for adult patients with hepatocellular carcinoma in Korea: comparison between cadaveric donor and living donor liver transplantations. Liver Transpl 2005;11(10):1265-72.

Iacob S, Cicinnati V, Hilgard P, et al. Predictors of graft and patient survival in hepatitis C virus (HCV) recipients: model to predict HCV cirrhosis after liver transplantation. Transplantation 2007;84:56-63.

Iacobellis A, Siciliano M, Perri F, et al. Peginterferon alfa-2b and ribavirin in patients with hepatitis C virus and decompensated cirrhosis: a controlled study. J Hepatol 2007;46(2):185-8.

Jacob DA, Neumann UP, Bahra M, et al. Long-term follow-up after recurrence of primary biliary cirrhosis after liver transplantation in 100 patients. Clin Transplant 2006;20(2):211-20.

Jain A, Kashyap R, Demetris AJ, Eghstesad B, Pokharna R, Fung JJ. A prospective randomized trial of mycophenolate mofetil in liver transplant recipients with hepatitis C. Liver Tranpl 2002;8:47-9.

Jiao ZY, Jiao Z. Prophylaxis of recurrent hepatitis B in Chinese patients after liver transplantation using lamivudine combined with hepatitis B immune globulin according to the titer of antibody to hepatitis B surface antigen. Transplant Proc 2007;39:1533-6.

Jimenez C, Marques E, Manrique A, al. Incidence and risk factors of development of lung tumors after liver transplantation. Transplant Proc 2005;37:3970-2.

John PR, Thuluvath PJ. Outcome of patients with new-onset diabetes mellitus after liver transplantation compared with those without diabetes mellitus. Liver Transpl 2002;8:708-13.

Kaihara S, Kiuchi T, Ueda M, et al. Living donor liver transplantation for hepatocellular carcinoma. Transplantation 2003;75:S37-40.

Kapoor D, Guptan RC, Wakil SM, et al. Beneficial effects of lamivudine in hepatitis B virus-related decompensated cirrhosis. J Hepatol 2000;33:308-12.

Kawasaki S, Makuuchi M, Matsunami H, et al. Living related liver transplantation in adults. Ann Surg 1998;227:269-74.

Khashab M, Tector AJ, Kwo PY, et al. Epidemiology of acute liver failure. Curr Gastroenterol Rep 2007;9(1):66-73.

Kilpe VE, Krakauer H, Wren RE. An analysis of liver transplant experience from 37 transplant centers as reported to Medicare. Transplantation 1993;56:554-61.

Kizilisik TA, Sebayel M, Hammad A, al-Traif I, Ramirez CG,Abdulla A. Hepatitis C recurrence in liver transplant recipients. Transplant Proc 1997;29:2875-7.

Kneteman NM, Oberholzer J, Al Saghier M, et al. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. Liver Transpl. 2004;10(10):1301-11.

Kniepeiss D, Iberer F, Grasser B, Schaffellner S, Tscheliessnigg KH. Sirolimus and mycophenolate mofetil after liver transplantation. Transpl Int 2003;16(7):504-9.

Knight SR, Friend PJ, Morris PJ. Role of transplantation in the management of hepatic malignancy. Br J Surg 2007;94(11):1319-30.

Konrad T, Steinmüller T, Vicini P, et al. Regulation of glucose tolerance in patients after liver transplantation: impact of cyclosporin versus tacrolimus therapy. Transplantation 2000;69(10): 2072-8.

Lake JR. The role of immunosuppression in recurrence of hepatitis C. Liver Transpl 2003;9:S63-6.

Lake KD. New prophylactic treatment strategy for cytomegalovirus disease. Am J Health Syst Pharm 2003;60(Suppl 8):S13-6.

Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. Gastroenterology 2007;133:1445-51.

Lautenschlager I, Hockerstedt K, Linnavuori K et al. Human herpesvirus-6 infection after liver transplantation. Clin Infect Dis 1998;26:702-7.

Lee SG, Hwang S, Moon DB, et al. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. Liver Transpl 2008; 14: 935-45.

Lehnert T. Liver transplantation for metastatic neuroendocrine carcinoma: an analysis of 103 patients. Transplantation 1998;66:1307-12.

Levy G, Schmidli H, Punch J, et al. Safety, tolerability, and efficacy of everolimus in de novo liver transplant recipients: 12- and 36-month results. Liver Transpl 2006;12(11):1640-8.

Liermann Garcia RF, Evangelista GC, McMaster P, Neuberger J. Transplantation for primary biliary cirrhosis: retrospective analysis of 400 patients in a single center. Hepatology 2001;33:22-7.

Lo CM, Fan ST, Liu CL, Chan SC, Wong J. The role and limitation of living donor liver transplantation for hepatocellular carcinoma. Liver Transpl 2004;10:440-7.

Lo CM, Fan ST, Liu CL, et al. Lessons learned from one hundred right lobe living donor liver transplants. Ann Surg 2004;240: 151-8.

Lo CM, Liu CL, Lau GK, Chan SC, Ng IO, Fan ST. Liver transplantation for chronic hepatitis B with lamivudine-resistant YMDD mutant using add-on adefovir dipivoxil plus lamivudine. Liver Transpl 2005;11:807-13.

Luan FL, Ding R, Sharma VK, Chon WJ, Lagman M, Suthanthiran M. Rapamycin is an effective inhibitor of human renal cancer metastasis. Kidney Int 2003;63:917-26.

Maheshwari A, Torbenson MS, Thuluvath PJ. Sirolimus monotherapy versus sirolimus in combination with steroids and/or MMF for immunosuppression after liver transplantation. Dig Dis Sci. 2006;51(10):1677-84.

Malago M, Sotiropoulos GC, Nadalin S, et al. Living donor liver transplantation for hepatocellular carcinoma: a single center preliminary report. Liver Transpl 2006;12:934-40.

Marsh JW, Finkelstein SD, Demetris AJ, et al. Genotyping of hepatocellular carcinoma in liver transplant recipients adds predictive power for determining recurrence-free survival. Liver Transpl 2003:9:664-71.

Martin AP, Bartels M, Redlich J, Hauss J, Fangmann J. A single center experience with liver transplantation for Wilson's disease. Clin Transplant 2008;22:216-21.

Martin P, Busuttil RW, Goldstein RM, et al. Impact of tacrolimus versus cyclosporine in hepatitis C virus-infected liver transplant recipients on recurrent hepatitis: a prospective, randomized trial. Liver Transpl 2004;10:1258-62.

Martin-Davila P, Fortun J, Gutierrez C, et al. Analysis of a quantitative PCR assay for CMV infection in liver transplant recipients: an intent to find the optimal cut-off value. J Clin Virol 2005;33:138-44.

Marzano A, Salizzoni M, Debernardi-Venon W, et al. Prevention of hepatitis B virus recurrence after liver transplantation in cirrhotic patients treated with lamivudine and passive immunoprophylaxis. J Hepatol 2001;34:903-10.

Marzano A, Lampertico P, Mazzaferro V, et al. Prophylaxis of hepatitis B virus recurrence after liver transplantation in carriers of lamivudine-resistant mutants. Liver Transpl 2005; 11: 532-8.

McCaughan GW, Spencer J, Koorey D, et al. Lamivudine therapy in patients undergoing liver transplantation for hepatitis B virus precore mutant-associated infection: high resistance rates in treatment of recurrence but universal prevention if used as prophylaxis with very low dose hepatitis B immune globulin. Liver Transpl Surg 1999;5:512-9.

McCaughan GW, Zekry A. Impact of immunosuppression on immunopathogenesis of liver damage in hepatitis C virus-infected recipients following liver transplantation. Liver Transpl 2003;9:S21-7.

Merion RM, Schaubel DE, Dykstra DM, et al. The survival benefit of liver transplantation. Am J Transplant 2005;5:307-13.

Molmenti EP, Netto GJ, Murray NG, et al. Incidence and recurrence of autoimmune/alloimmune hepatitis in liver transplant recipients. Liver Transpl 2002;8(6):519-26.

Moreno JM, Rubio E, Pons F, et al. Usefulness of mycophenolate mofetil in patients with chronic renal insufficiency after liver transplantation. Transplant Proc 2003;35:715-7.

Moreno Planas JM, Cuervas-Mons Martinez V, Rubio Gonzalez E, et al. Mycophenolate mofetil can be used as monotherapy late after liver transplantation. Am J Transplant 2004;4:1650-5.

Morioka D, Egawa H, Kasahara M et al. Outcomes of adult-to-adult living donor liver transplantation. A single institution`s experience with 335 consecutive cases. Ann Surg 2007;245:315-25.

Morioka D, Egawa H, Kasahara M, et al. Impact of human leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. Liver Transpl 2007;13(1):80-90.

Mutimer D, Dusheiko G, Barrett C, et al. Lamivudine without HBIg for prevention of graft reinfection by hepatitis B: long-term follow-up. Transplantation 2000;70:809-15.

Naoumov NV, Lopes AR, Burra P, et al. Randomized trial of lamivudine versus hepatitis B immunoglobulin for long-term prophylaxis of hepatitis B recurrence after liver transplantation. J Hepatol 2001;34:888-94.

Nath DS, Kalis A, Nelson S, Payne WD, Lake JR, Humar A. Hepatitis B prophylaxis post-liver transplant without maintenance hepatitis B immunoglobulin therapy. Clin Transplant 2006;20:206-10.

Neff GW, Montalbano M, Lapak-Green G, et al. Sirolimus therapy in orthotopic liver transplant recipients with calcineurin inhibitor related chronic renal insufficiency. Transplant Proc 2003;35:3029-31.

Neff GW, Nery J, Lau DT, et al. Tenofovir therapy for lamivudine resistance following liver transplantation. Ann Pharmacother 2004;38:1999-2004.

Neff GW, Montalbano M, O'Brien CB, et al. Treatment of established recurrent hepatitis C in liver transplant recipients with pegylated interferon-alfa-2b and ribavirin therapy. Transplantation 2004;78:1303-7.

Neff GW, Kemmer N, Kaiser TE, et al. Combination therapy in liver transplant recipients with hepatitis B virus without hepatitis B immune globulin. Dig Dis Sci 2007;52:2497-500.

Nelson DR, Soldevila-Pico C, Reed A, et al. Anti-interleukin 2 receptor therapy in combination with mycophenolate mofetil is associated with more severe hepatitis recurrence after liver transplantation. Liver Transpl 2001;8:123-31.

Neuberger J. Incidence, timing, and risk factors for acute and chronic rejection. Liver Transpl Surg 1999;5:S30-6.

Neuberger J. Public and professional attitudes to transplanting alcoholic patients. Liver Transplantation 2007;13(11Suppl2):S65-8.

Neuhaus P, Clavien PA, Kittur D, et al. Improved treatment response with basiliximab immunoprophylaxis after liver transplantation: results from a double-blind randomized placebo-controlled trial. Liver Transpl 2002;8:132-42.

Nikolaidis N, Vassiliadis T, Giouleme O, et al. Effect of lamivudine treatment in patients with decompensated cirrhosis due to anti-HBe positive/HBeAg-negative chronic hepatitis B. Clin Transplant 2005;19:321-6.

O'Grady JG. Acute liver failure. Postgrad Med J 2005;81:148-54.

Ono SK, Kato N, Shiratori Y, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J Clin Invest 2001;107:449-55.

Opelz G, Daniel V, Naujokat C, Fickenscher H, Döhler B. Effect of cytomegalovirus prophylaxis with immunoglobulin or with antiviral drugs on post-transplant non-Hodgkin lymphoma: a multicentre retrospective analysis. Lancet Oncol 2007;8:212-8.

Orr DW, Portmann BC, Knisley AS, et al. Anti-interleukin 2 receptor antibodies and mycophenolate mofetil for treatment of steroid-resistant rejection in adult liver transplantation. Transplant Proc 2005;37:4373-9.

Pabón V, Dumortier J, Gincul R, et al. Long-term results of liver transplantation for Wilson's disease. Gastroenterol Clin Biol. 2008;32(4):378-81.

Pascher A, Sauer IM, Walter M, et al. Donor evaluation, donor risks, donor outcome, and donor quality of life in adult-to-adult living donor liver transplantation. Liver Transpl 2002;8:829-37.

Patel R, Snydman DR, Rubin RH, et al. Cytomegalovirus prophylaxis in solid organ transplant recipients. Transplantation 1996;61:1279-89.

Patel S, Orlaoff M, Tsoulfas G, et al. Living donor liver transplantation in the United States: identifying donors at risk for perioperative complications. Am J Transplant 2007;7:2344-9.

Paya C, Humar A, Dominguez E, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant 2004;4(4):611-20.

Perkins JD. Biliary tract complications: The most common postoperative complication in living liver donors. Liver Transpl 2008:14(9):1372-7.

Perlmutter DH. Alpha-1-antitrypsin deficiency. Semin Liver Dis 1998; 18: 217-25.

Perney P, Bismuth M, Sigaud H, et al. Are preoperative patterns of alcohol consumption predictive of relapse after liver transplantation for alcoholic liver disease? Transpl Int 2005;18:1292-7.

Picciotto FP, Tritto G, Lanza AG, et al. Sustained virological response to antiviral therapy reduces mortality in HCV reinfection after liver transplantation. J Hepatol 2007:46(3):459-65.

Pfitzmann R, Schwenzer J, Rayes N, Seehofer D, Neuhaus R, Nüssler NC. Long-term survival and predictors of relapse after orthotopic liver transplantation for alcoholic liver disease. Liver Transpl 2007;13(2):197-205.

Prados E, Cuervas-Mons V, de la Mata M, et al. Outcome of autoimmune hepatitis after liver transplantation. Transplantation 1998;66:1645-50.

Qiu J, Ozawa M, Terasaki PI. Liver transplantation in the United States. Clin Transpl 2005:17-28.

Raimondo ML, Dagher L, Papatheodoridis GV, et al. Long-term mycophenolate mofetil monotherapy in combination with calcineurin inhibitors for chronic renal dysfunction after liver transplantation. Transplantation 2003;75:186-90.

Rea DJ, Heimbach JK, Rosen CB. Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. Ann Surg 2005;242(3):451-8.

Ringe B, Wittekind C, Bechstein WO, et al. The role of liver transplantation in hepatobiliary malignancy. A retrospective analysis of 95 patients with particular regard to tumor stage and recurrence. Ann Surg 1989;209:88-98.

Roayie K, Feng S. Allocation policy for hepatocellular carcinoma in the MELD era: room for improvement? Liver Transpl 2007;13:S36-43.

Rosenau J, Bahr MJ, Tillmann HL, et al. Lamivudine and low-dose hepatitis B immune globulin for prophylaxis of hepatitis B reinfection after liver transplantation possible role of mutations in the YMDD motif prior to transplantation as a risk factor for reinfection. J Hepatol 2001;34:895-902.

Rudraraju M, Osowo AT, Singh V, Carey EJ. Do patients need more frequent colonoscopic surveillance after liver transplantation? Transplant Proc 2008;40(5):1522-4.

Saab S, Niho H, Comulada S, et al. Mortality predictors in liver transplant recipients with recurrent hepatitis C cirrhosis. Liver Int 2005;25:940-5.

Samonakis DN, Cholongitas E, Triantos CK, et al. Sustained, spontaneous disappearance of serum-HCV under immunosuppression after liver transplantation for hepatitis C cirrhosis. J Hepatol 2005;43:1091-3.

Sanchez EQ, Martin AP, Ikegami T, et al. Sirolimus conversion after liver transplantation: improvement in measured glomerular filtration rate after 2 years. Transplant Proc 2005;37:4416-23.

Schacherer D, Zeitoun M, Buttner R, et al. Sirolimus-induced drug fever and ciclosporin-induced leukencephalopathia with seizures in one liver transplant recipient. World J Gastroenterol 2007;13:6090-3.

Seaberg EC, Belle SH, Beringer KC, et al. Liver transplantation in the United States from 1987-1998: updated results from the Pitt-UNOS Liver Transplant Registry. In: Cecka JM, Terasaki PI, eds. Clinical Transplants 1998. Los Angeles: UCLA Tissue Typing Laboratory; 1999:17-37.

Seehofer D, Rayes N, Steinmuller T, et al. Occurrence and clinical outcome of lamivudine-resistant hepatitis B infection after liver transplantation. Liver Transpl 2001;7:976-82.

Schiff E, Lai CL, Hadziyannis S, et al. Adefovir dipivoxil for wait-listed and post-liver transplantation patients with lamivudine-resistant hepatitis B: final long-term results. Liver Transpl 2007;13:349-60.

Schlitt HJ, Barkmann A, Boker KH, et al. Replacement of calcineurin inhibitors with mycophenolate mofetil in liver-transplant patients with renal dysfunction: a randomised controlled study. Lancet 2001;357:587-91.

Sevmis S, Karakayali H, Aliosmanoglu I, et al. Transplantation for Wilson's disease. Transplant Proc 2008;40(1):228-30.

Shakil A O, Lilly L, Angus P, et al. Entecavir significantly reduces viral load in liver transplant recipients failing lamivudine therapy for HBV. J Hepatol 2002;36:122.

Sheiner PA, Boros P, Klion FM et al. The efficacy of prophylactic interferon alfa-2b in preventing recurrent hepatitis after liver transplantation. Hepatology 1998;28:831-8.

Shergill AK, Khalili M, Straley S, et al. Applicability, tolerability, and efficacy of preemptive antiviral therapy in hepatitis C infected patients undergoing liver transplantation. Am J Transplant 2005;5:118-24.

Shimoda M, Farmer DG, Colquhoun SD, et al. Liver transplantation for cholangiocellular carcinoma: analysis of a single-center experience and review of the literature. Liver Transpl 2001;7(12):1023-33.

Singh N, Gayowski T, Wannstedt CF, et al. Interferon-alpha for prophylaxis of recurrent viral hepatitis C in liver transplant recipients: a prospective, randomized, controlled trial. Transplantation 1998;65:82-6.

Singh N. Fungal infections in the recipients of solid organ transplantation. Infect Dis Clin North Am 2003:17(1):113-34.

Smets F, Sokal EM. Epstein-Barr virus-related lymphoproliferation in children after liver transplant: role of immunity, diagnosis, and management. Pediatr Transplant 2002;6:280-7.

Sotiropoulos GC, Molmanti EP, Lösch C, Beckebaum S, Brolesch CE, Lang H. Meta-analysis of tumor recurrence after liver transplantation for hepatocellular carcinoma based on 1,198 cases. Eur J Med Res 2007;12(10):527-34.

Sotiropoulos GC (b), Lang H, Sgourakis G, et al. Liberal policy in living donor liver transplantation for hepatocellular carcinoma: Lessons learned. Dig Dis Sci. 2008. Epub ahead of print.

Sotiropoulos CG(a), Beckebaum S, Lang H, et al. Single-center experience on liver transplantation for hepatocellular carcinoma arising in alcoholic cirrhosis: results and ethical issues. Eur Surg Res 2008; 40(1):7-13.

Starzl TE, Marchioro TL, Von Kaulla KN, et al. Homotransplantation of the liver in humans. Surg Gynecol Obstet 1963;117:659-76.

Starzl TE, Groth CG, Brettschneider L, et al. Orthotopic homotransplantation of the human liver. Ann Surg 1968; 168 (3):392-415.

Stewart SF, Hudson M, Talbot D, Manas D, Day CP. Mycophenolate mofetil monotherapy in liver transplantation. Lancet 2001;357:609-10.

Study 109. Medical review baraclude (entecavir): Antiviral activity of entecavir in patients receiving liver transplant due to chronic hepatitis B virus infection. (Accessed December 14, 2007, at http://www.clinicaltrials.gov.)

Sugawara Y, Makuuchi M. Technical advances in living-related liver transplantation. J Hepatobiliary Pancreat Surg 1999;6:245-53.

Sugawara Y, Makuuchi M, Matsui Y et al. Preemptive therapy for hepatitis C virus after living-donor liver transplantation. Transplantation 2004;78:1308-11.

Sugawara Y, Makuuchi M. Living donor liver transplantation: present status and recent advances. Br Med Bull 2005;75-76:15-28.

Sutcliffe RP, Maguire DD, Muiesan P, et al. Liver transplantation for Wilson's disease: long-term results and quality-of-life assessment. Transplantation 2003;75(7):1003-6.

Sylvestre PB, Batts KP, Burgart LJ, Poterucha JJ, Wiesner RH. Recurrence of primary biliary cirrhosis after liver transplantation: Histologic estimate of incidence and natural history. Liver Transpl 2003;9:1086-93.

Tan J, Lok AS. Antiviral therapy for pre- and post-liver transplantation patients with hepatitis B. Liver Transpl 2007;13:323-6.

Testa G, Crippin JS, Netto GJ, et al. Liver transplantation for hepatitis C: recurrence and disease progression in 300 patients. Liver Transpl 2000;6:553-61.

Thomas RM, Brems JJ, Guzman-Hartman G, Yong S, Cavaliere P, van Thiel DH. Infection with chronic hepatitis C virus and liver transplantation: a role for interferon therapy before transplantation. Liver Transpl. 2003;9(9):905-15.

Tillmann HL. Antiviral therapy and resistance with hepatitis B virus infection. World J Gastroenterol 2007;13:125-40.Trotter JF. Sirolimus in liver transplantation. Transplant Proc 2003;35(3 Suppl): 193S-200S.

Toniutto P, Fabris C, Fumo E, et al. Pegylated versus standard interferon-alpha in antiviral regimens for post-transplant recurrent hepatitis C: comparison of tolerability and efficacy. J Gastroenterol Hepatol 2005;20:577-82.

Toso C, Meeberg GA, Bigam DL, et al. De novo sirolimus-based immunosuppression after liver transplantation for hepatocellular carcinoma: long-term outcomes and side effects. Transplantation. 2007;83(9):1162-8.

Triantos C, Samonakis D, Stigliano R, Thalheimer U, Patch D, Burroughs A. Liver transplantation and hepatitis C virus: systematic review of antiviral therapy. Transplantation 2005;79:261-8.

Tung BY, Kimmey MB. Biliary complications of orthotopic liver transplantation. Dig Dis 1999;17:133-44.

Ulrich C, Degen A, Patel MJ, Stockfleth. Sunscreens in organ transplant patients. Nephrol Dial Transplant 2008;23:1805-8.

Valentin-Gamazo C, Malago M, Karliova M, et al. Experience after the evaluation of 700 potential donors for living donor liver transplantation in a single center. Liver Transpl 2004; 10: 1087-96.

Vallejo GH, Romero CJ, de Vicente JC. Incidence and risk factors for cancer after liver transplantation. Crit Rev Oncol Hematol 2005;56:87-99.

van Bommel F, Wunsche T, Mauss S, et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40:1421-5.

van Bommel F, De Man RA, Stein K, et al. A multicenter analysis of antiviral response after one year of tenofovir monotherapy in HBV-monoinfected patients with prior nucleos(t)ide analog experience. J Hepatol 2008;48: 73.

Vassiliadis T, Giouleme O, Koumerkeridis G, et al. Adefovir dipivoxil plus lamivudine combination treatment is superior to adefovir dipivoxil monotherapy in lamivudine-resistant hepatitis B e antigen-negative chronic hepatitis B patients. Hepatology 2007;46:662A.

Vennarecci G, Gunson BK, Ismail T, et al. Transplantation for end stage liver disease related to alpha 1 antitrypsin. Transplantation 1996;61:1488-95.

Vera A, Moledina S, Gunson B, et al. Risk factors for recurrence of primary sclerosing cholangitis of liver allograft. Lancet 2002;360:1943-4.

Villeneuve JP, Condreay LD, Willems B, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000;31:207-10.

Vogel A, Heinrich E, Bahr MJ, et al. Long-term outcome of liver transplantation for autoimmune hepatitis. Clin Transplant 2004;18:62-9.

Walsh TJ, Groll AH. Emerging fungal pathogens: Evolving challenges to immunocompromised patients for the twenty first century. Transpl Infect 1999;1:247-61.

Washington K. Update on post-liver transplantation infections, malignancies, and surgical complications. Adv Anat Pathol 2005;12:221-6.

Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. Hepatology 2003; 38:1282-88.

Watson CJ, Friend PJ, Jamieson NV, et al. Sirolimus: a potent new immunosuppressant for liver transplantation. Transplantation 1999;67:505-9.

Weiss KH, Gotthardt D, Schmidt J, et al. Liver transplantation for metabolic liver diseases in adults: indications and outcome. Nephrol Dial Transplant 2007;22(Suppl 8):viii9-12.

Welch CS. A note on transplantation of the whole liver in dogs. Transplant Bull 1955;2:54-5.

Wiesner RH, Shorr JS, Steffen BJ, Chu AH, Gordon RD, Lake JR. Mycophenolate mofetil combination therapy improves long-term outcomes after liver transplantation in patients with and without hepatitis C. Liver Transpl 2005;11:750-9.

Wigg AJ, Gunson BK, Mutimer DJ. Outcomes following liver transplantation for seronegative acute liver failure: experience during a 12-year period with more than 100 patients. Liver Transpl 2005;11:27-34.

Wong F. Hepatorenal syndrome: current management. Curr Gastroenterol Rep 2008;10(1):22-9.

Wong PY, Portmann B, O'Grady JG, et al. Recurrence of primary biliary cirrhosis after liver transplantation following FK506-based immunosuppression. J Hepatol 1993;17:284-7.

Wong SN, Chu CJ, Wai CT, et al. Low risk of hepatitis B virus recurrence after withdrawal of long-term hepatitis B immunoglobulin patients receiving maintenance nucleos(t)ide analogue therapy. Liver Transpl 2007;13:374-81.

Yamagiwa S, Ichida T. Recurrence of primary biliary cirrhosis and primary sclerosing cholangitis after liver transplantation in Japan. Hepatol Res 2007;37(Suppl 3):S449-54.

Yan ML, Yan LN, Li B, et al. Intramuscular hepatitis B immune globulin combined with lamivudine in prevention of hepatitis B recurrence after liver transplantation. Hepatobiliary Pancreat Dis Int 2006;5:360-3.

Yao FY, Osorio RW, Roberts JP, et al. Intramuscular hepatitis B immune globulin combined with lamivudine for prophylaxis against hepatitis B recurrence after liver transplantation. Liver Transpl Surg 1999;5:491-6.

Yao FY, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. Hepatology 2001;34:411-6.

Yao FY, Gautam M, Palese C, et al. De novo malignancies following liver transplantation: a case-control study with long-term follow-up. Clin Transplant 2006;20(5):617-23.

Yao FY, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. Am J Transpl 2007;7(11):2587-96.

Yamagiwa S, Ichida T. Recurrence of primary biliary cirrhosis and primary sclerosing cholangitis after liver transplantation in Japan. Hepatol Res 2007;37(Suppl 3):S449-54.

Yokoyama I, Todo S, Iwatsuki S, Starzl TE. Liver transplantation in the treatment of primary liver cancer. Hepatogastroenterology 1990;37:188-93.

Yoo HY, Patt CH, Geschwind JF, et al. The outcome of liver transplantation in patients with hepatocellular carcinoma in the United States betweeen 1988 and 2001: 5-year survival has improved significantly with time. J Clin Oncol 2003;21:4329-35.

Yoshida H, Kato T, Levi DM, et al. Lamivudine monoprophylaxis for liver transplant recipients with non-replicating hepatitis B virus infection. Clin Transplant 2007;21:166-71.

Zavaglia C, De Carlis L, Alberti AB, et al. Predictors of long term survival after liver transplantation for hepatocellular carcinoma. Am J Gastroenterol 2005;100:2708-16.

Zheng S, Chen Y, Liang T, et al. Prevention of hepatitis B recurrence after liver transplantation using lamivudine or lamivudine combined with hepatitis B immunoglobulin prophylaxis. Liver Transpl 2006;12:253-8.

Zimmerman MA, Trotter JF, Wachs M, et al. Sirolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. Liver Transpl 2008;14(5):633-8.

Ziolkowski J, Paczek L, Senatorski G, et al. Renal function after liver transplantation: calcineurin inhibitor nephrotoxicity. Transplant Proc 2003;35(6):2307-9.

Zoepf T, Maldonado-Lopez EJ, Hilgard P, et al. Balloon dilatation vs. balloon dilatation plus bile duct endoprostheses for treatment of anastomotic biliary strictures after liver transplantation. Liver Transpl 2006;12:88-94.

Chapter 23: Management of ESLD in HIV coinfection

José M. Miró, Fernando Agüero, Montserrat Laguno, Christian Manzardo, Montserrat Tuset, Carlos Cervera, Asuncion Moreno, Juan-Carlos García-Valdecasas, Antonio Rimola, Hospital Clinic OLT in HIV Working Group.

Introduction

Liver disease due to chronic hepatitis B and C is currently one of the leading causes of morbidity and mortality among HIV-positive patients in the developed world. Non-coinfected patients with chronic hepatitis C tend to progress to end-stage liver disease (ESLD) in 20-30 years, whereas coinfected patients have higher rates of progression (Mohsen 2003; Poynard 2003). One meta-analysis demonstrated a higher overall adjusted relative risk (RR) of histological cirrhosis or decompensated liver disease in patients coinfected with HIV and the hepatitis C virus (HCV) than in HCV-monoinfected patients (Graham 2001). ESLD is common in coinfected patients. We review different approaches to the condition.

Epidemiology

Of the approximately 40 million persons infected with HIV globally, 2 to 4 million are chronically infected with the hepatitis B virus (HBV) and 4 to 5 million have chronic HCV (Alter 2006).

The prevalence of HCV and/or HBV coinfection is high in developed countries. Studies performed in European HIV-positive patients showed rates of 33% and 9%, respectively (Rockstroh 2003; Konopnicki 2005), while in the USA figures are very similar, 28% and 9% (Fung 2004). Other authors have addressed the significance of HCV as a cause of non–AIDS-related death (Palella 2006; Crum 2006; Lewden 2005). One single-centre study (Martínez 2007) in Spain analysed the cause of 235 deaths in 4471 patients (5%) on combination antiretroviral therapy (cART) from 1997 until 2004. The number of patients who died from ESLD increased from 8% in 1997 to 41% in 2004, and in recent years this condition has become the leading cause of death in HIV-positive patients. In comparison with the general population of a similar age, deaths due to liver disease were 11 times more frequent in HIV-positive patients. Another prospective multicenter study (Rosenthal 2007) in France determined mortality due to ESLD in a nationwide population of HIV-positive patients. The authors followed a total of 20,940 HIV-positive patients, 4005 (19.9%) of whom were coinfected, and showed that, in 2003, mortality due to ESLD represented 23.7% of non–AIDS-related deaths. In this population, ESLD was fatal in 1.5% of patients in 1995, 6.6% in 1997, 14.3% in 2001, and 12.6% in 2003, and 92.6% of patients who died from ESLD had chronic HCV infection. A prospective, observational study of 11 cohorts carried out in Europe, the United States and Australia included 23,441 HIV-1–infected patients (22.5% were HCV-positive) and followed them from December 1999 until February 2004. This study showed that, of the 1246 deaths recorded, those related to AIDS were the most frequent (31.1%), while liver disease was the most frequent non–AIDS-related cause (14.5%). HCV infection was shown to be an

independent predictor of liver-related death (D:A:D 2006). As for hepatocellular carcinoma (HCC), a recent study comparing liver-related deaths in HIV-positive patients (Salmon-Ceron 2009) described an increase in mortality due to HCC from 15% in 2000 to 25% in 2005 (p=0.04).

Clinical features of coinfected patients with ESLD

One recent prospective study (Pineda 2009) that enrolled 154 patients with a new diagnosis of Child-Turcotte-Pugh class A compensated cirrhosis found that 36 patients (23.4%) developed a first hepatic decompensation during follow-up (mean 36 months). The probability of developing decompensated cirrhosis at 3 and 5 years was 26% and 33%, respectively. A multivariate analysis revealed that the factors predicting the emergence of an episode of hepatic decompensation at 5 years were Child-Turcotte-Pugh stage (p=0.007; HR, 3.33 [95% CI, 1.39-7.69]), lack of anti-HCV therapy (p=0.035; HR, 3.38 [95% CI, 1.14-5.04]), and baseline CD4 cell count below 300 cells/mm^3 (p=0.021; HR, 2.40 [95% CI, 1.09-10.53]).

In a retrospective study, the same authors (Pineda 2005) described the frequency of specific events, such as the first decompensation and cause of death in HIV-negative and HIV/HCV-coinfected subjects. Ascites and jaundice were more frequent among HIV-positive patients, while upper gastrointestinal bleeding and HCC were more frequent in monoinfected patients. Hepatic encephalopathy (HE) as both first decompensation and cause of death was higher in coinfected patients.

The clinical characteristics and outcome of spontaneous bacterial peritonitis (SBP) were evaluated in an HIV-positive population with cirrhosis (Shaw 2006). Thirty-five HIV-positive patients with cirrhosis were compared with 70 HIV-negative patients with cirrhosis. An aetiologic diagnosis was made in almost 80% of the HIV-positive cases and bacteraemia was present in more than 50%, with both rates being higher than those observed in HIV-negative patients. An important bacteriological finding in this study was the high incidence of Streptococcus pneumoniae as the etiologic agent of SBP among HIV-positive patients, second only to Escherichia coli.

HCC has a faster and worse outcome in HIV/HCV-coinfected patients than in HCV-monoinfected patients (Puoti 2004; Bruno 2006).

The findings on HCC in 41 HIV-positive and 2384 HIV-negative patients were compared in an Italian study (Puoti 2004). The authors found a more aggressive course of HCC in HIV-positive patients, with an independent association between HIV infection and a more advanced stage of HCC at clinical presentation, in addition to a higher rate of infiltrating neoplasm and extrahepatic-extranodal metastasis. Furthermore, portal vein thrombosis was more frequent among HIV-positive patients with HCC.

A retrospective study from Canada and the USA compared 63 HIV-positive patients with HCC and 226 HIV-negative patients with HCC (Bräu 2007) and revealed that HIV+ patients were younger and more frequently symptomatic. In this cohort, median survival was similar between HIV-positive (6.9 months) and HIV-negative patients (7.5 months, p=0.44, log-rank), as was tumour stage.

Survival of HBV/HCV-monoinfected patients with HCC detected by screening has improved in recent years due to the advent of liver transplantation and radiofrequency ablation (RFA) (Chan 2008).

Prognosis after decompensation

The survival rate of HIV-positive patients with decompensated cirrhosis is much lower than that of HIV-negative patients—approximately 50% at 1 year of follow-up (Pineda 2005; Merchante 2006; Murillas 2009). In a multicentre case-control study (Pineda 2005), the outcome of cirrhosis after the first decompensation in coinfected patients was much worse than in the monoinfected population. Survival at 1, 2, and 5 years for the coinfected/monoinfected population was 54%/74%, 40%/61%, and 25%/44%, respectively. In another study (Merchante 2006) severity of liver disease (Child-Turcotte-Pugh score or HE as the first hepatic decompensation) and the level of cellular immunosuppression (< 100 CD4 cells) were identified as independent predictors of poor outcome in coinfected patients. On the other hand, cART was associated with a reduced mortality rate.

Another study followed the outcome of 104 HIV-positive patients with HCC or cirrhosis after their first hepatic decompensation (Murillas 2009). The median survival time of this cohort was 14 months, similar to that observed by Merchante (13 months). This study included HCV and non–HCV-infected patients, and it did not find significant differences in survival based on the aetiology of cirrhosis, suggesting that HIV-positive patients have an overall poor outcome regardless of the nature of their liver disease. Furthermore, the MELD score and the inability to reach an undetectable plasma HIV-1 viral load at any time during follow-up were the only variables independently associated with the risk of death ($p<0.001$). This is particularly relevant because recently the MELD score has been increasingly used to establish the prognosis of patients with cirrhosis and, consequently, to indicate liver transplantation.

Another recent prospective study (Girón-González 2007) that enrolled 92 HIV-positive patients with compensated and decompensated cirrhosis observed that the overall probability of death was 25% and 37% at 1 and 2 years, respectively. Independent factors associated with mortality due to liver cirrhosis were Child-Turcotte-Pugh score at inclusion, progression of this score at follow-up, more than one decompensation during follow-up, and absence of cART at follow-up.

HIV-positive patients with cirrhosis have a poor prognosis after the development of SBP (Shaw 2006). HIV infection was associated with a more than 6-fold increase in the probability of dying within a month of the first episode of SBP. Impaired renal function at diagnosis and severity of liver disease were identified as predictors of death. HIV-positive patients also had a dramatically shorter survival time than HIV-negative patients: only 50% of patients were still alive 3 months after the first episode of SBP and only 23% were alive after 1 year. Death was mostly related to complications of advanced liver disease rather than to AIDS-related conditions.

High mortality rates among coinfected patients with ESLD waiting for liver transplantation have also been reported in observational studies (Maida 2005; Prieto 2008; Murillas 2009). In one study (Maida 2005), death due to ESLD occurred in 25% of patients during the evaluation period. Another study analysed 18 patients who were accepted for OLT and placed on the waiting list. Eight (44%) received a transplant, 8 (44%) died while on the waiting list, and 2 (12%) were still on the waiting list at the end of the study (Prieto 2008). 10 (67%) out of 15 patients on the transplant waiting list died after a median follow-up of 5 months, and 5 (33%) underwent liver transplantation (Murillas 2009).

Two case-control studies have analysed mortality rates among coinfected patients with ESLD waiting for liver transplantation. In the first (Ragni 2005), mortality rates during the pre-transplant evaluation in HIV-positive (N=58) and HIV-negative (N=1359) patients were 36% and 15%, respectively ($p<0.001$), although these data were not confirmed by a recent American multicentre study (Subramanian 2009). Waiting list mortality was 14.4% in patients with HIV infection (N=167) and 11.1% in the control group (N=792) ($p=0.30$). In the multivariate analysis, a MELD score higher than 25 was the only variable related to death on the waiting list (Subramanian 2009).

A recent French study (Carmona 2009) identified factors associated with the mortality of HIV/HCV-coinfected patients on the waiting list. The authors analysed different scores: MELD, MELD-Na, Child-Turcotte-Pugh, and ASA. Multivariate analysis showed that presence of cART at the time of the first referral ($p=0.04$), ASA score ($p=0.04$), and Child-Turcotte-Pugh score ($p=0.02$) were associated with mortality. The findings suggest that the Child-Turcotte-Pugh score rather than the MELD score should be used to predict mortality in these patients.

Physicians attending HIV-positive patients with cirrhosis should follow patients prospectively and evaluate them early for OLT after the first clinical decompensation of liver disease. Similarly, patients whose cirrhosis is associated with HCC should also be evaluated (Llovet 2004). Both prevention and effective treatment of these complications may improve the likelihood of survival until OLT, and this should be performed also with the HIV-negative patients (Agüero 2007; Merchante 2007).

Management of cirrhosis complications

Management of the complications of cirrhosis (portal hypertension, ascites, gastrointestinal bleeding, encephalopathy, SBP, HCC, and hepatorenal syndrome) must be planned, just as in the HIV-negative population (Cardenas 2005; Han 2006; Arroyo 2008). Medical management also includes prevention of infection. In view of the short survival associated with the development of SBP, primary antibiotic prophylaxis with quinolones or trimethoprim-sulfamethoxazole should be considered (Fernández 2007).

Another study (Pineda 2009) shows that transient elastometry could be used to select HIV/HCV-coinfected patients undergoing screening with upper gastrointestinal endoscopy for oesophageal varices. This study found that HIV/HCV-coinfected patients with cirrhosis who harbour oesophageal varices requiring preventive therapy for bleeding had liver stiffness values higher than those who did not require treatment. Liver stiffness values lower than 21 kPa were highly predictive of varices not at risk for bleeding.

As far as HCC is concerned, patients may benefit from more frequent imaging, i.e., every 3 months (Bräu 2007). Treatment of HCC may not be successful, depending on the stage. HCC is incurable in advanced stages.

In patients with hepatorenal syndrome, haemodialysis can be used as a bridge to liver transplantation; otherwise, it is usually fatal (Han 2006). The molecular adsorbent recirculating system (MARS) could be a new therapeutic tool in this setting, and may prove highly effective if combined with transplant/retransplantation (Gaspari 2006), although more evidence in coinfected patients with ESLD and hepatorenal syndrome is needed.

Other issues that may delay the progression of liver disease, such as avoidance of hepatotoxic drugs (e.g., didanosine) and vaccination for hepatitis A and B, should be heeded.

Substance abuse

Smoking has been linked to HCC (Kuper 2000) and increased hepatic fibrosis (Pessione 2001) and it may also increase histological activity in chronic HCV patients irrespective of alcohol consumption (Hezode 2003).

According to one study (Rosenthal 2007), alcohol consumption was more frequent among coinfected patients who died from ESLD (92%), and another study suggested that excess alcohol consumption increases HCV RNA levels (Cooper 2005).

In addition, a recent study found that daily cannabis smoking was significantly associated with the presence of moderate to severe fibrosis in patients with chronic HCV infection and recommend that those with hepatitis C cirrhosis should abstain from or reduce cannabis use (Ishida 2008).

HCV/HBV management

Specific treatment for infection with HBV or HCV is possible, although more difficult, in patients with advanced cirrhosis, especially for HCV infection (Soriano 2007; Rockstroh 2008).

One of the objectives when treating HCV-monoinfected patients with advanced liver cirrhosis using pegylated-interferon plus ribavirin is to obtain undetectable plasma HCV RNA levels at the time of OLT in order to reduce the risk of HCV recurrence post-transplant. One study (Everson 2005) using a low accelerating dosage regimen (LADR) of anti-HCV therapy in monoinfected patients on the OLT waiting list showed that 30 (24%) of 124 patients achieved a sustained virological response (SVR) and 12 (80%) of 15 patients who were HCV RNA–negative before OLT remained HCV RNA–negative 6 months or more after transplantation. This approach has not yet been addressed in coinfected patients, although safety data can be extrapolated from the APRICOT substudy (Mauss 2004). Hepatic decompensation was observed only in HIV/HCV-coinfected patients with markers of advanced cirrhosis, and its incidence was 10.4% (14/134). However, 6 (43%) of the 14 patients died as a result of hepatic decompensation. One of the associated risk factors was antiretroviral treatment with didanosine. In contrast, no hepatic decompensation was noted in HIV/HCV-coinfected patients without cirrhosis. Therefore, anti-HCV treatment during the pre-transplant evaluation or while patients are on the waiting list should be individualized (e.g., patients with Child-Turcotte-Pugh class A and HCC or genotypes 2/3) and patients must be monitored closely because of their high risk of hepatic decompensation and death.

According to the latest AADSL Practice Guidelines for HCV infection (Ghany 2009), HIV-infected patients with decompensated liver disease (CTP class B or C) should not be treated with peg-interferon a + ribavirin, and should be considered candidates for liver transplantation (Grading IIa, C).

In a recent case-control study (Iacobellis 2007) comprising 129 HCV-monoinfected patients with decompensated cirrhosis, 66 were treated with peg-interferon plus ribavirin for 24 weeks and compared with the untreated control group (n=63). Thirteen patients discontinued treatment due to intolerance. SVR was observed in 82.6%, 43.5%, 30.2%, and 7% for HCV genotypes 2, 3, 1, and 4, respectively. In the treated group, the odds ratio for severe infection or death due to infection was higher, whereas ascites, encephalopathy, and oesophageal bleeding decreased. During follow-up, there were 15 deaths in the

controls and 9 in the non-responders. All patients who experienced SVR survived and did not need to undergo OLT. The authors concluded that HCV clearance via therapy is life-saving and reduces disease progression in HCV-monoinfected patients.

Since HBV replication is a contraindication for OLT and only patients without HBV viraemia are accepted for OLT, treatment of this infection should be a priority. HIV-positive patients who require antiretroviral therapy and have chronic HBV infection can be treated with lamivudine (or emtricitabine) and tenofovir as part of their triple antiretroviral therapy (Soriano 2007; GESIDA/PNS Panel of Experts 2009).

Adefovir and tenofovir have proven useful against HBV and could be used in cases of resistance to lamivudine (Soriano 2007).

Combination antiretroviral therapy (cART)

The role of cART in progression of liver disease and in overall mortality in HCV/HIV-coinfected patients remains controversial (Tedaldi 2003; Qurishi 2003). The use of protease inhibitors may offer protection from the progression of HCV-related fibrosis (Benhamou 2001; Macías 2006). Antiretroviral drug regimens should be carefully planned in persons with HIV and ESLD. These patients should follow general recommendations (GESIDA/PNS Panel of Experts 2009; Panel on Antiretroviral Guidelines for Adults and Adolescents 2009, Hammer 2008) and their liver function must be closely monitored for signs of hepatotoxicity. Careful consideration of drug prescriptions and possible interactions is essential.

Furthermore, some antiretroviral drugs may be contraindicated in cirrhotic patients (e.g., didanosine, nevirapine, full-dose ritonavir) and their dosing should be adjusted according to the degree of hepatic impairment (Wyles 2005; Back 2009; Tuset 2009).

Therapeutic drug monitoring (TDM) may be useful for efavirenz and protease inhibitors. Indinavir and atazanavir can increase unconjugated bilirubin levels by inhibiting UDP-glucuronosyltransferase. As total bilirubin is a component of both the Child-Turcotte-Pugh and MELD scores, results in patients taking these drugs should be interpreted with caution.

It is noteworthy that the new antiretroviral drug raltegravir, which is not a substrate of CYP450, can be used in HIV-1 OLT recipients. A recent French study (Tricot 2009) enrolled 13 patients with HIV-1 infection who underwent solid organ transplantation (8 liver and 5 kidney) and received raltegravir. The authors found a lack of significant interaction between raltegravir and calcineurin inhibitors that allowed simplified management of immunosuppressive treatment, excellent tolerability, and no events related to outcome (acute rejection) or HIV infection.

Finally, given the speed with which new antiretrovirals appear and thus new interactions, physicians should consult updated databases on drug interactions (Back 2009; Tuset 2009).

Orthopic liver transplant (OLT)

OLT is the only therapeutic option for patients with ESLD. HIV infection is not a contraindication for liver transplantation (Miro 2007; Stock 2007; Samuel 2008; Norris 2008). There are 3 different classes of criteria for including HIV-positive patients on the liver transplant waiting list: liver disease, HIV infection, and other criteria.

Liver disease criteria

These are the same as for the non–HIV-infected population; the main indication for OLT in HIV-positive patients is ESLD caused by HCV coinfection. Less frequent indications are HBV coinfection (either acute or ESLD) and liver cancer.

In the UK guidelines (O'Grady 2005), indications for liver transplantation include acute liver failure, decompensated liver disease—with ascites, encephalopathy (it is important to exclude HIV-related dementia), variceal bleeding that is difficult to manage with standard therapy, and poor liver function (albumin <30 g/l, INR >1.5, and elevated serum bilirubin >450 mmol/l)—and HCC detected during regular tumour surveillance. In the Eurotransplant region these criteria have been replaced by the MELD score. The criteria for liver transplantation in patients with HCC are as follows: no more than 3 tumour nodules, no nodule greater than 5 cm in diameter, absence of macroscopic portal vein invasion, and absence of recognizable extrahepatic disease.

A new indication for liver transplant in HIV+ patients has been described in a recent French study (Tateo 2009) in which 3 patients underwent liver transplantation and the cause of ESLD was nodular regenerative hyperplasia (NRH). OLT is the only therapeutic option in cases of severe portal hypertension such as that observed in these patients.

HIV infection criteria

Most liver transplant groups from Europe and North America use similar HIV criteria. These are summarized in Table 1 (O'Grady 2005; Grossi 2005; Miro 2007; Anonymous 2004).

	Spain [Miro 2005]	Italy [Grossi 2005]	UK [O'Grady 2005]	USA [Anon 2004]
Previous C events				
Opportunistic infections	Some*	None in the previous year	None after ART-induced immunological reconstitution	Some**
Neoplasms	No	No		No
CD4 cell count/mm^3	>100***	>200 or >100 if decompensated cirrhosis	>200 or >100 if portal hypertension	>100***
Plasma HIV-1 RNA viral load BDL on HAART**	Yes	Yes	Yes	Yes

** In Spain, patients with previous tuberculosis, Pneumocystis jiroveci pneumonia (PCP) or esophageal candidiasis can be evaluated for OLT; ** In USA, PCP and esophageal candidiasis were not exclusión criteria; *** Patients with previous OIs should have >200 CD4 cells/mm^3; **** If PVL was detectable, post-OLT supression with cART should be predicted in all patients.*

Table 1. HIV criteria for OLT in some European countries and the US.

Clinical criteria

Some authors are in favour of withdrawing exclusion criteria for some opportunistic infections that can be efficaciously treated and prevented, such as tuberculosis, candidiasis, and Pneumocystis jiroveci pneumonia (Roland 2003; Neff 2004; Radecke 2005). In fact, the NIH-sponsored study has recently updated the inclusion criteria for opportunistic complications and only untreated diseases are still an exclusion criteria for liver transplantation (e.g., progressive multifocal leukoencephalopathy, chronic cryptosporidiosis, multidrug-resistant systemic fungal infections, primary CNS lymphoma, and visceral Kaposi's sarcoma) (Roland 2006).

Immunological criteria

All groups agree that the CD4+ lymphocyte count should be above 100 cells/mm^3 for OLT (Roland 2003; Neff 2004). This figure is lower than that for kidney transplantation (CD4 >200 cells/mm^3), because patients with cirrhosis often have lymphopenia due to hypersplenism, which leads to a lower absolute CD4+ count, despite high CD4 percentages and good virologic control of HIV. In Spain and the USA, the CD4+ count must be greater than 200 cells/mm^3 in patients with previous opportunistic infections (Miro 2005; Anonymous 2004).

In Italy (Grossi 2005) and the UK (O'Grady 2005) the CD4+ cut-off is 200 cells/mm^3, unless patients have decompensated cirrhosis or portal hypertension. In these scenarios, they use the same CD4+ cell threshold as in Spain and the USA (100 cells/mm^3).

Virological criteria

The essential criterion for OLT is that the patient must be able to have effective, safe and long-lasting cART during the post-transplant period (Neff 2004; Fung 2003). The ideal situation is one in which the patient tolerates cART before transplant and is ready for the transplant with undetectable HIV viral load by ultra-sensitive techniques (<50 copies/ml). Some patients do not have an indication for cART, as they are long-term non-progressors with no immunological criteria (CD4+ lymphocyte count above 350 cells/mm^3) or clinical criteria to start cART and a detectable plasma viral load. In this setting, it is unknown whether and when (pre-transplant or post-transplant) it would be beneficial to initiate cART in order to reach an undetectable plasma viral load.

Other criteria

To be included on the OLT waiting list, an HIV-infected patient must have a favourable psychiatric evaluation. One recent observational prospective study found that ESLD patients with HIV-1 infection improved on all the items of a psychometric score (MADRS) at the follow-up evaluation (Barbanti 2009). In this study, the score variation was 10.20 at baseline and 4.09 at follow-up ($p<0.001$).

Patients who actively consume drugs should not be placed on the waiting list. In Spain, patients must have undergone a 2-year consumption-free period for heroin and cocaine (Miro 2005), and 6 months with no consumption of other drugs (e.g., alcohol). Patients who are on stable methadone maintenance programmes can be included and can continue on the maintenance programme after the procedure (Liu 2003). Fi-

nally, as is the case with any transplant candidate, HIV-positive patients must show an appropriate degree of social stability in order to ensure adequate care in the post-transplant period.

Outcome of OLT in HIV-positive patients

Overall short-term survival rates of HIV-positive patients who undergo OLT have been reported to be similar to those of HIV-negative patients when there is no HCV coinfection (Fung 2004; Roland 2002; Ragni 2003; Neff 2003; Norris 2004; Duclos-Vallee 2006; De Vera 2006; Schreibman 2007; Coffin 2007; Grossi 2008; Tateo 2009) (Table 2).

Author	Year	Country	Nº cases	Virus	Follow-up (months)	Survival rate
Roland	2002	International	19	Most HCV	10	15 (79%)
Ragni	2003	International	24	HCV, 62% HBV, 29%	17	18 (75%)
Neff	2003	US	16	HCV or HVB	12	14 (87%)
Fung	2004	US	29	HCV, 90%	18	20 (69%)
Norris	2004	UK	14	HCV, 50% HBV/OH, 50%	12 19	2 (29%) 7 (100%)
Duclos-Vallée	2006	France	41	HCV, 88% HBV, 12%	18	29 (81%) 5 (100%)
De Vera	2006	US	27	HCV, 100%	27	13 (48%)
Schreibman	2007	US	15	HCV, 40% HBV, 33%	74	10 (67%)
Coffin	2007	US	16	HBV 100%	8.5	14(86%)
Grossi	2008	Italy	60	HCV 65% HBV 12%	12	41 (58.3%)
Tateo	2009	France	13	HBV 100%	32	13 (100%)
Spanish study*	2010	Spain	200	HCV, 96%	35	57 (71.5%)

Table 2. Liver transplantation in HIV infected patients: main cohorts of cases (≥10) in the late cART era (2002-2009).

HIV-positive patients have not been shown to have an increased risk of post-operative complications or a higher incidence of opportunistic infections or tumours than HIV-negative patients (Samuel 2008; Norris 2008). Although findings from a recent case-control study (81 HIV/HCV-coinfected liver transplant recipients vs. 213 control patients) found that coinfected individuals were about twice as likely to have treated acute rejection than HCV-monoinfected patients (35% vs 18%, p=0.001) (Terrault, 2009). Bacterial infections were common in liver (43%) and kidney recipients (35%), and HCV infection was the only factor associated with an increased risk of bacterial

infection (liver recipients only) (Blumberg 2008). A recent study (Moreno 2009) that included 84 HIV/HCV-coinfected patients who underwent liver transplantation found bacterial infections in 39 patients (46%), CMV infection in 21 (25%), Herpes virus infection in 13 (15%), and fungal infections in 14 patients (17%) (5 were invasive cases). Fungal infection was associated with death (p=0.01).

HIV/HCV Coinfection

Mid-term survival is affected by recurrent hepatitis C (De Vera 2006). After OLT, recurrence of HCV infection is universal, regardless of whether the patient is infected by HIV or not. In fact, it is currently the number one cause of death. Some studies have suggested that recurrence of HCV in coinfected patients tends to be more severe and occurs earlier (De Vera 2006; Castells 2006). A recent French study (Antonini 2009) that included 68 HIV/HCV-coinfected patients who underwent OLT found that 19% of recipients developed cirrhosis and 13% developed fibrosing cholestatic hepatitis with a mean delay of 18.9 months and 6 months, respectively.

One study compared the outcomes of 27 coinfected patients with 54 HCV-monoinfected patients who underwent OLT (De Vera 2006). The researchers found that HIV-positive patients had a higher likelihood of developing cirrhosis or dying of an HCV-related complication than HIV-negative patients (RR = 2.6; 95%CI, 1.06-6.35; p=0.03). Cumulative 1-, 3- and 5-year survival for coinfected and monoinfected patients was 67% vs. 76%, 56% vs. 72%, and 33% vs. 72%, respectively (p=0.07).

In a recent retrospective study (Mindikoglu 2008) in the USA that enrolled 138 HIV-positive patients who underwent liver transplant during the cART era (1996-2006), the rate of survival at years 2 and 3 was significantly lower in HIV-positive patients (70% and 60%) than in the general population (n=30,520) (81% and 77%), although this difference was observed only in the HCV/HIV-HBV/HIV coinfected group. None of the 24 HIV-monoinfected recipients died. Therefore, liver transplant in HIV-positive patients does not have higher short-term mortality (1-2 years). Nevertheless, the management and outcome of HCV reinfection could affect survival in the medium term (3-5 years) and long term (5-7 years).

In France, data from 35 HIV/HCV-coinfected patients were analysed and compared with those of 44 HCV-monoinfected patients. The rates of survival at 2 and 5 years were 81%/91% and 51%/73% in HIV/HCV-coinfected patients/HCV-monoinfected patients, respectively (p=0.004) (Duclos-Vallée 2008).

In Spain, data from a multicentre case-control study show that survival of HIV/HCV-coinfected patients (N=84) at 1 year was similar to that of HCV-monoinfected patients (N=252)— 88% vs. 89% (NS)—but it was significantly lower at 3 and 5 years: 62% vs. 77% and 48% vs. 75%, respectively (p<0.01). In multivariate analysis, the variables independently associated with mortality were HCV genotype 1 infection, non-traumatic donor death, number of units of blood transfused during surgery, and development of invasive fungal infection after transplant (Miró 2009). The role of HCV infection is also well demonstrated in another recent case-control study performed in the UK that included 33 HIV-infected patients (0.6% of total LT activity), 847 HCV-monoinfected patients (15% of LT activity), and 5435 HIV-negative patients (Joshi 2009). Compared with the HCV group, survival rates at 1 year and 5 years differed significantly

in the HIV-positive patients (73% and 53% [HIV-positive/HCV] vs. 100% and 100% [HIV-positive/other] vs. 87% and 69% [HCV], log-rank test, p=0.04). No difference in survival rates at 1 and 5 years was demonstrated between the HIV-negative and HIV-positive groups (86.5% and 74% vs. 87.1% and 78%, log-rank test, p=NS). However, a recent Italian case-control study (Baccarani 2009) that included 27 HIV-positive and 24 HIV-negative recipients found that patient and graft survival at 1, 2, and 4 years were 90%, 82.5%, and 82.5% for HIV-positive patients vs. 100%, 94%, and 79% for HIV-negative patients (p=0.64), and 95%, 87%, and 87% for HIV-positive patients vs. 95%, 89%, and 82% for HIV-negative patients (p=0.89), respectively. However, the median follow-up was only 21 months (range 2-47) and 29 months (range 3-39) for HIV-positive recipients and HIV-negative recipients (p=0.93), respectively, and the aetiology of ESLD was HCV in most cases (70% vs. 61% respectively).

However, additional cohort studies analysing donor and recipient characteristics and issues related to the activity of both viruses and the efficacy and safety of antiviral therapies are necessary in order to determine the long-term prognosis of this procedure.

Rapid progression of HCV-related liver disease in HIV-positive recipients would represent a major drawback and would shorten life expectancy in this group. In fact, it is currently the number one cause of death. A French study observed that progression to fibrosis (≥F2) was significantly higher in HIV-positive patients (p<0.0001) (Duclos-Vallée 2008) and MELD was the only significant predictor of mortality, although donor age was of borderline significance (p=0.06).

Other negative survival factors reported by a multicentre Spanish study were histological progression to cirrhosis (F4) and donor age (Miro 2008).

Author, Year of Publication	**HIV/HCV coinfected patients**		**Non-HIV HCV monoinfected patients (Control Group)**	
	No. of cases	SVRa No (%)	No. of cases	SVRa No (%)
Fung, 2004	12	2 (17%)	-	-
Duclos-Vallee, 2006	13	2 (15%)	-	-
de Vera, 2006b	15	4 (27%)	27	7 (28%)c
Vennarecci, 2006d	9	0 (0%)	-	-
Castells, 2007e	5	1 (20%)	9	1 (11%)
Spanish study, 2009	41	9f (22%)	-	-
Total	**90**	**17 (18.8%)**	**-**	**-**

aSVR: *sustained virological response; bMost cases were genotype 1. Three patients were treated with classical interferon plus ribavirin; cRate of sustained virological response was not specified. Data show the rate of virologic response (clearance of HCV RNA from serum); d The authors did not specify the type of interferon used; and, aThese patients were included in the Spanish study and were not taken into account for the overall response rate. f 3/27 (11%) Genotype1-4 and 6/13 (46%) genotype 2-3. Modified from Miro et al. J HIV ther. 2007; 12(1):24-35.*

Table 3. Summary of the studies evaluating the effectiveness of the treatment of HCV reinfection in OLT with pegylated-interferon + ribavirin.

There is insufficient experience on the efficacy and safety of therapy with pegylated-interferon and ribavirin in coinfected transplant patients. One study (Miró 2007) summarized the reports evaluating the effectiveness of treatment of HCV reinfection in OLT with pegylated-interferon + ribavirin (Fung 2004; Duclos-Vallée 2008; De Vera 2006; Vennarecci 2006; Castells 2007; Miró 2009). These patients were treated when they had histological criteria. Only 12 (18.5%) out of 65 HCV/HIV-coinfected patients achieved an SVR. Krishnan et al (2008) investigated SVR-associated factors in 23 HIV/HCV-coinfected liver recipients and found (using logistic regression analysis) that donor age <60 years (p=0.02), genotype other than 1 (p=0.001), and use of cyclosporin A (p=0.002) were independently correlated with SVR. New strategies are necessary to improve the outcome of HCV recurrence in this setting. In this sense, a recent German study showed that SVR was obtained in the 6 out of 7 patients treated within the first 3 months after OLT (Emmelkamp 2007). Finally, a recent study has described 2 cases of spontaneous clearance of HCV RNA after OLT. This phenomenon is very infrequent and its mechanism is not known (Bhagat 2008).

On the other hand, in two recent genome-wide association studies (Ge 2009; Thomas 2009), a single nucleotide polymorphism (rs12979860) 3 kilobases upstream of the IL28B gene, which encodes the type III interferon-l, was shown to be associated with natural clearance of HCV among HIV-negative individuals of both European and African ancestry and with more than a twofold difference in response to anti-HCV drug treatment with pegylated-interferon and ribavirin in HCV mono-infected patients. In a Swiss study (Rauch 2010), this antiviral effect was stronger in patients with HCV genotypes 1 or 4. Similar results have been recently communicated in HCV/HIV coinfected patients (Rallon 2010). The role of IL28B polymorphysms of the donor and their impact on the natural history of HCV recurrence and response to antiviral therapy in liver transplant recipients is not yet understood.

HIV/HVB coinfection

Cohorts of HIV/HBV-coinfected patients are not as large as those of HIV/HCV-infected patients. Nevertheless, the outcome of HBV infection after OLT is much better (Terrault 2006; Coffin 2007; Grossi 2008; Joshi 2009; Tateo 2009). The survival rate in the short and medium term in HBV/HIV-coinfected patients is high and similar to that observed in HBV-monoinfected patients, probably due to the low incidence of HBV reinfection. A recent French study (Tateo 2009) that included 13 HIV/HBV-coinfected patients (3 out of 6 patients with positive anti-HCV serology had HCV RNA detectable before OLT), revealed 100% graft and patient survival after a mean follow-up of 32 months. Another recent study (Joshi 2009) described a 5-year survival rate of 100% in 6 HIV/HBsAg-positive patients. The presence of HBV resistance to lamivudine at the time of transplantation was a potential risk for recurrence of HBV after OLT (Terrault 2006).

Hepatocellular carcinoma

Preliminary Italian experience showed good results in 7 HIV-1–infected patients with HCC who underwent OLT. They observed an 86% overall patient and graft survival rate after a mean follow-up of 8 months, and recommend OLT in HIV-positive pa-

tients with early stage HCC (Di Benedetto 2006; Di Benedetto 2008). A recent Italian study found no recurrence in 7 HIV-1 OLT recipients after a median follow-up of 13 months (Di Benedetto 2008).

A French study (Vibert 2009) compared the histological characteristics of HCC in the explanted liver from 21 HIV/HCV-coinfected recipients and 53 HCV-monoinfected recipients who underwent OLT due to HCC. For pathologically confirmed HCC, no significant differences between HIV-positive patients and non–HIV-infected patients were noted for the number of nodules (2.0 vs. 3.1, p=0.23), the maximum diameter of nodules (30 mm vs. 29 mm, p=0.88), the sum of nodule diameters (45 mm vs. 54 mm, p=0.46), presence of satellite nodules (8/15 vs 24/53, p=0.69), microvascular invasion (7/15 vs 22/53, p=0.3), mean tumour necrosis (35% vs 42%, p=0.50), and Edmonson grade (2.9 vs 2.8, p=0.56). Another French study (Vibert 2008) compared HIV-positive and HIV-negative patients with HCC. They found that the proportion of excess Milan criteria were similar (2/20 [19%] vs. 11/61 [18%]). The time on the waiting list was similar (7 vs. 4 months). Thirteen HIV-1–infected patients and 55 HIV-negative patients had a transplant. The pathological findings were similar in both groups for number of nodules (2.3 vs. 2.6, p=0.70), maximum diameter (27 mm vs. 28 mm p=0.82), satellite nodules (4/13 vs. 21/49, p=0.39), and vascular invasion (6/13 vs. 22/48, p=0.98). It is noteworthy that the Edmonson grade was higher in HIV-positive patients (3.1 vs. 2.5, p=0.04). After a mean follow-up of 16 months and 24 months (p=0.11) tumour recurrence was observed in 4/13 (30%) vs. 2/49 (4%) OLT recipients, respectively. This is the only study to have noted such a high recurrence rate. Further studies with a higher number of cases and longer follow-up are needed in order to know the efficacy of OLT in HIV-1–positive recipients with HCC.

Conclusions

ESLD is an increasingly frequent clinical scenario in the setting of HIV/HCV coinfection, and its relevance has grown since cART became available.

Early diagnosis of ESLD complications is particularly important and should be actively monitored and treated. In general terms, the management of ESLD in HIV-positive patients should be the same as in those without HIV infection.

Physicians attending ESLD patients should follow them prospectively and evaluate them for OLT after the first clinical decompensation of the liver disease.

OLT is a life-saving procedure in this population, and is safe and effective in patients with HBV infection. However, recurrence of HCV infection in coinfected patients can affect both graft and patient survival in the medium and long term. Prospective and larger studies with a longer duration must be carried out to determine the benefit of OLT in this setting.

References

Agüero F, Laguno M, Moreno A, et al. Management of end-stage liver disease in HIV-infected patients. Curr Op HIV/AIDS. 2007, 2:474–481.

Alter M. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol 2006;44,S6-S9

Anonymous. Solid organ transplantation in the HIV-infected patient. Am J Transplant. 2004 Nov;4 Suppl 10:83-8.

Antonini T, Roque-Afonso A, Sebagh M, Roche B; Samuel D, Duclos-Vallee J. Prognosis of severe recurrence of HCV infection after liver transplantation in a large cohort of HIV infected patients. Tranplant International 2009 Suppl. 22: 27-27.

Arroyo V, Fernandez J, Ginés P. Pathogenesis and treatment of hepatorenal syndrome. Semin Liver Dis. 2008;28(1):8.

Baccarani U, Adani G, Tavio M, Lorenzin D; Londero A, Bragantini F, et al. Liver transplantarion in HIV positive patients: a case control study. Transplant International 2009. Suppl. 22: 1-1 01.

Baccarani U, Tavio M, Adani G, Lorenzin D Londero A, Bragantini F, et al. Feasibility of liver transplantation in HIV patients. Liver Transp. 2008 Suppl. 14 (7): S220-S220.

Back D, Gibbons S. The University of Liverpool HIV drug interactions website. Available at: http://www.hiv-druginteractions.org. Accessed 7 th Decenber 2009

Barbanti Silva V, Ferrara M, Mazzi F, Pingani L, Cocchi S, Romano A,et al. What is the biopsychosocial role of human immunodeficiency virus positivity in patients with end-stage liver disease who undergo orthotopic liver transplantation? Transplant Proc. 2008. 40(6):1910-2.

Benhamou Y, Di Martino V, Bochet M, et al. Factors affecting liver fibrosis in HIV and hepatitis C coinfected patients: impact of protease inhibitor therapy. Hepatology 2001; 34: 283–287.

Bhagat V, Foont J, Schiff E, et al. Spontaneous Clearance of Hepatitis C Virus After Liver Transplantation in Two Patients Coinfected with Hepatitis C Virus and Human Immunodeficiency Virus. Liver Transpl 14:92-95, 2008

Blumberg E, Barin B, Olthoff K, et al. Bacterial Infections in HIV Positive Transplant Recipients. American Transplant Congress. May 31- June 4 2008. Toronto Canada. Abstract# 17

Bräu N, Fox R, Xiao P, et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a U.S.-Canadian multicenter study. H Hepatol. 2007; 47(4): 447-50

Bruno R, Puoti M, Sacchi P et al. Management of hepatocellular carcinoma in human immunodeficiency virus-infected patients J Hepatol 2006; 44: S146-S150

Cardenas A, Gines P. Management of complications of cirrhosis in patients awaiting liver transplantation. J Hepatol 2005;42 Suppl 1: S124-S133.

Carmona D, Delvart V, Antonini T, Sobesky R, Roche B, Teicher E et al. Analyse of predictive factors of mortality of HIV/HCV infected patients on the waiting list of liver transplantation. Liver transplantation. 2009. Suppl.15 (7): S157-S157.

Castells L, Escartin A, Bilbao I, et al. Liver Transplantation in HIV-HCV Coinfected Patients: A Case-Control Study. Transplantation. 2007; 83:354-358

Castells L, Esteban J, Bilbao I et al. Early antiviral treatment of hepatitis C virus recurrence after liver transplantation in HIV-infected patients. Antiviral Therapy 2006; 11:1061-1070

Chan A, Poo R ,Ng K et al. Changing Paradigm in the Management of Hepatocellular Carcinoma Improves the Survival Benefit of Early Detection by Screening. Ann Surg 2008;247: 666–673

Coffin CS, Berg CL, Dove LM. Survival and risk of hepatitis B virus (HBV) recurrence in HIV-HBV coinfected liver transplant recipients: preliminary findings from the HIV-TR study. 58th Annual Meeting of the American Association for the Study of Liver Diseases. Boston, MA. November 2-6, 2007. Abstract 28

Cooper C, Cameron DW: Effect of alcohol use on the impact of effective antiretroviral therapy for HIV co-infection on plasma HCV levels. Clin Infect Dis 2005, 41:S105-109.

Crum NF, Riffenburgh RH, Wegner S, et al. Comparisons of causes of death and mortality rates among HIV-infected persons: analysis of the pre, early, and late HAART (highly active antiretroviral therapy) eras. J Acquir Immune Defic Syndr 2006; 41:194–200.

De Vera ME, Dvorchik I, Tom K et al. Survival of liver trasplant patients coinfected with HIV and HCV is adversely impacted by recurrent Hepatitis C. Am J Transplant 2006; 6:2983-2993

Di Benedetto F, De Ruvo N, Berretta N, et al. Hepatocellular carcinoma in HIV patients treated by liver transplantation. EJSO 34 (2008) 422e427

Di Benedetto F, de Ruvo N, Berretta M, Massetti M, Montalti R, di Sandro S et al. Don't Deny Liver Transplantation to HIV Patients With Hepatocellular Carcinoma in the Highly Active Antiretroviral Therapy Era. J Clin Oncol. 2006; 24: e26-e27.

Duclos-Vallée J, Féray C, Sebagh M, et al. Survival and recurrence of hepatitis C after liver transplantation in patients coinfected with human immunodeficiency virus and hepatitis C virus. Hepatology. 2008 Feb;47(2):407-17

Duclos-Vallee JC, Teicher E, Feray C, et al. Liver Transplantation of HIV-HCV and HIV-HBV Coinfected Patients: A Large Experience in a Single Centre. Liver Transpl 2006, 12, No. 5 C-103.

Emmelkamp J, Guaraldi G, Cocchi S et al. Antiviral therapy for HCV-recurrence after liver transplantation in HIV/HCV-infected individuals. 11th European AIDS Conference/EACS. October 2007, Madrid

Everson G. Treatment of Hepatitis C in Patients Who Have Decompensated Cirrhosis. Clin Liver Dis 9 (2005) 473– 486

Fernández J, Navasa M, Planas R, et al. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. Gastroenterology. 2007 Sep;133(3):818-24.

Fung J, Eghtesad B, Patel-Tom K et al. Liver transplantation in patients with HIV infection. Liver Transpl 2004;10 (Suppl 2): S39-S53.

Fung LT, Roland ME, Stock PG. Review of solid-organ transplantation in HIV-infected patients. Transplantation 2003;75: 425-429.

Gaspari R, Avolio AW, Zileri Dal Verne L. Molecular adsorbent recirculating system in liver transplantation: safety an efficacy. Transplant Proc 2006; 38(10):3544-51.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009 Sep 17;461(7262):399-401.

GESIDA/PNS Panel of Experts 2009, Panel de expertos de Gesida y Plan Nacional sobre el Sida. Recomendaciones de Gesida/Plan Nacional sobre el Sida respecto al tratamiento antirretroviral en adultos infectados por el virus de la inmunodeficiencia humana (actualización febrero de 2009). Enferm Infecc Microbiol Clin. 2009;27(4):222–235.

Ghany M, Strader D, Thomas D, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, Management, and Treatment of Hepatitis C: An Update. 2009. Hepatology. 2009 Apr;49(4):1335-74.

Girón-gonzález J, Brun F, Terrón A, et al. Natural history of compensated and decompensated HCV-related cirrhosis in HIV-infected patients: a prospective multicentre study. Antivir Ther 2007;12(6):899-907

Graham CS, Baden LR, Yu E, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis 2001; 33:562-9

Grossi P, Gabbrielli F, De Cillia C, et al. The Italian Experience of Liver Transplantation in HIV-Infected Individuals. 43rd annual meeting of the European Association for the Study of the Liver (EASL 2008). Milan, Italy. April 23-27, 2008

Grossi P, Gabbrielli F, De Cillia C, et al. The Italian Experience of Liver Transplantation in HIV-Infected Individuals. 43rd annual meeting of the European Association for the Study of the Liver (EASL 2008). Milan, Italy. April 23-27, 2008

Grossi PA, Tumietto F, Costigliola P, et al. Liver Transplantation In HIV-Infected Individuals: Results Of The Italian National Program. Transplant International 2005:18 (suppl 1): 11.

Hammer S, Eron J, Reiss P, et al. Antiretroviral Treatment of Adult HIV infection: 2008 Recomendations of the International AIDS Society USA Panel. JAMA. 2008; 300 (5): 555-570.

Han MK, Hyzy R. Advances in critical care management of hepatic failure with insufficiency. Crit Care Med 2006; 9: S225-31

Hezode C, Lonjon I, Roudot-Thoraval F, et al. Impact of smoking on histological liver lesions in chronic hepatitis C. Gut 2003, 52:126-129

Iacobellis A, siciliano M, Perri F, at el. Peginterferon alfa-2b and ribavirin in patients with hepatitis C virus and decompensated cirrhosis: a controlled study. J Hepatol. 2007 Feb;46(2):206-12)

Ishida JH, Peters MG, Jin C, Louie K, Tan V, Bacchetti P, Terrault NA. Influence of cannabis use on severity of hepatitis C disease. Clin Gastroenterol Hepatol. 2008 ;6(1):69-75.

Joshi D, Aluvihare V, Belgaumkar A, O'Grady J, Suddle A, Heneghan M, Agarwal K. Liver transplantation for HIV: Long term outcome from the UK. ILTS. New York, NY, July 8-11, 2009. Abstract 0-11

Konopnicki D, Mocroft A, de Wit S, et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. AIDS. 2005;19: 593-601.

Krishnan K, Poordad F, Reddy R et al. Safety and Efficacy of Antiviral Therapy in Hepatitis C Virus (HCV)-Human Immunodeficiency Virus (HIV) Coinfected Liver Transplant Recipients: Preliminary HIVTR Results. American Transplant Congress 2008. May 31- June 4 2008. Toronto Canada. Abstract# 1618

Kuper H, Tzonou A, Kaklamani E, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer 2000, 85:498-502.

Lewden C, Salmon D, Morlat P, et al. Causes of death among human immunodeficiency virus (HIV)-infected adults in the era of potent antiretroviral therapy: emerging role of hepatitis and cancers, persistent role of AIDS. Int J Epidemiol 2005; 34:121–130.

Liu LU, Schiano TD, Lau N et al. Survival and risk of recidivism in methadone-dependent patients undergoing liver transplantation. Am J Transplant 2003;3: 1273-1277.

Llovet JM, Fuster J, Bruix J; Barcelona-Clinic Liver Cancer Group. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. Liver Transpl 2004;10(2 Suppl 1): S115-S120.

Macias J, Mira J, Lopez Cortes L. Antiretroviral therapy based on protease inhibitors as a protective factor against liver fibrosis progression in patients with chronic hepatitis C. Antiviral therapy 2006; 11: 839-846.

Maida I, Nunez M, Gonzalez-Lahoz J, Soriano V. Liver transplantation in HIV-HCV coinfected candidates: what is the most appropriate time for evaluation? AIDS Res Hum Retroviruses 2005; 21: 599-601.

Manitpisitkul W, McCann E, Lee S and Weir M. Drug interactions in transplant patients: what everyone should know. Curr Opin Nephrol Hypertens 2009, 18:404–411.

Martinez E, Milinkovic A, Buira E et al. Incidence and causes of death in HIV-infected persons receiving highly active antiretroviral therapy compared with estimates for the general population of similar age and from the same geographical area. HIV Medicine 2007;8: 251-258

Mauss S, Valenti W, DePhamphilis J. Risk factors for hepatic decompensation in patients with HIV/HCV coinfection and liver cirrhosis during interferon-based therapy. AIDS 2004; 18:F21-F25

Merchante N, Giron-Gonzalez JA, Gonzalez-Serrano M et al. Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. AIDS. 2006 Jan 2;20(1):49-57.

Merchante N, Jiménez-Saenz M, Pineda JA. Management of HCV-related end-stage liver disease in HIV-coinfected patients. AIDS Rev. 2007; 9:131-9

Mindikoglu A, Regev A, Magder L. Impact of human immunodeficiency virus on survival after liver transplantation: analysis of United Network for Organ Sharing database. Transplantation. 2008 Feb 15;85(3):359-68).

Miro JM, Aguero F, Laguno M et al. Liver trasplantation in HIV/Hepatitis coinfection. J HIV ther. 2007; 12(1):24-35.

Miro JM, Montejo M, Castells LL, et al. Treatment of Spanish HIV-infected patients with recurrent hepatitis C virus (HCV) after liver transplantation (OLT) with pegylated interferon (PEG-INF) plus ribavirin (RBV): Preliminary results of the FIPSE OLT-HIV-05 - GESIDA 45-05 Cohort Study (2002-06). 14th Conference on Retroviruses and Opportunistic Infections. Los Angeles, California. February 25-28, 2007. Abstract 890.

Miró JM, Montejo M, Castells L et al. 3-year survival of HCV-HIV coinfected liver transplant recipients (OLT) is similar to that of HCV monoinfected recipients. 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Chicago, 2007. V-1732.

Miró JM, Montejo M, Castells L et al. 5-year survival of HCV-HIV coinfected liver transplant recipients (OLT): a case control study. 16th Interscience Conference on Antimicrobial Agents and Chemotherapy (CROI). Montreal, 2009. Abstract H-124.

Miró JM, Torre-Cisneros J, Moreno A, et al. GESIDA/GESITRA-SEIMC, PNS and ONT consensus document on solid organ transplant (SOT) in HIV-infected patients in Spain – March, 2005. Enferm Infecc Microbiol Clin. 2005; 23: 353-362.

Mohsen AH, Easterbook PJ, Taylar C et al. Impact of human immunodeficiency virus (HIV) on the progression of liver fibrosis in Hepatitis C virus infected patients. Gut 2003; 52:1035-40.

Moreno A, Fortun J, Blanes M, Cervera C, Montejo E, Meneu J et al. Epidemiology and Outcome of Infections in 84 Spanish HCV/HIV-Coinfected Liver Transplant Recipients: A FIPSE/GESIDA Prospective Cohort Study. ICAAC San Francisco, CA. 2009. Abstract K-974.

Neff GW, Bonham A, Tzakis AG et al. Orthotopic liver transplantation in patients with human immunodeficiency virus and end-stage liver disease. Liver Transpl. 2003;9(3):239-47.

Neff GW, Sherman KE, Eghtesad B, Fung J. Review article: current status of liver transplantation in HIV-infected patients. Aliment Pharmacol Ther 2004;20: 993-1000.

Norris S, Houlihan D. Liver transplantation in HIV-positive patients. Expert Rev Gastroenterol Hepatol 2008, 2, 39-46.

Norris S, Taylor C, Muiesan P et al. Outcomes of liver transplantation in HIV-infected individuals: the impact of HCV and HBV infection. Liver Transpl 2004;10: 1271-1278.

O'Grady J, Taylor C, Brook G. Guidelines for liver transplantation in patients with HIV infection (2005). HIV Med. 2005; 6 Suppl 2:149-53.

Palella FJ Jr, Baker RK, Moorman AC, et al. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 2006; 43:27–34.

Panel on Antiretroviral Guidelines for Adults and Adolescentes. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Department of Health and Human Services. December 1, 2009; 1-161. Availabel at http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed December 7th 2009.

Pessione F, Ramond MJ, Njapoum C, et al. Cigarette smoking and hepatic lesions in patients with chronic hepatitis C. Hepatology 2001, 34:121-125.

Pineda JA, Aguilar-Guisado M, Rivero A, Girón-González JA, Ruiz-Morales J, Merino D, et al. Natural history of compensated hepatitis C virus-related cirrhosis in HIV-infected patients. Clin Infect Dis. 2009 15;49(8):1274-82.

Pineda JA, Recio E, Camacho A, Macías J, Almodóvar C, González-Serrano M, et al. Liver stiffness as a predictor of esophageal varices requiring therapy in HIV/hepatitis C virus-coinfected patients with cirrhosis. J Acquir Immune Defic Syndr. 2009;51(4):445-9.

Pineda JA, Romero-Gomez M, Diaz-Garcia F, Giron-Gonzalez JA, Montero JL, Torre-Cisneros J, et al. HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. Hepatology. 2005; 41: 779-789.

Poynard T, Mathurin P, Lai CL, et al. A comparison of fibrosis progression in chronic liver diseases. J Hepatol 2003; 38:257-65

Prieto J, Laguno M, Agüero F, Freixa N, Monras M, Cervera C, et al. Evaluation of cirrhotic patients with HIV infection (HIV+) as candidates for liver transplantation. Liver transplantation. 2008. 14 (7) S223- S223.

Puoti M, Bruno R, Soriano V, et al. Hepatocellular carcinoma in HIV-infected patients: epidemiological features, clinical presentation and outcome. AIDS 2004; 18: 2285-93

Qurishi N, Kreuzberg C, Luchters G, et al. Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C virus coinfection. Lancet 2003; 362: 1708–1713.

Radecke K, Fruhauf NR, Miller M, et al. Outcome after orthotopic liver transplantation in five HIV-infected patients with virus hepatitis-induced cirrhosis. Liver Int. 2005 Feb;25(1):101

Ragni MV, Belle SH, Im K, et al. Survival of human immunodeficiency virus-infected liver transplant recipients. J Infect Dis 2003;188: 1412-1420

Ragni MV, Eghtesad B, Schlesinger KW, et al. Pretransplant survival is shorter in HIV-positive than HIV-negative subjects with end-stage liver disease. Liver Transpl. 2005 (11):1425-30.

Rallon N, Naggie S, Benito J, Medrano J, Restrepo C, Goldstein D, et al. Strong Association of a Single Nucleotide Polymorphism Located Near the Interleukin-28b Gene with Response to Hepatitis C Therapy in HIV/HCV Co-infected Patients. 17th Conference on Retroviruses and Opportunistic Infections. San Francisco, CA. February 16-19, 2009. Abstract 165LB.

Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al; Swiss Hepatitis C and HIV Cohort Studies. Genetic Variation in IL28B Is Associated With Chronic Hepatitis C and Treatment Failure: A Genome-wide Association Study. Gastroenterology. 2010 Jan 11. [Epub ahead of print].

Rockstroh J, Bhagani S, Benhamo Y, et al. EACS Executive Committee European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of chronic hepatitis B and C coinfection in HIV-infected adults. HIV Med 2008 Feb;9(2):82-8.

Rockstroh J, Mocroft A, Soriano V, et al. Influence of Hepatitis C Coinfection on HIV Disease Progression within the Eurosida Cohort. 9th European AIDS Conference. October 25 - 29, 2003 Warsaw (Poland). Abstract F12/4.

Roland M, Carlson L, Ragni M, et al. Solid organ transplantation in HIV-infected recipients: 47 cases in the HAART era (Abstract MoOrB1060). XIV International AIDS Conference. Barcelona, Spain 2002.

Roland M, Stock PG. Liver Transplantation in HIV-Infected Recipients. Seminar in Liver Disease. 2006; 26:273-284.

Roland ME, Stock PG. Review of solid-organ transplantation in HIV-infected patients. Transplantation 2003;75: 425-429.

Rosenthal E, Pialoux G, Bernard N, et al. Liver-related mortality in human-immunodeficiency-virus-infected patients between 1995 and 2003 in the French GERMIVIC Joint Study Group Network (MORTAVIC 2003 Study). J Viral Hepat, 2007, 14:183-88

Salmon-Ceron D, Rosenthal E, Lewden C, Bouteloup V, May T, Burty C et al. Emerging role of hepatocellular carcinoma among liver-related causes of deaths in HIV-infected patients: The French national Mortalité 2005 study. J Hepatol. 2009 Apr;50(4):736-45.

Samuel D, Weber R, Stock P et al. Are HIV-infected patients candidates for liver transplantation? J Hepatol. 2008 May;48(5):697-707

Schreibman I, Gaynor J, Jayaweera D et al. Outcomes after orthotopic liver trasnplantation in 15 HIV-infected patients. Transplantation 2007; 84: 697-705

Shaw E, Castellote J, Santín M et al. Clinical features and outcome of spontaneous bacterial peritonitis in HIV-infected cirrhotic patients: a case-control study. Eur J Clin Microbiol Infect Dis 2006; 25: 291–298

Soriano V, Puoti M, Sulkowski M et al.Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. AIDS. 2007; 21:1073-89.

Stock PG, Roland M. Evolving clinical strategies for transplantation in the HIV-positive recipient. Transplantation 2007;84:563-571

Subramanian A, Sulkowski M, Barin B, Stablein D, Curry M, Nissen N et al. MELD Score Is an Important Predictor of Pretransplantation Mortality in HIV-Infected Liver Transplant Candidates. Gastroenterology. 2009 Sep 30. (Epub ahead of print).

Tateo M, Roque-Afonso AM, Antonini TM, et al. Long-term follow-up of liver transplanted HIV/hepatitis B virus coinfected patients: perfect control of hepatitis B virus replication and absence of mitochondrial toxicity. AIDS. 2009 23(9):1069-76.

Tateo M, Sebagh M, Bralet MP, Teicher E, Azoulay D, Mallet V et al. A new indication for liver transplantation: nodular regenerative hyperplasia in human immunodeficiency virus-infected patients. Liver Transpl. 2008 14(8):1194-8.

Tedaldi EM, Baker RK, Moorman AC et al. Influence of coinfection with hepatitis C virus on morbidity and mortality due to human immunodeficiency virus infection in the era of highly active antiretroviral therapy. Clin Infect Dis 2003; 36: 363–367.

Terrault N, Barin B, Schiano T et al. Survival and Risk of Severe Hepatitis C Virus (HCV) Recurrence in Liver Transplant (LT) Recipients Coinfected with Human Immunodeficiency Virus (HIV) and HCV. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2009). Boston. October 30-November 1, 2009. Abstract 195.

Terrault NA, Carter JT, Carlson L, et al. Outcome of Patients with Hepatitis B Virus and Human Immunodeficiency Virus Infections Referred for Liver Transplantation. Liver Transpl. 2006; 12:801-807.

The Data Collection on Adverse Events of Anti-HIV Drugs Study group. Liver-related deaths in persons infected with the human immunodeficiency virus, The D:A:D Study. 2006. Arch Intern Med; 166:1632-41.

Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009 Oct 8;461(7265):798-801.

Tricot, L, Teicher E, Peytavin G, Zucman D, Conti F and Calmus Y. Safety and efficacy of Raltegravir in HIV-infected transplant patients cotreated with immunosuppressive drugs. Am J Transplant 2009;9: 1-7.

Tuset M, Miró JM, Codina C, Ribas J, Ed. Guía de interacciones en HIV. Available at: http://www.interaccionesHIV.com. Accessed December 7 th 2009.

Vennarecci G, Mutimer D, Ettorre G, al. Liver Transplantation in HIV positive Patients. The 2006 Joint International Congress of ILTS, ELITA, LICAGE. Milan (Italy) May 3-6, 2006. Abstract number 457. Published at: Liver Transplantation, Vol 12, No. 5, Supplement 1, 2006: pp C-115.

Vibert E, Adam R, Tralhao J, Azoulay D, Castaing D, Samuel D. Evolution after listing and results of liver transplantation for hepatocellular carcinoma in HIV/HCV and HIV/HBV coinfected patients. Liver Transp 2008 Suppl.14 (7): S220-S221.

Vibert E, Ghigna M, Adam R, Azoulay D, Castaing D, Samuel D. Pathological description of native liver in HIV-HCV coinfected patients transplanted for hepatocellular carcinoma: a comparative study with HCV monoinfected patients. Liver transp. 2009. Suppl. 15 (7): S196-S196.

Wyles DL, Gerber J. Antiretroviral drug pharmacokinetics in hepatitis with hepatic dysfunction. Clin Infect Dis 2005;40:174-181.

Part 8

Autoimmune and metabolic liver diseases

Chapter 24: Metabolic liver diseases: Haemochromatosis

Claus Niederau

Definition and classification of iron overload diseases

Hereditary haemochromatosis is classified into 4 subtypes (Table 1) of which type 1 is the one of clinical importance in Caucasian populations. Type 1 is the well known form of iron overload due to an autosomal-recessive genetic metabolic malfunction; the homozygous C282Y mutation of the HFE gene on chromosome 6 accounts for more than 90% of clinical phenotypes in populations of Caucasian origin (Feder 1996). The mutation leads to an inadequately high intestinal iron absorption that after decades may cause iron overload and damage to various organs (Figure 1). Types 2a and 2b of genetic haemochromatosis are juvenile forms of iron overload that lead to a severe outcome prior to age 30, with cardiomyopathy and hypogonadism. The corresponding mutations are located in the haemojuveline and hepcidin genes, respectively (Roetto 1999). Type 3 has mainly been described in Italian families and refers to a mutation in the transferrin receptor 2 gene (Girelli 2002). Clinical consequences of type 3 haemochromatosis are similar to type 1. Types 2 and 3 are autosomal-recessive traits. The mutations of the autosomal-dominant type 4 haemochromatosis are located in the gene coding for the basolateral iron transporter ferroportin 1 (Njajou 2001). In contrast to the other types, iron is accumulated in type 4 mainly in macrophages; ferritin values are markedly elevated although transferrin saturation is only slightly higher. Secondary haemochromatosis is usually caused by multiple blood transfusions in haemolytic anaemias such as thalassaemia, sickle cell anaemia and myelodysplasia syndrome. Iron first accumulates in RES macrophages and is later transferred to parenchymal cells. With frequent blood transfusions, iron may accumulate faster when compared to genetic haemochromatosis; thus, iron overload often leads to severe cardiomyopathy and liver cirrhosis, limiting effective prognosis. Therapy consists of iron chelators because phlebotomies cannot be done due to the underlying anaemia. This review will focus on type 1 HFE haemochromatosis, the most prevalent genetic form in Germany. Most consequences of iron overload are similar, whatever the cause. Thus, the pathophysiology of tissue and organ damage by iron excess is discussed in detail only for HFE haemochromatosis.

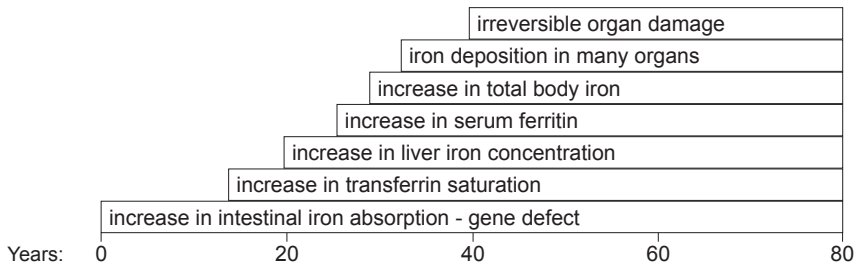


Figure 1. Scheme of natural history of type 1 genetic haemochromatosis.

I) Genetic haemochromatosis

Types	Gene defect on	Affected gene	Inheritance	High prevalence
type 1	chromosome 6	HFE Gene	autosomal-recessive	of Caucasian origin
type 2a	chromosome 1	haemojuveline	autosomal-recessive	juvenile form
type 2b	chromosome 19	hepcidin	autosomal-recessive	juvenile form
type 3	chromosome 7	transferrin receptor 2	autosomal-recessive	Italy
type 4	chromosome 2	ferroportin 1	autosomal-dominant	Italy
neonatal	unknown	unknown	unknown	very rare
others	unknown	unknown	unknown	of non-Caucasian origin

II) secondary haemochromatosis

a) chronic anaemias (thalassaemia, sickle cell disease, MDS, other rare haemolytic anaemias)
b) multiple blood transfusions in general
c) long-term oral intake of high amounts of iron (diet-related or IV)

III) non-classified, ill-defined iron overload syndromes

a) iron overload in the Bantu Africans
b) iron overload in aceruloplasminemia

Table 1. Classification of haemochromatosis.

Type 1 HFE haemochromatosis

History

The association between liver cirrhosis, pigment deposits in the liver, and diabetes mellitus was recognized over a century ago (Trosseau 1865; Troisier 1871; Hanot and Schachmann 1886). The term haemochromatosis was first introduced by Recklinghausen in 1889 (Recklinghausen 1889), but was not generally accepted until used by Sheldon as the title of his classic monograph in 1935 (Sheldon 1935). The controversy over whether haemochromatosis is merely a form of alcoholic liver cirrhosis (MacDonald 1960) or an genetic error of iron metabolism (Sheldon 1935; Crosby 1966) lasted almost a century until Simon described the association between special HLA haplotypes and haemochromatosis which recognized the genetic nature of the disease (Simon 1975). The mode of inheritance was identified as an autosomal recessive disorder (Simon 1977). Finally, the major mutation on the HFE gene associated with clinical manifestations was identified (Feder 1996).

Epidemiology

Type 1 haemochromatosis is probably the most prevalent genetic metabolic error in Caucasian populations (Adams 2005). The prevalence of C282Y homozygotes is approximately 0.5% in central Europe and in the Caucasian population of North America; the prevalence of C282Y and H63D heterozygotes approaches 40% in similar populations (Adams 2005). Phenotypic expression also depends on several non-genetic factors such the amount of dietary iron and blood loss (Figure 2). For example, females develop clinical consequences of iron overload 5-8 times less frequently and 10-20 years later than males due to menses. It is now widely accepted that not all C282Y homozygous men will develop the full clinical manifestation of haemochromatosis. It is unknown, however, whether 5% or 50% will show clinical disease during their lifetime and which other factors determine that phenotype.

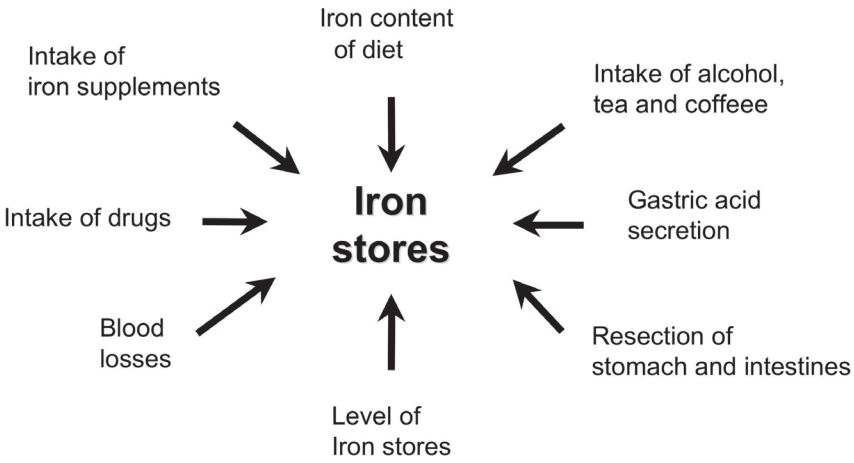


Figure 2. Non-genetic factors that may influence iron absorption.

As mentioned previously, the homozygous C282Y mutation accounts for more than 90% of the clinical phenotype in populations of Caucasian origin (Feder 1996; Adams 2005) (Table 2). A point mutation at H63D is also frequently identified in the HFE gene as well as other less frequent mutations. None of these gene alterations or polymorphisms, found in up to 40% of subjects with a Celtic background, correlates with the phenotype. A subject with a C282Y variation on one allele and a H63D variation on the other is called a "compound heterozygote" (Table 2). Only a small percentage of such compound heterozygotes are at risk for clinical consequences of iron overload. C282Y and H63D heterozygotes are at no risk of iron overload (Table 2). In non-Caucasian populations other genes may be involved in causing iron overload.

Mutations/ polymorphisms	Prevalence in Caucasian populations	Risk of advanced clinical phenotype
C282Y/C282Y	85-95%	low if ferritin is <1000 ng/ml
H63D/C282Y	3-8%	very low
C282Y/wild type	-	none
H63D/wild type	-	none
Others	1%	unknown

Table 2. Genotype/phenotype correlation in haemochromatosis.

Aetiology and pathogenesis

Intestinal iron absorption and iron losses are finely balanced under physiological conditions. Approximately 10% of the total daily intake (10-20 mg) is absorbed by the small intestine (1-2 mg). However, subjects with the homozygous C282Y mutation may absorb up to 20% of iron intake; i.e., up to 2-4 mg/day. Thus, homozygotes have an excessive iron intake of approximately 1 mg/day. It may therefore take several decades until iron stores approach 10 g above which organ damage is considered to be induced. Many patients at the clinical end stage of haemochromatosis, including liver cirrhosis and diabetes mellitus, have total body iron stores of 20-30 g. Their intestinal iron absorption is down regulated when iron stores increase, as it is in patients with genetic haemochromatosis. This downregulation, however, occurs on an increased level when compared to subjects without the HFE gene mutation. Correspondingly, intestinal iron absorption is massively increased in patients with haemochromatosis when iron stores have been depleted by phlebotomy. Phlebotomies should be continued after iron depletion in order to prevent reaccumulation. These regulatory processes however do not explain how HFE gene mutations cause the increase in intestinal iron absorption since the HFE gene product is neither an iron transporter nor an iron reductase or oxidase. Only recently have carriers and regulators of cellular iron uptake and release been identified (Pietrangelo 2002; Fleming 2002; Townsend 2002; Fletcher 2002). It has also become increasingly evident that some of them interact with the HFE gene product in the regulation of intestinal iron absorption (Pietrangelo 2002; Fleming 2002; Townsend 2002; Fletcher 2002). Recent studies have shown that the Nramp2 protein is the luminal iron carrier. Shortly thereafter, the luminal iron reductase was identified as the Dcytb protein (duodenal cytochrome B) (Pietrangelo 2002; Fleming 2002; Townsend 2002; Fletcher 2002). At the same time, the basolateral iron transporter ferroportin 1 (also named Ireg1 or MTP1) was identified (Donovan 2000; Abboud 2000) as well as the basolateral iron oxidase hephaestin (Vulpe 1999). Mutations in some of these proteins are responsible for the rarer types 2-4 of genetic haemochromatosis, although none of these genes is altered in type 1 haemochromatosis. Recently, two other proteins have been shown to act as important iron regulating proteins, transferrin receptor 2 and hepcidin (Pietrangelo 2002; Fletcher 2002; Fleming 2005). Mutations in the transferrin receptor 2 gene may lead to the rare type

3 haemochromatosis, and mutations in the ferroportin 1 gene to type 4 haemochromatosis. More recent studies also indicate that hepcidin may be the most important regulator of iron metabolism, involved in iron deficiency and overload. Hepcidin has been shown to down regulate the basolateral iron carrier ferroportin. It has also been demonstrated that hepcidin itself is up regulated by HFE. Thus, an HFE mutation may reduce the up regulation of hepcidin that then does not down regulate ferroportin; the corresponding increase in ferroportin expression finally causes the increase in intestinal iron uptake (DeDomenico 2007). There may be further interactions between HFE, transferrin receptor 2, Nramp2, Dcytb, ferroportin, hephaestin and hepcidin, all of which are currently being studied.

Diagnosis

Laboratory tests. Any increase in serum iron should start with the exclusion of haemochromatosis so as not to overlook early disease. Normal serum iron, however, does not exclude haemochromatosis and increased serum iron often occurs in the absence of haemochromatosis. Serum iron values are highly variable and should not be used either for diagnosis or for screening of haemochromatosis. The determination of transferrin saturation is a better indicator of iron overload than serum iron. The increase in transferrin saturation usually precedes the ferritin increase (Figure 1). Transferrin saturation is more sensitive and specific for detection of haemochromatosis when compared to serum ferritin. For screening, a threshold of 50% for transferrin saturation may be optimal under fasting conditions. Ferritin on the other hand is a good indicator of largely increased iron stores and reliably indicates iron deficiency. It has less value for early detection of haemochromatosis. In haemochromatosis a slightly increased serum ferritin (300-500 ng/ml) is usually accompanied by transferrin saturations exceeding 80-90%. Unfortunately, serum ferritin is also increased, often in the presence of infections and malignancies, and thus has a low specificity for indicating haemochromatosis (Niederau 1998). Ferritin increases not due to genetic haemochromatosis are usually associated with normal or only slightly elevated transferrin saturation. Therefore, transferrin saturation should be measured in order to correctly interpret ferritin increases.

Liver biopsy and determination of liver iron concentration. Although simultaneous increases of both serum ferritin and transferrin saturation strongly indicate a risk for haemochromatosis, diagnosis needs to be confirmed by genetic testing or by liver biopsy with a determination of iron content in the liver. Hepatic iron concentration also increases with time in subjects with an HFE gene mutation. Thus, it is recommended to divide liver iron concentrations by the patient's age in order to obtain the "liver-iron-index" (Summers 1990). The semi-quantitative estimation of liver iron stores by the Berlin-Blue colour is less sensitive and specific than the chemical quantification of liver iron concentration. In case of a homozygous C282Y gene test, liver biopsy is not required for the diagnosis of genetic haemochromatosis (Table 3).

There may, however, be other reasons to perform a liver biopsy in iron overload: (1) subjects with biochemical or clinical evidence of iron overload in the absence of the homozygous C282Y mutation should have a liver biopsy to substantiate iron

overload; (2) in C282Y homozygotes the risk for liver fibrosis and cirrhosis increases at ferritin values >1000 ng/ml (Loreal 1992); in those patients liver biopsy is recommended because the presence of liver cirrhosis markedly increases later HCC risk and thus warrants HCC screening.

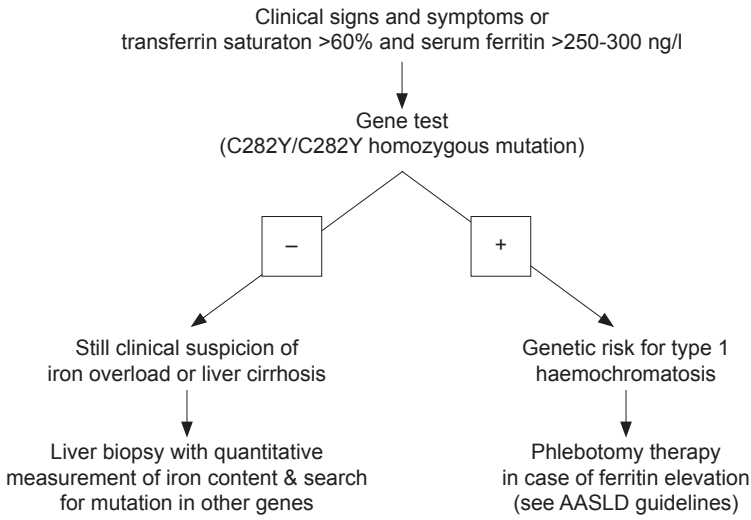


Table 3. Diagnosis and treatment algorithm for type 1 haemochromatosis.

Deferoxamine testing and ferrokinetic measurements. Determinations of urinary excretion of iron after administration of deferoxamine allows some estimation of total body iron stores. The deferoxamine test, however, often only shows pathological results when serum ferritin and transferrin saturation are markedly increased and does not allow diagnosis of early disease. Ferrokinetic measurements today are only done for scientific research or in difficult diagnostic situations.

Computed tomography (CT), magnetic resonance tomography (MRT) and biomagnetometry. CT density measurements of the liver allow a semi-quantitative estimation of iron concentration in the liver. This method however is associated with radiation and therefore not allowed in many countries where alternative methods are available. MRT, on the other hand, allows a reliable measurement of liver iron content, provided that special software is used and the equipment is calibrated for such measurement. In clinical practice most MRT do not fulfill these criteria. Biomagnetometry allows the most accurate non-invasive measurement of liver iron concentration. However, this equipment is expensive and only allows measurement of iron concentration. Consequently, biomagnetometry is done only at a few centers worldwide and is primarily used for scientific studies and not in daily clinical practice. With the availability of reliable and inexpensive genetic testing, CT, MRT, and biomagnetometry do not need to be done for most patients.

Genetic tests. As outlined previously, in Caucasian populations the homozygous C282Y mutation accounts for more than 90% of patients with the clinical phenotype of type 1 haemochromatosis (Adams 2005; Erhardt 1999). Approximately 5% of patients with the clinical phenotype are C282Y/H63D compound heterozygotes; the prevalence of C282Y or H63D heterozygosity in patients with the clinical phenotype of haemochromatosis is considerably lower than in the general population. Thus, a subject who is heterozygous for C282Y or H63D per se has no risk of iron overload. In subjects homozygous for C282Y, both serum ferritin and transferrin saturation are frequently increased; however, only male subjects have an increased risk for liver disease when compared to subjects without HFE gene alterations in a recent large screening study. It is unknown how many C282Y homozygotes will later develop clinical signs and symptoms due to iron overload. It is increasingly evident that only a minority of C282Y homozygotes progress to end stage iron overload with liver cirrhosis and diabetes mellitus. In subjects who are not C282Y homozygotes but have laboratory, histological or clinical evidence of iron overload, further genes may be analysed for mutations such as haemojuveline, transferrin receptor 2, ferroportin 1 and hepcidin.

Early diagnosis and screening

The prevalence of C282Y homozygotes is 0.5% in Caucasian populations (Adams 2005; Erhardt 1999). Clinical manifestation however is variable and depends on non-genetic factors such as dietary iron intake and blood loss. Until 1980 most patients with haemochromatosis were detected with late irreversible complications such as liver cirrhosis and diabetes mellitus. With a better understanding of the disease, the broad use of ferritin and transferrin saturation measurements and the availability of a reliable genetic test, diagnostic efforts have concentrated on the detection of early disease in the absence of liver cirrhosis and diabetes mellitus. Several studies have shown that iron removal by phlebotomy is associated with normal life expectancy in patients diagnosed early (Niederau 1985; Niederau 1996; Fargion 1992) (Figure 3). Thus, several other studies have focused on screening procedures in order to diagnose more subjects with early disease (Edwards 1988). These studies include populations with special risks, family members, as well as the general population (Table 4) (for further literature see Niederau 2002). It has also been shown that an increasing number of patients are now diagnosed in early stages and that this trend increases survival (Figure 4).

A large number of studies have shown that screening is useful for detection of asymptomatic C282Y homozygotes by using transferrin saturation and serum ferritin as well a genetic test for the C282Y mutation (Edwards 1988; Phatak 1998; Niederau 1998). A broad screening of the general population however is as yet not recommended by WHO and CDC mainly because its is unknown how many of the asymptomatic C282Y homozygotes will later develop clinical disease (for further literature see US Preventive Services Task Force 2007). The largest screening study analyzed HFE gene mutations in almost 100,000 subjects in North America. In Caucasian subjects, C282Y homozygosity was found in 0.44%, a value similar to many previous studies in other populations with a Caucasian background. Asian or Black people in contrast almost never have an HFE gene mutation (Adams 2005). Among the Caucasian C282Y homozygotes only males had a significant increase in liver disease when compared

to subjects without an HFE gene variation (Adams 2005). Only further prospective follow-up studies will determine how many asymptomatic C282Y homozygotes will develop clinical consequences of iron overload.

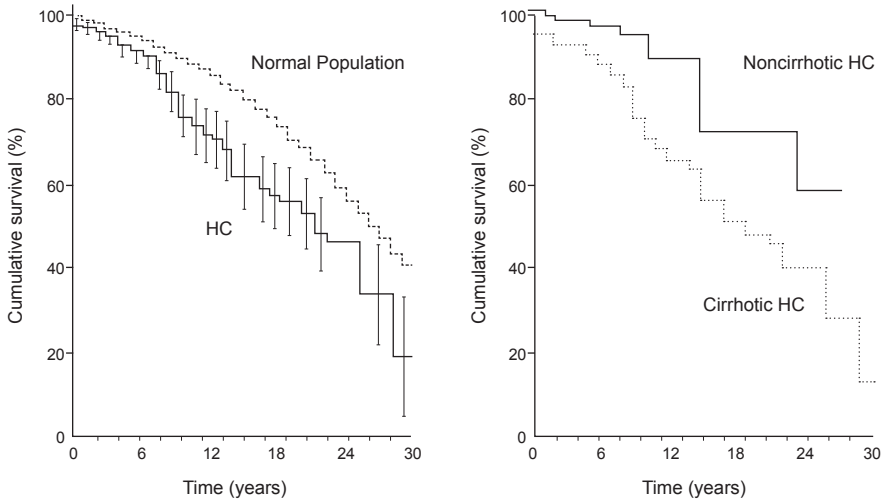


Figure 3. Survival of 251 patients with genetic haemochromatosis (with and without cirrhosis) in comparison with matched general population. Modified from Niederau 1996.

It is also unknown at which ferritin values phlebotomy treatment should be initiated in asymptomatic C282Y homozygotes (Table 5). The values recommended by the AASLD (American Association for the Study of Liver Diseases) are based more on the judgment of experts than on solid data. The only solid data shows that the risk for liver fibrosis and cirrhosis increases above the threshold of 1000 ng/ml for serum ferritin (Loreal 1996). The value of screening family members is obvious when a first-degree relative has clinical haemochromatosis. Such family screening is easy to do with the genetic test. Heterozygous family members are not at risk for haemochromatosis unless they have other risk factors.

The clinical phenotype of haemochromatosis is detected in 1-2% of patients with newly diagnosed diabetes mellitus and in 3-15% of patients with liver cirrhosis (Niederau 1999). These latter patients should be screened for iron overload although such screening obviously does not aim at a very early diagnosis. Nevertheless, cirrhotic and diabetic patients with haemochromatosis can benefit significantly from phlebotomy therapy. Little is known about the prevalence of haemochromatosis in patients with arthropathy or cardiomyopathy of unclear etiology. Several smaller studies indicate that arthropathy may be a rather early clinical sign of iron overload, whereas cardiomyopathy usually occurs in severe iron overload.

1. Screening in the general population not recommended
A screening of HFE gene alterations is not recommended in the general population because it remains unknown how many of the C282Y homozygotes will develop clinical manifestations. Sch screening would be meaningful only in Caucasian populations.

2. Family screening
Genetic testing can reliably determine who, among the first-degree relatives of a aemochromatotic patient, is a heterozygote or homozygote. Heterozygotes are healthy and do not need follow-up. C282Y homozygotes should be followed and treated by phlebotomy if ferritin increases >300 ng/ml in men and >200 ng/ml in women.

3. Haemochromatosis should be excluded in patients with
- newly diagnosed diabetes mellitus
- chronic liver disease of unknown aetiology
- elevation of iron, transferrin saturation or serum ferritin
- cardiomyopathy of unknown aetiology
- arthropathy of unknown aetiology
- loss of potency/libido and amennorhea of unknown aetiology

4. Every liver biopsy needs to be checked for iron deposits

Table 4. Methods for early diagnosis of haemochromatosis.

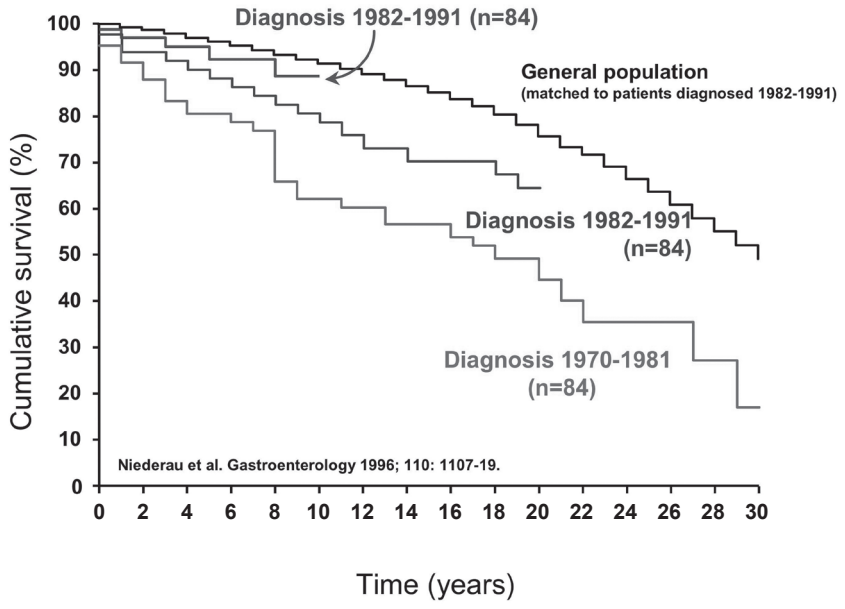


Figure 4. Cumulative survival in 251 patients with genetic haemochromatosis according to the time of diagnosis. Modified from Niederau 1996.

1. Phlebotomy

a) In symptomatic genetic haemochromatosis

- aims: complete iron depletion in 12-24 months;
- treatment: 1-2 phlebotomies of 500 ml each week until serum ferritin is in the range of 20-50 ng/ml long-term therapy with 4-8 phlebotomies per year to keep ferritin between 20-50 ng/ml and thus prevent reaccumulation of iron

b) In asymptomatic C828Y homozygotes therapy should be initiated above these ferritin values:

- subjects <18 years >200 ng/ml
- men >300 ng/ml
- women (not pregnant) >200 ng/ml
- women (pregnant) >500 ng/ml

2. Therapy with iron chelators in secondary haemochromatosis and anaemia

- aims: removal of iron overload by increase of iron excretion in faeces and urine
- in case of further blood transfusions at high frequency at stabilisation of iron balance and reduction of further iron accumulation
- treatment: until recently, 25-50 mg deferoxamine/kg as SC infusion for 10-12 h daily; today, deferoxamine is largely replaced by the oral chelator deferasirox - 20 mg/kg deferasirox once daily to prevent iron accumulation up to 800 ml erythrocytes concentrates/month
- long-term treatment necessary
- normalisation of ferritin and liver iron concentration is often not possible

3. Diet

- recommended: avoidance of food with very high iron content (e.g., liver) and iron-supplemented food
- a further strict iron-depleted diet is very difficult to adhere to and not recommended
- a single phlebotomy of 500 ml blood is as effective for iron removal as a very rigid iron-restricted diet for a whole year

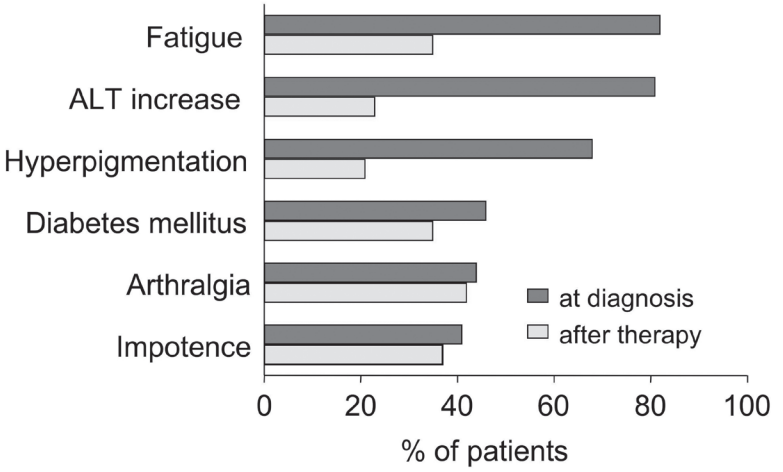
Table 5. Iron overload therapy.

Complications of iron overload

Liver cirrhosis, diabetes mellitus, and increased skin pigmentation are the classical trio of genetic haemochromatosis. Cardiomyopathy, cardiac arrhythmias, and impotence are also typical complications of advanced iron overload. Arthropathy in contrast may be an early sign of haemochromatosis, which may help with diagnosis in the precirrhotic stage (Niederau 1996).

Liver disease. The liver is the organ that is affected by genetic iron overload most early and heavily. At early stages excess iron stores are mainly found in periportal parenchymal cells as ferritin and haemosiderin. When iron excess further increases, there is development of perilobular fibrosis and iron stores are also found in bile ducts and Kupffer cells. Septal fibrosis eventually progresses towards complete cirrhosis.

The stage of fibrosis is closely associated with the degree of excess of iron. In many affected symptomatic patients with type 1 haemochromatosis there are some signs of liver disease at the time of diagnosis (Niederau 1985; Niederau 1996). Many nonspecific symptoms such as abdominal discomfort and fatigue may also be due to liver involvement. In asymptomatic patients diagnosed by a screening procedure, signs of liver disease are infrequent. Complications due to cirrhosis such as ascites, jaundice and portal hypertension are seen only rarely and only in cases of advanced severe iron overload (Niederau 1985; Niederau 1996). The risk for liver cirrhosis increases at ferritin values >1000 ng/ml (Loreal 1996). Similar to insulin-dependent diabetes, liver cirrhosis cannot be reversed by removal of iron (Niederau 1996). However, less advanced stages like hepatic fibrosis and abnormalities in liver enzymes and function respond well to iron removal (Niederau 1996) (Figure 5). Survival is significantly reduced in the presence of liver cirrhosis whereas patients diagnosed in the precirrhotic stage have a normal life expectancy when treated by phlebotomy (Niederau 1996) (Figure 3).



Niederau et al. Gastroenterology 1996;110:1107-1119.

Figure 5. Signs and symptoms in 185 patients with genetic haemochromatosis prior to and after iron removal. Modified from Niederau 1996.

Association of haemochromatosis with other liver diseases. Some studies indicate that C282Y heterozygosity may aggravate the progression of concomitant liver diseases such as porphyria cutanea tarda, chronic hepatitis C, alcoholic hepatitis and non-alcoholic steatohepatitis (NASH). In the latter patients one might find slightly elevated liver iron concentrations and serum ferritin levels when they are C282Y heterozygotes (for review see Erhardt 2003). Most studies however have shown that these associations are of only minor importance in the clinical course of the disease. Phlebotomy as yet has only been proven meaningful in porphyria cutanea tarda because it can ameliorate the cutaneous manifestations.

Liver carcinoma. Liver carcinoma develops in approximately 30% of patients with haemochromatosis and cirrhosis independent of iron depletion (Niederau 1996); the interval between complete iron depletion and reported diagnosis of liver cancer is approximately 9 years in large cohorts of German patients (Niederau 1985; Niederau 1996). The risk of liver cancer is increased in patients with haemochromatosis by 100-200 times when compared to the general population (Figure 6). Among liver cancers there are hepatocellular carcinomas (HCC) as well as cholangiocellular carcinomas. Most liver cancers develop in patients with cirrhosis. Thus, cancer screening by ultrasound and APF (twice a year) is only recommended for cirrhotic patients. Patients who develop liver cancer usually have the largest amount of mobilisable iron among various subgroups (Niederau 1996; Niederau 1999).

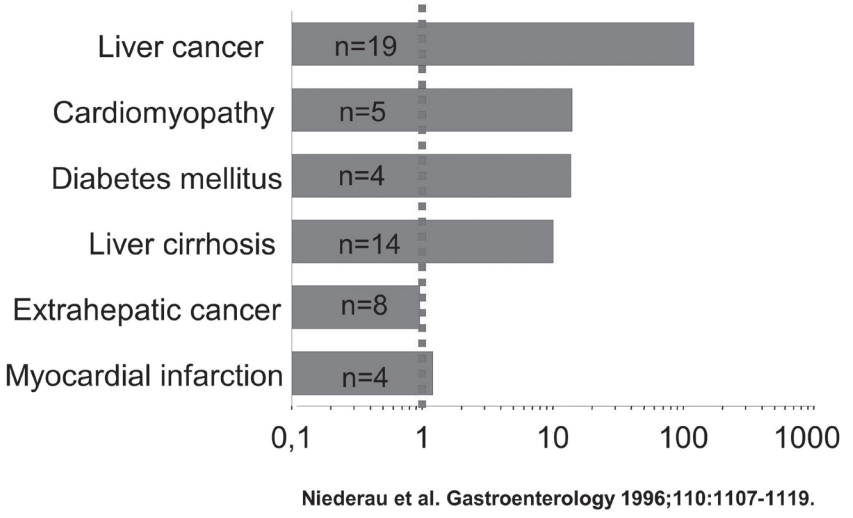


Figure 6. Relative mortality risk of 251 patients with genetic haemochromatosis in comparison to the general population. Modified from Niederau 1996.

Diabetes mellitus. In recent studies the prevalence of diabetes in hereditary haemochromatosis ranges from 20-50% (Niederau 1996; Adams 1991). The prevalence and stage of diabetes is related to the degree of iron deposition in the pancreas. Patients with diabetes have a twofold higher mobilisable iron content than non-diabetics (Yaouanq 1995). Investigations into the prevalence of unrecognized genetic haemochromatosis in diabetic patients show some variation in Europe vs. elsewhere; i.e., screening revealed a prevalence of 5-8 per 1000 unrecognized cases in Europe (Singh 1992) and 9.6 per 1000 in Australia (Phelps 1989). Diabetes mellitus and impaired glucose tolerance are frequent features in several chronic liver diseases (Creutzfeldt 1970; Blei 1982). This author's study (Niederau 1984) showed hyperinsulinaemia and hence insulin resistance without impaired glucose tolerance in noncirrhotic haemochromatosis. The increase in circulating insulin concentrations is likely to be due to a decrease

in diminished hepatic extraction of insulin. With the progression of iron overload and destruction of beta-cells, insulin secretion becomes impaired (Dymock 1972; Bierens de Haan 1973). In end-stage haemochromatosis, insulin deficiency is associated with severe reduction in the mass of beta-cells (Rahier 1987). Insulin resistance observed in early iron overload may be partially reversible after phlebotomy therapy (Niederau 1985; Niederau 1996) whereas insulin-dependent diabetes is irreversible (Niederau 1996). Survival is significantly reduced in patients with diabetes mellitus at diagnosis compared to patients without diabetes (Niederau 1996). Survival of non-diabetic patients is virtually identical to that of a matched normal population.

Heart disease. Cardiomyopathy and cardiac arrhythmias are specific complications of haemochromatosis caused by iron deposition in the heart (Buja and Roberts 1971; Short 1981). Clinical or electrocardiographic signs of heart disease may be found in 20-35% of patients with HFE haemochromatosis (Niederau 1985). Arrhythmias usually respond well to iron removal (Short 1981; Niederau 1996). In type 1 haemochromatosis cardiomyopathy is rare and usually associated with advanced iron overload and an older patient population. However, particularly in young patients who present with cardiac disease due to haemochromatosis, cardiomyopathy is a frequent cause of death (Finch 1966; Short 1981). Only recently has it become clear that young patients with severe cardiomyopathy may be affected by juvenile type 2 haemochromatosis; these patients may show severe iron overload, hypogonadism, cardiomyopathy, liver cirrhosis, and amennorrhea by ages 15-24. The type 2 associated cardiomyopathy is often irreversible despite initiation of phlebotomy or chelation therapy and may require an immediate transplant of the heart and potentially of the liver as well (von Herbay 1996; Jensen 1993).

Arthropathy. Joint changes in genetic haemochromatosis may occur in two different ways (Schuhmacher 1964; Dymock 1970; Niederau 1985; Niederau 1996). The most prevalent changes are seen in the metacarpophalageal joints II and III, in the form of cystic and sclerotic changes, cartilage damage and a narrowing of the intraarticular space. Sometimes other joints of the hands and the feet are affected. Large joints, i.e., of the knees and hips, may be affected in the form of chondrocalcinosis. The pathogenesis of joint changes in haemochromatosis remains unclear. Arthropathy is one of the few complications not associated with the degree of iron overload. It has been speculated that iron may inhibit pyrophosphatase and may thereby lead to a crystallisation of calcium pyrophosphates. Alternatively, iron may have direct toxic effects on the joints. Arthropathy may be an early sign of haemochromatosis and may help to make the diagnosis at a precirrhotic stage (Niederau 1996). Haemochromatosis should therefore been considered in all patients with an arthropathy of unknown etiology.

Endocrine abnormalities. In contrast to the early onset of arthropathic changes, endocrine abnormalities are a late consequence of iron overload. Sexual impotence and loss of libido may occur in up to 40% of male patients (Niederau 1985). The endocrine abnormalities in haemochromatosis are mainly, if not exclusively, due to pituitary

failure. This is in contrast to alcoholic cirrhosis where testicular failure is predominant (Kley 1985a; Kley 1985b). In contrast to alcoholic cirrhosis, where estrogen levels are usually increased, estrogen levels were found decreased in haemochromatosis (Kley 1985a). Most endocrine changes are late and irreversible complications of genetic haemochromatosis and do not respond well to phlebotomy treatment (Niederau 1996). Iron overload only infrequently affects other endocrine organs such as the thyroid and adrenal glands. Severe hypogonadism with amennorrhea in young women and impotence in young men is today thought to be due to type 2 haemochromatosis.

Skin. Increased skin pigmentation is mainly seen in areas exposed to sunlight. A large part of the darkening of pigmentation is thought to be due to an increase in melanin and not due to iron excess itself. The increase in skin pigmentation is reversible on iron removal (i.e., phlebotomy).

Other potential complications. Iron overload has been speculated to aggravate atherosclerosis; however, the evidence for that speculation is rather weak (for review see Niederau 2000). There have also been reports that extrahepatic malignancies may be increased in HFE haemochromatosis (Amman 1980; Fracanzani 2001) while other studies have not found extrahepatic associations (Bain 1984; Niederau 1996; Elmberg 2003). It is not clear whether HFE gene mutations are involved in the pathogenesis of porphyria cutanea tarda since the prevalence of both risk factors vary greatly in different parts of the world; associations between HFE gene mutations and pophyria have often been described in southern Europe but not in northern Europe (for literature, see Toll 2006).

Therapy

Phlebotomy treatment. Phlebotomy treatment is the standard of care to remove iron in genetic haemochromatosis. One phlebotomy session removes approximately 250 mg iron from the body. Since patients with the classical clinical phenotype may have an excess of 10-30 g iron, it may take 12-24 months to remove the iron overload when phlebotomies of 500 ml blood are done weekly (Table 5). Phlebotomy treatment is generally well tolerated and haemoglobin usually does not drop below 12 g/dl. Several studies have shown that liver iron is completely removed at such low ferritin values; thus the effect of therapy can be checked by ferritin measurements and a control liver biopsy is not necessary. After complete removal of excess iron the intervals of phlebotomies may be increased to once every 2-3 months; serum ferritin should be kept in the lower normal range, between 20-50 ng/ml. Phlebotomy should not be interrupted for longer intervals; there is a risk of reaccumulation of iron due to the genetic autosomal-recessive metabolic malfunction

Iron removal by chelators. Deferoxamin therapy for genetic haemochromatosis is not recommended because phlebotomy is more effective with less side effects and lower cost. Recently, a phase II study has started, looking for safety and effectiveness of the new oral iron chelator deferasirox in genetic haemochromatosis. As yet, deferasirox is only approved for secondary haemochromatosis.

Diet. An iron-low diet is not recommended for patients with genetic haemochromatosis. One phlebotomy of 500 ml blood removes approximately 250 mg iron. A difficult to follow rigid iron-restricted diet for a complete year would have the effect of a single phlebotomy. It is thus recommended that patients simply do not eat excessive amounts of food with very high iron content (such as liver) and that they do not eat food to which iron has been added (Table 5).

Liver transplantation. Advanced liver cirrhosis and carcinoma may be indications for a liver transplant in haemochromatosis (Kowdley 1995; Brandhagen 2000). The prognosis of patients who have a liver transplant for haemochromatosis is markedly worse than that for patients with other liver diseases; a considerable number of patients with haemochromatosis die after transplant from infectious complications or heart failure (Brandhagen 2000). Liver transplantation does not heal the original genetic defect.

Prognosis

Untreated haemochromatosis often has a bad prognosis in the presence of liver cirrhosis and diabetes mellitus. The prognosis is markedly worse in patients with cirrhosis than in those without cirrhosis at diagnosis (Figure 3); the same is true for diabetes mellitus. It is generally accepted that phlebotomy therapy improves the prognosis. Patients diagnosed and treated in the early non-cirrhotic stage have a normal life expectancy (Figure 3) (Niederau 1985; Niederau 1996). Thus, early diagnosis markedly improves the prognosis (Figure 4). Iron removal by phlebotomy also improves the outcome in patients with liver cirrhosis. The prognosis of liver cirrhosis due to haemochromatosis is markedly better than those with other types of cirrhosis (Powell 1971). Hepatomegaly and elevation of aminotransferases often regress after iron removal (Niederau 1985; Niederau 1996) (Figure 5). Insulin-dependent diabetes mellitus and hypogondism are irreversible complications despite complete iron removal (Niederau 1996) (Figure 5). Earlier changes in glucose and insulin metabolism, however, may be ameliorated after iron removal. For unknown reasons arthropathy does not respond well to phlebotomy treatment although it may be an early sign of iron overload (Figure 5). The AASLD consensus guidelines recommend to start phlebotomy treatment at ferritin values >300 ng/ml in men and >200 ng/ml in women. The risk for liver fibrosis and cirrhosis is increased only at ferritin levels >1000 ng/ml. Further studies need to determine whether asymptomatic C282Y homozygotes with ferritin values between 300 and 1000 ng/ml need to be treated or whether one might wait and monitor ferritin at that stage.

Juvenile hereditary haemochromatosis

Two genes have been shown to be associated with juvenile haemochromatosis: 90% of cases are associated with mutations in hemojuveline (HJV) (locus name HFE2A), which encodes HJV, while 10% of cases are associated with HAMP (locus name HFE2B), which encodes hepcidin. Despite the nomenclature of HFE2A and HFE2B, juvenile haemochromatosis is not associated with HFE mutations. In order to avoid confusion most physicians use the terms type 2A (haemojuvelin mutations) and type 2B (HAMP mutations). Mutations in haemojuvelin are associated with low levels of hepcidin in urine suggesting that haemojuvelin regulates hepcidin. Hepcidin is the

key regulator of intestinal iron absorption and iron release from macrophages. Hepcidin facilitates ferroportin internalisation and degradation. Hepcidin mutations may thereby lead to an increase in ferroportin and thus iron uptake from the intestine. Juvenile haemochromatosis is very rare. A clustering of HJV mutations is seen in Italy and Greece although few families account for this phenomenon. Mutations in HJV represent the majority of worldwide cases of juvenile haemochromatosis. Only a small number of patients have been identified with HAMP-related juvenile haemochromatosis. Juvenile haemochromatosis is characterized by an onset of severe iron overload in the first to third decades of life. Clinical features include hypogonadism, cardiomyopathy, and liver cirrhosis (Diamond 1989; Vaiopoulos 2003). The main cause of death is cardiomyopathy (De Gobbi 2002; Filali 2004). In contrast to HFE type 1 haemochromatosis, both sexes are equally affected. Mortality can be reduced in juvenile haemochromatosis when it is diagnosed early and treated properly. Phlebotomy is the standard therapy in juvenile haemochromatosis as well and is treated similarly to HFE haemochromatosis (Tavill 2001). In patients with juvenile haemochromatosis and anaemia or severe cardiac failure, administration of chelators such as deferoxamine have been tried to reduce mortality; some case reports suggest that this might improve left ventricular ejection fraction (Kelly 1998).

Transferrin receptor 2 (TFR2)-related type 3 haemochromatosis

TFR2-related haemochromatosis is defined as type 3 and is also known as HFE3; however, the term HFE3 should not be used because the HFE gene is not affected in type 3 haemochromatosis. TFR2-related haemochromatosis is inherited in an autosomal recessive manner. TFR2 is a type II 801-amino-acid transmembrane glycoprotein expressed in hepatocytes and at lower levels in Kupffer cells (Zhang 2004). A finely regulated interaction between TFR2, TFR1 and HFE is now thought to affect the hepcidin pathway, and, consequently, iron homeostasis (Fleming 2005). Patients with homozygous TFR2 mutations have increased intestinal iron absorption that leads to iron overload. Hepcidin concentrations in urine are low in TFR2 haemochromatosis (Nemeth 2005). TFR2-related haemochromatosis is very rare with only about 20 patients reported worldwide (Mattman 2002). Age of onset in TFR2-related type 3 haemochromatosis is earlier than in HFE-associated type 1 (Piperno 2004; Girelli 2002; Hattori 2003). Progression is, however, slower than in juvenile type 2 (De Gobbi 2002; Roetto 2001; Girelli 2002). The phenotype is similar to type 1. Many patients present with fatigue, arthralgia, abdominal pain, decreased libido, or with biochemical signs of iron overload (Roetto 2001; Girelli 2002; Hattori 2003). Complications of type 3 haemochromatosis include cirrhosis, hypogonadism, and arthropathy. Cardiomyopathy and diabetes mellitus appear to be rather rare. Hepatocellular carcinoma has not been observed in the small number of cases diagnosed. Most individuals with type 3 haemochromatosis have an Italian or Japanese genetic background. Some of the Japanese males have had liver cirrhosis at diagnosis (Hattori 2003). Similar to type 1 haemochromatosis, the penetration of type 3 haemochromatosis is also considerably less than 100% (Roetto et al. 2001). Standard therapy is iron removal by weekly phlebotomy similar to the management of type 1 disease. Individuals with increased ferritin should be treated similar to those with HFE haemochromatosis.

Type 4 haemochromatosis – Ferroportin disease

Ferroportin-associated iron overload (also called Ferroportin disease) was first recognised by Pietrangelo (1999) who described an Italian family with an autosomal dominant non-HFE haemochromatosis. Many family members had iron overload resulting in liver fibrosis, diabetes, impotence, and cardiac arrhythmias. In addition to autosomal dominant inheritance, features distinguishing this from HFE haemochromatosis included early iron accumulation in reticuloendothelial cells and a marked increase in ferritin earlier than what is seen in transferrin saturation (Pietrangelo 1999; Rivard 2003; Montosi 2001; Wallace 2004; Fleming 2001). Several patients showed a reduced tolerance to phlebotomy and became anaemic despite elevated ferritin (Pietrangelo 1999; Jouanolle 2003). In 2001 this form of non-HFE haemochromatosis was linked to mutations of ferroportin (Montosi 2001) that had just been identified as the basolateral iron transporter (Abboud 2000; Donovan 2000). Since that time, numerous mutations in the gene have been implicated in patients from diverse ethnic origins with previously unexplained haemochromatosis. Iron overload disease due to ferroportin mutations has been defined as type 4 haemochromatosis or Ferroportin Disease (for review see Pietrangelo 2004). The iron export is tightly regulated because both iron deficiency and iron excess are harmful. The main regulator of this mechanism is the peptide hepcidin which binds to ferroportin, induces its internalization and degradation, thereby reducing iron efflux (Nemeth 2004). Increase in iron absorption may be caused either by hepcidin deficiency or its ineffective interaction with ferroportin. All recent studies have shown that hepcidin deficiency appears to be the common characteristic of most types of genetic (mutations in HFE, transferrin receptor 2, haemojuvelin, or hepcidin itself). The remaining cases of genetic iron overload are due to heterozygous mutations in the hepcidin target, ferroportin. Because of the mild clinical penetrance of the genetic defect there were doubts about the rationale for iron-removal therapy. However, a recent study shows that there may be clinically relevant iron overload with organ damage and liver cancer in patients carrying the A77D mutation of ferroportin (Corradini 2007). Treatment schemes are similar to those described for other types of genetic haemochromatosis.

Secondary haemochromatosis

Pathophysiology

Most forms of secondary haemochromatosis are due to haemolytic anaemia associated with polytransfusions such as thalassaemia, sickle cell disease, and MDS. Most of these patients need blood transfusions on a regular basis for survival. However, in the long run, multiple blood transfusions often lead to iron overload if patients are not treated with iron chelators. In general, iron overload due to blood transfusions is similar to genetic haemochromatosis; however, secondary iron overload develops much faster than the genetic forms (McLaren 1983), sometimes as soon as after 10-12 blood transfusions (Porter 2001). Subsequently secondary iron overload can result in more rapid organ damage when compared with genetic haemochromatosis. Secondary iron overload can obviously not be treated by phlebotomy because a marked anaemia is the clinical marker of the disease. Secondary iron overload often limits the prognosis of patients with thalassaemia; life expectancy deteriorates with increasing

iron concentrations in the liver (Telfer 2000). Therapy with iron chelator may reduce the transfusional iron burden if the frequency of transfusion is not too high. The development of HFE versus secondary haemochromatosis does not only differ in terms of the speed of iron accumulation but also in the type of organ damage; in secondary haemochromatosis cardiomyopathy is often the complication that limits the prognosis (Liu 1994). It is interesting that heart disease is also very frequent in juvenile genetic haemochromatosis where there is also a rapid iron accumulation. In general, serum ferritin values closely reflect liver iron concentration and may be used as an indication for timing of therapy as well as to check the effects of iron chelation.

Until recently deferoxamine was the only iron chelator available in most countries; in some countries the drug deferiprone is approved for patients who do not tolerate deferoxamine (Hoffbrandt 2003). The clinical use of deferiprone was limited due to side effects such as agranulocytosis and neutropaenia (Refaie 1995). Long-term data prove that deferoxamine can reduce iron overload and its organ complications (Olivieri 1994; Cohen 1981). Deferoxamine, however, needs to be given daily subcutaneously or by IV infusion for several hours. Thus, patients with thalassaemia often consider the deferoxamine treatment worse than thalassaemia itself (Goldbeck 2000). There are minor compliance problems that often limit the beneficial effects of this iron chelator (Cohen 1989).

Without iron chelation, children with thalassaemia often develop a severe cardiomyopathy prior to age 15 (Cohen 1987). After that age, liver cirrhosis is also a significant complication in secondary iron overload due to thalassaemia (Zurlo 1992). Iron chelation should start early to prevent complications of iron overload. By the ages of 3-5 years old, liver iron concentration may reach values associated with a significant risk for liver fibrosis in severe thalassaemia (Angelucci 1995). Children younger than 5 should therefore be cautiously treated with chelators if they have received transfusions for more than one year (Olivieri 1997). Deferoxamine can reduce the incidence and ameliorate the course of iron-associated cardiomyopathy (Olivieri 1994; Brittenham 1994; Miskin 2003).

Deferasirox is a new oral iron chelator with high selectivity for iron III (Nick 2003). Deferasirox binds iron in a 2:1 proportion with a high affinity and increases the biliary iron excretion (Nick 2003). This chelator is able to reduce iron overload in hepatocytes and cardiomyocytes (Nick 2003; Hershko 2001). Due to its half-life of 11-18 hours it needs to be taken only once daily (Nisbet-Brown 2003). Deferasirox exerted a similar iron chelation when compared with deferoxamine in patients with thalassaemia; the effect of 40 mg/kg deferoxamine was similar to that of 20 mg/kg deferasirox (Piga 2006). Both in adults and children 20-30 mg/kg/day deferasirox significantly reduced liver iron concentration and serum ferritin (Cappellini 2006). Magnetic resonance imaging showed that 10-30 mg/day deferasirox may also reduce iron concentration in the heart within one year of maintaining therapy. Deferasirox may cause minor increases in serum creatinine as well as gastrointestinal discomfort and skin exanthema which are both usually self-limiting. Considering the compliance problems with deferoxamine, deferasirox has a better cost-effectiveness ratio (Vichinsky 2005). Deferasirox is defined as standard therapy both in the guidelines of the National Comprehensive Cancer Network (NCCN) (USA) and in the international guidelines on MDS (Greenberg 2006; Gattermann 2005).

References

Abboud S, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 2000;275:19906-19912

Adams PC, Speechley M, Kertesz AE. Long-term survival analysis in hereditary hemochromatosis. Gastroenterology 1991;101:368-372

Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005; 352:1769-1778

Angelucci E, Baronciani D, Lucarelli G, et al. Needle liver biopsy in thalassaemia: analyses of diagnostic accuracy and safety in 1184 consecutive biopsies. Br J Haematol 1995;89:757-761

Bain C, Bradbear R, Siskind V, et al. Cohort study of the risk of malignancy in haemochromatosis and other nonalcoholic liver diseases. Hepatology 1984;4:A1020

Biasiotto G, Belloli S, Ruggeri G, et al. Identification of new mutations of the HFE, hepcidin, and transferrin receptor 2 genes by denaturing HPLC analysis of individuals with biochemical indications of iron overload. Clin Chem 2003;49:1981-1988

Bierens deHaan B, Scherrer JR, Stauffacher W, et al. Iron excess, early glucose intolerance, and impaired insulin secretion in idiopathic hemochromatosis. Eur J Clin Invest 1973;3:179-187

Blei AT, Robbins DC, Drobny E, et al. Insulin resistance and insulin receptors in hepatic cirrhosis. Gastroenterology 1982;83:1313-1318

Brandhagen DJ, Alvarez W, Therneau TM, et al. Iron overload in cirrhosis-HFE genotypes and outcome after liver transplantation. Hepatology 2000;31:456-460

Brittenham GM, Griffith PM, Nienhuis AW, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med 1994;331:567-573

Buja L, Roberts W. C Iron in the heart. Am J Med 1971;51:209-221

Cappellini MD, Cohen A, Piga A, et al. A Phase III study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 2006;107:3455-3462

Cohen A, Martin M, Schwartz E. Response to long-term deferoxamine therapy in thalassemia. J Pediatr 1981;99:689-694

Cohen AR, Mizanin J, Schwartz E. Rapid removal of excessive iron with daily, high-dose intravenous chelation therapy. J Pediatr 1989;115:151-155

Cohen A. Management of iron overload in the pediatric patient. Hematol Oncol Clin North Am 1987;1:521-544

Corradini E, Ferrara F, Pollicino T, et al. Disease progression and liver cancer in the ferroportin disease. Gut 2007;56:1030-1032

Creutzfeldt W, Frerichs H, Sickinger K. Liver diseases and diabetes mellitus. Prog Liver Dis 1970;3:371-407

Crosby WH. Hereditary hemochromatosis. In: Ingelfinger FJ (ed) Controversy in internal medicine. Saunders, Philadelphia, 1966:261-270

De Domenico I, Diane M, Ward DM, et al. Hepcidin regulation: ironing out the details. J Clin Invest 2007;117:1755-1758

De Gobbi M, Pasquero P, Brunello F, et al. Juvenile hemochromatosis associated with B-thalassemia treated by phlebotomy and recombinant human erythropoietin. Haematologica 2000;85:865-867

Diamond T, Stiel D, Posen S. Osteoporosis in hemochromatosis: iron excess, gonadal deficiency, or other factors? Ann Intern Med 1989;110:430-436

Donovan A, Brownlie A, Zhou Y, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 2000;403:778-781

Dymock W, Hamilton EBD, Laws JW, et al. Arthropathy of hemochromatosis: clinical and radiological analysis of 73 patients with iron overload. Ann Rheum Dis 1970;29:469-476

Dymock W, Cassar J, Pyke DA, et al. (1972) Observations on the pathogenesis, complications, and treatment of diabetes in 115 cases of hemochromatosis. Am J Med 52:203-210

Edwards CQ, Griffen LM, Goldgar D, et al. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med 1988;318:1355-1362

Elmberg M, Hultcrantz R, Ekbom A, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. Gastroenterology 2003;125:1733-2741

Erhardt A, Niederau C, Osman Y, et al. Demonstration of HFE polymorphism in German patients with hereditary hemochromatosis. Dtsch Med Wochenschr 1999;124:1448-1452

Erhardt A, Maschner-Olberg A, Mellenthin C, et al. HFE mutations and chronic hepatitis C: H63D and C282Y heterozygosity are independent risk factors for liver fibrosis and cirrhosis. J Hepatol 2003;38:335-342

Fargion S, Mandelli C, Piperno A, et al. Survival and prognostic factors in 212 italian patients with genetic hemochromatosis. Hepatology 1992;15:655-659

Feder JN, Gnirke A, Thomas W, et al. A novel MCH class I-like gene is mutated in patients with hereditary haemochromatosis. Nature Genetics 1996;13:399-407

Filali M, Le Jeunne C, Durand E, et al. Juvenile hemochromatosis HJV-related revealed by cardiogenic shock. Blood Cells Mol Dis 2004;33:120-124

Finch SC, Finch CA. Idiopathic hemochromatosis, an iron storage disease. Medi-cine 1966;34:381-430

Fleming RE, Sly WS. Ferroportin mutation in autosomal dominant hemochromatosis: loss of function, gain in understanding. J Clin Invest 2001;108:521-522

Fleming RE, Sly WS.. Mechanisms of iron accumulation in hereditary hemochromatosis. Ann Rev Physiol 2002;4:663-680

Fletcher LM, Halliday JW. Haemochromatosis: Understanding the mechanism of disease and implications for diagnosis and patient management following the recent cloning of novel genes involved in iron metabolism. J Intern Med 2002;251:181-192

Gattermann N. Consensus statement on iron overload in myelodysplastic syndromes. Hematol Oncol Clin N Am 2005;19:S18-S25

Girelli D, Bozzini C, Roetto A. Clinical and pathological finding in hemochromatosis type 3 due to a novel mutation in transferrin receptor 2 gene. Gastroenterology 2002;122:1295-1302

Goldbeck L, Baving A, Kohne E. Psychosocial aspects of beta-thalassemia: distress, coping and adherence. Klin Pädiatr 2000;212:254-259

Greenberg PL, Baer MR, Bennett JM, et al. Myelodysplastic syndromesclinical practice guidelines in oncology. J Natl Compr Canc Netw 2006;4:58-77

Hanot V, Schachmann M. Sur la cirrhose pigmentaire dans le diabe`te. sucré. Arch Physiol Norm Pathol 1886;7:50-72

Hattori A, Wakusawa S, Hayashi H, et al. AVAQ 594-597 deletion of the TfR2 gene in a Japanese family with hemochromatosis. Hepatol Res 2003;26:154-156

Herbay von A, Niederau C, Pelichowska M, et al. Kardiomyopathie als Todesursache bei genetischer Hämochromatose. Z Gastroenterol 1996;34:178-182

Hershko C, Konijn AM, Nick HP, et al. ICL670A: a new syntheticoral chelator: evaluation in hypertransfused rats with selective radio iron probes of hepatocellular and reticuloendothelial iron stores and in ironloaded-rat heart cells in culture. Blood 2001 ;97:1115-1122

Jensen PD, Bagger JP, Jensen FT, et al. Heart transplantation in a case of juvenile hereditary haemochromatosis followed up by MRI and endomyocardial biopsies. Eur J Haematol 1993;51:199-205

Jouanolle AM, Douabin-Gicquel V, Halimi C, et al. Novel mutation in ferroportin 1 gene is associated with autosomal dominant iron overload. J Hepatol 2003;39:286-289

Kelly AL, Rhodes DA, Roland JM, et al. Hereditary juvenile haemochromatosis: a genetically heterogeneous life-threatening iron-storage disease. QJM 1998;91:607-618

Kley HK, Stremmel W, Niederau C, et al. Androgen and estrogen response to adrenal and gonadal stimulation in idiopathic hemochromatosis: evidence for decreased estrogen formation Hepatology 1985a:251-256

Kley HK, Niederau C, Stremmel W, et al. Conversion of androgens to estrogens in idiopathic hemochromatosis: comparison with alcoholic cirrhosis. J Clin Endocrinol Metabol 1985b;61:1-6

Kowdley K, Hassanein T, Kaur S, et al. Primary liver cancer and survival in patients undergoing liver transplantation for hemochromatosis. Liver Transpl Surg 1995;1:237-241

Liu P, Olivieri N. Iron overload cardiomyopathies: new insights into an old disease. Cardiovasc Drugs Ther 1994;8:101-110

Loreal O, Deugnier Y, Moirand R. Liver fibrosis in genetic hemochromatosis. Respective roles of iron and non-iron related factors in 127 homozygous patients. J Hepatol 1992;16:122-127

MacDonald RA, Mallory GK. Hemochromatosis and hemosiderosis. Study in 211 autopsied cases. Arch Intern Med 1960;105:686-700

Mattman A, Huntsman D, Lockitch G, et al. Transferrin receptor 2 (TfR2) and HFE mutational analysis in non-C282Y iron overload: identification of a novel TfR2 mutation. Blood 2002;100:1075-1077

McLaren GD, Muir WA, Kellermeyer RW. In overload disorders: natural history, pathogenesis, diagnosis, and therapy. Crit Rev Clin Lab Sci 1983;19:205-266

Miskin H, Yaniv I, Berant M, et al. Reversal of cardiac complications in thalassemia major by long-term intermittent daily intensive iron chelation. Eur J Haematol 2003;70:398-403

Montosi G, Donovan A, Totaro A, et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. J Clin Invest 2001;108:619-623

Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306:2090-2093

Nemeth E, Roetto A, Garozzo G, et al. Hepcidin is decreased in TFR2-Hemochromatosis. Blood 2005;105:1803-1806

Nick H, Acklin P, Lattmann R, et al. Development of tridentate iron chelators: from desferrithiocin to ICL670. Curr Med Chem 2003;10:1065-1076

Niederau C, Berger M, Stremmel W, et al. Hyper-insulinaemia in non-cirrhotic hae-mo-chromatosis: impaired hepatic insulin degradation? Diabetologia 1084; 26:441-444

Niederau C, Fischer R, Sonnenberg A, et al. Survival and causes of death in cirrhotic and noncirrhotic patients with primary haemochromatosis. New Engl J Med 1985;313:1256-1262

Niederau C, Fischer R, Pürschel A, et al. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-1119

Niederau C, Niederau CM, Littauer A, et al. Screening for iron overload and iron deficiency. Ann Int Med 1998;128:337-345

Niederau C. Diabetes mellitus bei Hämochromatose. Z Gastroenterol 1999;37:22-32

Niederau C. Iron overload and atherosclerosis. Hepatology 2000;32:569-674

Nisbet-Brown E, Olivieri F, Giardina PJ, et al. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet 2003;361:1597-1602

Njajou OT, Vaessen N, Joosse M, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nat Genet 2001;28:213-214

Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood 1997;89:739-761

Olivieri NF, Nathan DG, Macmillan JH, et al. Survival in medically treated patients with homozygous beta-thalassemia. N Engl J Med 1994;331:574-578

Phatak PD, Sham RL, Raubertas RF, et al. Prevalence of hereditary hemochromatosis in 16,031 primary care patients. Ann Intern Med 1998;129:954-961

Phelps G, Chapman I, Hall P, et al. Prevalence of genetic haemochromatosis among diabetic patients. Lancet 1989;2:233-234

Pietrangelo A, Montosi G, Totaro A. Hereditary hemochromatosis in adults without pathogenic mutations in the hemochromatosis gene. N Engl J Med 1999;341:725-732

Pietrangelo A. Physiology of iron transport and the hemochromatosis gene. Am J Physiol Gastrointest Liver Physiol 2000;282:G403-G414

Pietrangelo A. Hereditary hemochromatosis -a new look at an old disease. N Engl J Med 2004;350:2383-2397

Pietrangelo A. The ferroportin disease. Blood Cells Mol Dis 2004;32:131-138

Piga A, Galanello R, Forni GL, et al. Randomized phase II trial of deferasirox (Exjade, ICL670), a once-daily, orally-administered iron chelator, in comparison to deferoxamine in thalassemia patients with transfusional iron overload. Haematologica 2006;91:873-880

Piperno A, Roetto A, Mariani R, et al. Homozygosity for transferrin receptor-2 Y250X mutation induces early iron overload. Haematologica 2004; 89:359-360

Porter JB Practical management of iron overload. Br J Haematol 2001;115:239-252

Powell LW, Mortimer R, Harris OD. Cirrhosis of the liver: A comparative study of the four major aetiological groups. Med J Aust 1971;58:1941-1950

Rahier J, Loozen S, Goebbels RM, et al. The haemochromatosis human pancreas: a quantitative immunochemical and ultrastructural study. Diabetologia 1987;30:5-12

Recklinghausen von FD. Über Hämochromatose. Berl Klin Wochenschr 1898;26:925

Rivard SR, Lanzara C, Grimard D, et al. Autosomal dominant reticuloendothelial iron overload (HFE type 4) due to a new missense mutation in the FERROPORTIN 1 gene (SLC11A3) in a large French-Canadian family. Haematologica 2003;88:824-826

Roetto A, Totaro A, Cazzola M, et al. Juvenile hemochromatosis locus maps to chromosome 1q. Am J Hum Genet 1999;64:1388-1393

Roetto A, Totaro A, Piperno A, et al. New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. Blood 2001;97:2555-2560

Schuhmacher HR. Hemochromatosis and arthritis. Arthr Rheum 1964;7:41-50

Sheldo JH. Haemochromatosis. Oxford University Press, 1935, London.

Short EM, Winkle RA, Billingham ME. Myocardial involvment in idiopathic hemochromatosis. Am J Med 1979;70:1275-1279

Singh BM, Grunewald RA, Press M, et al. Prevalence of haemochromatosis amongst patients with diabetes mellitus. Diabet Med 1992 ;9:730-731

Simon M, Pawlotsky Y, Bourel M, et al. Hemochromatose idiopathique. Maladie associee a l'antigen HLA-A3? Nouv Press Med 1075;4:1432

Simon M, Bourel M, Genetet B. Idiopathic hemochromatosis: demonstration of recessive transmission and early detection by family HLA typing. N Engl J Med 1977;297:1017-1021

Summers KM, Halliday JW, Powell LW. Identification of homozygous hemochromatosis subjects by measurement of hepatic iron index. Hepatology 1990;12:20-25

Tavill AS. Diagnosis and management of hemochromatosis. Hepatology 2001;33:1321-1328

Telfer PT, Prestcott E, Holden S, et al. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. Br J Haematol 2000;110:971-977

Toll A, Celis R, Ozalla MD, et al. The prevalence of HFE C282Y gene mutation is increased in Spanish patients with porphyria cutanea tarda without hepatitis C virus infection. J Eur Acad Dermatol Venereol 2006;20:1201-1206

Troisier M. Diabete sucre. Bull Soc Anatom (Paris) 1871;16:231-235

Trosseau A. Glucosurie: Diabete sucre. Bull Soc Anatom (Paris) 1865;2:663

U.S. Preventive Services Task Force. Screening for Hemochromatosis: Recommendation Statement. Am Fam Phys 2007;75:11-21

Vaiopoulos G, Papanikolaou G, Politou M, et al. Arthropathy in juvenile hemochromatosis. Arthritis Rheum 2003;48:227-230

Vichinsky E, Fischer R, Pakbaz Z, et al. Satisfaction and convenience of chelation therapy in patients with sickle cell disease (SCD): comparison between deferasirox (Exjade®, ICL670) and deferoxamine (DFO). Blood 2005;106:A2334

Vulpe CD, Kuo YM, Murphy TL, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat Genet 1999;21:195-199

Wallace DF, Clark RM, Harley HA, et al. Autosomal dominant iron overload due to a novel mutation of ferroportin1 associated with parenchymal iron loading and cirrhosis. J Hepatol 2004;40:710-713

Yaouanq JM. Diabetes and hemochromatosis: current concepts, management and prevention. Diabete et Metabolisme (Paris) 1995;21:219-329

Zhang AS, Xiong S, Tsukamoto H, et al. Localization of iron metabolism-related mRNAs in rat liver indicate that HFE is expressed predominantly in hepatocytes. Blood 2004;103:1509-1514

Zurlo MG, De Stefano P, Borgna-Pignatti C, et al. Survival and causes of death in thalassaemia major. Lancet 1989;2:27-30

Chapter 25: NAFLD and NASH

Claus Niederau

Introduction

Both non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steato-hepatitis (NASH) are often associated with obesity, diabetes mellitus and asymptomatic elevations of serum ALT and gamma-GT. Ultrasound monitoring can suggest the presence of a fatty infiltration of the liver; differentiation between NAFLD and NASH, however, requires a liver biopsy. Such differentiation may be important because NASH is associated with a much higher risk of liver fibrosis and cirrhosis than NAFLD. Moderate loss of weight due to dietary and life-style modifications is the only therapy proven to be effective in NASH. Complete alcohol abstinence and good control of diabetes mellitus are probably also important to reduce the risk of severe liver disease in NASH.

Prevalence

NAFLD is present in the general population of industrialized countries in 20 to 40% and is the most prevalent chronic liver disease (Browning 2004; Chitturi 2004; McCullough 2005). It is more prevalent in obese and diabetic subjects (Bellentani 1994; Wanless 1990; Clark 2002; Chitturi 2004). Among all subjects with NAFLD, features of non-alcoholic steato-hepatitis (NASH) can be seen in 10-20%. The prevalence of NASH in western countries is approximately 2-6%. In the US, NASH was estimated to affect 5-6% of the general population (McCullough 2005). It has been suggested that NASH accounts for more than 50% of cryptogenic cirrhosis (Ratziu 2002). NAFLD may progress to NASH with fibrosis, cirrhosis, and hepatocellular carcinoma (Marchesini 2003; Caldwell 2004). The term NASH was introduced by Ludwig (Ludwig 1980) who described 20 Mayo Clinic patients with a hitherto unnamed disease associated with hepatomegaly, abnormal ALT, a fatty liver histology, lobular hepatitis, and fibrosis mimicking alcoholic hepatitis in the absence of alcohol intake; most patients had obesity and diabetes mellitus.

Demographics and risk factors

In the US, NAFLD is 3-5 times more prevalent in men than in women; such differences in gender might partly be explained by the fact that men have a higher BMI and that some male patients with NAFLD drink more alcohol than they report drinking (Schwimmer 2005; Bahcecioglu 2006; Loguercio 2001). The NAFLD prevalence in the US is particularly high in people of Hispanic (28%) or Asian origin (20-30%) (Schwimmer 2005; Weston 2005). Due to the dramatic increase in obesity in the US and many other industrialized countries, there is also a dramatic increase in the prevalence of NAFLD and NASH. In the US almost 50% of obese boys have NAFLD (Schwimmer 2005). In many countries more than 80% of NAFLD patients have an increased BMI and 30-40% are obese; approximately 50% show signs of insulin resistance, 20-30% have type 2 diabetes, 80% show hyperlipidemia, and 30-60% have arterial hypertension. Correspondingly there is a strong association between NAFLD

and NASH and the metabolic syndrome throughout the world (Marchesini 1999; Bedogni 2005). In comparison with NAFLD patients, NASH patients are older, more obese and more often have high serum liver enzymes, diabetes mellitus and metabolic syndrome (Ratziu 2002; Adams 2005; Hamaguchi 2005; Fassio 2004).

Pathogenesis

The degree of fatty infiltration in NAFLD is graded according to the percentage of hepatocytes with fat deposits: mild NAFLD involves less than 30% hepatocytes, moderate NAFLD up to 60%, and severe NAFLD more than 60% (Ploeg 1993). NAFLD may regress if the cause is eliminated. NASH is associated with insulin resistance, increased circulating levels of leptin, adiponectin, tumour necrosis factor and some interleukins (Friedman 1998; Marra 2004). It is thought that there is an increased flow of free fatty acids from visceral fat to the liver contributing to abnormalities in intracellular lipid metabolism (Hashimoto 1999; Vendemiale 2001). Insulin resistance and increased free fatty acids may both affect mitochondrial oxidation of fatty acids causing free radical generation in hepatocytes (Grattagliano 2003). Thus, NASH is caused by two mechanisms or toxic "hits"; the first mechanism is the hepatic accumulation of triglycerides (NAFLD) due to insulin resistance and the second is thought to be the generation of free radicals with subsequent release of mediators and cytokines (McCullough 2006). Insulin resistance has been closely linked to non-alcoholic fatty liver disease in both clinical trials and laboratory-based studies (McCullough 2006; Marchesini 2001; Sanyal 2001). The actual process by which NAFLD turns into NASH however remains ill defined despite this double-hit theory. Likely, genetic factors (similar to those responsible for the metabolic syndrome) as well as exogenic factors (like drugs, moderate amounts of alcohol, and other toxins) may contribute to the evolution of NAFLD into NASH. The role of hepatic iron in the progression of NASH remains controversial, but in some patients, iron may have a role in the pathogenesis of NASH by promoting oxidative stress. Iron overload has been shown to cause lipid peroxidation and to activate hepatic stellate cells (Lee 1995). In some reports, an increased prevalence of the Cys282Tyr HFE gene mutation in patients with NASH has been reported (George 1998). The presence of the Cys282Tyr mutation was associated with increased hepatic iron concentration that in turn is associated with the severity of the fibrosis. Other studies have shown that other heterozygote HFE gene mutations are more prevalent in NASH patients when compared with controls (Bonkowsky 1999). In another clinical cohort, there was no association between hepatic iron and histological or clinical outcome (Younoussi 1999).

Natural history

The natural history of NAFLD in the general population is not well-defined since most data come from selected patients and tertiary centres (Dam-Larsen 1996; Lee 1989; Teli 1995). Correspondingly, published mortality and morbidity in hospitalized NAFLD are approximately 5 times higher than what is seen in the general population (Matteoni 1999). In the general population the risk for liver-related death in NAFLD appears to be associated mainly with age, insulin resistance, and histological evidence of hepatic inflammation and fibrosis (Adams 2005). Probably around 10% of NAFLD patients will progress to NASH over a period of 10 years (Figure 1). Cirrhosis later develops in 5-25% of patients

with NASH and 30-50% of these patients die from liver-related causes over a 10-year period (McCollough 2005; Matteoni 1999). Cirrhosis in patients with NASH can also decompensate into subacute liver failure, progress to hepatocellular cancer (HCC), and recur after liver transplantation (McCollough 2005). Steatosis alone is reported to have a more benign clinical course, with cirrhosis developing in only 1-3% of patients (Day 2004; Day 2005; McCollough 2005; Matteoni 1999). Patients with NASH and fibrosis also have a significant risk for hepatocellular carcinoma (El-Serag 2004) (Figure 1).

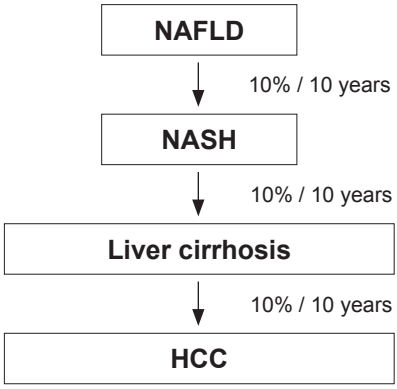


Figure 1. Natural history of NASH.

HAIR index (hypertension; ALT >40 U/l; insulin resistance) ≥2 are 80% sensitive, 89% specific for NASH (Dixon 2001)
BAAT index (BMI >28; Age >50 years; ALT >2x UNL; increased triglycerides) ≤1 has 100% negative predictive value for NASH (Ratziu 2000)

Table 1. Non-invasive predictors of NASH.

Diagnosis

NAFLD and NASH require valid reporting about alcohol consumption. Since only approximately 10% of western populations are completely abstinent from alcohol, one needs to set a threshold above which one assumes that alcohol at least contributes to the pathogenic process of NAFLD and NASH. Most authors use a daily alcohol ingestion of 20 g as such a threshold (Figure 2); others use lower values such as 10 g/day or as high as 40 g/day for men. The workup of NAFLD and NASH also includes checking into drug abuse, HBV and HCV infections, haemochromatosis, autoimmune liver disease and, in younger patients, Wilson's Disease. In special groups of

patients NASH may be accompanied by drug- and alcohol-induced liver disease and by HCV and HBV infections. The combination of NAFLD/NASH and HCV infection plays a particularly important clinical role because in this situation the rate of liver fibrosis is increased and the success of antiviral therapy is diminished (Ramesh 2004). NASH can be induced by various drugs and toxins including corticosteroids, amiodarone, methotrexate, tetracycline, tamoxifen, and valproate (Pessayre 2002). Thus, one needs to carefully assess the full clinical history of patients. In practice NAFLD is often diagnosed by combining elevated levels of ALT and gamma-GT with the sonographic appearance of an increase in the echodensity of the liver. However, a considerable number of patients with NAFLD and even with NASH and fibrosis have normal serum liver enzymes (Abrams 2004). Usually ALT is higher that AST unless there is already severe fibrosis or cirrhosis. Fasting serum glucose should be checked in all patients with NAFLD and NASH; one will also often find elevated serum insulin, insulin resistance, and/or diabetes (Table 2).

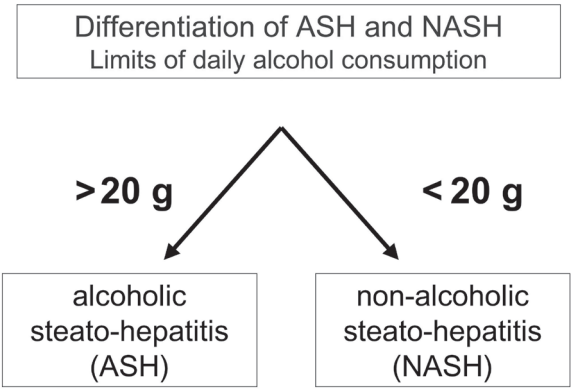


Figure 2. Differentiation of alcoholic liver disease (ASH) and NASH.

Moderate weight loss
Drugs for treatment of obesity (e.g., orlistat or sibutramine)
Complete abstinence from alcohol
Good control of diabetes mellitus
Insulin sensitizers (e.g., glitazones)
Surgery for massive obesity (e.g., gastric bypass surgery)
Liver transplant (LTX)

Table 2. Treatment options for NASH.

Many authors also recommend to routinely look for metabolic syndrome, which is diagnosed when three of the following features are seen (Greenland 2003):

- waist circumference ≥102 cm for men and ≥88 cm for women,
- fasting glucose level ≥6.1 mmol/l,
- triglyceridemia ≥1.7 mmol/l,
- increase in high-density lipoprotein cholesterol (>1.3 mmol/l in women; >1.03 mmol/l in men)
- hypertension ≥135/80 mm Hg.

Ultrasound of the liver has a high sensitivity and specificity (both approaching 90%) for detection of fatty infiltration but does not allow assessment of the presence or degree of inflammation and fibrosis (Davies 1991). Therefore, diagnosis of fat in the liver is easily made by ultrasound but diagnosis of NAFLD or NASH can not be made without a liver histology. In addition liver biopsy is still the only way to reliably differentiate NASH from NAFLD (Harrison 2003). Today most pathologists use the Brunt description to score the histological degree of NASH (Brunt 1999) (Figure 3). Since NAFLD is a very frequent but also relatively benign disease, one aims to identify some risks factors for NASH in order to avoid doing liver biopsies in all NAFLD patients. Risk factors for NASH include older age, excessive obesity, diabetes mellitus, other hepatotoxins, and clinical, laboratory or sonographic signs suggesting severe liver disease; two non-invasive scores have been used to predict NASH and might be used to identify patients who should have a liver biopsy (Dixon 2001; Ratziu 2000). Combinations of various serum markers of liver fibrosis and the results from liver stiffness measured by the fibroscan have been suggested to predict the presence of NASH and fibrosis (Rosenberg 2004; Suzuki 2005). These tests have not yet replaced the liver biopsy.

Grade	Steatosis	Ballooning of hepatocytes	Degree of inflammation
1	<33%	Minimal	Mild
2	34-66%	Present	Moderate
3	>66%	Marked	Portal moderate, lobular moderate

Stage	Fibrosis
1	Perisinusoidal
2	Perisinusoidal and portal/periportal
3	Bridging septa
4	Extensive bridging fibrosis, cirrhosis

Figure 3. Histological Brunt score (Brunt 1999).

Diet and lifestyle recommendations

Today, the only effective treatment for NAFLD and NASH is a slow and moderate weight loss, usually associated with other lifestyle modifications. Several studies have shown that rapid weight loss (very low calorie diet or starving) increases the risk of progression of liver disease and even liver failure (Grattagliano 2000; James 1998; Neuschwander-Tetri 2003). Patients should therefore be educated not to induce rapid weight loss, but to aim at a weight loss of less than 10% of their body weight over 6-12 months (Okita 2001). It is unclear whether special diets are helpful; probably it is more important that the patients simply eat healthy foods like vegetables and fruits, rich in fibre and complex carbohydrates with a low glycemic index; they should avoid meat, saturated fat and products with less complex carbohydrates. Lifestyle modifications should include an increase in physical activity and sports as well as abstinence from alcohol. With the results of recent studies, coffee consumption does not need to be limited.

Pharmacological treatment

There is no drug proven to be beneficial in NAFLD and NASH; therefore no drug has been approved by FDA or EMEA. In general, drugs that might reverse insulin resistance such as metformin and thiazolidinediones (rosiglitazone, pioglitazone) are the most promising (Angelico 2007); in smaller studies these drugs have shown some histologic improvement of NASH (Bugianesi 2004; Belfort 2006).

In general all drugs that induce weight loss might be beneficial in NAFLD and NASH, in particular when diet and life-style modification do not work. Both sibutramine and orlistat have shown to improve some characteristics of NAFLD and NASH such as the sonographic degree of liver steatosis as well as the histological degree of steatosis and fibrosis (Sabuncu 2003; Derosa 2004, Hussein 2007; Harrison 2007).

Antioxidants and cytoprotective substances have also been proposed to treat NAFLD and NASH including vitamin E, vitamin C, glutathione, betaine, acetylcysteine, S-adenosyl-L-methionine and ursodesoxycholic acid. After a recent Cochrane analysis, none of these substances has shown significant benefit in validated randomized studies (Lirussi 2007).

Surgery for obesity

Gastric bypass has also recently been shown to improve NASH (Liu 2007; de Almeida 2006; Furuya 2007); however, surgery is usually restricted to patients with massive obesity.

Liver transplantation (LTX) for NASH

LTX is the final option for patients with end-stage liver disease due to cirrhosis and complications of portal hypertension with NASH. Due to the increase in the prevalence of NASH, there is also an increase in LTX done for end-stage liver disease caused by NASH (Burke 2004). However, NASH can recur after LTX, particularly if patients have previously undergone jejunoileal bypass surgery (Kim 1996; Requart 1995; Weston 1998; Contos 2001; Burke 2004). LTX does not cure the metabolic defect that causes NASH.

References

Abrams GA, Kunde SS, Lazenby AJ, Clements RH. Portal fibrosis and hepatic steatosis in morbidly obese subjects: a spectrum of nonalcoholic fatty liver disease. Hepatology 2004;40:475–83.

Adams LA, Lymp JF, Sauver SJ, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–21.

Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005;42:132–8.

Angelico F, Burattin M, Alessandri C, Del Ben M, Lirussi F. Drugs improving insulin resistance for non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis. Cochrane Database Syst Rev 2007;24:CD005166.

Bahcecioglu IH, Koruk M, Yilmaz O, et al. Demographic and clinicopathological characteristics of nonalcoholic fatty liver disease in the east-southeastern Anatolia regions in Turkey. Med Princ Pract 2006;15:62–8.

Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:44–52.

Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 2006;355:2297-2307.

Bellentani S, Tiribelli C, Saccoccio G, et al. Prevalence of chronic liver disease in the general population of northern Italy: the Dionysos Study. Hepatology 1994;20:1442–9.

Bonkovsky HL, Jawaid Q, Tortorelli K, et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. J Hepatol. 1999;31:421-429.

Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387-1395.

Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74.

Burke A, Lucey MR. Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis and orthotopic liver transplantation. Am J Transplant 2004;4:686-93.

Caldwell SH, Crespo DM. The spectrum expanded: cryptogenic cirrhosis and the natural history of non-alcoholic fatty liver disease. J Hepatol 2004;40:578–84.

Chitturi S, Farrell GC, George J. Non-alcoholic steatohepatitis in the Asia-Pacific region: future shock. J Gastroenterol Hepatol 2004;19:368-374.

Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology 2002;122:1649–57

Contos MJ, Cales W, Sterling RK et al. Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. Liver Transpl 2001;7:363-73.

Davies RJ, Saverymuttu SH, Fallowfield M, Joseph AE. Paradoxical lack of ultrasound attenuation with gross fatty change in the liver. Clin Radiol 1991;43:393–6.

Day CP. The potential role of genes in nonalcoholic fatty liver disease. Clin Liver Dis 2004;8:673–91.

Day CP. Natural history of NAFLD: remarkably benign in the absence of cirrhosis. Gastroenterology 2005;129:375–8.

Dam-Larsen S, Franzmann M, Andersen IB, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut 2004;53:750–5

de Almeida SR, Rocha PR, Sanches MD, et al. Roux-en-Y gastric bypass improves the nonalcoholic steatohepatitis (NASH) of morbid obesity. Obes Surg 2006;16:270-8.

Derosa G, Cicero AF, Murdolo G, Ciccarelli L, Fogari R. Comparison of metabolic effects of orlistat and sibutramine treatment in Type 2 diabetic obese patients. Diabetes Nutr Metab 2004;17:222-9.

Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001; 21:91-100.

El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460–8

Fassio E, Alvarez E, Dominguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. Hepatology 2004;40:820–6.

Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.

George DK, Goldwurm S, MacDonald GA, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology. 1998;114:311-318.

Grattagliano I, Caraceni P, Portincasa P, et al. Adaptation of subcellular glutathione detoxification system to stress conditions in choline-deficient diet induced rat fatty liver. Cell Biol Toxicol 2003;19:355–66.

Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Managing nonalcoholic fatty liver disease. Recommendations for family physicians. Can Fam Physician 2007;53:857-63.

Furuya CK Jr, de Oliveira CP, de Mello ES, et al. Effects of bariatric surgery on nonalcoholic fatty liver disease: preliminary findings after 2 years. J Gastroenterol Hepatol. 2007;22:510-4.

Harrison SA, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. Am J Gastroenterol 2003;98:2042–7

Harrison SA, Fincke C, Helinski D, Torgerson S, Hayashi P.A pilot study of orlistat treatment in obese, nonalcoholic steatohepatitis patients. Aliment Pharmacol Ther 2004;20:623-8.

Hashimoto T, Fujita T, Usuda N, et al. Peroxisomal and mitochondrial fatty acid beta-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. J Biol Chem 1999;274:19, 228–36.

Hussein O, Grosovski M, Schlesinger S, Szvalb S, Assy N. Orlistat reverse fatty infiltration and improves hepatic fibrosis in obese patients with nonalcoholic steatohepatitis (NASH). Dig Dis Sci 2007;52:2512-9.

Grattagliano I, Vendemiale G, Caraceni P, et al. Starvation impairs antioxidant defense in fatty livers of rats fed a choline-deficient diet. J Nutr 2000;130:2131–6.

Greenland P, Knoll MD, Stamler J, et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 2003;290:891–7.

Hamaguchi M, Kojima T, Takeda N, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 2005;143:722–8.

Hussein O, Grosovski M, Schlesinger S, Szvalb S, Assy N. Orlistat reverse fatty infiltration and improves hepatic fibrosis in obese patients with nonalcoholic steatohepatitis (NASH). Dig Dis Sci 2007;52:2512-9.

James OF, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol 1998;29:495–501.

Kim WR, Poterucha JJ, Porayko MK, Dickson ER, Steers JL, Wiesner RH. Recurrence of nonalcoholic steatohepatitis following liver transplantation. Transplantation 1996;62:1802-1805.

Lee RG. Nonalcoholic steatohepatitis: a study of 49 patients. Hum Pathol 1989;20:594–8.

Lee KS, Buck M, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF_ and collagen type I is mediated by oxidative stress through c-myb expression. J Clin Invest 1995;96:2461-2468.

Lirussi F, Azzalini L, Orando S, Orlando R, Angelico F. Antioxidant supplements for non-alcoholic fatty liver disease and/or steatohepatitis. Cochrane Database Syst Rev 2007;24:CD004996.

Liu X, Lazenby AJ, Clements RH, Jhala N, Abrams GA. Resolution of nonalcoholic steatohepatits after gastric bypass surgery. Obes Surg 2007;17:486-92.

Loguercio C, De Girolamo V, de Sio I, et al. Non-alcoholic fatty liver disease in an area of southern Italy: main clinical, histological, and pathophysiological aspects. J Hepatol 2001;35:568–74.

Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434-8.

Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844-1850.

Marchesini G, Brizi M, Morselli-Labate AM, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107:450–5.

Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–23.

Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullogh AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-1419.

Marra F. NASH: are genes blowing the hits? J Hepatol 2004;40:853–6

McCullough AJ. Pathophysiology of nonalcoholic steatohepatitis. J Clin Gastroenterol 2006;40:1:S17-S29.

McCullough AJ. The epidemiology and risk factors of NASH. In: Farrell GC, George J, Hall P, McCullough AJ eds. Fatty liver disease: NASH and related disorders. Malden, MA: Blackwell, 2005:23-37.

Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37:1202–19.

Okita M, Hayashi M, Sasagawa T, et al. Effect of a moderately energy-restricted diet on obese patients with fatty liver. Nutrition 2001;17:542–7

Pessayre D, Berson A, Fromenty B, Mansouri A. Mitochondria in steatohepatitis. Semin Liver Dis 2001;21:57–69.

Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, et al. Risk factors for primary dysfunction after liver transplantation—a multivariate analysis. Transplantation 1993;55:807–13

Ramesh S, Sanyal AJ. Hepatitis C and nonalcoholic fatty liver disease. Semin Liver Dis 2004;24:399–413

Ratziu et al. Liver Fibrosis in Overweight Patients. Gastroenterology 2000;118:1117-1123.

Ratziu V, Bonyhay L, Di Martino V, et al. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 2002;35:1485–93.

Requarth JA, Burchard KW, Colacchio TA, et al. Long-term morbidity following jejunoileal bypass: the continuing potential need for surgical reversal. Arch Surg 1995;130:318-325.

Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 2004;127:1704–13.

Sabuncu T, Nazligul Y, Karaoglanoglu M, Ucar E, Kilic FB.The effects of sibutramine and orlistat on the ultrasonographic findings, insulin resistance and liver enzyme levels in obese patients with non-alcoholic steatohepatitis. Rom J Gastroenterol 2003;12:189-92.

Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183-1192.

Schwimmer JB, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. Pediatrics 2005 115:561–5.

Suzuki A, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. Liver Int 2005;25:779–86

Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. Hepatology 1995;22:1714–9

Vendemiale G, Grattagliano I, Caraceni P, et al. Mitochondrial oxidative injury and energy metabolism alteration in rat fatty liver: effect of the nutritional status. Hepatology 2001;33:808-15

Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12:1106–10.

Weston S, Charlton MR, Lindor KD. Liver transplantation for nonalcoholic steatohepatitis. Gastroenterology 1998;114:A1364.

Weston SR, Leyden W, Murphy R, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. Hepatology 2005;41:372–9.

Younossi ZM, Gramlich T, Bacon BR, et al. Hepatic iron and nonalcoholic fatty liver disease. Hepatology 1999;30:847-850.

Chapter 26: Wilson's Disease

Claus Niederau

Introduction

In 1912, Kinnear Wilson was the first to describe an inherited lethal disease associated with progressive lenticular degeneration, chronic liver disease and cirrhosis (Wilson 1912). In the same year, Kayser and Fleischer detected that patients with Wilson's Disease (WD) often have brownish corneal copper deposits now called Kayser-Fleischer rings (Fleischer 1912).

WD is an autosomal recessive error of the metabolism. Its gene ATP7B encodes a copper-transporting ATPase (Bull 1993; Tanzi 1993; Petrukhin 1993; Yamaguchi 1993). The genetic defect of the ATP7B protein reduces biliary copper excretion leading to copper accumulation in the cornea and various organs including the liver, brain and kidney. The alteration of the ATP7B protein also reduces the incorporation of copper into ceruloplasmin. The corresponding presence of apoceruloplasmin (ceruloplasmin with no copper incorporation) leads to a decrease in circulating levels of ceruloplasmin due to the reduced half-life of the apoprotein. Thus, despite copper accumulation in many organs, circulating levels of copper and ceruloplasmin are decreased in most WD patients.

The prevalence of WD is rare, estimated at 3 per 100,000 population (Frysman 1990). The clinical presentation may vary: some WD patients are diagnosed with liver problems while others present with neurologic or psychiatric symptoms; many patients show both hepatic and neurological disease (Figure 1). Episodes of hemolysis and renal abnormalities may also occur. WD typically affects children and younger adults, and is rarely seen in adults older than 40. WD is fatal unless appropriately treated. Drugs for treatment of WD are copper chelators such as penicillamine, and trientine (Walshe 1956). More recently, zinc has been used to reduce intestinal copper absorption and to detoxify free circulating copper. Patients with fulminant liver failure or decompensated cirrhosis may have to undergo liver transplantation (LTX), which cures WD.

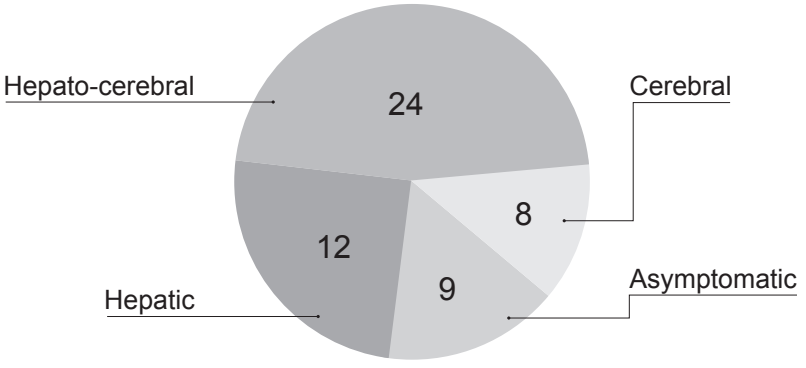


Figure 1. Clinical course of WD in 53 patients (modified from Stremmel 1991).

Clinical Presentation

Screening for WD is useful only in families with an affected member. In all other circumstances diagnostic procedures are only done when symptoms and findings suggest WD. These include liver disease, neurological symptoms, renal abnormalities and episodes of hemolysis. WD is diagnosed in the vast majority of patients between the ages of 5 and 35. There are rare reports of patients diagnosed at ages 3-5 (Kalach 1993; Wilson 2000) and at ages of up to about 60 years (Gow 2000). Late-onset WD is a frequently overlooked condition (Ferenci 2007). Diagnostic workup does not rely on a single test but includes identification of corneal Kayser-Fleischer rings, reduced serum ceruloplasmin and copper as well as a quantitative determination of liver copper concentration (Scheinberg 1952; Walshe 1956; Saito 1987; Stremmel 1991; Roberts 2003) (Figure 2).

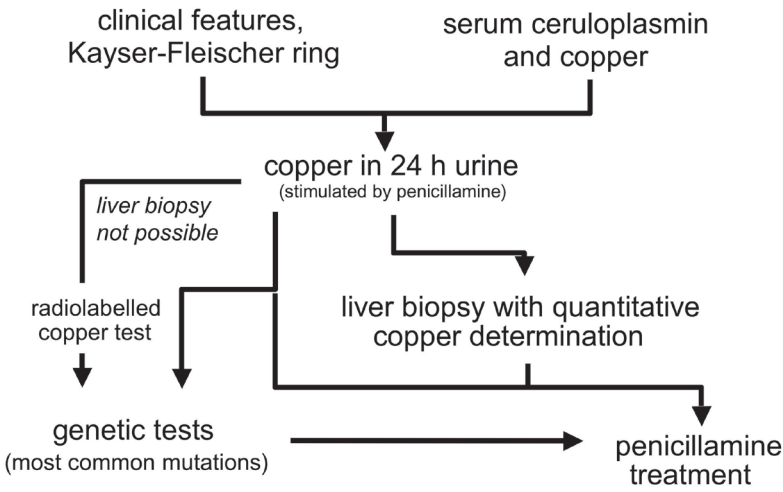


Figure 2. Diagnostic workup for WD.

Genetic tests are usually only done in relatives of a confirmed WD patient. It is easy to diagnose WD in subjects who present with liver cirrhosis, typical neurologic manifestations and Kayser-Fleischer rings; many of these patients present at ages 5 to 35 and have decreased serum copper and ceruloplasmin (Sternlieb 1990). However, a considerable number of WD patients present only with liver disease and may not have Kayser-Fleischer rings or decreased serum levels of ceruloplasmin (Steindl 1997). Under these circumstances diagnosis may be difficult; measurement of 24-hr urinary copper excretion often helps to support the suspicion of WD. Liver biopsy with measurement of quantitative copper concentration should be done to corroborate the diagnosis (Stremmel 1991; Roberts 2003).

In general, WD patients who are diagnosed primarily with liver disease are children and adolescents and are younger than those diagnosed due to neurological symptoms (Merle 2007). Many patients who present only with CNS symp-

toms are 20-40 years old. Patients with WD may present with a wide spectrum of liver disease ranging from asymptomatic elevation of serum aminotransferases to fulminant liver failure. Serum aminotransferases are elevated in most WD patients irrespective of age (Schilsky 1991). Other WD patients may present with findings and symptoms of autoimmune hepatitis including autoimmune antibodies and elevated IgG (Scott 1978; Milkiewicz 2000). The clinical picture might also resemble acute or chronic viral hepatitis, without the viral serum markers. Even liver histology is not predictive or typical for WD unless copper concentration is measured. Histological findings may range from fatty liver changes to severe necro-inflammatory and fibrotic disease and complete cirrhosis. In particular, children and adolescents with chronic active hepatitis of unknown aetiology or autoimmune hepatitis and adult patients with a suspicion of autoimmune hepatitis or non-response to immunosuppressants should be evaluated for WD (Roberts 2003).

WD has to be excluded in patients with fulminant liver failure of unknown aetiology, especially at ages under 35 years; WD patients with such presentation usually have some sort of liver disease (Rector 1984; Ferlan-Maroult 1999; Roberts 2003) associated with coombs-negative hemolytic anemia and severely increased prothrombine time non-responsive to vitamin K and progressive renal failure (Sallie 1992). Some patients have bilirubin levels of more than 40 mg/dl while serum alkaline phosphatase is normal or just slightly elevated (Berman 1991). In contrast to many types of toxic liver failure, liver failure in WD usually does not start with high increases in aminotransferases. In many WD patients AST levels exceed ALT levels (Emre 2001; Berman 1991). In most cohorts, for unexplained reasons, the ratio of females to males is approximately 2:1 (Roberts 2003). Serum ceruloplasmin may be decreased while serum copper and 24-hour urinary excretion of copper is usually elevated. It is extremely helpful when one can identify Kayser-Fleischer rings in this situation; these patients need to be studied with a slit lamp by an experienced ophthalmologist. Patients with acute liver failure need a diagnostic workup as rapidly as possible; if there is a strong suspicion or diagnosis of WD, the patient should be transferred to a transplant center the same day.

Neurological symptoms in WD often resemble those seen in Parkinson's disease including tremor and rigour. Many patients report that symptoms start with problems in handwriting and dysarthria. Neurological symptoms may be associated with slight behavioural alterations, which may later proceed to manifest psychiatric disease including depression, anxiety and psychosis. With the progression of CNS involvement WD patients may develop seizures and pseudobulbar palsy associated with severe dysphagia, aspiration and pneumonia. Although many older WD patients present with neurological disease, the diagnostic workup often shows significant liver involvement or even complete liver cirrhosis.

Renal involvement of WD may present with aminoaciduria and nephrolithiasis (Azizi 1989; Nakada 1994; Cu 1996). There may be various other non-neurological and non-hepatic complications of WD such as osteoporosis and arthritis, cardiomyopathy, pancreatitis, hypoparathyroidism, and miscarriages (for literature, see Roberts 2003).

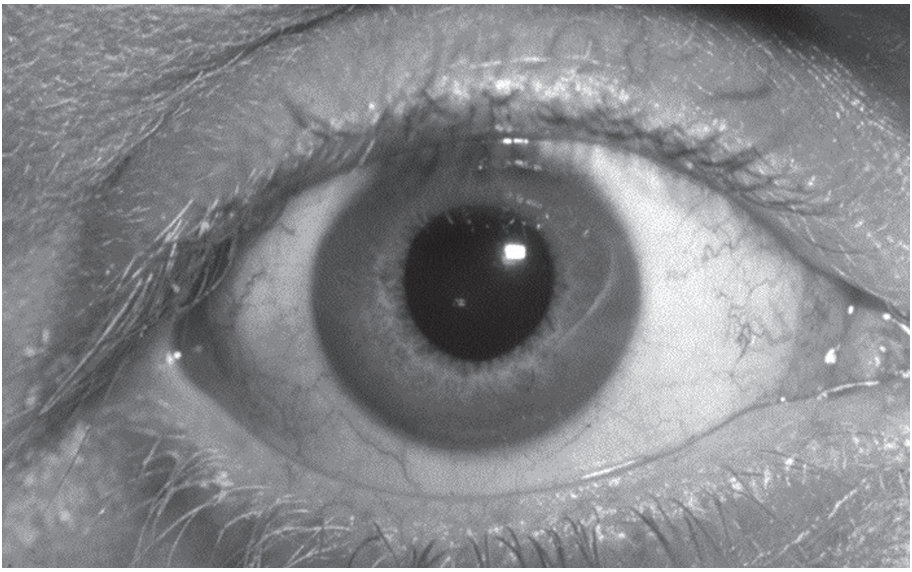


Figure 3. Kayser-Fleischer ring in a patient with WD.

Kayser-Fleischer rings are caused by corneal copper deposition (Figure 3). Sometimes, one can see the rings directly as a band of brown pigment close to the limbus. In other patients the ring can only be identified using a slit lamp. Very rarely similar rings may be seen in non-WD patients, e.g., in some patients with neonatal or chronic cholestasis (Tauber 1993). Kayser-Fleischer rings are detectable in 50-60% of WD patients in most large cohorts (Tauber 1993; Roberts 2003). Many young WD patients with liver disease do not have such rings (Giacchino 1997) whereas almost all patients with primarily neurologic symptoms do have them (Steindl 1997). WD patients may also have other less specific eye changes including sunflower cataracts (Cairns 1963). Kayser-Fleischer rings usually regress with chelation therapy or after LTX (Stremmel 1991; Schilsky 1994).

Diagnosis

Serum ceruloplasmin

Ceruloplasmin, the major circulating copper transporter, is synthesized and secreted mainly by hepatocytes. The 132-kd protein consists of six copper atoms per molecule of ceruloplasmin (holoceruloplasmin) while the remaining part of the protein does not carry copper (apoceruloplasmin). Ceruloplasmin acts as an acute phase reactant and may thus be increased by any inflammatory process; it may also rise in pregnancy and with the use of estrogens and oral contraceptives. One also needs to remember that the normal range of serum ceruloplasmin is age-dependent: it is usually low in infants until the age of 6 months; in older children it may be some-

what higher than in adults. As explained previously, serum levels of ceruloplasmin are generally decreased in WD; however, this finding alone is unreliable because low serum ceruloplasmin may be seen without WD and serum ceruloplasmin may even be increased in severe WD and liver failure. Non-specific reductions of ceruloplasmin are usually associated with protein deficiency or any end-stage liver disease. Long-term parenteral nutrition may also lead to decreased levels of ceruloplasmin. Low serum ceruloplasmin is also a hallmark of Menkes' disease, a very rare X-linked inborn error of metabolism that leads to a defect in copper transport due to mutations in ATP7A (Menkes 1999). Very rarely, one cannot measure serum ceruloplasmin at all. This aceruloplasminemia is a very rare genetic disease caused by mutations in the ceruloplasmin gene; however, patients with aceruloplasminemia develop iron and not copper overload (Harris 1998).

Most patients with WD have a serum ceruloplasmin lower than 20 mg/dl; this finding is diagnostic for WD however only when there are other findings such as a Kayser-Fleischer corneal ring. In one prospective screening study, ceruloplasmin was measured in 2867 patients presenting with liver disease: only 17 of them had reduced ceruloplasmin levels and only 1 of these subjects had WD (Cauza 1997). Thus decreased ceruloplasmin had a positive predictive value of only 6% in the 2867 patients tested. In two cohorts, about 20% of WD had normal ceruloplasmin and no Kayser-Fleischer rings (Steindl 1997; Gow 2000). Most reports, however, show that more than 90% of WD patients have a reduced serum ceruloplasmin (Walshe 1989; Lau 1990; Stremmel 1991). Measurement of ceruloplasmin as a single marker cannot reliably differentiate homozygotes from heterozygotes.

Serum copper

Corresponding to the decrease in serum ceruloplasmin, total serum copper is also usually decreased in WD. Similar to the diagnostic problems in interpreting ceruloplasmin data in WD patients with fulminant liver failure, serum copper may also be normal in this situation – even if serum ceruloplasmin is decreased. In acute liver failure circulating copper may in fact be elevated because it is massively released from injured hepatocytes. If ceruloplasmin is reduced, a normal or elevated serum copper usually suggests that there is an increase in free serum copper (not bound to ceruloplasmin). The free copper concentration calculated from total copper and ceruloplasmin values has also been proposed as a diagnostic test and for monitoring of WD. It is elevated above 25 mg/dl in most untreated patients (normal values are below 10-15 mg/dl). The amount of copper associated with ceruloplasmin is 3.15 mg of copper per mg of ceruloplasmin. Thus free copper is the difference between the total serum copper in mg/dl and 3 times the ceruloplasmin concentration in mg/dl (Roberts 1998).

Increases in serum free copper, however, are not specific for WD and can be seen in all kinds of acute liver failure as well as in marked cholestasis (Gross 1985; Martins 1992). The calculation of the free copper concentration critically depends on the adequacy of the methods used for measuring total serum copper and ceruloplasmin; often labs simply state that one of the tests is below a certain value, which makes it impossible to calculate the amount of free copper.

Urinary copper excretion

Most WD patients have an increase in urinary copper excretion above 100 mg/24 hours, which is thought to represent the increase in circulating free copper (not bound to ceruloplasmin). Some studies suggest that about 20% of WD patients may have 24-hr urinary copper excretion between 40-100 mg/24 h (Steindl 1997; Giacchino1997; Gow 2000; Roberts 2003). However, some increase in urinary copper excretion can be found in severe cholestasis, chronic active hepatitis and autoimmune hepatitis (Frommer 1981). It has been suggested that urinary copper excretion stimulated by penicillamine may be more useful than the non-stimulating test. In children 500 mg of oral penicillamine is usually given at the beginning and then at 12 hours during the 24-hour urine collection. All WD children looked at had levels above 1600 mg copper/24 h and all patients with other liver diseases including autoimmune hepatitis and cholestatic liver disease had lower values. It is not clear whether this test has a similar discriminative power in adults where it has been used in various modifications (Tu 1967; Frommer 1981).

Hepatic copper concentration

Hepatic copper content above 250 mg/g dry weight liver is still the gold standard for diagnosis of WD and is not seen in heterozygotes or other liver diseases with the exception of Indian childhood cirrhosis (Martins 1992). Biopsies (larger than 1 cm in length) for measurements of hepatic copper determination should be taken with a disposable Tru-Cut needle, placed dry in a copper-free container and shipped frozen (Song 2000; Roberts 2003).

Radiolabelled copper

In WD, incorporation of radiolabelled copper into ceruloplasmin is significantly reduced. This test is rarely used because of the difficulty in obtaining the isotope and because of legal restrictions.

Liver biopsy findings

Histological findings in WD range from some steatosis and hepatocellular necrosis to the picture as seen in severe autoimmune hepatitis with fibrosis and cirrhosis. Patients diagnosed at a young age usually have extensive liver disease; older patients who first present with neurological symptoms often have abnormalities in liver biopsy as well (Stremmel 1991; Steindl 1997; Merle 2007). Detection of copper in hepatocytes, e.g., by staining with rhodamin using routine histochemistry does not allow a diagnosis of WD (Geller 2000) (Figure 4).

Neurology and MRI of the CNS

Neurologic symptoms in WD include Parkinson's-like abnormalities with rigidity, tremor and dysarthria. In more severely affected patients there may be muscle spasms, contractures, dysphonia, and dysphaegia. In patients with pronounced neurological symptoms magnetic resonance imaging (MRI) often identifies abnormalities in basal ganglia such as hyperintensity on T2 weighted imaging (Aisen 1995; van Wassanaer 1996). MRI of the CNS is superior to computed tomography to diagnose WD.

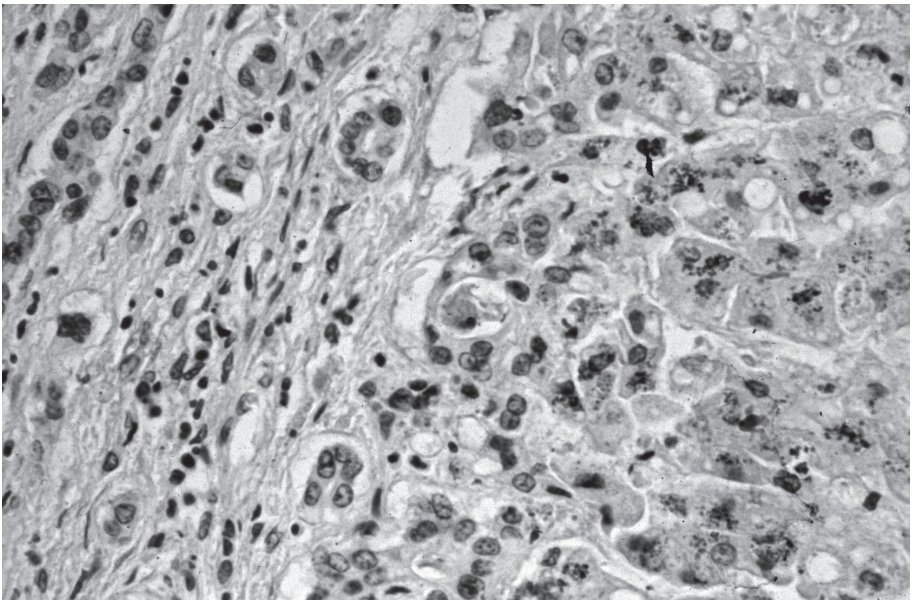


Figure 4. Liver histology (rhodamine staining for copper) in a WD patient.

Genetic Studies

The use of mutation analysis in WD is limited by the fact that more than 200 ATP7B mutations have been described (see www.medgen.med.ualberta.ca/database.html). When the mutation is known in a specific patient, gene analysis may be useful for family screening or prenatal analysis (Thomas 1995; Shab 1997; Loudianos 1994). Some populations in Eastern Europe show predominance of the H1069Q mutation (for literature, see Roberts 2003).

Treatment

Before 1948, all patients with Wilson's Disease died shortly after diagnosis. In 1948, intramuscular administration of the copper chelator BAL (dimercaprol) was introduced as the first treatment of WD (Cumming 1951; Denny-Brown 1951) followed by the oral chelators penicillamine (1955), trientine (1969) and tetrathiomolybdate (1984). Other treatment modalities include oral zinc salts (1961) and liver transplantation (1982). Today, most patients with WD remain on a lifelong pharmacologic therapy usually including a copper chelator and/or a zinc salt (Figure 5). LTX is reserved for fulminant liver failure and irreversible decompensation of liver cirrhosis. Patients with a successful LTX do not need WD treatment because LTX heals the biochemical defect. Today, most doctors use oral chelators for initial treatment of symptomatic patients; many physicians start therapy with penicillamine while some prefer trientine. Both drugs are probably equally effective, with trientine having fewer side effects. In patients with advanced neurological disease some authors recommend tetrathiomolybdate for primary therapy.

Combination therapy of chelators and zinc salts might have additive effects, acting on both urinary copper excretion and its intestinal absorption. After removal of most accumulated copper and regression of the most severe clinical problems the chelator dose may be reduced and later replaced by zinc. Patients presenting without symptoms may be treated with a rather low dose of a chelator or with a zinc salt from the beginning. Compliance problems have been shown to regularly cause recurrence of symptomatic WD and may lead to fulminant liver failure, need for LTX or death.

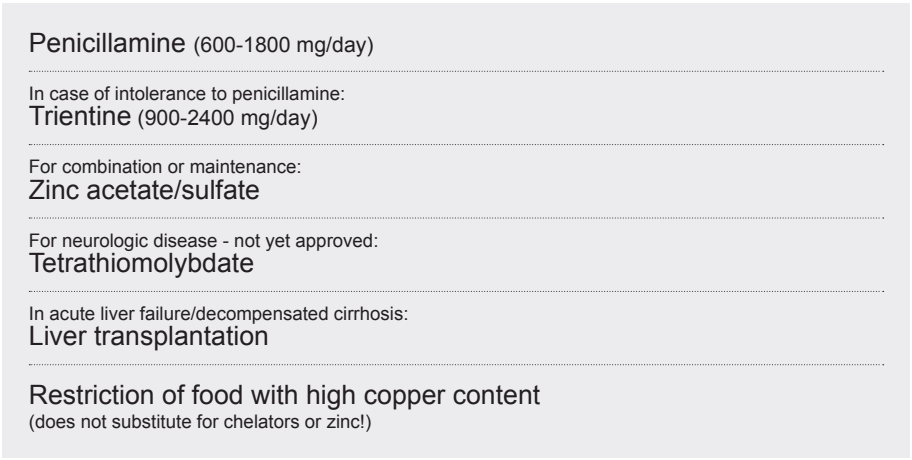


Figure 5. Treatment options in WD.

Penicillamine. Penicillamine was the first oral copper chelator shown to be effective in WD (Walshe 1955). Total bioavailability of oral penicillamine ranges between 40 and 70% (Bergstrom 1981). Many studies have shown that penicillamine reduces copper accumulation and provides clinical benefit in WD (Walshe 1973; Grand 1975; Sternlieb 1980). Signs of liver disease often regress during the initial 6 months of treatment. Non-compliance has been shown to cause progression of liver disease, liver failure, death and LTX (Scheinberg 1987). However, neurological symptoms may deteriorate at the start of penicillamine treatment; it remains controversial how often this neurological deterioration occurs and whether it is reversible; the rate of neurological worsening ranges from 10-50% in different cohorts (Brewer1987; Walshe 1993). Some authors even recommend not using penicillamine at all in WD patients with neurological disease (Brewer 2006). Penicillamine is associated with many side effects that lead to its discontinuation in up to 30% of patients (for further literature, see Roberts 2003). An early sensitivity reaction may occur during the first 3 weeks including fever, cutaneous exanthema, lymphadenopathy, neutropaenia, thrombocytopaenia, and proteinuria. In such early sensitivity, penicillamine should be replaced by trientine immediately. Nephrotoxicity is another frequent side effect of penicillamine, which occurs later and includes proteinuria and signs of tubular damage. In this case

penicillamine should be immediately discontinued. Penicillamine may also cause a lupus-like syndrome with haematuria, proteinuria, positive antinuclear antibody, and Goodpasture's Syndrome. More rarely the drug can damage the bone marrow leading to thrombocytopenia or total aplasia. Dermatologic side effects include elastosis perforans serpiginosa, pemphigoid lesions, lichen planus, and aphthous stomatitis. There have also been reports of myasthenia gravis, polymyositis, loss of taste, reduction of IgA, and serous retinitis due to administration of penicillamine.

In order to minimize its side effects pencillamine should be started at 250 mg daily; the dose may be increased in 250 mg steps every week to a maximal daily amount of 1000 to 1500 mg given in 2 to 4 divided doses daily (Roberts 2003). Maintenance doses range from 750 to 1000 mg/d given as 2 divided doses. In children the dose is 20 mg/kg/d given in 2 or 3 divided doses. Penicillamine should be given 1 hour before or 2 hours after meals because food may inhibit its absorption. After starting penicillamine therapy serum ceruloplasmin at first may decrease. Treatment success is checked by measuring 24-hr urinary copper, that should range between 200-500 mg/day. In the long run ceruloplasmin should increase and free copper should regress towards normal with penicillamine therapy (Roberts 2003).

Trientine (triene). The chemical structure of the copper chelator trientine (triethylene tetramine dihydrochloride - short name, triene) differs from penicillamine. Trientine has usually been used as an alternative or substitute for penicillamine, in particular when penicillamine's major side effects are not tolerable (Walshe 1982). Triene only rarely has side effects. Similar to penicillamine long-term treatment with trientine may cause hepatic iron accumulation in persons with WD. Trientine is poorly absorbed from the gastrointestinal tract, and only 1% appears in the urine (Walshe 1982). Doses range from 750 to 1500 mg/d given in 2 or 3 divided doses; 750 or 1000 mg are given for maintenance therapy (Roberts 2003). In children a dose of 20 mg/kg/d is recommended. Similar to penicillamine, trientine should be given 1 hour before or 2 hours after meals. The effectiveness of copper chelation by triene is measured as described for penicillamine. Triene chelates several metals such as copper, zinc, and iron by urinary excretion and it effectively removes accumulated copper from various organs in persons with WD as well as in severe liver disease (Walshe 1979; Scheinberg 1987; Santos 1996; Saito 1991). It is still unclear whether penicillamine is a more effective copper chelator when compared to triene; probably the difference in effectiveness is small (Walshe 1973; Sarkar 1977). Potential deterioration of neurological disease may also be seen after starting triene therapy; the worsening however is less frequent and less pronounced than that seen after starting with penicillamine.

Zinc. Most physicians substitute penicillamine or triene with zinc for maintenance therapy when most copper accumulation has been removed. Zinc can also be given as initial therapy in asymptomatic patients diagnosed by family screening. A recent report however shows that WD symptoms may occur despite zinc prophylaxis in asymptomatic patients (Mishra 2008). In a recent study from India, 45 WD patients were on both penicillamine and zinc sulfate. The majority of patients (84%) had neuropsychiatric manifestations. The mean duration of treatment with penicillamine and zinc, before

stopping penicillamine, was 107 months. All patients had to stop penicillamine due to financial burden. The patients then only received zinc sulfate for 27 months and 44 of the 45 patients (98%) remained stable. Only one patient reported worsening in dysarthria (Sinha 2008). Zinc does not act as an iron chelator but inhibits intestinal copper absorption and has also been suggested to bind free toxic copper (Brewer 1983; Schilksky 1989; Hill 1987). Zinc rarely has any side effects. It is still unclear whether zinc as monotherapy is an effective "decoppering" agent in symptomatic patients. There are some hints that hepatic copper may accumulate despite zinc therapy including reports about hepatic deterioration with a fatal outcome (Lang 1993; Walshe 1995). Therefore some authors use zinc in combination with a chelator. Neurological deterioration is rather rare under zinc therapy (Brewer 1987; Czlonkowska 1996). The recommended doses of zinc vary in the literature: according to AASLD practice guidelines dosing is in milligrams of elemental zinc (Roberts 2003). For larger children and adults, 150 mg/d is administered in 3 doses. Compliance with doses given thrice daily may be problematic; zinc has to be taken at least twice daily to be effective (Brewer 1998). Other authors recommend using zinc sulfate at 150 mg thrice daily as a loading dose and 100 mg thrice daily for maintenance. Further recommendations suggest giving 50 mg as zinc acetate thrice daily in adults. The type of zinc salt used has been thought to make no difference with respect to efficacy (Roberts 2003). However, zinc acetate has been suggested to cause the least gastrointestinal discomfort. When zinc is combined with a chelator the substances should be given at widely spaced intervals, potentially causing compliance problems. Effectiveness of the zinc treatment should be checked as described for penicillamine and zinc (Roberts 2003).

Tetrathiomolybdate. Tetrathiomolybdate is an experimental copper chelator not approved by FDA or EMEA. It has been suggested as the initial treatment of WD patients with neurological involvement. Early reports state that tetrathiomolybdate stabilizes the neurological disease and reduces circulating free copper in a matter of weeks (Brewer 1994; Brewer 1996). A more recent randomized study supports this view and also suggests that zinc monotherapy is insufficient for treatment of neurological WD (Brewer 2006).

Vitamin E, other antioxidants and diet. Since serum and hepatic concentrations of vitamin E levels may be reduced in WD (von Herbay 1994; Sokol 1994) it has been suggested to complement vitamin E intake. Some authors have also recommended taking other antioxidants; studies have not proven their effectiveness as yet.

WD patients should avoid food with high copper content (nuts, chocolate, shellfish, mushrooms, organ meats, etc.). Patients living in older buildings should also check whether the water runs through copper pipes. Such dietary and lifestyle restrictions do not replace chelator or zinc therapy (Roberts 2003).

Fulminant hepatic failure and LTX. Most WD patients with fulminant liver failure need LTX urgently in order to survive (Sokol 1985; Roberts 2003). However, in a long-term cohort study only two patients died prior to LTX being available (Stremmel 1991). It is a difficult clinical question whether WD patients with liver

failure can survive without LTX. The prognostic score used to help with this difficult decision includes bilirubin, AST, and INR (Nazer 1986). In any case, WD patients with signs of fulminant liver failure need to be transferred immediately (same day!) to a transplant center.

WD patients with a chronic course of decompensated cirrhosis follow the usual rules for LTX. LTX cures the metabolic defects and thus copper metabolism returns to normal afterwards (Groth 1973). Prognosis for WD after LTX is excellent, in particular when patients survive the first year (Eghtesad 1999). It is still unclear under which circumstances LTX may be helpful for WD patients with neurological complications, which do not respond to drug therapy. In some patients CNS symptoms regress after LTX while other patients do not improve (for detailed literature see Brewer 2000).

Asymptomatic patients. All asymptomatic WD subjects - usually identified by family screening - need to be treated by chelators or zinc in order to prevent life-threatening complications (Walshe 1988; Brewer 1989; Roberts 2003). It is unclear whether therapy should begin in children under the age of 3 years.

Maintenance therapy. After initial removal of excessive copper by chelators, some centres replace the chelators with zinc for maintenance therapy. It is unclear when such change is advisable and whether it might be better to reduce the dose of chelators instead of replacing them with zinc. It is generally accepted that replacement of chelators with zinc should only be done in patients who are clinically stable for some years, have normal aminotransferase and liver function, a normal free copper concentration and a 24-hr urinary copper repeatedly in the range of 200-500 mg while on chelators (Roberts 2003). Long-term treatment with zinc may be associated with fewer side effects than chelator treatment. Many patients on trientine, however, do not have significant side effects, and this author believes one does need to replace trientine with zinc in such patients. In any case, therapy either with a chelator or with zinc needs to be maintained indefinitely; any interruption may lead to lethal liver failure (Walshe 1986; Scheinberg 1987).

Pregnancy. Treatment must be maintained during pregnancy because an interruption has been shown to carry a high risk of fulminant liver failure (Shimono 1991). Maintenance therapy with chelators (penicillamine, trientine) or with zinc usually results in a good outcome for mother and child, although birth defects have (rarely) been documented (for detailed literature see Sternlieb 2000). It is recommended that the doses of both chelators be reduced, if possible by about 50%, in particular during the last trimester to avoid potential problems in wound healing (Roberts 2003). Zinc does not need to be reduced.

Monitoring of treatment

Monitoring should be done closely during initial treatment in all WD patients to look for efficacy (Figure 6) and side effects. During the maintenance phase patients should be checked at least twice a year.

Clinical improvement (Neurologic features, liver disease, hematology)

Regression of Kayser-Fleischer Ring

Circulating free copper <10 µg/dl

24-hr urinary copper excretion (200-500 µg/day on chelators)

Decrease in liver copper content

Figure 6. Monitoring the treatment efficacy in WD.

Clinical examinations include neurological, ophthalmologic and psychiatric consultations (Figure 7). Patients with liver involvement need to be checked carefully for signs of liver failure.

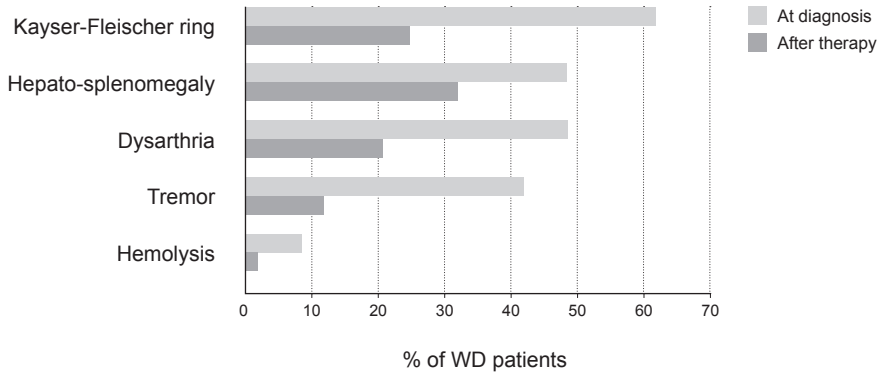


Figure 7. Findings prior to and after beginning chelating therapy in 53 WD patients (modified from Stremmel 1991).

Laboratory tests include measurements of serum copper and ceruloplasmin, calculation of free (nonceruloplasmin-bound) copper (see above), and 24-hr urinary copper excretion (Roberts 2003). While on chelating therapy 24-hr urinary copper excretion should initially range between 200 and 500 mg; such a value can also suggest that the patient is adherent to the drug. After removal of copper accumulation, urinary copper excretion may be lower. Prognosis of WD is dependent on the initial severity of the disease and then on adherence to the life-long treatment. Patients treated prior to severe and potentially irreversible neurological and hepatic complications have a good prognosis approaching a normal life expectancy (Figure 8). Irreversible liver disease often can be treated successfully by LTX while some patients with severe neurological disease do not get better despite optimal therapy.

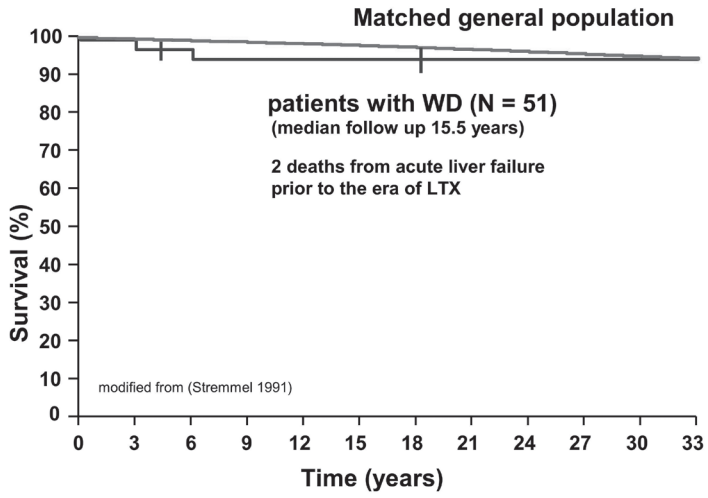


Figure 8. Cumulative survival in 51 WD patients versus a matched general population (modified from Stremmel 1991).

References

Aisen AM, Martel W, Gabrielsen TO, et al. Wilson disease of the brain: MR imaging. Radiology 1985;157:137-141.

Azizi E, Eshel G, Aladjem M. Hypercalciuria and nephrolithiasis as a presenting sign in Wilson disease. Eur J Pediatr 1989;148:548-549.

Bergstrom RF, Kay DR, Harkcom TM, Wagner JG. Penicillamine kinetics in normal subjects. Clin Pharmacol Ther 1981;30:404-413.

Berman DH, Leventhal RI, Gavaler JS, Cadoff EM, Van Thiel DH. Clinical differentiation of fulminant Wilsonian hepatitis from other causes of hepatic failure. Gastroenterology 1991;100:1129-1134.

Brewer GJ, Hill GM, Prasad AS, Cossack ZT, Rabbani P. Oral zinc therapy for Wilson's disease. Ann Intern Med 1983;99:314-319.

Brewer GJ, Terry CA, Aisen AM, Hill GM. Worsening of neurologic syndrome in patients with Wilson's disease with initial penicillamine therapy. Arch Neurol 1987;44:490-493.

Brewer GJ, Yuzbasiyan-Gurkan V, Young AB. Treatment of Wilson's disease. Semin Neurol 1987;7:209-220.

Brewer GJ, Yuzbasiyan-Gurkan V, Lee DY, Appelman H. Treatment of Wilson's disease with zinc. VI. Initial treatment studies. J Lab Clin Med 1989;114:633-638.

Brewer GJ, Dick RD, Johnson V, et al. Treatment of Wilson's disease with ammonium tetrathiomolybdate. I. Initial therapy in 17 neurologically affected patients. Arch Neurol 1994;51:545-554.

Brewer GJ, Johnson V, Dick RD, Kluin KJ, Fink JK, Brunberg JA. Treatment of Wilson disease with ammonium tetrathiomolybdate. II. Initial therapy in 33 neurologically affected patients and follow-up with zinc therapy. Arch Neurol 1996;53:1017-1025.

Brewer GJ, Dick RD, Johnson VD, Brunberg JA, Kluin KJ, Fink JK. Treatment of Wilson's disease with zinc: XV long-term follow-up studies. J Lab Clin Med 1998;132:264-278.

Brewer GJ, Askari F. Transplant livers in Wilson's disease for hepatic, not neurologic, indications. Liver Transpl 2000;6:662-664.

Brewer GJ, Askari F, Lorincz MT, et al. Treatment of Wilson disease with ammonium tetrathiomolybdate. IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease. Arch Neurol 2006;63:521-527

Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet 1993;5:327-337.

Cairns JE, Williams HP, Walshe JM. “Sunflower cataract” in Wilson’s disease. Br Med J 1969;3:95-96.

Cauza E, Maier-Dobersberger T, Polli C, Kaserer K, Kramer L, Ferenci P. Screening for Wilson’s disease in patients with liver diseases by serum ceruloplasmin. J Hepatol 1997;27:358-362.

Chu CC, Huang CC, Chu NS. Recurrent hypokalemic muscle weakness as an initial manifestation of Wilson’s disease. Nephron 1996;73:477-479.

Cumings JN. The effect of BAL in hepatolenticular degeneration. Brain 1951;74:10-22.

Czlonkowska A, Gajda J, Rodo M. Effects of long-term treatment in Wilson’s disease with D-penicillamine and zinc sulphate. J Neurol 1996; 243:269-273.

Denny-Brown D, Porter H. The effect of BAL (2,3 dimercaptopropanol) on hepatolenticular degeneration (Wilson’s disease).NEngl J Med 1951;245:917-925.

Eghtesad B, Nezakatgoo N, Geraci LC, et al. Liver transplantation for Wilson’s disease: a single-center experience. Liver Transpl Surg 1999;5:467-474.

Emre S, Atillasoy EO, Ozdemir S, et al. Orthotopic liver transplantation for Wilson’s disease: a single-center experience. Transplantation 2001;72:1232-1236.

Ferenci P, Cz_onkowska A, Merle U, et al. Late-onset Wilson’s disease. Gastroenterology 2007;132:1294-1298.

Ferlan-Marolt V, Stepec S. Fulminant Wilsonian hepatitis unmasked by disease progression: report of a case and review of the literature. Dig Dis Sci 1999;44:1054-1058.

Fleischer B. Ueber einer der “Pseudosklerose” nahestehende bisher unbekannte Krankheit (gekennzeichnet durch Tremor, psychische Stoerungen, braeunliche Pigmentierung bestimmter Gewebe, insbesondere der Hornhautperipherie, Lebercirrhose). Deutsch Z Nerven Heilk 1912;44:179-201.

Frommer DJ. Urinary copper excretion and hepatic copper concentrations in liver disease. Digestion 1981;21:169-178.

Frydman M. Genetic aspects of Wilson’s disease. J Gastroenterol Hepatol 1990;5:483-490.

Geller SA, Petrovic LM, Batts KB, et al. Histopathology of end-stage Wilson disease. Mod Pathol 2000; 13:184A.

Giacchino R, Marazzi MG, Barabino A, et al. Syndromic variability of Wilson’s disease in children. Clinical study of 44 cases. Ital J Gastroenterol Hepatol 1997;29:155-161.

Grand RJ, Vawter GF. Juvenile Wilson disease: histologic and functional studies during penicillamine therapy. J Pediatr 1975;87:1161-1170.

Gross JB, Jr., Ludwig J, Wiesner RH, McCall JT, LaRusso NF. Abnormalities in tests of copper metabolism in primary sclerosing cholangitis. Gastroenterology 1985;89:272-278.

Groth CG, Dubois RS, Corman J, et al. Metabolic effects of hepatic replacement in Wilson’s disease. Transplant Proc 1973;5:829-833.

Gow PJ, Smallwood RA, Angus PW, Smith AL, Wall AJ, Sewell RB. Diagnosis of Wilson’s disease: an experience over three decades. Gut 2000;46:415-419.

Harris ZL, Klomp LW, Gitlin JD. Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. Am J Clin Nutr 1998;67:972S-977S.

Hill GM, Brewer GJ, Prasad AS, Hydrick CR, Hartmann DE. Treatmentof Wilson’s disease with zinc. I. Oral zinc therapy regimens. Hepatology 1987;7:522-528.

Kalach N, Seidman EG, Morin C, et al. Acute liver failure from Wilson’s disease in a five year-old child. Can J Gastroenterol 1993;7:610-612.

Lang CJ, Rabas-Kolominsky P, Engelhardt A, Kobras G, Konig HJ. Fatal deterioration of Wilson’s disease after institution of oral zinc therapy. Arch Neurol 1993;50:1007-1008.

Lau JY, Lai CL, Wu PC, Pan HY, Lin HJ, Todd D. Wilson’s disease: 35 years’ experience. Q J Med 1990;75:597-605.

Loudianos G, Figus AL, Loi A, et al. Improvement of prenatal diagnosis of Wilson disease using microsatellite markers. Prenat Diagn 1994;14:999-1002.

Martins da Costa C, Baldwin D, Portmann B, Lolin Y, Mowat AP, Mieli-Vergani G. Value of urinary copper excretion after penicillamine challenge in the diagnosis of Wilson’s disease. Hepatology 1992;15: 609-615.

Menkes JH. Menkes disease and Wilson disease: two sides of the same copper coin. Part I: Menkes disease. Europ J Paediatr Neurol 1999;3: 147-158.

Merle U, Schaefer M, Ferenci P, Stremmel W.Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut 2007;56:115-120.

Milkiewicz P, Saksena S, Hubscher SG, Elias E. Wilson's disease with superimposed autoimmune features: report of two cases and review. J Gastroenterol Hepatol 2000;15:570-574.

Mishra D, Kalra V, Seth R. Failure of prophylactic zinc in Wilson disease. Indian Pediatr 2008;45:151-153

Nakada SY, Brown MR, Rabinowitz R. Wilson's disease presenting as symptomatic urolithiasis: a case report and review of the literature. J Urol 1994;152:978-979.

Nazer H, Ede RJ, Mowat AP, Williams R. Wilson's disease: clinical presentation and use of prognostic index. Gut 1986;27:1377-1381.

Petrukhin K, Fischer SG, Pirastu M, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. Nat Genet 1993;5:338-343.

Rector WG, Uchida T, Kanel GC, Redeker AG, Reynolds TB. Fulminant hepatic and renal failure complicating Wilson's disease. Liver 1984;4:341-347.

Roberts EA, Cox DW. Wilson disease. Baillieres Clin Gastroenterol 1998;12:237-256.

Roberts EA, Schilsky ML. A Practice Guideline on Wilson Disease. Hepatology 2003;37:1475-92.

Saito T. Presenting symptoms and natural history of Wilson disease. Eur J Pediatr 1987;146:261-265.

Saito H, Watanabe K, Sahara M, Mochizuki R, Edo K, Ohyama Y. Triethylene-tetramine (trien) therapy for Wilson's disease. Tohoku J Exp Med 1991;164:29-35.

Sallie R, Katsiyiannakis L, Baldwin D, et al. Failure of simple biochemical indexes to reliably differentiate fulminant Wilson's disease from other causes of fulminant liver failure. Hepatology 1992;16:1206-1211.

Santos Silva EE, Sarles J, Buts JP, Sokal EM. Successful medical treatment of severely decompensated Wilson disease. J Pediatr 1996;128:285-287.

Sarkar B, Sass-Kortsak A, Clarke R, Laurie SH, Wei P. A comparative study of in vitro and in vivo interaction of D-penicillamine and triethylene-tetramine with copper. Proc R Soc Med 1977;70:13-18.

Scheinberg IH, Gitlin D. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). Science 1952;116:484-485.

Scheinberg IH, Jaffe ME, Sternlieb I. The use of trientine in preventing the effects of interrupting penicillamine therapy in Wilson's disease. N Engl J Med 1987;317:209-213.

Schilsky M, Blank RR, Czaja MJ, Scheinberg IH, Stockert RJ, Sternlieb I. Hepatocellular copper toxicity and its attenuation by zinc. J Clin Invest 1989;84:1562-1568.

Schilsky ML, Scheinberg IH, Sternlieb I. Prognosis of Wilsonian chronic active hepatitis. Gastroenterology 1991;100:762-767.

Schilsky ML, Scheinberg IH, Sternlieb I. Liver transplantation for Wilson's disease: indications and outcome. Hepatology 1994;19:583-587.

Scott J, Gollan JL, Samourian S, Sherlock S. Wilson's disease, presenting as chronic active hepatitis. Gastroenterology 1978;74:645-651.

Shah AB, Chernov I, Zhang HT, et al. Identification and analysis of mutations in the Wilson disease gene (ATP7B): population frequencies, genotype-phenotype correlation, and functional analyses. Am J Hum Genet 1997;61:317-328.

Shimono N, Ishibashi H, Ikematsu H, et al. Fulminant hepatic failure during perinatal period in a pregnant woman with Wilson's disease. Gastroenterol Jpn 1991;26:69-73.

Sinha S, Taly AB. Withdrawal of penicillamine from zinc sulphate-penicillamine maintenance therapy in Wilson's disease: promising, safe and cheap. J Neurol Sci 2008;264:129-132

Sokol RJ, Francis PD, Gold SH, Ford DM, Lum GM, Ambruso DR. Orthotopic liver transplantation for acute fulminant Wilson disease. J Pediatr 1985;107:549-552.

Sokol RJ, Twedt D, McKim JM, et al. Oxidant injury to hepatic mitochondria in patients with Wilson's disease and Bedlington terriers with copper toxicosis. Gastroenterology 1994;107:1788-1798.

Song YM, Chen MD. A single determination of liver copper concentration may misdiagnose Wilson's disease. Clin Biochem 2000;33:589-590.

Steindl P, Ferenci P, Dienes HP, Grimm G, Pabinger I, Madl C, et al.Wilson's disease in patients presenting with liver disease: a diagnostic challenge. Gastroenterology 1997;113:212-218.

Sternlieb I. Copper and the liver. Gastroenterology 1980;78:1615-1628.

Sternlieb I. Perspectives on Wilson's disease. Hepatology 1990;12:1234-1239.

Sternlieb I. Wilson's disease and pregnancy. Hepatology 2000;31:531-532.

Stremmel W, Meyerrose KW, Niederau C, Hefter H, Kreuzpaintner G, Strohmeyer G. Wilson disease: clinical presentation, treatment, and survival. Ann Intern Med 1991;115:720-726.

Tanzi RE, Petrukhin K, Chernov I, et al. The Wilson disease gene is a copper transporting ATPase with homologyto the Menkes disease gene. Nat Genet 1993;5:344-350.

Tauber J, Steinert RF. Pseudo-Kayser-Fleischer ring of the cornea associated with non-Wilsonian liver disease. A case report and literature review.Cornea 1993;12:74-77.

Thomas GR, Roberts EA, Walshe JM, Cox DW. Haplotypes and mutations in Wilson disease. Am J Hum Genet 1995;56:1315-1319.

Tu JB, Blackwell RQ. Studies on levels of penicillamine-induced cupriuresis in heterozygotes of Wilson's disease. Metabolism 1967;16:507-513.

van Wassenaer-van Hall HN, van den Heuvel AG, Algra A, Hoogenraad TU, Mali WP. Wilson disease: findings at MR imaging and CT of the brain with clinical correlation. Radiology 1996;198:531-536.

von Herbay A, de Groot H, Hegi U, Stremmel W, Strohmeyer G, Sies H. Low vitamine E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease. J Hepatol 1994;20:41-46.

Walshe JM. Wilson's disease. New oral therapy. Lancet 1956;i:25-26.

Walshe JM. Copper chelation in patients with Wilson's disease. A comparison of penicillamine and triethylene tetramine dihydrochloride. Q J Med 1973;42:441-452.

Walshe JM. The management of Wilson's disease with trienthylene tetramine 2HC1 (Trien 2HC1). Prog Clin Biol Res 1979;34:271-280.

Walshe JM. Treatment of Wilson's disease with trientine (triethylenetetramine) dihydrochloride. Lancet 1982;1:643-647.

Walshe JM, Dixon AK. Dangers of non-compliance in Wilson's disease. Lancet 1986;1:845-847.

Walshe JM. Diagnosis and treatment of presymptomatic Wilson's disease. Lancet 1988;2:435-437.

Walshe JM. Wilson's disease presenting with features of hepatic dysfunction: a clinical analysis of eighty-seven patients. Q J Med 1989;70:253-263.

Walshe JM, Yealland M. Chelation treatment of neurological Wilson's disease. Q J Med 1993;86:197-204.

Walshe JM, Munro NA. Zinc-induced deterioration in Wilson's disease aborted by treatment with penicillamine, dimercaprol, and a novel zero copper diet. Arch Neurol 1995;52:10-11.

Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain 1912;34:295-507.

Wilson DC, Phillips MJ, Cox DW, Roberts EA. Severe hepatic Wilson's disease in preschool-aged children. J Pediatr 2000;137:719-722.

Yamaguchi Y, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. Biochem Biophys Res Commun 1993;197:271-277.

Chapter 27: Autoimmune liver diseases: AIH, PBC and PSC

Christian P. Strassburg

Autoimmune hepatitis (AIH)

Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory disease in which a loss of tolerance of hepatic tissue is presumed. AIH was first defined in 1950 when a form of chronic hepatitis in young women showing jaundice, elevated gammaglobulin levels and amenorrhea eventually leading to liver cirrhosis was described (Waldenström 1950). It was later seen in combination with other extrahepatic autoimmune syndromes, particularly in the presence of antinuclear antibodies (ANA) leading to the term lupoid hepatitis (Mackay1956). Systematic evaluations of the cellular and molecular immunopathology of clinical symptoms and of laboratory features has subsequently led to the establishment of autoimmune hepatitis as a separate clinical entity, serologically heterogeneous, treatable by a specific therapeutic strategy (Strassburg 2000). An established and recently revised scoring system allows for a reproducible and standardised approach to diagnosing AIH in a scientific context (Alvarez 1999). The use and interpretation of seroimmunological and molecular biological tests permits a precise discrimination of autoimmune hepatitis from other etiologies of chronic hepatitis, in particular from chronic viral infection, the most common cause of chronic hepatitis worldwide (Strassburg 2002).

Definition and diagnosis of autoimmune hepatitis

At the time of diagnosis, AIH has normally been present for more than 6 months. In 1992, an international panel met in Brighton, UK, to establish diagnostic criteria for AIH, because it was recognized that several features including histological changes and clinical presentation are also prevalent in other chronic liver disorders (Johnson 1993). In this and a later revised report the group noted that there is no single specific test for AIH diagnosis; a set of diagnostic criteria was suggested in the form of a diagnostic scoring system designed to classify patients as having probable or definite AIH (Table 1).

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 2).

AIH predominantly affects women of any age group and is characterized by a marked elevation of serum globulins, in particular gammaglobulins 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 (Table 3) such as rheumatoid arthritis, autoimmune thyroiditis, ulcerative colitis, diabetes mellitus and a family history of autoimmune or allergic disorders has been reported (Strassburg 1995).

PARAMETER	SCORE
Gender	
Female	+2
Male	0
Serum biochemistry	
Ratio of elevation of serum alkaline phosphatase vs aminotransferase	
>3.0	-2
1.5-3	0
<1.5	+2
Total serum globulin, y-globulin or IgG	
Times upper limit of normal	
>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	
>1:80	+3
1:80	+2
1:40	+1
<1:40	0
Antimitochondrial antibody	
Positive	-4
Negative	0
Hepatitis viral markers	
Negative	+3
Positive	-3
Other etiological factors	
History of drug use	
Yes	-4
No	+1
Alcohol (average consumption)	
<25gm/day	+2
>60 gm/day	-2
Genetic factors: HLA DR3 or DR4	
Other autoimmune diseases	+2
Response to therapy	
Complete	+2
Relapse	+3
Liver histology	
Interface hepatitis	+3
Predominant lymphoplasacytic infiltrate	+1
Rosetting of liver cells	+1
None of the above	-5
Biliary changes	-3
Other changes	-3
Seropositivity for other defined autoantibodies	+2

Interpretation of aggregate scores: definite AIH - greater than 15 before treatment and greater than 17 after treatment; probable AIH - 10 to 15 before treatment and 12 to 17 after treatment.

Table 1. International criteria for the diagnosis of autoimmune hepatitis (Alvarez 1999).

SUSPECTED DIFFERENTIAL DIAGNOSIS	TEST PERFORMED TO EXCLUDE
Hepatitis C (HCV)	Anti-HCV (HCV RNA)
Hepatitis B and D (HBV, HDV)	-HBsAg, anti-HBc (HBV DNA) -anti-HDV, HDV RNA only when HBsAg positive
Hepatitis A virus (HAV)	Antibodies, serology: IgG, IgM
Hepatitis E virus (HEV)	Only if suspected
Epstein-Barr virus (EBV)	Only if suspected
Herpes simplex virus (HSV)	Only if suspected
Cytomegalovirus (CMV)	Only if suspected
Varicella zoster zirus (VZV)	Only if suspected
Drug-induced hepatitis	History; if applicable, withdrawal of drug LKM-2, LM autoantibody in selected cases
Primary biliary cirrhosis (PBC)	Anti-mitochondrial antibodies (AMA) Specificity of reactivity: PDH-E2, BCKD-E2 Liver histology: copper deposition in bile ducts Unresponsive to steroids
Primary sclerosing cholangitis (PSC)	Cholangiography
Wilson's Disease	Coeruloplasmin, urine copper, eye examination, quantitative copper in liver biopsy
Haemochromatosis	Serum ferritin, serum iron, transferrin saturation, liver histology: iron staining, quantitative iron in biopsy Genetic testing: C282Y, H63D mutation of HFE gene in Caucasians
Alpha-1-antitrypsin deficiency	Phenotype testing: PiZZ/PiSS/PiMZ/PiSZ

Table 2. Differential diagnosis of autoimmune hepatitis and diagnostic tests.

Frequent
Autoimmune thyroid disease
Ulcerative colitis
Synovitis
Rare or individual reports
Rheumatoid arthritis
Lichen planus
Diabetes mellitus
CREST syndrome
Autoimmune-thrombozytopenic purpura
Vitiligo
Nail dystrophy
Alopecia

Table 3. Extrahepatic associations of autoimmune hepatitis are present in 10% to 50% of patients.

Autoantibodies are one of the distinguishing features of AIH. The discovery of autoantibodies directed against different cellular targets (Table 4), 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 antinuclear antibodies (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 (Manns 1991; Manns 1989) and with lower frequency against UDP-glucuronosyltransferases (UGT) (Strassburg 1996). AIH type 3 (Manns 1987; Stechemesser 1993) is characterized by autoantibodies against a soluble liver antigen (SLA/LP) identified as UGA suppressor serine tRNA-protein complex (Gelpi 1992; Volkmann 2001; Wies 2000).

Antibody	kDa	Target antigen	Disease
Autoantigens of the endoplasmic reticulum (microsomal autoantigens)			
LKM-1	50	Cytochrome P450 2D6	Autoimmune hepatitis Type 2 Hepatitis C
LKM-2	50	Cytochrome P450 2C9	Ticrynafen-induced hepatitis
LKM-3	55	UGT1A	Hepatitis D-associated autoimmunity Autoimmune hepatitis type 2
LKM	50	Cytochrome P450 2A6	Autoimmune polyendrocrine syndrome type 1 (APS-1) Hepatitis C
LM	52	Cytochrome P450 1A2	Dihydralazine-induced hepatitis Hepatitis with autoimmune polyendocrine syndrome type 1 (APS-1)
	57	Disulfidisomerase	Halothane hepatitis
	59	Carboxylesterase	Halothane hepatitis
	35	?	Autoimmune hepatitis
	59	?	Chronic hepatitis C
	64	?	Autoimmune hepatitis
	70	?	Chronic hepatitis C
Autoantigens of the cytosol (soluble liver proteins)			
LC1	58-62	Formiminotransferase	Autoimmune hepatitis type 2
		Cyclodeaminase	Autoimmune hepatitis Hepatitis C?
SLA/LP	50	UGA repressor tRNA-associated protein	Autoimmune hepatitis (type 3)

Table 4. Heterogeneity of autoimmune hepatitis based on serological findings: Molecular definitions of the most important autoantigens in serological diagnostics.

Although the histological appearance of AIH is characteristic, there is no specific histological feature that can be used to confirm the diagnosis (Dienes 1989). Percutaneous liver biopsy should be performed for grading, staging and therapeutic monitoring. Histological 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 viral infections is sufficient in most cases. The exclusion of other hepatotropic viruses such as cytomegalovirus, Epstein-Barr virus, and herpes group viruses may only be required in cases where such infections are suspected 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, histological signs of cholangiopathy and detection of AMA. If one or more components of the scoring system are not evaluated, a direct score pointing to a probable diagnosis can be compiled (Table 1). A simplified system is currently in development and will be published shortly.

Epidemiology of AIH

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 and myasthenia gravis, which also have an autoimmune etiology (Nishioka 1997; Nishioka 1998). Among the North American and Western European Caucasian population AIH accounts for about up to 20% of cases with chronic hepatitis (Cancado 2000). 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 needed to comprehensively elucidate the prevalence and geographical distribution of AIH.

Autoantibodies and aetiology of AIH

There is no doubt that a loss of self-tolerance is the pathophysiological process driving AIH, which leads to the observed sequelae. A number of concepts have been pursued to elucidate the causative agents or mechanisms leading to AIH. Autoantibodies directed against the endoplasmatic reticulum, in particular against members of the cytochrome P450 (CYP) superfamily of proteins also occur as markers of serological autoimmunity in hepatitis C and hepatitis D virus infection (Strassburg 1996), as well as frequent transient markers of drug-mediated allergic hepatic disease (Beaune 1996), and even in the context of genetically determined autoimmune disease such as the autoimmune poly-glandular syndrome (APS) type 1 (Obermayer-Straub 2001).

The exact immunological basis of AIH still remains unresolved despite the awareness of its serological features and considerable research efforts invested into the identified autoantigen targets. When AIH is diagnosed the disease is usually not in its early stages and the initiating events are therefore not available for detailed analysis. The parallel serological features of virus-associated autoimmunity and genuine autoimmune hepatitis have led to the hypothesis of an external trigger (infectious or chemical), suggesting that the susceptibility inherent to the host is a required co-factor. The epidemiology of autoimmune hepatitis unfortunately offers very little information suggesting genetic susceptibility mainly because familial risk is understudied. Apart from individual reports and in the absence of twin studies AIH itself does not appear to cluster in families but the prevalence of other autoimmune diseases including autoimmune thyroid disease, celiac disease, ulcerative colitis etc., is increased in relatives. This feature is the basis of inclusion of first degree relatives with autoimmune diseases into the revised international scoring system for autoimmune hepatitis, a score that describes - for scientific purposes - the likelyhood of the presence of AIH. The hypothesis of trigger and genetic susceptibility is strengthened by a significant body of evidence linking major histocompatibility complex (MHC) genes with AIH (Donaldson 2002). MHC class I and II antigens are critical players in T cell immunity in their ability to present short antigenic peptides for recognition by antigen-specific T cells. Variants of MHC encoded proteins therefore influence the precise interplay of T cell receptor and HLA molecule including the possibility of determining immunological susceptibility and resistance. The study of autoantibodies and autoantigens on the one hand offers a window to identify the relevant antigenic determinants involved in the loss of tolerance, while the study of genetic associations (Strassburg 2000) on the other hand leads to the definition of the permissive genetic profile, and the combination represents the stage upon which the pathophysiology of AIH unfolds.

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 (Strassburg 2002; Strassburg 2000). 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 group of cellular proteins (Tan 1988). To date, subtyping of the various ANA antigens offers no diagnostic or prognostic advantage. ANA are also detected in PBC, 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 culture monolayer slides.

Most commonly, a homogeneous (Figure 1) or speckled immunofluorescence pattern is encountered. ANA have been found to be reactive with centromers, ribonucleoproteins, and cyclin A (Figure 1) (Strassburg 1996). They represent the most common autoantibody in AIH and occur in high titers usually exceeding 1:160.

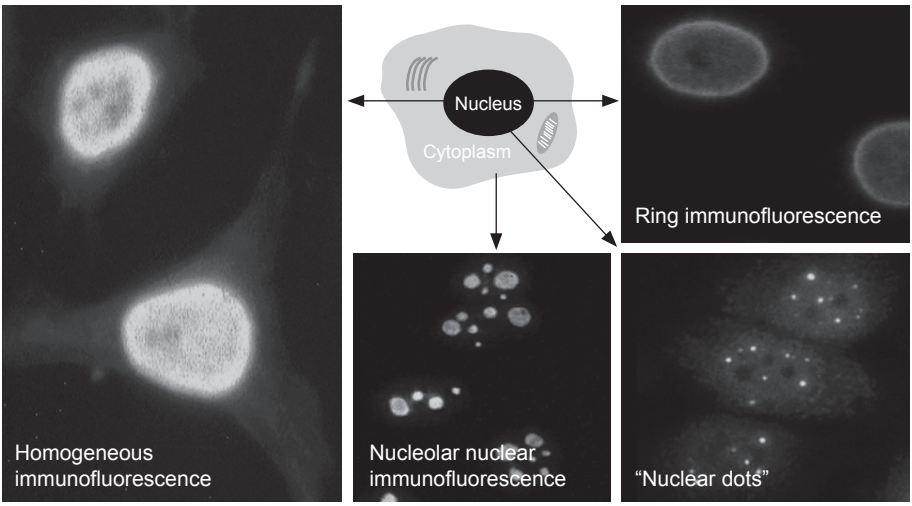


Figure 1. Indirect immunofluorescence micrographs of a variety of ANA found in autoimmune hepatitis and other autoimmune diseases, detected on immobilized Hep.2 cells. Aspect of the nuclear membranous (rim) immunofluorescence pattern (top right) found in a patient with autoimmune hepatitis type 1 at 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 SLE clearly distinguished from a homogeneous pattern (top left). The middle panel demonstrates a nucleolar ANA fluorescence pattern. This pattern is rarely seen in autoimmune hepatitis, but is common in rheumatological diseases such as scleroderma and polymyositis. If present in autoimmune hepatitis type 1, it can be indicative of overlap syndromes with rheumatological disorders. The lower right panel shows multiple nuclear dots. This pattern is not typical for autoimmune hepatitis and can be found in about 20% of patients with PBC. Usually AMA are present at the same time but can be missing in cases of ANA-positive, AMA-negative PBC. These autoantibodies are directed against the SP100 nuclear antigen (100 kDa).

Anti-smooth muscle antibodies (SMA) are directed against components of the cytoskeleton such as actin, troponin and tropomyosin (Dighiero 1990; Kurki 1980; Lidman 1976). They frequently occur in high titers in association with ANA. However, SMA autoantibodies 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 (Figure 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.

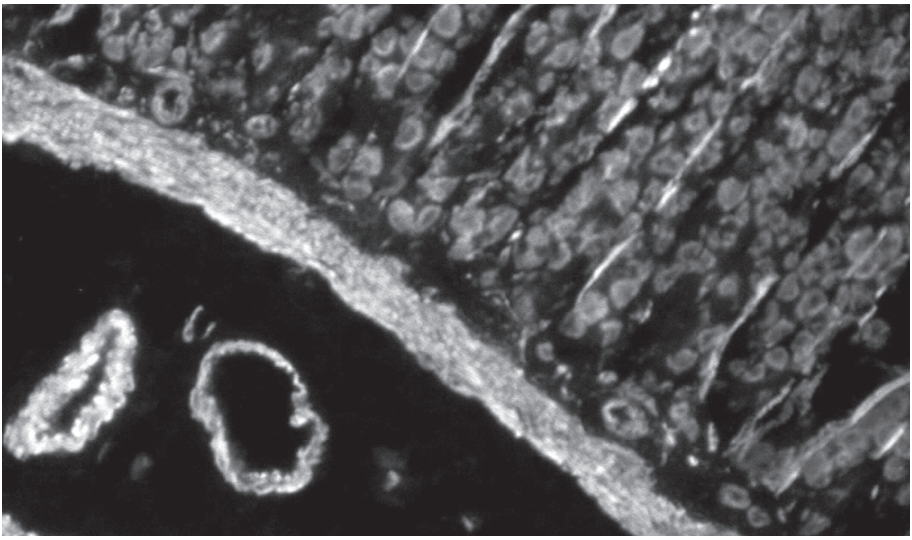


Figure 2. Typical immunofluorescence pattern of SMA autoantibodies detected on rat stomach cryostat sections. This serum shows immunoreactivity with the muscularis mucosae and muscularis propria layers of rat stomach. Note that the mucosa is excluded from reactivity. This autoantibody is often detected in conjunction with ANA in autoimmune hepatitis type 1.

Liver/kidney microsomal antibodies (LKM) are directed against proteins of the endoplasmic reticulum (microsomal protein). In 1973, Rizzetto discovered autoantibodies reactive to the proximal renal tubulus and the hepatocellular cytoplasm by indirect immunofluorescence (Figures 3 A and B) (Rizzetto 1973). 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 p450 2D6 (CYP 2D6).

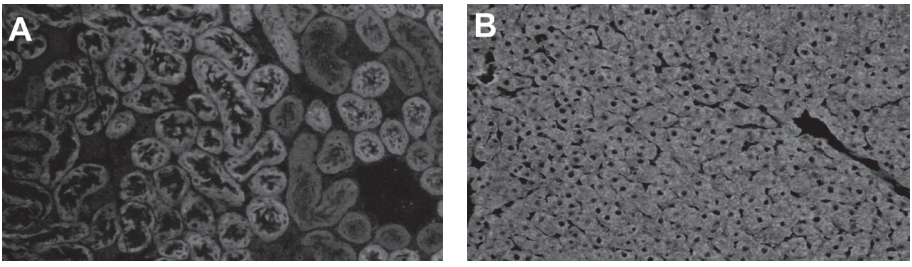


Figure 3. Indirect immunofluorescence showing LKM-1 autoantibodies on rat kidney and liver cryostat sections. Serum of a patient with autoimmune hepatitis type 2. A. Typical indirect immunofluorescence pattern of LKM-1 autoantibodies detecting the proximal (cortical) renal tubules but excluding the distal tubules located in the renal medulla, which corresponds to the tissue expression pattern of the autoantigen CYP 2D6. B. Using rat hepatic cryostat sections a homogeneous cellular immunofluorescence staining is visualized excluding the hepatocellular nuclei (LKM-1).

LKM-1 autoantibodies recognize a major linear epitope between amino acids 263 and 270 of the CYP 2D6 protein (Guenguen 19991; Guenguen 1988; Manns 1984; Manns 1991; Zanger 1988). These autoantibodies inhibit CYP 2D6 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 (Sugimura 2002). However, the recognition of epitopes located between amino acids 257 and 269 appears to be a specific autoimmune reaction of autoimmune hepatitis and discriminatory against LKM-1 autoantibodies associated with chronic HCV infection. The endoplasmic reticulum-based CYP 2D6 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 4; Figure 4).

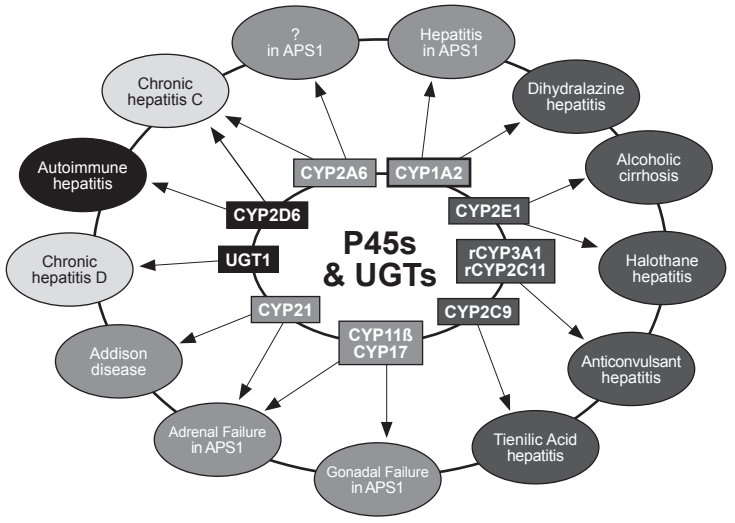


Figure 4. Diversity of autoantibodies against endoplasmatic reticulum (microsomal) targets in autoimmune hepatitis, drug induced hepatitis, viral hepatitis and genetic disease (autoimmune polyglandular syndrome type 1; APECED/APS-1). CYP, cytochrome P450; UGT, uridine diphosphate glucuronosyltransferase.

LKM autoantibodies against CYP 1A2 as well as 2A6 are found in patients with APECED and hepatic involvement. Anti-CYP 2A6 autoantibodies also occur in HCV infection. LKM autoantibodies, characterised by an immunofluorescence pattern selectively staining the hepatocellular but not renal cell cytoplasm, have been found to be directed against CYP 1A2. These autoantibodies are also found in APECED syndrome with hepatic involvement and occur as well in dihydralazine-induced hepatitis. A second

type of LKM autoantibodies, LKM-2, are directed against CYP 2C9 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) (Crivelli 1983). These autoantibodies are directed against family 1 UDP-glucuronosyltransferases (UGT1A) (Philipp 1994), which are also a superfamily of drug metabolizing proteins located in the endoplasmic reticulum membrane (Turkey 2001). LKM-3 autoantibodies have been identified in HDV infection but also in AIH type 2 patients (Strassburg 1996). They can also occur in LKM-1-negative and ANA-negative AIH. In addition, LKM positive sera display reactivity with a number of as yet undefined antigens with molecular weights of 35 kDa, 57 kDa, 59 kDa, and 70 kDa (Durazzo 1995). These autoantibodies are predominantly found in AIH, HCV infection and halothane hepatitis (Figure 4). 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 (SLA) were detected in a patient with ANA-negative AIH (Manns 1987). It is now clear that the description of liver pancreas (LP) antibodies recognize the same target protein structure leading to the designation SLA/LP autoantibodies (Stechemesser 1993; Wies 2000). Anti-SLA/LPs were found to be highly specific for AIH and are detectable in about 10-30% of all patients with AIH. In 1992, specific autoantibodies were identified in patients with a severe form of autoimmune chronic hepatitis (Gelpi 1992). These antibodies precipitated a UGA suppressor serine tRNA-protein complex, which is probably involved in cotranslational selenocyteine 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 cytoceratins 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.

Antibodies against liver-cytosol type 1 (LC1) were found in up to 50% of patients with AIH type 2 (Muratori 1995). Less frequently, anti-LC1 may be associated with SMA and ANA in sera from patients with AIH type 1 and chronic hepatitis C infection. In addition anti-LC1 proved to be the only serological marker in 10% of patients with AIH. Anti-LC1 are visualized by indirect immunofluorescence, however their characteristic staining may be masked be the more diffuse pattern of LKM-1 antibodies. The antigen recognized by anti-LC1 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 link. Anti-LC1 sera recognize distinct epitopes on FTCD preferentially localized to the FT domain of FTCD (Muratori 2001). Contrary to most other autoantibodies in AIH, anti-LC1 seems to correlate with disease activity and may be useful as a marker of residual hepatocellular inflammation in AIH.

Antibodies against the asialoglycoprotein receptor (ASGPR) (Treichel 1990) are seen in up to 90% of 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 hepati-

tis, 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 (pANCA) were detected in 65–95% of sera from patients with AIH type 1, and additionally in sera from patients with PSC (Figure 5). pANCA are detected by immunofluorescene, 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 immunmofluorescene pattern but do not show reactivity with myeloperoxidase, one of the mayor autoantigens of classical ANCA. The discrimination of ANCA is difficult, because ANA frequently also stain ethanol-fixed neutrophils. The target antigen of 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 may be useful in identifying patients formerly classified as having cryptogenic hepatitis (Álvarez 1999).

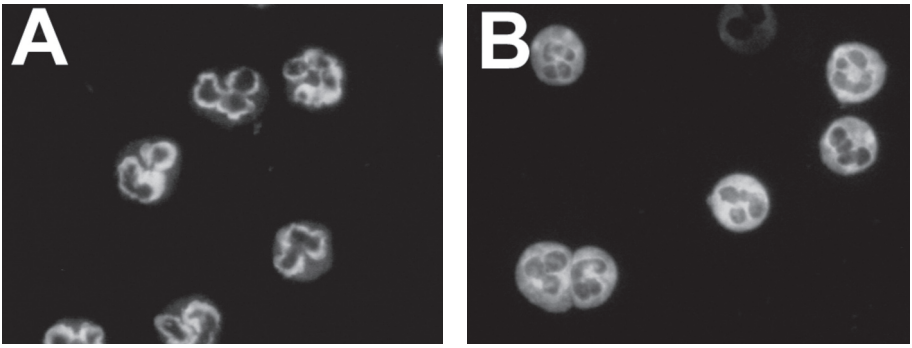


Figure 5. Immunofluorescence study showing anti-neutrophil cytoplasmatic antibodies (ANCA) with a typical pANCA (A) and cANCA (B) distinction. These autoantibodies are found in autoimmune hepatitis type 1 (ANA- and SMA-positive) in up to 95% but are not considered to be a specific diagnostic finding in AIH. When further analyzed they frequently do not exhibit reactivity with myeloperoxidase (pANCA) or proteinase 3 (cANCA) in AIH.

Aetiology of AIH

Conclusive evidence of a single aetiology of AIH has not yet been presented. Many findings point towards a viral aetiology, which has been investigated in numerous studies (Lenzi 1995; Manns 1990); this remains, however, a matter of controversy (Voguel 2002).

In anecdotal reports a relationship of the hepatitis A virus, hepatitis B virus, Epstein-Barr virus and herpes simplex virus with autoimmune hepatitis has been implicated (Vento 1996; Vento 1997; Vento 1991; Vento 1995). 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 CYP 2D6, which is targeted by LKM-1 autoantibodies, displays homology with the immediate early antigen IE175 of herpes simplex

virus (HSV). A case has been reported, in which the only difference in HLA identical twins with discordant manifestation of AIH was HSV exposition (Manns 1990).

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 serologic autoimmunity differs with respect to recognition of antigen targets (CYP 2D6 and CYP 2A6), recognition of epitopes (in AIH mainly 257-269, in HCV more diverse and also more conformation-dependent epitopes) and the clinical presentation (Table 5). From these considerations it is unlikely that HCV is etiologically responsible for AIH (Czaja 1993).

	Autoimmune hepatitis	**Viral hepatitis**
Autoantibody titer	↑↑↑	↑
Linear autoepitopes	+ + +	+
Conformational epitopes	+	+ + + +
Inhibitory antibodies	+ +	+ +
Autoimmune response	homogenous	heterogeneous
Treatment	Immunosuppression	(antiviral)

Table 5. Differences between genuine autoimmune disease and virus-induced serological autoimmunity.

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 a complex trait like most other human diseases, which means that there are one or more 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 autoimmune hepatitis is related to the major histocompatibility complex alleles. Approximately 1000 human leukocyte antigens (HLA) have been identified to date. The MHC is encoded on a 4000 kbp portion of chromosome 6p21.3, is characterized by considerable genetic polymorphism and is divided into 3 regions: MHC class I and II encode HLA A, B, Cw, Dr, Dq and DP, the MHC class II region encompasses several immune-reactive proteins including the complement proteins C2, C4A, CaB, the heat shock protein (HSP-70) family, tumor necrosis factor (TNF) alpha and beta, and MHC class I chain-related proteins (MICA, MICB). Interest in HLA-association in AIH stems from the fact that the molecular structure and variation of the MHC-peptide alpha-helical region at the floor of the peptide binding groove determines antigen presentation to the T cell receptor and that most of the interindividual variability in HLA alleles is relevant to the amino acid sequence of this peptide binding groove. 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 at 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 includes shaping of the T cell repertoire, peptide selection and presentation as well as peptide transport. HLA A1 and B8 association and AIH has been reported (Mackay 1972).

Genetic variability is not limited to the HLA I and II genes but equally affects TNFa and b, complement genes, MICA, and MICB. Although the functional changes of TNF gene promoter polymorphisms are not clear the role of the molecule in inflammation, cell death, apoptosis and the upregulation of MHC expression make it an interesting candidate gene.

Association of HLA A1, Cw7, B8 and DR3 - which is inherited as a haplotype – as well as DR4 with AIH and other autoimmune diseases has been conclusively demonstrated in a number of studies (Donaldson 1991; Mackay 1980). In turn, an association with HLA B, Cw, TNF-a have been found not to be the major factor. Studies from Europe and the US have identified DRB1*0301 and DRB1*0401 as susceptibility alleles, and DRB1*1501 as a resistance allele (Table 2) (Doherty 1994; Strettell 1997). However, immunogenetic findings appear not to apply universally and it was noted that significant geographic differences exist. In Japan DR2 (DRB1*1501) is a weak susceptibility rather than a resistance allele (Ota 1992) and in South American children DRB1*1301 is a strong susceptibility allele (Pando 1999) not found in any of the other studies. Molecular comparison of amino acid residues at the a helical binding groove region of the HLA molecule have suggested that in Japanese patients histidine at position 13, in US and European as well as Japanese, Mexican and Argentinian patients lysine at position 71, and for South American children valine at position 86 appears to confer AIH risk. These data illustrate that genetic association varies in study populations (Table 6).

HLA	DR3	DR4
Genotype	DR B1*0301	DR B1*0401 (DR B1*0405 in Japan)
Age at onset	<30	>40
Disease activity	+++	+
Treatment response	++	++++
Relapse after treatment	+++	+
Liver transplantation	+++	+
DRß chain amino acid as risk factor	lysine at amino acid 71	?

Table 6. Heterogeneity of autoimmune hepatitis based on genetic markers.

A number of explanations may account for this finding. An exogenous factor present in a distinct population may be necessary (molecular footprint) which is in line with the current hypothesis of environmental trigger plus genetic susceptibility, but the model may just be too simple altogether in its assumption of the relevance of single amino acid residue differences. AIH is not likely to be monogenetic or oligogenetic disease. It is obvious that a polygenetic profile of factors yet to be elucidated will define predisposition for this disease.

Non-MHC genes and autoimmune hepatitis

The CD152 (cytotoxic lymphocyte antigen-4, CTLA-4) molecule on immune regulatory (CD25 positive) T cells interacts with CD80 and CD86 on the antigen-presenting cell with up to 50 fold higher affinity than CD28. Corecognition of CD152 results in a reduction of the immune response. The CTLA-4 gene has been shown to exhibit more than 16 single nucleotide polymorphisms, and the CTLA-4 A+49G allele was found to be associated with diabetes, primary biliary cirrhosis and autoimmune thyroiditis. An association with AIH makes biological sense and is indeed the strongest non-MHC association yet (Agarwal 2000). The study of interleukin 1 and 10 did not reveal associations. Variants of the vitamin D receptor, associated with a number of autoimmune diseases as well as with the tyrosine phosphatase CD45 showed a week association with AIH (Vogel 2003; Vogel 2002).

Autoimmune hepatitis in the autoimmune polyendocrine syndrome (APS) type 1: a model disease?

The APS-1 syndrome is characterized by a number of autoimmune disorders involving endocrine and non-endocrine organs including muco-cutaneous candidiasis, hypoparathyroidism and adrenal insufficiency (establishing the diagnosis when two of the latter are present) (Obermayer-Straub 2001). In 10% of patients autoimmune hepatitis is present. APS-1 has greatly increased our understanding of autoimmune diseases since it has a monogenic association with mutations in the autoimmune regulator (AIRE) gene. AIRE is expressed in medullary epithelial cells of the thymus accounting for less than 0.1% of thymic cells (Pitkanen 2001). The transcription factor encoded by the AIRE gene regulates the expression of a multitude of antigens required for the negative selection of autoreactive T cells in the thymus. In AIRE-deficient mice less autoantigen is expressed in thymic medullary epithelial cells resulting in a higher number of reactive T cells in the periphery, which contributes to the establishment of autoimmune disease (Ramsey 2002). AIH in APS-1 syndrome leads to the formation of autoantibodies against CYP1A2 and CYP2A6. AIH can be the first clinically apparent component of this syndrome particularly in children (Lankisch 2005). However, a restrospective analysis of adult patients with AIH has not detected an increased frequency of variant AIRE alleles (Vogel 2001).

Clinical presentation

Systematically, autoimmune hepatitis is part of the syndrome of chronic hepatitis, characterized by a sustained hepatocellular inflammation for at least 6 months accompanied by an elevation of ALT and AST of 1.5 times the upper limit of normal.

In about 49% of AIH patients an acute onset of AIH is observed; rare cases of fulminant AIH have been reported. In most cases, however, the clinical presentation is not spectacular and characterized by fatigue, right upper quadrant pain, jaundice and occasionally by palmar erythema and spider naevi. In later stages, the consequences of portal hypertension dominate, including ascites, bleeding aesophageal 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 3).

Subclassification

Immunoserologic parameters assume a central role in the subclassification of AIH (Table 4) and allow the discrimination of clinically distinct groups of patients. The IAIHG has not recommended these subdivisions for anything more than research purposes, because autoantibodies do not define distinct therapeutic groups. However, they note that the distinction between AIH type 1 and type 2 has already been widely adopted in clinical practice (Manns 2001).

Autoimmune hepatitis type 1 is characterized by ANA and in most cases also SMA autoantibodies. In 97% of patients hypergammaglobulinemia with elevated immunoglobulin G is present. Representing 80% of the cases of AIH, this most prevalent subclass was originally described as lupoid, classical or idiopathic AIH. 70% of patients are female with a peak incidence between the ages of 16 and 30. 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 heading the list. The clinical course is often not spectacular and acute onset is very rare. About 25% have cirrhosis at the time of diagnosis.

Autoimmune hepatitis type 2 is characterized by the presence of LKM-1 autoantibodies against CYP 2D6. In 10% of patients LKM-3 autoantibodies against UDP-glucuronosyltransferases are also present. In contrast to AIH type 1, additional organ-specific autoantibodies are present such as anti-thyroid, anti-parietal cell, and anti-Langerhans cell autoantibodies. The number of extrahepatic immune syndromes such as diabetes, vitiligo and autoimmune thyroid disease is more prevalent. Serum immunoglobulin levels are moderately elevated with a reduction of IgA. AIH type 2 is a rare disorder that affects 20% of AIH patients in Europe but only 4% in the US. There is a female predominance. The maximum age is around 10 years but AIH type 2 is also observed in adults, especially in Europe. AIH type 2 carries a higher risk of progression to cirrhosis with a fulminant course.

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, affects female patients in 90% of cases and has a maximum age of between 20 and 40 years. This subclass of AIH is a matter of debate and further evaluations are needed 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 marker. This should decrease the likelihood of misclassification.

Cryptogenic hepatitis and overlap syndromes

Cryptogenic hepatitis is an aetiologically-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 patients initially tested by indirect immunofluorescence for ANA, SMA and LKM, it is possible to detect SLA autoantibodies and contribute to a 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 additional markers and symptoms point to other diseases. Among these are PBC in 8% with serum AMA and histological signs of cholangitis, PSC in 6% with typical changes of the cholangiography, and autoimmune cholangitis in 10% with ANA, SMA and histological inflammation of the biliary system (Czaja 1998). 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. Differences reported may reflect the differences between serologic overlap and genuine clinical overlap of two autoimmune diseases. The latter appears to be very rare (Strassburg 2004).

A clinically significant association is virus-associated autoimmunity, which describes the coexistence of autoantibodies and viral infection (Strassburg 1995; Strassburg 1996). 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 5). LKM autoantibodies in viral infections are present at lower titers and recognise more conformational and diverse epitopes than in genuine AIH. This discrimination is relevant since it forms the basis for mutually exclusive therapeutic strategies: immunosuppression in AIH and interferon in chronic viral hepatitis (Dalekos 1999).

Natural history and prognosis

Data describing the natural history of AIH are scarce. The last placebo-controlled immunosuppressive treatment trial was published in 1980 (Kirk 1980). The value of these studies is limited considering that these patients were only screened for epidemiological risk factors for viral hepatitis and were not characterised by standardised diagnostic criteria. Nevertheless these studies reveal that untreated AIH had a very poor prognosis and 5- and 10-year survival rates of 50% and 10% are reported. They furthermore demonstrate that immunosuppressive treatment significantly improves survival.

Recent data reveals that up to 30% of adult patients have histological features of cirrhosis at diagnosis. In 17% of patients with periportal hepatitis cirrhosis develops 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 10-year survival (90%) and those patients require a similarly aggressive treatment strategy (Geall 1968; Soloway 1972).

Almost half of the children with AIH already have cirrhosis at the time of diagnosis. Long-term follow-up revealed that few children can completely stop all treatment and about 70% of children receive long-term treatment (Gregorio 1997; Homberg 1987). Most of these patients relapse when treatment is discontinued, or if the dose of the immunosuppressive drug is reduced. About 15% of patients develop chronic liver failure and need to be transplanted before the age of 18.

In elderly patients, a more severe initial histological grading has been reported, but the frequency of definite cirrhosis seems not to differ from younger patients. At follow-up, about 30% of patients develop cirrhosis. Response to immunosuppression is similar in older and younger 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 in younger, usually treated, patients (Newton 1997).

The risk of hepatocellular carcinoma varies considerably between PBC, PSC and AIH. Particularly, PCS can be complicated by cholangiocarcinoma, gall bladder carcinoma and hepatocellular carcinoma. In contrast occurrence of HCC in patients with AIH is a rare event and develops only in long-standing cirrhosis.

AIH therapy

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 limited effects.

Independent of clinically or immunoserologically defined types of AIH, treatment is implemented with predniso(lo)ne alone or in combination with azathioprine. Both strategies are effective (Manns 2001). 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. Important is the exact differentiation between viral infection and autoimmune hepatitis. Treatment of replicative viral hepatitis with corticosteroids must be prevented as well as administration of interferon in AIH, which can lead to dramatic disease exacerbation.

An indication for treatment is present when aminotransferases are elevated 2-fold, gamma-globulin levels are elevated 2-fold 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, histological signs of severe inflammation and necrosis, and upon disease progression.

The treatment regimen and suggested follow-up examinations are summarised in Table 7. Therapy is usually administered over the course of 2 years. The decision between monotherapy and combination therapy is guided by principle considerations: Long-term steroid therapy leads to cushingoid side effects. These visible side effects decrease patient compliance considerably. Serious complications such as steroid diabetes, osteopenia, aseptic bone necrosis, psychiatric symptoms, hypertension and cataract formation also have to be anticipated with long-term treatment. Side effects are seen in 44% of patients after 12 months and in 80% of patients after 24 months of treatment. Predniso(lo)ne monotherapy is possible in pregnant

patients. Azathioprine, on the other hand, leads to a decreased dose of prednisone. It bears a theoretical risk of teratogenicity. In addition, abdominal discomfort, nausea, cholestatic hepatitis, rash and leukopenia can be encountered. These side effects are seen in 10% of patients receiving a dose of 50 mg per day. From a general point of view, a postmenopausal woman with osteoporosis, hypertension and elevated blood glucose is a candidate for combination therapy. In women of childbearing age, pregnant women or patients with hematological abnormalities, prednisone monotherapy may be the treatment of choice.

Treatment is initiated according to the regimen in Table 7. A strict administration is essential since most cases of relapse are the result of erratic changes of medication and/or dose. Dose reduction is aimed at finding a tolerable maintenance dose. Since histology lags 3 to 6 months behind the normalisation of serum parameters therapy has to be continued beyond the normalisation of aminotransferase levels. Usually, maintenance doses of prednisone range between 10 and 25 mg. After 12-24 months of therapy predniso(lo)ne can be tapered down over a 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. AIH relapse and risk of progression to fibrosis is almost universal when immunosuppression is tapered when there is still residual histological inflammation.

	A. monotherapy	**B. combination therapy**
Prednis(ol)one	60 mg Reduction within 4 weeks to maintenance dose 20 mg or lower	30 mg Reduction within 4 weeks to maintenance dose 10 mg or lower
Azathioprine	n.a.	50 mg
	(maintenance with azathioprine: monotherapy: 2 mg/kg body weight)	
After remission is reached treatment length 12-24 months, histology, avoid premature reduction:		
Prednis(ol)one	Reduction of daily dose by 2.5 mg per week	
Azathioprine	n.a.	Reduction: 25 mg every 3 weeks

n.a. - not applicable. In the elderly patient with low inflammatory activity the indication to treat must be weighed against side effects - many of these patients may best remain untreated. The table reflects the Mayo approach (Manns 2001). In our own experience monotherapy with prednisone beginning with 50 mg and tapered by 10 mg every 10 days to a maintenance dose of 15 to 20 mg; alternatively, combination therapy with 1 mg/kg bodyweight of azathioprine for 3 weeks and tapering to 50 mg daily combined with prednisone therapy tapered to 10 mg daily is equally effective (Manns 2001). 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 young patient without severe symptoms and with low inflammatory activity (biopsy, ALT <5 x upper limit of normal) treatment can be initiated with maintenance doses.

Examination	Before therapy	During therapy before remission q 4 weeks	Remission under therapy q 3-6 months	Cessation of therapy q 3 weeks (x 4)	Remission post-therapy q 3-6 months	Evaluation of relapse
Physical	+		+	+	+	+
Liver biopsy	+		(+/-)			+
Blood count	+	+	+	+	+	
Amino-transferases	+	+	+	+	+	+
Gamma gluta-myltransferase	+	+	+			
Gammaglobulin	+	+	+	+	+	+
Bilirubin	+	+	+	+	+	+
Coagulation studies	+	+	+	+	+	
Autoantibodies	+	+/-				+
Thyroid function tests	+	+/-				+

Table 7. Treatment regimen and follow-up examinations of autoimmune hepatitis regardless of autoantibody type.

The four outcomes: remission, relapse, treatment failure and stabilization

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 of 2 mg/kg bodyweight (Johnson 1995). 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 3-fold increase of aminotransferase levels and the reccurrence of clinical symptoms. Relapse is seen 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 a long-term maintenance dose with predniso(lo)ne or azathioprine monotherapy.

Treatment failure characterizes a progression of clinical, serological and histological 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 experimental regimens can be administered; otherwise, liver transplantation will become necessary.

Stabilisation is the achievement of partial remission. Since 90% of patients reach remission within 3 years, the benefit of standard therapy has to be reevaluated in this subgroup of patients. Liver transplantation provides a definitive treatment option for this group.

If standard treatment fails or drug intolerance occurs, alternative therapies such as cyclosporine, tacrolimus, cyclophosphamide, mycophenolate mofetil, rapamycin,

UDCA, and budesonide can be considered. The efficacy of these options has not been clearly defined.

Budesonide is a synthetic steroid with high first-pass metabolism in the liver, which should limit systemic side effects compared to conventional steroids. In a study treating 13 AIH patients with budesonide over a period of 9 months the drug was well tolerated and aminotransferase levels were normalized (Danielson 1994). Our own experiences have confirmed that budesonide is effective but does not offer an advantage over conventional steroids when cirrhosis and porto-systemic shunts are present (Schüler 1995). However, in a more recent study budesonide therapy was associated with a low frequency of remission and high occurrence of side effects (Czaja 2000). The main advantage of budesonide for the future treatment of autoimmune hepatitis may be to replace prednisone in long-term maintenance therapy to reduce steroid side effects. The potential benefit of budesonide is currently being evaluated in clinical trials.

Deflazacort has been proposed as an alternative corticosteroid for immunosuppression with fewer side effects than conventional glucocorticoids. In a recent study, 15 patients with AIH type I were treated with deflazacort who had previously been treated with prednisone with or without azathioprine until biochemical remission was obtained. Remission was sustained during 2 years of follow-up. However, the long-term role of second-generation corticosteroids to sustain remission in AIH patients with reduced treatment-related side effects requires further controlled studies (Rebollo 1999).

Cyclosporine A (CyA) is a lipophylic 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 alternative agents, the greatest experience to date has been with CyA. CyA has been successfully used for AIH treatment and has been well tolerated (Alvarez 1999; Debray1999). The principal difficulty in advocating widespread use of CyA 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) (Alvarez 1999; Debray1999; Frazer 1985; Heneghan 2002).

Tacrolimus is a macrolide lactone compound with immunosuppressive capabilities exceeding those of CyA. The mechanism of action is similar to that of CyA but it binds to a different immunophilin. The application of tacrolimus in 21 patients treated for one year led to an improvement of aminotransferase and bilirubin levels with a minor increase in serum BUN and creatinine levels (Van Thiel 1995). Although tacrolimus represents a promising immunosuppressive candidate drug, larger randomized trials are required to assess its role in AIH therapy.

Mycophenolate has attracted attention as a transplant immunosuppressant with an important role in the steroid-free immunosuppressive therapy of patients transplanted for chronic hepatitis C infection. Mycophenolate is a noncompetitive 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. In a recent pilot study 7 patients with AIH type 1 who either did not tolerate azathioprine or did not respond to standard therapy with a complete normalization of aminotransferase levels were treated with mycophenolate in addition to steroids. In five out of seven patients normalization of aminotransferase levels was achieved within three months.

These preliminary data suggest that mycophenolate may represent another promising treatment strategy (Richardson 2000).

The induction of remission with 1-1.5 mg per kg per day of cyclophosphamide in combination with steroids has been reported. However the need for a continued application of cyclophosphamide with its potentially severe hematological side effects renders it a highly experimental treatment option (Kanzler 1996).

Ursodeoxycholic acid 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 histological abnormalities, clinical and biochemical improvement but not a reduction of fibrosis in 4 patients with AIH type 1 (Calmus 1990; Czaja 1999; Nakamura 1998). Its role in AIH therapy or in combination with immunosuppressive therapy is still unclear.

Liver transplantation in AIH

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 varicees and coagulation abnormalities despite adequate immunosuppressive therapy (Ahmed 1997; Neuberger 1984; Prados 1998; Sanchez-Urdazpal 1991; Tillmann 1999; Vogel 2004). 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 after 4 years of continuous therapy. Indicators of a high mortality associated with liver failure are histological evidence of multilobular necrosis and progressive hyperbilirubinemia. In Europe, 4% of liver transplants are for AIH (European Liver Transplant Registry 1996). The long-term results of liver transplantat due to AIH are excellent. The 5-year survival is up to 92% (Prados 1998; Rea 2005; Sanchez-Urdazpal 1991) and well within the range of other indications for liver transplantation.

The potential of AIH to recur after liver transplantation is a matter of debate. The first case of recurrent AIH after liver transplantation reported (Neuberger 1984) was based upon serum biochemistry, biopsy findings and steroid reduction. Studies indicate that the rate of recurrence of AIH ranges between 10-35%, and that the risk of AIH recurrence is perhaps as high as 68% at 5 years (Devlin 1995; Götz 1999; Manns 2000; Milkiewicz 1999; Vogel 2004; Wright 1992). 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 histological evidence of recurrent AIH may be missed. It is therefore suggested that all patients with suspected recurrence of autoimmune hepatitis receive a liver biopsy, biochemical analyses of aminotransferases as well as a determination of immunoglobulins and autoantibody titers (Vogel 2004). Significant risk factors for the recurrence of AIH have not yet been identified although it appears that the presence of fulminant hepatic failure before transplantation offers protection against the development of recurrent disease. A potential risk factor for the development of recurrent AIH is the presence of specific HLA antigens that may predispose towards 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 not been 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 steroids 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 (Vogel 2004). Steroid withdrawal should therefore be undertaken with great caution. In addition to AIH recurrence the development of de novo autoimmune hepatitis after liver transplant has been reported (Kerkar 1998).

Serum autoantibodies are an integral part of the diagnosis of autoimmune hepatitis. Autoantibody prevalence and titers have been studied in patients receiving liver transplants for autoimmune hepatitis and primary biliary cirrhosis. In general, autoantibody types persist in the majority of patients after transplantation. In PBC antimitochondrial antibodies persisted, albeit at lower titers in almost 100% of patients, later confirmed by several groups (Gouw 1994). In AIH, autoantibodies of the specific subtype present before transplant were detected at lower titers in 77% post-transplant 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 titers exceeding levels detected prior to transplant may be indicative of AIH recurrence. The majority of published data and our own experience do not support a prognostic role of autoantibodies in AIH and liver transplantation (Vogel 2004).

Primary biliary cirrhosis

Introduction

Primary biliary cirrhosis (PBC) is a chronic inflammatory, cholestatic disease of the liver with an unknown cause. The clinical observation of a broad array of immune-mediated symptoms and phenomena suggests the disease to be of autoimmune etiology, in the course of which progressive and irreversible destruction of small interlobular and septal bile ducts progressively and irreversibly ensues (Table 8).

90% female sex
Age 40-59 years
Pruritus
Jaundice
Skin pigmentation
Elevation: alkaline phosphatase (AP), aspartate aminotransferase (AST), bilirubin, IgM
Antimitochondrial antibodies (AMA)
Associated immune-mediated syndromes
Liver biopsy
• Cellular bile duct infiltration • Granulomas possible • Copper deposits

Table 8. Clinical Profile of Primary Biliary Cirrhosis (PBC).

As in other autoimmune diseases PBC affects women in over 90% of cases and is associated with varying extrahepatic autoimmune syndromes in up to 84%. These extrahepatic manifestations of immune-mediated disease include the dry gland syndrome (sicca syndrome with xerophthalmia and xerostomia) but also collagen diseases, autoimmune thyroid disease, glomerulonephritis and ulcerative colitis (Table 9).

- Dry gland "sicca" syndrome
- Sjögren's syndrome
- Rheumatoid arthritis
- Autoimmune thyroid disease
- Renal tubular acidosis
- Mixed connective tissue disease (MCTD)
- Polymyositis
- Polymyalgia rheumatica
- Pulmonary fibrosis
- CREST syndrome
- Systemic lupus erythematosus (SLE)
- Pernicious anemia
- Ulcerative colitis
- Exogenous pancreatic insufficiency
- Myasthenia gravis

Table 9. PBC extrahepatic immune-mediated syndromes and overlap with rheumatic diseases.

The striking female predominance (Donaldson 1996; Mackay 1997; Uibo 1999) and family clustering of PBC (Jones 1999; Kato 1981; Tsuji 1999) suggest that inheritable genetic factors play a role. This has focussed attention on the immunogenetics of PBC in order to better define host risk factors (Strassburg 2000). Studies have suggested an instability of lymphocytic DNA in PBC patients (Notghi 1990). Immunogentic analyses, however, have come up with only relatively weak associations with specific human leukocyte antigen haplotypes. PBC appears to be associated with the class 2 DR8 haplotype and a combination of DR8 and the nullallele of the complement factor C4A: C4AQ0 (Donaldson 1996; Manns 1991; Mehal 1994; Onishi 1994). The role of tumor necrosis factor a gene or promoter polymorphisms in the etiology of PBC is a matter of evaluation (Donaldson 1999; Gordon 1999). Furthermore, a role of the cytotoxic T-lymphocytic antigen 4 (CTLA-4) is being evaluated as a risk factor. The genetic basis of PBC is most likely heterogeneous and polygenic in nature. Apart from host factors external trigger mechanisms are likely to define a considerable proportion of the risk in developing PBC.

Definition and prevalence of PBC

Primary biliary cirrhosis is an inflammatory, primarily T cell-mediated, chronic destruction of intrahepatic microscopic bile ducts of unknown etiology (Strassburg 2000). It affects women in 90% of cases who exhibit elevated immunoglobulin M, antimitochondrial antibodies directed against the E2-subunit of pyruvate dehydrogenase (PDH-E2), a cholestatic liver enzyme profile with elevated alkaline phosphatase, gamma glutamytransferase as well as serum bilirubin levels, and a variable course of disease leading to cirrhosis over the course of years or decades. A prominent feature is the presence of extrahepatic immune-mediated disease associations, which include autoimmune thyroid disease, sicca syndrome, rheumatoid arthritis, inflammatory bowel disease, and less frequently celiac disease and CREST syndrome. Extrahepatic syndromes frequent-

ly precede hepatic disease manifestation. In later stages pronounced fatigue, pruritus, marked hyperbilirubinemia and the consequences of portal hyprtension such as ascites, bleeding esophageal varices, and encephalopathy develop (Strassburg 2004).

The prevalence is estimated at 65 per 100,000 in women and 12 per 100,000 in men with an incidence of five per 100,000 in women and one per 100,000 in men. The prevalence and incidence appear to vary regionally. An increase of PBC incidence in recent years may be the result of more specific testing of antimitochondrial antbody reactivity (Strassburg 2004).

Pathophysiology of PBC

The progressive destruction of bile ducts is mediated by T cells. Biopsies show pericholangiolar infiltrates of mononuclear cells including lymphocytes, plasma cells, eosinophils and macrophages, in addition to granulomas. The homology of E. coli PDH-E2, human PDH-E2, which represents the major B-cell autoantigen recognised by anitmitochondrial antibodies in PBC patients, and HLA-A may suggest mimicry effects. In cell culture PBC patient-derived lymphocytes lead to an increased expression of the B-cell autoantigen PDH-E2 on the surface of cholangiocytes indicating transmissible factors. The association of a water reservoir and a cluster of PBC patients in England may suggest an infectious etiology which is also hypothesized for E. coli rough mutants as well as a number of bacterial and viral pathogens. However, conclusive and reproducible evidence for any of these hypotheses has not been provided.

Diagnostic principles of PBC

Suspicion of PBC arises when cholestasis and cirrhosis are present in middle-aged women (Figure 6). Ultrasound is employed to rule out mechanical cholestasis. The presence of antimitochondrial antibodies (AMA) against PDH-E2 is diagnostic of PBC. AMA against E2 subunits of members of the inner mitochondrial membrane expressed oxoacid dehydrogenase complex (PDH, branched chain ketoacid dehydrogenase-BCKD, and ketoglutarate dehydrogenase-OADC) are present in 95% of PBC patients. AMA-negative PBC can exhibit antinuclear autoantibodies with specificity against nuclear dot antigen (SP100), a 210 kDa nuclear membrane protein (gp210), or nucleoporin p62. In AMA-negative PBC a biopsy is indicated to contribute to the establishment of the diagnosis, while in the presence of AMA against PDH-E2 histology is used primarily for the staging of cirrhosis and is not necessary (Strassburg 2004).

Antimitochondrial antibodies in PBC

It is generally believed that the autoimmune attack on the small intrahepatic bile ducts in PBC is mediated by cellular mechanisms and that this process is the main contributor to the pathophysiology of PBC (Joplin 1999; Lohr 1993; Van Hoogstraten 2000). While cellular autoimmunity is the defining process of patient survival and hepatic function, humoral autoimmunity is the main diagnostic feature of this disease. High titer AMA were first described in 1958 (Mackay 1958). In 1967 the target antigen of AMA was localized within the inner mitchondrial membrane and termed M2 (Berg 1967). In 1985, further analysis of M2 antigens led to their subdivision into individual antigen fractions between 36 and 74 kDa molecular weight (Berg 1986; Frazer 1985; Ishii 1985; Linden-

born-Fotinos 1985; Manns 1987; Manns 1982). The molecular cloning of the 74 kDa antigen led to the identification of the ketoacid dehydrogenase multiprotein complex (OADC) as the major autoantigen of PBC-associated AMA (Gershwin 1987). Autoantibodies directed against members of the OADC represent those previously defined as anti-M2 autoantibodies. These AMA are PBC-specific and can be separated from non-specific AMA using molecularly defined seroimmunological methods (Table 10).

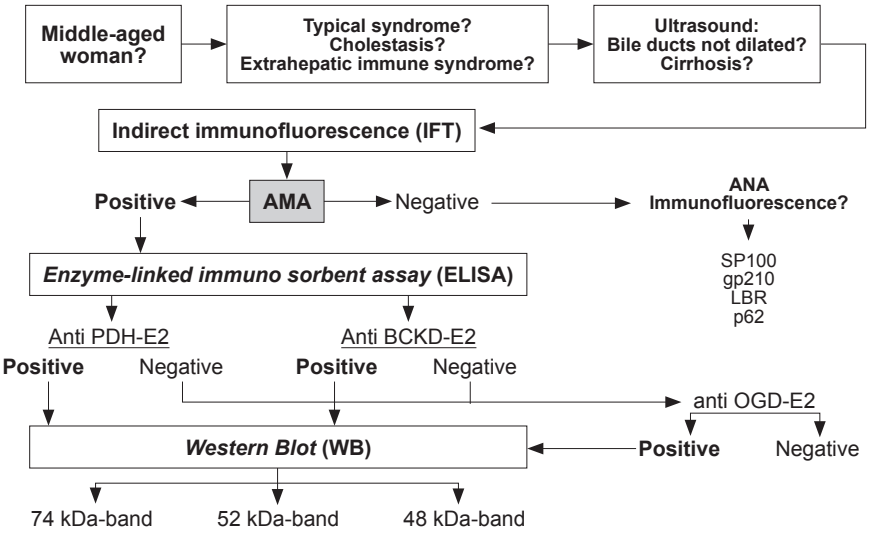


Figure 6. PBC diagnostic algorithm including clinical presentation, ultrasound and serology.

		kDa	occurence	old M-classification
Pyruvate dehydrogense (PDH)				
PDH-E2	(Pyruvate decarboxylase)	74	95%	M2 a
PDH-E1 a	(Pyruvate decarboxylase)	41	41-66%	M2 d
PDH-E1 b	(Pyruvate decarboxylase)	36	2-7%	M2 e
Protein X	(Lipoid pomponent of PDH)	52	95%	M2 c
Branched-chain ketoacid dehydrogenase (BCKD)				
BCKD-E2	(Acyltransferase)	50	53-55%	M2 c
BCKD-E1 a	(Acyldecarboxylase)	46	?	
BCKD-E1 b	(Acyldecarboxylase)	38	?	
Ketoglutarate dehydrogenase (KGD)				
KGD-E2	(Succinyltransferase)	48	39-88%	M2 c
KGD-E1	(Ketoglutarate decarboxylase)	110	low	
E3	(Lipoamide dehydrogenase)	55	38%	M2 c

Table 10. Classification and heterogeneity of mitochondrial autoantigens (modified according to Gershwin 1991).

AMA against ketoacid dehydrogenase complex antigens (OADC)

The OADC consists of three major antigens: pyruvate dehydrogenase (PDH), branched chain ketoacid dehydrogenase (BCKD), and ketoglutarate dehydrogenase (OGD) (Strassburg 2000) (Table 3). Every enzyme in itself consists of three subunits with individual enzymatic activities: E1 (decarboxylase), E2 (dihydro lipoamide acyltransferase), and E3 (lipoamide dehydrogenase) (Table 10).

In 95% of all North American and European, and 65% of all Japanese, PBC sera, AMA are directed against the E2 subunit of PDH (PDH-E2). PDH-E2 represents the 74 kDa autoantigen identified first as part of the M2 antigen fraction. AMA mainly belong to the IgM class of immunoglobulins, but IgA, IgG1 and IgG3 class autoantibodies are also regularly detected. The further analysis of PBC sera has demonstrated that 53-55% are reactive with the E2 subunit of BCKD (BCKD-E2), which corresponds to the earlier identified 52 kDa antigen of M2. In addition, 39-88% of PBC sera display autoantibodies directed against the E2 unit of OGD (OGD-E2), corresponding to the 48 kDa component of M2. Reactivity of these three major subspecies of PBC-specific AMA has a number of common features: immunoreactivity favors epitopes on the E2 subunit in all three cases, the recognized epitopes are of considerable size and are conformation-dependent, and they are localized within the lipoyl domain of the molecules. Epitopes have been characterized for PDH-E2 (93 amino acids) (Gershwin 1991; Van De Water 1988), BCKD-E2 (227 amino acids) (Leung 1995), and OGD-E2 (81 amino acids) (Moteki 1996). Autoantibodies against PDH-E2 occur together with anti-BCKD-E2 in 60% of cases. In about 10-20% of PBC patients anti-BCKD-E2 autoantibodies are detected alone, the significance of which is not clear.

Autoantibodies directed against the other components of OADC are of minor diagnostic importance. Anti-PDH-E1a autoantibodies have been detected in 41-66% of PBC patients and have been implicated as a serological indicator of coexisting systemic sclerosis (Fujimoto 1995). However, this test is not routinely employed. Autoantibodies against protein X, a 56 kDa autoantigen, have been described and found to be completely cross-reactive with PDH-E2 antibodies (Leung 1996; Palmer 1999).

In 89% of PBC patients AMA have also been detected in the bile. These were directed against PDH-E2 (79%), BCKD-E2 (32%) and OGD-E2 (5%), and were always found when AMA of the same reactivity were also present in the serum (Nishio 1997). Almost half of these biliary AMA were of the IgA subtype, which were directed against the same autoepitopes as serum AMA. Interestingly, the presence of PDH-E2, BCKD-E2, and OGD-E2 antigen was detected in bile of PBC patients indicating that the humoral response in these patients may be antigen driven by OADC antigen or proteins cross-reactive with this antigen. AMA of the IgA subtype, the expression of PDH-E2 antigen (or a cross reactive antigen) on biliary epithelial cells (Joplin 1995; Leung 1996) in PBC patients, may indicate that PBC could represent a mucosal disease entity. AMA and PDH-E2 or cross-reactive antigens are also detected in the saliva of PBC patients, which may represent additional evidence for this hypothesis (Reynoso-Paz 2000). AMA in saliva and bile are not part of the routine determination of AMA in PBC patients, and their diagnostic significance is unknown.

Non PBC-specific AMA

While AMA directed against the OADC of the inner mitochondrial membrane are disease specific for PBC a number of AMA exist in extrahepatic diseases. Based on differentially centrifuged mitochondrial antigen preparations a more descriptive antigen system consisting of 9 fractions has been established (M1-M9, M2 contains OADC antigens) (Berg 1986). This nomenclature comprises as yet unidentified antigens of the inner mitochondrial membrane (M1, M2, M5a, M7) and of the outer mitochondrial membrane (M3, M4, M5b, M6, M8, M9). Anti-M1autoantibodies (anti-cardiolipin) have been found in syphilis, anti-M7 directed against sarcosine dehydrogenase in acute myocarditis, anti-M3 in venocuran drug-induced pseudolupus, anti-M6 in iproniacide drug-induced hepatitis and anti-M5 in a number of patients with collagen disorders (Berg 1995).

A controversy exists concerning the prognostic value of this antigen fraction-based autoantibody classificiation (Klein 1991; Klein 1997). It has been suggested, that anti-M4 and anti-M8 autoantibodies are indicative of a more severe course of PBC requiring earlier transplantation. It has also been suggested that M4 autoantibodies are directed against sulfite oxidase (Klein 1991) and M9 autoantibodies against glycogen phosphorylase (Berg 1995), which other authors have been unable to confirm (Davis 1992; Palmer 1993). At present a prognostic significance of M-based AMA remains speculative.

The diagnostic role of AMA in PBC

The main aim of AMA determinations is the detection of PBC-specific AMA and the exclusion of AMA of low diagnostic relevance for the disease. As a screening test the determination of AMA using indirect immunofluorescence testing on rat kidney cryostat sections or immobilised HEp.2 cells (Strassburg 1999). The indirect immunofluorescence on rat kidney sections leads to the staining of the distal and proximal tubuli (note: proximal staining is only indicative of liver/kidney microsomal antibodies, LKM). When positive AMA immunofluorescence is detected a further analysis should include subclassification using molecularly-defined antigen preparations. The detection of PDH-E2, BCKD-E2 can be achieved by ELISA using recombinant antigen or reference sera. If both are negative, testing should include OGD-E2. The final step is performed using Western blot analyses to confirm the findings. By Western Blot the indicative 74 kDa (PDH-E2), 52 kDa (BCKD-E2) and 48 kDa (OGD-E2) bands can be visualized. This multi-step regimen secures a rational and reliable diagnosis of PBC-specific AMA exluding those found in drug-induced and infectious diseases.

In the majority of cases the determination of anti-PDH-E2 is sufficient to confirm the diagnosis. Studies will have to evaluate whether the future application of a single PDH-E2 ELISA as highly specific screening test in suspected PBC represents an efficient and economical diagnostic approach.

The predictive value of AMA

The search for reliable predictive parameters in PBC has led to the evaluation of AMA in this respect. The question is whether certain AMA can predict a more severe course of disease with histological progression or a relapse of disease after transplantation.

It has been suggested that higher AMA titers may indicate more severe inflammatory activity. In a recent study the titers of AMA against OADC antigens were evaluated and correlated with disease progression. Titers varied over 200-fold between individuals but stayed relatively constant within individual PBC patients. A correlation with stage, histology or progression of PBC could not be established (Van Norstrand 1997). Quantification of AMA titers is therefore not a useful tool in the staging of PBC. AMA have been observed to persist after orthotopic liver transplantation for PBC (Haagsma 1987; Mattalia 1997). In a recent evaluation it was demonstrated that in a number of patients AMA levels decreased after transplantation. However there is no consistent overall pattern and it is questionable whether AMA can serve as predictive indicators of transplantation outcome (Gouw 1994).

The rate of recurrence of PBC is still a matter of investigation. While it is recognized that recurrence of PBC does occur in a subset of patients (Haagsma 1999) the clinical implications for the management of these patients are still minor although they will perhaps increase as survival increases beyond 10 years post transplant. At present, it is questionable whether the qualitative or the quantitative determinations of AMA carry any significance beyond their diagnostic importance.

Antinuclear antibodies (ANA) in PBC

Antinuclear antibodies (ANA) are routinely determined as a diagnostic marker in a large number of immune-mediated diseases including autoimmune liver diseases (Strassburg 2000), and rheumatological diseases (Tan Em 1988). ANA have also been identified as a serological parameter in up to 52% of patients with PBC (Table 11; Figure 1). The question is whether these antibodies can contribute to the diagnosis of PBC by identifying AMA-negative cases of PBC. Antigens of the nuclear pore complex have emerged as secondary antigens in the serologcial diagnosis of PBC (Bloch 1999; Worman 1994). Autoantibodies against a 210 kDa glycoprotein of the nuclear membrane (gp 210) (Las-soued 1990; Nickowitz 1994) which are highly PBC-specific and occur in 10-47% of patients (Bandin 1996) are well-characterized. Although these autoantibodies have been found to be exhibit a high specificity for PBC, they persist after orthotopic liver transplantation, and do not appear to indicate disease recurrence (Dubel 1998; Luettig 1998; Mattalia 1997). The epitope has been mapped to the carboxyterminus of the protein and is recognized by all gp210 positive sera (Nickowitz 1993).

Anti-gp210
anti-nucleoporin p62
Anti-SP100
Anti-lamin B receptor
Anti-cyclin A
Anti-promyelocytic leukemia protein (PML)

Table 11. PBC-associated antinuclear antibodies.

Nucleoporin p62 is targeted in 32% of PBC sera and also appears to be disease specific (Manns 2000). In about 20% of sera autoantibodies are detected against SP100, a 100 kDa nucleoprotein (Szostecki 1987; Szostecki 1992). SP100 appears to exhibit a high specificity for PBC and has also been found to persist after orthotopic liver transplantation for PBC (Luettig 1998). The prognostic significance of these autoantibodies is most likely similarly low to that found for PBC-specific AMA (Zuchner 1997). Molecular analyses have identified linear SP100 epitopes in PBC sera (Bluthner 1999). A recently reported study identified cyclin A as human autoantigen in hepatic and extrahepatic diseases (Strassburg 1996). Anti-cyclin A autoantibodies were detected in 7% of patients with PBC and more frequently in autoimmune hepatitis type 1. Other antinuclear autoantibodies with specificity for PBC include the lamin B receptor (Lin 1996) and promyelocytic leukemia associated protein PML (Sternsdorf 1995).

When ANA are detected in PBC, they frequently display unique immunofluorescence patterns such as nuclear dots (i.e., SP100) or a nuclear ring-like pattern (Laminins, gp210) (Figure 1). While in autoimmune hepatitis the predominant ANA pattern is a homogeneous or speckled immunofluorescence appearance, ANA in PBC or AMA-negative PBC are frequently distinguishable on screening by immunofluorescence for nuclear dots or ring patterns. Cases of these autoantibodies in patients with clinical presentation of PBC and absence of AMA are rare but may be the only sero-immunological clue to establishing the diagnosis of PBC in a select number of patients.

Therapeutic principles in PBC

A treatment leading to a cure of PBC is not available (Strassburg 2004). Ursodeoxycholic acid (UDCA) (15 mg/kg per day) has been shown to improve serum biochemistry, histology and survival but has no effect on fatigue and osteoporosis. It has immunomodulatory properties, alters cell signal transduction and modifies hydrophilicity of the bile. UDCA should not be given in severe cholestasis and during the first trimester of pregnancy. Immunosuppression in PBC has disappointing results. Symptomatic therapy of the complications of PBC includes management of pruritus (colestyramine, induction with rifampicin, opioid antagonists, serotonin antagonists), ascites (diuretics, beta blockers to control portal hypertension), osteoporosis (vitamin D and calcum supplementation, biphosphonates in osteoporosis, as well as endoscopic intervention for bleeding esophageal varices. Fat soluble vitamin replacement is suggested. On liver cirrosis-induced liver failure liver transplantation remains a definitive therapeutic option. Ten-year survival rates are 75-80% and recurrence of PBC after transplant occurs in 10-40%.

Immunosuppression in PBC

Corticosteroids: Treatment with prednisolone can improve serum aminotransferase activities, alkaline phosphatase and elevated immunoglobulins. It does not lead to significant improvement of bilirubin, pruritus, or histology. In a one-year placebo-controlled study with 36 asymptomatic patients osteopenia and cushingoid side effects were noted (Mitchison 1992).

Azathioprin: The classical immunosuppressant azathioprin, which has a pronounced effect in AIH did not show significant effects in 2 studies and is not used in PBC (Christensen 1985).
Cyclosporine A: In a large study of 346 patients with a median observation time of 2.5 years this classical transplant immunosuppressant did not show significant effects on histological progression (Lombard 1993). Contrasting these findings in a small study with 20 patients who were treated for 2 years histology improved, which should however be viewed with caution (Wiesner 1990). Because of the possibility of severe side effects cyclosporine A is not a recommended therapeutic option.
D-penicillamine: Because PBC is characterized by copper accumulation in the bile ducts the chelator d-penicillamine was studied. D-penicillamine also has immunosuppressive and antifibrotic properties. It was tested on a total of 748 patients in 6 studies, without leading to a positive therapeutic effect. However, 30% of treated patients had severe side effects (Bodenheimer 1985). D-penicillamine for PBC is not recommended.
Colchicine: Because of its antifibrotic and antiinflammatory properties colchicine was investigated in 3 studies in the 1980s. Despite improvement of albumin, bilirubin, aminotransferases and alkaline phosphatase an improvement of clinical symptoms and histology was not observed (Bodenheimer 1988; Kaplan 1986; Warnes 1987). Severe side effects were not reported but an effect on long-term prognosis was also not found.
Methotrexate: Despite its known hepatotoxicity methotrexate was used as an immunosuppressant in PBC. In a placebo-controlled study of 60 patients, low dose methotrexate (7.5 mg/week) led to an improvement of biochemical parameters except for bilirubin but no effect was reported regarding necessity of liver transplantation or survival (Hendrickse 1999). Hepatotoxicity was not observed. Interstitial pneumonitis, which affects about 3-5% of rheumatoid arthritis patients, was observed in 14% of PBC patients. Methotrexate cannot be recommended outside scientific evaluations or studies.

In principle other immunosuppressants (Table 12) such as mycophenolic acid (mycophenolate-mofetil), tacrolimus (FK506) or even monoclonal antibodies against interleukin-2 receptor may represent interesting candidate strategies. However, study data is currently lacking.

	Biochemical improvement	Histological improvement	Survival	Side effects/ toxicity
Corticosteroids	++	++	-	++
Azathioprin	-	-	+	+
Cyclosporine A	++	-	++	++
D-penicillamine	-	-	-	++
Colchicine	++	-	+	-
Methotrexate	++	+	-	+

Table 12. Effects of immunosuppressants in PBC.

Ursodeoxycholic acid in PBC (UDCA)

Leuschner was the first to observe, in 1981, a positive effect of UDCA on elevated liver parameters, the exact mechanism of which was unclear (Leuschner 1996). On the one hand UDCA leads to a modification of the bile acid pool to a more hydrophilic environment with lower detergent-like properties, and it leads to increased bile flow. On the other hand immunomodulatory activity is suggested regarding HLA antigens expressed on biliary epithelial cells and altered signal transduction (Paumgartner 2002). The optimal dose in PBC patients appears to be 13-15 mg/kg. In a meta-analysis of three studies in 548 patients with this dose, biochemical improvement and retarded histological progression to fibrosis was observed (Poupon 1997). These effects were only evident when follow-up continued to 4 years. These data rely heavily on the positive effects of a single study and a subsequent meta-analysis of 8 studies with 1114 patients failed to find positive associations with UDCA therapy (Goulis 1999). There are a number of problems with this. Doses varied, protocols included patients with insufficient dosing, and follow-up was less than two years in some cases. In a recently published analysis of 367 patients from four clinical cohorts an initiation of UDCA therapy in the early stages of PBC (stage I-II) and a treatment duration of two years led to a retardation of histological progression, which argues for an early initiation of UDCA therapy after diagnosis even in the absence of fibrosis or cirrhosis. UDCA was also shown to improve biochemistry, delay portal hypertension and varices, and currently has no therapeutic alternative (Poupon 2003). No convincing effect was demonstrable on osteopenia and extrahepatic manisfestations of PBC. An interesting side effect appears to be the significant reduction of colonic epithelial proliferation. UDCA therapy is not associated with a higher prevalence of colonic polyps and appears to delay their reappearance after polypectomy (Serfaty 2003).

Therapy in non-responders and combination strategies

Non-response can be defined as the unaltered progression of PBC to cirrhosis and portal hyptension in the presence of UDCA therapy. Several factors can contribute to this. Inadequate dosing of UDCA, non-compliance, the presence of an overlapping syndrome with AIH, other co-existing liver diseases but also arterio-portal fistulas, thyroid disease and celiac sprue may be responsible. The most frequent reason for a non-response is an overlap syndrome. In cases of progression during therapy combination therapy can be considered.

Steroids and UDCA: The combination of immunosuppressants and UDCA was studied in four smaller studies and included the use of prednisolone (Leuschner 1996), azathioprine (Wolfhagen 1998) and budesonide (Angulo 2000; Leuschner 1996) (Table 13).

In a randomised controlled study with 30 patients who received 10 mg prednisolone/day an improvement of inflammatory activity was reported (Leuschner 1996). A study with 9 mg budesonide/day showed not only biochemical but also histological improvement in 39 patients (Leuschner 1999). In an open-label study with 22 patients a deterioration of osteopenia was noted (Angulo 2000).

Cholangiocarcinoma	10-20% of PSC patients Yearly risk 1.5% Frequent within 1 year of diagnosis Bilirubin, male gender, long standing ulcerative colitis, abdominal symptoms, smoking
Colorectal cancer	10-fold risk (PSC and ulcerative colitis) Yearly colonoscopies in ulcerative colitis In ulcerative colitis and AP-elevation: consider ERC
Pancreatic cancer	14-fold risk in PSC patients Abdominal ultrasound

Table 13. Cancer association of PSC.

S**ulindac and UDCA:** In an open-label study with 23 patients and incomplete response to UDCA over 12 months of treatment of UDCA or UDCA plus sulindac a trend towards histological improvement and biochemical improvement was reported in the combination group (Leuschner 2002).

Colchicine and UDCA: In three studies the combination of colchicine and UDCA were studied for 24 months on a total of 118 patients (Ikeda 1996; Poupon 1996; Raedsch 1992). Mild biochemical improvement was noted although the effect of longer treatment remains unclear. Because of the biliary elimination of colchicine combinations with bile acids may be potentially toxic.

Methotrexate and UDCA: Several pilot studies and three randomised studies have looked at methotrexate in combination with UDCA. In a recent randomised placebo-controlled protocol with 60 patients a high rate of side effects without therapeutic benefit was reported (Bach 2003; Van Steenbergen 1996).

Primary sclerosing cholangitis

Diagnosis of primary sclerosing cholangitis (PSC)

PSC is classically characterised by the progressive destruction of large intra- as well as extrahepatic bile ducts and – contrasting with AIH and PBC – preferably affects male patients with a maximum age of around 25-45 (Figure 7) (Strassburg 1996). In about 50-75% PSC is associated with ulcerative colitis. PSC is clinically characterized by upper quadrant pain, pruritus, anorexia and fever, but up to 50% of patients lack any symptoms (Weismüller 2008). The diagnosis is established by a typical biochemical profile of cholestasis with elevations of bilirubin, alkaline phosphatase and gamma glutamyl transferase. The characteristic findings upon cholangiography and a typical biopsy show ring fibrosis around the bile ducts, which is not present in all patients. Serology regularly identifies atypical antineutrophil cytoplasmatic autoantibodies (xANCA) in

up to 80% of patients (Terjung 2000), however these are not disease-specific and can occur in patients with ulcerative colitis without PSC. There is a significant association of PSC with cholangiocarcinoma (10-20%) and colorectal cancer (9% in 10 years). In a subgroup of patients small bile duct PSC may be present (Broome 2002), which lacks typical strictures and pruning of the biliary tree upon cholangiography. In these cases the diagnosis can be established in the presence of the typical association with ulcerative colitis in male patients by performing a liver biopsy.

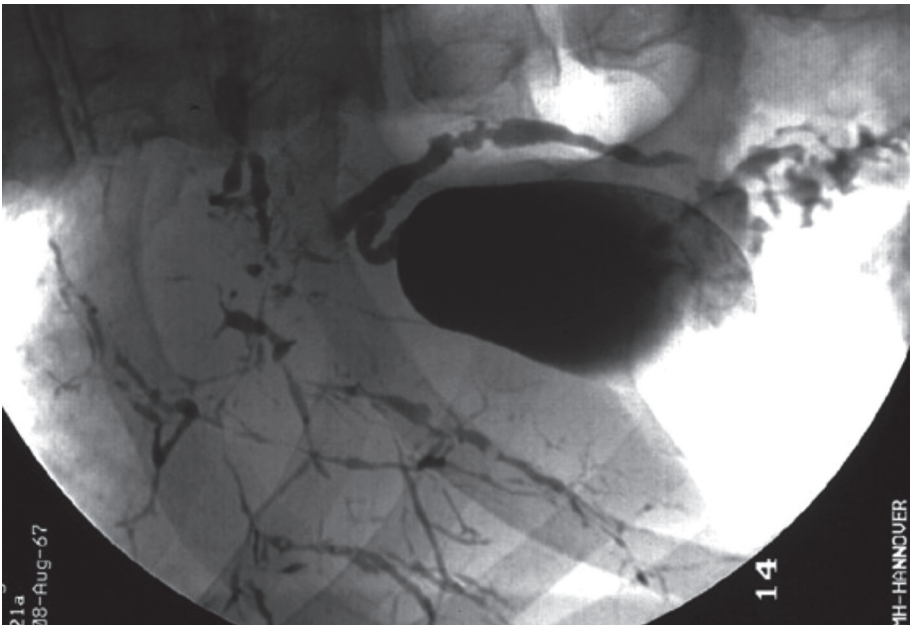


Figure 7. Typical cholangiography of intrahepatic and extrahepatic PSC.

Association of PSC with inflammatory bowel disease

A clinical hallmark of PSC is the high number of patients suffering from inflammatory bowel disease (IBD). In various studies with 605 PSC patients in the US (Mayo Clinic), England (King's College) and in Sweden, IBD was found in 71%, 73% and 81% of PSC cases (Bergquist 2002; Boberg 1998). In our own experience this is found in 52% (Tischendorf 2007). Ulcerative colitis is more often associated (England 71%, Sweden 72%) than Crohn's disease. IBD is usually diagnosed before PSC but owing to the symptomatic latency of both IBD and PSC it can also be diagnosed at the same time or later than PSC. Most commonly ulcerative colitis is diagnosed more than 1 year before PSC (67%), while in 22% the diagnoses occurred within 1 year of each other, and only in 11% the diagnosis of ulcerative colitis was reached more than 1 year after PSC was established. All IBD patients with elevated liver biochemistry represent a risk group and require careful hepatological work-up for PSC. About 5% of all patients with ulcerative colitis have PSC.

PSC as risk factor for cancer

Apart from the risk of developing portal hypertension and cirrhosis PSC is a severe risk factor for cancer, which distinguishes this disease from AIH and PBC (Table 13). The increased risk of cholangiocarcinoma is well described (Bergquist 2001; Boberg 2002). The numbers reported vary because explanted livers during liver transplantation, autopsies and *in vivo* diagnosed cases are taken into account in different analyses. The diagnosis of cholangiocarcinoma (CC) in PSC patients continues to represent a difficult task because stenoses upon cholangiography may be caused by inflammatory activity as well as tumor, and because biochemical tests and biopsy procedures have a low sensitivity and specificity. Imaging studies are equally complicated by a lack of sensitivity since tumors frequently grow intramurally and are diagnosed in late stages precluding curative therapeutic approaches. Studies from Sweden show that 54% of CC ocurr within 1 year of diagnosis of PSC and 27% are diagnosed at liver transplantation. Overall 12.2% of Northern European PSC patients develop CC, which is corroborated by our data from Hannover (Boberg 2002; Tischendorf 2006). These patients suffer from jaundice, pruritus and abdominal pains and have a longer IBD history. Male gender and smoking is also a risk factor (Tischendorf 2006; Weismüller 2008). In a Dutch study there were similar findings of 18 CC out of 174 patients (10%) (Ponsioen 2002). The CC risk of a PSC patient amounts to 1.5% per year and is 161-fold higher than in healthy controls. It is also important to realize that the risk for colorectal cancer (CRC) is elevated10-fold, in addition to a 14-fold risk of pancreatic cancer (Bergquist 2002). These data justify that the diagnosis of PSC should lead to yearly colonoscopies and ultrasound studies to monitor the high potential for cancer development.

Medical therapy of PSC

Current data and clinical experience does not suggest that PSC represents a disease that is curable by medical therapy (Larusso 2006). A cure would include the improvement or normalisation of abnormal cholestatic biochemic features but more importantly the improvement of sclerosing changes to the intra- and extrahepatic biliary tree, ultimately leading to biliary cirrhosis, to episodes of cholangitis, and carrying the risk of cholangiocellular carcinoma. The only available drug that combines a favourable toxicity profile and can lead to a reduction of cholestatic serum parameters is currently ursodeoxycholic acid (UDCA) (Table 14).

Uncomplicated PSC	Ursodeoxycholic acid 15-30 mg/kg per day
Biliary strictures **Cholelithiasis**	Interventional endoscopy, dilatations (stents)
Cholangitis	Antibiotics (e.g., mezlocillin and metronidazole)

Table 14. PSC therapy.

Predictive scores, which have been developed to assess the progress of PSC in view of the clinical experiences of high interindividual variability and unpredictable acceleration episodes almost always contain serum bilirubin as a parameter (Broome 1996; Dickson 1992; Farrant 1991; Kim 2000; Okolicsanyi 1996; Wiesner 1989). Between 1998 and 2000 four such scores were reported employing bilirubin in addition to age, histology, variceal bleeding, hepatomegaly, inflammatory bowel disease, albumin, AST, and hemoglobin (Broome 1996; Dickson 1992; Kim 2000; Wiesner 1989). From this perspective, an improvement of the parameter bilirubin, common to these four scores, would be a plausible indicator of an improved prognosis. However, a number of controversies surround the use of UDCA. In two studies an improvement was documented using 20 mg/kg body weight, and 25-30 mg/kg body weight, respectively (Harnois 2001; Mitchell 2001). Both use UDCA doses considerably higher than the common dose (15 mg/kg body weight). From these data the higher dose appeared to be more beneficial in PSC. However, a study analysing UDCA in bile as a function of oral UDCA dose found that doses exceeding 25 mg/kg body weight are not likely to be useful since the maximum transport of UDCA into the bile levelled off at this dose with no further increase (Rost 2004).

After these and other initial reports a meta-analysis was published in 2002 (Chen 2003), which concluded that UDCA therapy improved biochemical parameters but overall beneficial effect in patients with PSC, in particular survival benefit, was uncertain. In 2005 a large study was reported that appeared to confirm this. 219 PSC patients were studied in a placebo-controlled trial (Olsson 2005). Treatment was carried out with 17-23 mg/kg body weight of UDCA and a trend towards better survival and less need for transplantation was seen, which did not reach statistical significance. A difference in the incidence of cholangiocarcinoma was not observed. However, statistical analyses reported in this study concluded that 346 patients would have been required to reach statistical significance. Based on the body of literature available, a positive effect of UDCA at present cannot be excluded, and clearly larger placebo-controlled studies are required. This will only be possible in multi-center approaches.

An additional effect of UDCA has been cited in two reports, which observed a decrease of the dysplasia in colon polyps associated with UDCA doses as low as 10-15 mg/kg bodyweight (Pardi 2003; Tung 2001). Although this requires confirmation in larger studies the association of PSC with ulcerative colitis in 75% of affected individuals would make this an interesting ancillary effect of UDCA therapy.

The issue of immunosuppression in PSC is controversial and the majority of centers and publications do not recommend the routine administration of corticosteroids and other immunosuppressants (Larusso 2006; Van Hoogstraten 2000). In PSC one of the most feared and unpredictable complicating factors is bacterial cholangitis and cholangiosepsis. Immunosuppression would be expected to aggravate this complication. In rare instances such as overlapping features of PSC and autoimmune hepatitis (AIH), immunosuppression may be of benefit but this requires rigorous documentation of AIH, which includes biopsies, autoimmune serology and suggestive biochemistry (Beuers 2005; Boberg 1996).

Therapy of IBD in PSC

Many PSC patients suffer from a milder course of IBD. Ulcerative colitis is frequently characterized by pancolitis without severe symptoms, rectal sparing and backwash ileitis. Nevertheless the risk of dysplasia and CRC remains significantly higher in PSC patients with ulcerative colitis. Therapeutic intervention is no different than for IBD without PSC. In this context UDCA appears to provide a beneficial effect for dysplasia development. In a study with 59 PSC patients with ulcerative colitis UDCA reduced the risk of colonic dysplasia (Serfaty 2003; Tung 2001). UDCA may therefore contribute to the positive modulation of CRC risk in PSC.

Endoscopic therapy

The most important factor determining the course of PSC is the development of biliary strictures, which carry and increase the risk of septic cholangitis driving biliary fibrosis. Endoscopic dilatation can improve cholestasis, which in some studies has been reached by biliary stenting (Weismüller 2008), which is not recommended by all gastroenterologists. The combination of endoscopic intervention and UDCA therapy appears to lead to a significant prolongation of transplant-free survival. UDCA alone does not lead to this effect.

Liver transplantation in PSC (OLT)

In PSC patient survival has been shown to be reduced both in symptomatic and in asymptomatic patients (Kim 2000; Larusso 2006), which is in part attributable to the inherent risk of cholangiocarcinoma in 10-20% of these patients, and renders decision making for liver transplantation a formidable challenge. In addition, PSC patients with advanced destructive cholangiopathy frequently exhibit only mild signs of liver failure based upon coagulation abnormalities, hypoalbuminemia, or complications of portal hypertension (Tischendorf 2007). The course of deterioration to liver failure is often observed after long periods of clinical stability, and frequently proceeds rapidly following septic biliary complications. This is not well predicted by the aforementioned PSC scores, which is also true for the model of end stage liver disease (MELD), which is used for organ allocation in the US and as of 2006 in the Eurotransplant member countries.

Two major problems define the challenges involved in the indication for liver transplantation in PSC. First, timing is difficult (Wiesner 1992). PSC patients are young and preemptive liver transplantation carries a higher short-term risk of OLT itself than the most likely short-term natural course of the disease. On the other hand, patients that urgently require OLT because of advanced biliary destruction frequently do not meet priority criteria calculated by the MELD system. Second, the 161-fold increase of cholangiocarcinoma risk (Bergquist 2002) is a risk that may eliminate the option of liver transplantation altogether when evidence of cholangiocarcinoma is detected by diagnostic imaging procedures. The diagnosis of early cholangiocarcinoma is difficult and presently no single diagnostic procedure characterized by high sensitivity and specificity is available (Tischendorf 2006). Moreover, those patients at risk cannot be reliably identified.

In terms of practical management the first point can only be addressed by careful clinical monitoring of PSC patients in experienced transplant hepatological centers, where the likelihood of early diagnosis and management, as well as the individual-

ized timing of listing for OLT is higher (Tischendorf 2007). The second point is addressed in two centers that established specific protocols for the management of hilar cholangiocarcinoma and OLT (Rea 2005; Sudan 2002). Rea et al. reported a rigorous algorithm for non-resectable hilar cholangiocarcinoma patients that were carefully selected and capable of surviving chemotherapy, radiation therapy and surgery. A multimodal approach including neoadjuvant chemo-/radiation therapy, brachytherapy, chemotherapy, laparotomy and OLT was employed resulting in a 5-year survival of 82%, which did not differ from results in PSC patients without cholangiocarcinoma (Rea 2005). However, although attractive, these interdisciplinary strategies are best limited to studies and experienced transplant hepatological centers.

Overall the results of liver transplantation in PSC are good, leading to 10-year survival rates of around 70% (Graziadei 1999). In our center the median survival of PSC patients with cholangiocarcinoma was 12.7 months, while all PSC patients irrespective of OLT had a mean survival of 112 months (Tischendorf 2006). Recurrence after OLT is difficult to diagnose but appears to occur in up to 25% of patients (Graziadei 1999). Liver transplantation continues to represent the only curative option in PSC. Future developments will have to address the missing sensitivity and specificity of early cholangiocarcinoma detection, the clinical prediction of the disease course, and consequently, specific allocation criteria for this group of patients.

References

Agarwal K, Czaja AJ, Jones De, et al. (2000) Cytotoxic T lymphocyteantigen-4 (CTLA-4) gene polymorphisms and susceptibility to type 1 autoimmune hepatitis. Hepatology 31:49-53.

Ahmed M, Mutimer D, Hathaway M, et al. (1997) Liver transplantation for autoimmune hepatitis: a 12-year experience. Transplant. Proc. 29:496

Alvarez F, Berg PA, Bianchi FB, et al. (1999) International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J. Hepatology 31:929-938.

Alvarez F, Ciocca M, Canero-Velasco C et al. (1999) Short-term cyclosporine induces a remission of autoimmune hepatitis in children. J. Hepatol. 30:222-227.

Angulo P, Smith C, Jorgensen Ra (2000) Budesonide in the treatment of patients with primary biliary cirrhosis with suboptimal response to ursodeoxycholic acid. Hepatology 20:471-490

Bach N, Bodian C, Bodenheimer H et al. (2003) Methotrexate therapy for primary biliary cirrhosis. Am J Gastroenterol 98:187-193.

Bandin O, Courvalin Jc, Poupon R et al. (1996) Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology 23:1020-1024

Beaune Ph, Lecoeur S, Bourdi M et al. (1996) Anti-cytochrome P450 autoantibodies in drug-induced disease. Eur J Haematol Suppl 60:89-92

Berg Pa, Doniach D, Roitt Im (1967) Mitochondrial antibodies in primary biliary cirrhosis. I. Localization of the antigen to mitochondrial membranes. J Exp Med 126:277-290

Berg Pa, Klein R (1995) Mitochondrial antigen/antibody systems in primary biliary cirrhosis: revisited. Liver 15:281-292

Berg Pa, Klein R (1986) Mitochondrial antigens and autoantibodies: from anti-M1 to anti-M9. Klin Wochenschr 64:897-909

Bergquist A, Broome U (2001) Hepatobiliary and extra-hepatic malignancies in primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol 15:643-656.

Bergquist A, Ekbom A, Olsson R et al. (2002) Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. J Hepatol 36:321-327.

Beuers U, Rust C (2005) Overlap syndromes. Semin Liver Dis 25:311-320

Bloch Db, Chiche Jd, Orth D et al. (1999) Structural and functional heterogeneity of nuclear bodies. Mol Cell Biol 19:4423-4430

Bluthner M, Schafer C, Schneider C et al. (1999) Identification of major linear epitopes on the sp100 nuclear PBC autoantigen by the gene-fragment phage-display technology. Autoimmunity 29:33-42

Boberg Km, Aadland E, Jahnsen J et al. (1998) Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. Scand. J. Gastroenterol. 33:99-103

Boberg Km, Bergquist A, Mitchell S et al. (2002) Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. Scand J Gastroenterol 37:1205-1211.

Boberg Km, Fausa O, Haaland T et al. (1996) Features of autoimmune hepatitis in primary sclerosing cholangitis: an evaluation of 114 primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. Hepatology 23:1369-1376

Bodenheimer H, Jr., Schaffner F, Pezzullo J (1988) Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 95:124-129.

Bodenheimer Hc, Jr., Schaffner F, Sternlieb I et al. (1985) A prospective clinical trial of D-penicillamine in the treatment of primary biliary cirrhosis. Hepatology 5:1139-1142.

Broome U, Glaumann H, Lindstom E et al. (2002) Natural history and outcome in 32 Swedish patients with small duct primary sclerosing cholangitis (PSC). J Hepatol 36:586-589.

Broome Uo, R. Loof, L. Bodemar, G. Hultcrantz, R. Danielsson, A. Prytz, H. Sandberg-Gertzen, H. Wallerstedt, S. Lindberg, G. (1996) Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 38:610-615

Calmus Y, Gane P, Rouger P et al. (1990) Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 11:12-15

Cancado Elr, Porta G (2000) Autoimmune hepatitis in South America. Kluwer Academic Publishers, Dordrecht, Boston, London

Chen W, Gluud C (2003) Bile acids for primary sclerosing cholangitis. Cochrane Database Syst Rev:CD003626

Christensen E, Neuberger J, Crowe J et al. (1985) Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. Gastroenterology 89:1084-1091.

Crivelli O, Lavarini C, Chiaberge E et al. (1983) Microsomal autoantibodies in chronic infection with HBsAg associated delta (delta) agent. Clin. Exp. Immunol. 54:232-238.

Czaja Aj (1998) Frequency and nature of the variant syndromes of autoimmune liver disease. Hepatology 28:360-365.

Czaja Aj, Carpenter Ha-, Santrach Pj et al. (1993) Evidence against hepatitis viruses as important causes of severe autoimmune hepatitis in the United States. J. Hepatol. 18:342-352.

Czaja Aj, Carpenter Ha, Lindor Kd (1999) Ursodeoxycholic acid as adjunctive therapy for problematic type 1 autoimmune hepatitis: a randomized placebo-controlled treatment trial. Hepatology 30:1381-1386

Czaja Aj, Lindor Kd (2000) Failure of budesonide in a pilot study of treatment-dependent autoimmune hepatitis. Gastroenterology 119:1312-1316.

Dalekos Gn, Wedemeyer H, Obermayer-Straub P et al. (1999) Epitope mapping of cytochrome P4502D6 autoantigen in patients with chronic hepatitis C during alpha-interferon treatment. J Hepatol 30:366-375

Danielson A, Prytz H (1994) Oral budesonide for treatment of autoimmune chronic hepatitis. Aliment. Pharmacol. Ther. 8:585-590.

Davis Pa, Leung P, Manns M et al. (1992) M4 and M9 antibodies in the overlap syndrome of primary biliary cirrhosis and chronic active hepatitis: epitopes or epiphenomena? Hepatology 16:1128-1136.

Debray D, Maggiore G, Giradet Jp et al. (1999) Efficacy of cyclosporin A in children with type 2 autoimmune hepatits. J. Pediatr. 135:111-114.

Devlin J, Donaldson P, Portman B et al. (1995) Recurrence of autoimmune hepatitis following liver transplantation. Liver Transpl. Surg. 1:162-165.

Dickson Er, Murtaugh Pa, Wiesner Rh et al. (1992) Primary sclerosing cholangitis: refinement and validation of survival models. Gastroenterology 103:1893-1901

Dienes Hp, Popper H, Manns M et al. (1989) Histologic features in autoimmune hepatitis. Z. Gastroenterol. 27:327-330.

Dighiero G, Lymberi P, Monot C et al. (1990) Sera with high levels of anti-smooth muscle and anti-mitochondrial antibodies frequently bind to cytoskeleton proteins. Clin Exp Immunol 82:52-56

Doherty Dg, Donaldson Pt, Underhill Ja et al. (1994) Allelic sequence variation in the HLA class II genes and proteins on patients with autoimmune hepatitis. Hepatology 19:609-615

Donaldson Pt (1996) Immunogenetics in liver disease. Baillieres Clin Gastroenterol 10:533-549

Donaldson Pt (1999) TNF gene polymorphisms in primary biliary cirrhosis: a critical appraisal [editorial; comment]. J Hepatol 31:366-368

Donaldson Pt, Czaja Aj (2002) Genetic effects on susceptibility, clinical expression, and treatment outcome of type 1 autoimmune hepatitis. Clin Liver Dis 6:419-437

Donaldson Pt, Doherty Dg, Hayllar Km et al. (1991) Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR 4 and A1-B8-DR-3 are independent risk factors. Hepatology 13:701-706

Dubel L, Farges O, Courvalin Jc et al. (1998) Persistence of gp210 and multiple nuclear dots antibodies does not correlate with recurrence of primary biliary cirrhosis 6 years after liver transplantation. J Hepatol 28:169-170

Durazzo M, Philipp T, Van Pelt Fn et al. (1995) Heterogeneity of liver-kidney microsomal autoantibodies in chronic hepatitis C and D virus infection. Gastroenterology 108:455-462

European Liver Transplant Registry J (1996)

Farr ant Jm, Hayllar Km, Wilkinson Ml et al. (1991) Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology 100:1710-1717

Fernandez Nf, Redeker Ag, Vierling Jm et al. (1999) Cyclosporine therapy in patients with steroid resistant autoimmune hepatitis. Am. J. Gastroenterol. 94:241-248.

Frazer Ih, Mackay Ir, Jordan Tw et al. (1985) Reactivity of anti-mitochondrial autoantibodies in primary biliary cirrhosis: definition of two novel mitochondrial polypeptide autoantigens. J Immunol 135:1739-1745

Fujimoto M, Sato S, Ihn H et al. (1995) Autoantibodies to pyruvate dehydrogenase complex in patients with systemic sclerosis. Possible role of anti-E1 alpha antibody as a serologic indicator for development of primary biliary cirrhosis. Arthritis Rheum 38:985-989

Geall Mg, Schoenfield Lj, Summerskill Whj (1968) Classification and treatment of chronic active liver disease. Gastroenterology 55:724-729.

Gelpi C Se, Rodriguez-Sanchez Jl (1992) Autoantibodies against a serine tRNA-protein complex implicated in cotranslational selenocysteine insertion. Proc Natl Acad Sci U S A 89:9739-9743

Gershwin Me, Mackay Ir (1991) Primary biliary cirrhosis: paradigm or paradox for autoimmunity. Gastroenterology 100:822-833

Gershwin Me, Mackay Ir, Sturgess A et al. (1987) Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 138:3525-3531

Gordon Ma, Oppenheim E, Camp Nj et al. (1999) Primary biliary cirrhosis shows association with genetic polymorphism of tumour necrosis factor alpha promoter region [see comments]. J Hepatol 31:242-247

Götz G, Neuhaus R, Bechstein Wo et al. (1999) Recurrence of autoimmune hepatitis after liver transplantation. Transplant. Proc. 31:430-431.

Goulis J, Leandro G, Burroughs Ak (1999) Randomised controlled trials of ursodeoxycholic acid therapy for primary biliary cirrhosis: a meta analysis. Lancet 354:1053-1060

Gouw As, Haagsma Eb, Manns M et al. (1994) Is there recurrence of primary biliary cirrhosis after liver transplantation? A clinicopathologic study in long-term survivors. J Hepatol 20:500-507

Graziadei Iw, Wiesner Rh, Batts Kp et al. (1999) Recurrence of primary sclerosing cholangitis following liver transplantation. Hepatology 29:1050-1056

Gregorio Gv, Portman B, Reid F et al. (1997) Autoimmune hepatitis in childhood: a 20-year experience. Hepatology 25:541-547

Guenguen M, Boniface O, Bernard O et al. (1991) Identification of the main epitope on human cytochrome P450IID6 recognized by anti-liver kidney microsome antibody. J. Autoimmun. 4:607-615

Guenguen M, Meunier-Rotival M, Bernard O et al. (1988) Anti-liver-kidney microsome antibody recognizes a cytochrome P450 from the IID subfamily. J. Exp. Med. 168:801

Haagsma Eb (1999) Clinical relevance of recurrence of primary biliary cirrhosis after liver transplantation. Eur J Gastroenterol Hepatol 11:639-642

Haagsma Eb, Manns M, Klein R et al. (1987) Subtypes of antimitochondrial antibodies in primary biliary cirrhosis before and after orthotopic liver transplantation. Hepatology 7:129-133

Harnois Dm, Angulo P, Jorgensen Ra et al. (2001) High-dose ursodeoxycholic acid as a therapy for patients with primary sclerosing cholangitis. Am J Gastroenterol 96:1558-1562.

Hendrickse Mt, Rigney E, Giaffer Mh et al. (1999) Low-dose methotrexate is ineffective in primary biliary cirrhosis: long-term results of a placebo-controlled trial. Gastroenterology 117:400-407.

Heneghan Ma, Mcfarlane Ig (2002) Current and novel immunosuppressive therapy for autoimmune hepatitis. Hepatology 35:7-13.

Homberg Jc, Abuaf N, Bernard O et al. (1987) Chronic active hepatitis associated with anti liver/kidney microsome type 1: a second type of “autoimmune” hepatitis. Hepatology 7:1333-1339

Ikeda T, Tozuka S, Noguchi O (1996) Effects of additional administration of colchicine in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: a prospective randomized study. j Hepatol 24:88-94

Ishii H, Saifuku K, Namihisa T (1985) Multiplicity of mitochondrial inner membrane antigens from beef heart reacting with antimitochondrial antibodies in sera of patients with primary biliary cirrhosis. Immunol Lett 9:325-330

Johnson Pj, Mcfarlane Ig (1993) Meeting report: International autoimmune hepatitis group. Hepatology 18:998-1005

Johnson Pj, Mcfarlane Ig, Williams R (1995) Azathioprine for long-term maintenance of remission in autoimmune hepatitis. N. Engl.J. Med. 333:958-963

Jones De, Watt Fe, Metcalf Jv et al. (1999) Familial primary biliary cirrhosis reassessed: a geographically-based population study. J Hepatol 30:402-407

Joplin R, Wallace Ll, Johnson Gd et al. (1995) Subcellular localization of pyruvate dehydrogenase dihydrolipoamide acetyltransferase in human intrahepatic biliary epithelial cells. J Pathol 176:381-390

Joplin Re, Neuberger Jm (1999) Immunopathology of primary biliary cirrhosis. Eur J Gastroenterol Hepatol 11:587-593

Kanzler S, Gerken G, Dienes Hp et al. (1996) Cyclophosphamide as alternative immunosuppressive therapy for autoimmune hepatitis - report of three cases. Z. Gastroenterol. 35:571-578.

Kaplan Mm, Alling Dw, Zimmerman Hj et al. (1986) A prospective trial of colchicine for primary biliary cirrhosis. N Engl J Med 315:1448-1454.

Kato Y, Suzuki K, Kumagai M et al. (1981) Familial primary biliary cirrhosis. Immunological and genetic study. Am J Gastroenterol 75:188-191

Kerkar N, Hadzic N, Davies Et et al. (1998) De-novo autoimmune hepatitis after liver transplantation. Lancet 351:409-413

Kim Wr, Therneau Tm, Wiesner Rh et al. (2000) A revised natural history model for primary sclerosing cholangitis. Mayo Clin Proc 75:688-694

Kirk Ap, Jain S, Pocock S et al. (1980) Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. Gut 21:7893.

Klein R, Berg Pa (1991) Anti-M4 antibodies in primary biliary cirrhosis react with sulphite oxidase, an enzyme of the mitochondrial inter-membrane space. Clin Exp Immunol 84:445-448

Klein R, Kloppel G, Garbe W et al. (1991) Antimitochondrial antibody profiles determined at early stages of primary biliary cirrhosis differentiate between a benign and a progressive course of the disease. A retrospective analysis of 76 patients over 6-18 years. J Hepatol 12:21-27

Klein R, Pointner H, Zilly W et al. (1997) Antimitochondrial antibody profiles in primary biliary cirrhosis distinguish at early stages between a benign and a progressive course: a prospective study on 200 patients followed for 10 years. Liver 17:119-128

Kurki P, Miettinen A, Linder E et al. (1980) Different types of smooth muscle antibodies in chronic active hepatitis and primary biliary cirrhosis: their diagnostic and prognostic significance. Gut 21:878-884

Lankisch To, Strassburg Cp, Debray D et al. (2005) Detection of autoimmune regulator gene mutations in children with type 2 autoimmune hepatitis and extrahepatic immune-mediated diseases. J Pediatr 146:839-842.

Larusso Nf, Shneider Bl, Black D et al. (2006) Primary sclerosing cholangitis: summary of a workshop. Hepatology 44:746-764

Lassoued K, Brenard R, Degos F et al. (1990) Antinuclear antibodies directed to a 200-kilodalton polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patients with primary biliary cirrhosis. Gastroenterology 99:181-186

Lenzi M, Manotti P, Muratori L et al. (1995) Liver cytosolic 1 antigen-antibody system in type 2 autoimmune hepatitis and hepatitis C virus infection. Gut 36:749-754

Leung Ps, Chuang Dt, Wynn Rm et al. (1995) Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. Hepatology 22:505-513

Leung Ps, Van De Water J, Coppel Rl et al. (1996) Molecular aspects and the pathological basis of primary biliary cirrhosis. J Autoimmun 9:119-128

Leuschner M, Guldutuna S, You T et al. (1996) Ursodeoxycholic acid and prednisolone versus ursodeoxycholic acid and placebo in the treatment of early stages of primary biliary cirrhosis. J Hepatol 25:49-57.

Leuschner M, Holtmeier J, Ackermann H et al. (2002) The influence of sulindac on patients with primary biliary cirrhosis that responds incompletely to ursodeoxycholic acid: a pilot study. Eur J Gastroenterol Hepatol 14:1369-1376.

Leuschner M, Maier Kp, Schlichting J et al. (1999) Oral budesonide and ursodeoxycholic acid for treatment of primary biliary cirrhosis: results of a prospective double-blind trial. Gastroenterology 117:918-925.

Lidman K, Biberfeld G, Fagraeus A et al. (1976) Anti-actin specificity of human smooth muscle antibodies in chronic active hepatitis. Clin Exp Immunol 24:266-272

Lin F, Noyer Cm, Ye Q et al. (1996) Autoantibodies from patients with primary biliary cirrhosis recognize a region within the nucleoplasmic domain of inner nuclear membrane protein LBR. Hepatology 23:57-61

Lindenborn-Fotinos J, Baum H, Berg Pa (1985) Mitochondrial antibodies in primary biliary cirrhosis: species and nonspecies specific determinants of M2 antigen. Hepatology 5:763-769

Lohr H, Fleischer B, Gerken G et al. (1993) Autoreactive liver-infiltrating T cells in primary biliary cirrhosis recognize inner mitochondrial epitopes and the pyruvate dehydrogenase complex. J Hepatol 18:322-327

Lombard M, Portmann B, Neuberger J et al. (1993) Cyclosporin A treatment in primary biliary cirrhosis: results of a long-term placebo controlled trial. Gastroenterology 104:519-526.

Luettig B, Boeker Kh, Schoessler W et al. (1998) The antinuclear autoantibodies Sp100 and gp210 persist after orthotopic liver transplantation in patients with primary biliary cirrhosis. J Hepatol 28:824-828

Mackay I, R, Taft Li, Cowling Dc (1956) Lupoid hepatitis. Lancet 2:1323-1326

Mackay Ir (1958) Primary biliary cirrhosis showing a high titer of autoantibody. N. Engl. J. Med. 258:707-713

Mackay Ir, Gershwin Me (1997) The nature of autoimmune disease. Semin Liver Dis 17:3-11

Mackay Ir, Morris Pj (1972) Association of autoimmune chronic hepatitis with HLA-A1-B8. Lancet 2:793-795

Mackay Ir, Tait Bd (1980) HLA association with autoimmune type chronic active hepatitis: identification of B8-DRw3 haplotype by family studies. Gastroenterology 79:95-98

Manns M, Gerken G, Kyriatsoulis A et al. (1987) Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. Lancet 1:292-294

Manns M, Gerken G, Trautwein C et al. (1987) Characterization of primary biliary cirrhosis (PBC) specific mitochondrial determinants by immunoblotting. Dtsch Z Verdau Stoffwechselkr 47:59-66

Manns M, Meyer Zum Buschenfelde Kh (1982) A mitochondrial antigen-antibody system in cholestatic liver disease detected by radioimmunoassay. Hepatology 2:1-7

Manns M, Meyer Zum Büschenfelde Kh, Slusarczyk J et al. (1984) Detection of liver-kidney microsomal autoantibodies by radioimmunoassay and their relation to antimitochondrial antibodies in inflammatory liver disease. Clin. Exp. Immunol. 54:600-608

Manns Mp, Bahr Mj (2000) Recurrent autoimmune hepatitis after liver transplantation - when non-self becomes self. Hepatology 32:868-870

Manns Mp, Bremm A, Schneider Pm et al. (1991) HLA DRw8 and complement C4 deficiency as risk factors in primary biliary cirrhosis. Gastroenterology 101:1367-1373.

Manns Mp, Griffin Kj, Sullivan Kf et al. (1991) LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase. J. Clin. Invest. 88:1370-1378

Manns Mp, Jentzsch M, Mergener K et al. (1990) Discordant manifestation of LKM-1 antibody positive autoimmune hepatitis in identical twins. Hepatology 12:840

Manns Mp, Johnson Ef, Griffin Kj et al. (1989) Major antigen of liver kidney microsomal antibodies in idiopathic autoimmune hepatitis is cytochrome P450db1. J. Clin. Invest. 83:1066-1072

Manns Mp, Kruger M (1994) Immunogenetics of chronic liver diseases. Gastroenterology 106:1676-1697.

Manns Mp, Strassburg Cp (2001) Autoimmune hepatitis: clinical challenges. Gastroenterology 120:1502-1517.

Mattalia A, Luttig B, Rosina F et al. (1997) Persistence of autoantibodies against recombinant mitochondrial and nuclear pore proteins after orthotopic liver transplantation for primary biliary cirrhosis. J Autoimmun 10:491-497

Mehal Wz, Gregory Wl, Lo Ym et al. (1994) Defining the immunogenetic susceptibility to primary biliary cirrhosis. Hepatology 20:1213-1219

Milkiewicz P, Hubscher Sg, Skiba G et al. (1999) Recurrence of autoimmune hepatitis after liver transplantation. Transplantation 68:253-256

Mitchell Sa, Bansi Ds, Hunt N et al. (2001) A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. Gastroenterology 121:900-907

Mitchison Hc, Palmer Jm, Bassendine Mf et al. (1992) A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol 15:336-344.

Moteki S, Leung Ps, Dickson Er et al. (1996) Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. Hepatology 23:436-444

Muratori L, Cataleta M, Muratori P et al. (1995) Detection of anti-liver cytosol antibody type 1 (anti-LC1) by immunodiffusion, counterimmunoelectrophoresis and immunoblotting: comparison of different techniques. J. Immunol. Meth. 187:259-264.

Muratori L, Sztul E, Muratori P et al. (2001) Distinct epitopes on formiminotransferase cyclodeaminase induce autoimmune liver cytosol antibody type 1. Hepatology 34:494-501

Nakamura K, Yoneda M, Yokohama S et al. (1998) Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis [see comments]. J Gastroenterol Hepatol 13:490-495

Neuberger J, Portmann B, Calne R et al. (1984) Recurrence of autoimmune chronic active hepatitis following orthotopic liver grafting. Transplantation 37:363-365

Newton Jl, Burt Ad, Park Jb et al. (1997) Autoimmune hepatitis in older patients. Age Ageing 26:441-444

Nickowitz Re, Worman Hj (1993) Autoantibodies from patients with primary biliary cirrhosis recognize a restricted region within the cytoplasmic tail of nuclear pore membrane glycoprotein Gp210. J Exp Med 178:2237-2242

Nickowitz Re, Wozniak Rw, Schaffner F et al. (1994) Autoantibodies against integral membrane proteins of the nuclear envelope in patients with primary biliary cirrhosis. Gastroenterology 106:193-199

Nishio A, Van De Water J, Leung Ps et al. (1997) Comparative studies of antimitochondrial autoantibodies in sera and bile in primary biliary cirrhosis. Hepatology 25:1085-1089

Nishioka M, Morshed Sa, Kono K et al. (1997) Frequency and significance of antibodies to P450IID6 protein in Japanese patients with chronic hepatiis C. J. Hepatol. 26:992-1000

Nishioka M, Morshed Sa, Mcfarlane Ig (1998) Geographical variation in the frequency and characteristics of autoimmune liver diseases. Elsevier, Amsterdam

Notghi A, Nestle U, Rittner G et al. (1990) Chromosomal aberrations in patients with primary biliary cirrhosis. Hum Genet 85:546-550

Obermayer-Straub P, Perheentupa J, Braun S et al. (2001) Hepatic autoantigens in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Gastroenterology 121:668-677

Okolicsanyi L, Fabris L, Viaggi S et al. (1996) Primary sclerosing cholangitis: clinical presentation, natural history and prognostic variables: an Italian multicentre study. The Italian PSC Study Group. Eur J Gastroenterol Hepatol 8:685-691

Olsson R, Boberg Km, De Muckadell Os et al. (2005) High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. Gastroenterology 129:1464-1472

Onishi S, Sakamaki T, Maeda T et al. (1994) DNA typing of HLA class II genes; DRB1*0803 increases the susceptibility of Japanese to primary biliary cirrhosis. J Hepatol 21:1053-1060

Ota M, Seki T, Kiyoshawa K et al. (1992) A possible association between basic amino acids of position 13 of DRB1 chains and autoimmune hepatitis. Immunogenetics 36:40-55.

Palmer Jm, Jones De, Quinn J et al. (1999) Characterization of the autoantibody responses to recombinant E3 binding protein (protein X) of pyruvate dehydrogenase in primary biliary cirrhosis. Hepatology 30:21-26

Palmer Jm, Yeaman Sj, Bassendine Mf et al. (1993) M4 and M9 autoantigens in primary biliary cirrhosis--a negative study. J Hepatol 18:251-254

Pando M, Larriba J, Fernandez Gc et al. (1999) Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. Hepatology 30:1374-1380

Pardi Ds, Loftus Ev, Jr., Kremers Wk et al. (2003) Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. Gastroenterology 124:889-893

Paumgartner G, Beuers U (2002) Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology 36:525-531.

Philipp T, Durazzo M, Trautwein C et al. (1994) Recognition of uridine diphosphate glucuronosyl transferases by LKM-3 antibodies in chronic hepatitis D. Lancet 344:578-581

Pitkanen J, Vahamurto P, Krohn K et al. (2001) Subcellular localization of the autoimmune regulator protein. characterization of nuclear targeting and transcriptional activation domain. J Biol Chem 276:19597-19602.

Ponsioen Cy, Vrouenraets Sm, Prawirodirdjo W et al. (2002) Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. Gut 51:562-566.

Poupon Re, Huet Pm, Poupon R et al. (1996) A randomized trial comparing colchicine and ursodeoxycholic acid combination to ursodeoxycholic acid in primary biliary cirrhosis. UDCA-PBC Study Group. Hepatology 24:1098-1103.

Poupon Re, Lindor Kd, Cauch-Dudek K (1997) Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 113:884-890

Poupon Re, Lindor Kd, Pares A et al. (2003) Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. J Hepatol 39:12-16.

Prados E, Cuervas-Mons V, De La Mata M et al. (1998) Outcome of autoimmune hepatitis after liver transplantation. Transplantation 66:1645-1650.

Raedsch R, Stiehl A, Walker S et al. (1992) [Combined ursodeoxycholic acid plus colchicine--treatment of primary biliary cirrhosis: results of a placebo-controlled double-blind study]. Z Gastroenterol 30 Suppl 1:55-57.

Ramsey C, Winqvist O, Puhakka L et al. (2002) Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. Hum Mol Genet 11:397-409

Ratziu V, Samuel D, Sebagh M et al. (1999) Long-term follow-up after liver transplantation for autoimmune hepatitis: evidence of recurrence of primary disease. J Hepatol 30:131-141

Rea Dj, Heimbach Jk, Rosen Cb et al. (2005) Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. Ann Surg 242:451-458; discussion 458-461

Rebollo Bernardez J, Cifuentes Mimoso C, Pinar Moreno A et al. (1999) Deflazacort for long-term maintenance of remission in type I autoimmune hepatitis. Rev Esp Enferm Dig 91:630-638.

Reynoso-Paz S, Leung Ps, Van De Water J et al. (2000) Evidence for a locally driven mucosal response and the presence of mitochondrial antigens in saliva in primary biliary cirrhosis. Hepatology 31:24-29

Richardson Pd, James Pd, Ryder Sd (2000) Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. J Hepatol 33:371-375

Rizzetto M, Swana G, Doniach D (1973) Microsomal antibodies in active chronic hepatitis and other disorders. Clin. Exp. Immunol. 15:331-344

Rost D, Rudolph G, Kloeters-Plachky P et al. (2004) Effect of high-dose ursodeoxycholic acid on its biliary enrichment in primary sclerosing cholangitis. Hepatology 40:693-698

Sanchez-Urdazpal L, Czaja Aj, Van Holk B (1991) Prognostic features and role of liver transplantation in severe corticoid-treated autoimmune chronic active hepatitis. Hepatology 15:215-221

Schüler A, Manns Mp (1995) Treatment of autoimmune hepatitis. In: Arroyo V, Bosch J, Rodés J (eds) Treatment in Hepatology. Masson, S.A., Paris, p 375-383

Serfaty L, De Leusse A, Rosmorduc O et al. (2003) Ursodeoxycholic acid therapy and the risk of colorectal adenoma in patients with primary biliary cirrhosis: an observational study. Hepatology 38:203-209.

Soloway Rd, Summerskill Wh, Baggenstoss Ah et al. (1972) Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. Gastroenterology 63:820-833

Stechemesser E, Klein R, Berg Pa (1993) Characterization and clinical relevance of liver-pancreas antibodies in autoimmune hepatitis. Hepatology 18:1-9

Sternsdorf T, Guldner Hh, Szostecki C et al. (1995) Two nuclear dot-associated proteins, PML and Sp100, are often co- autoimmunogenic in patients with primary biliary cirrhosis. Scand J Immunol 42:257-268

Strassburg Cp, Alex B, Zindy F et al. (1996) Identification of cyclin A as a molecular target of antinuclear antibodies (ANA) in hepatic and non-hepatic autoimmune diseases. J Hepatol 25:859-866

Strassburg Cp, Jaeckel E, Manns Mp (1999) Anti-mitochondrial antibodies and other immunological tests in primary biliary cirrhosis. Eur J Gastroenterol Hepatol 11:595-601

Strassburg Cp, Manns Mp (2002) Autoantibodies and autoantigens in autoimmune hepatitis. Semin Liver Dis 22:339-352

Strassburg Cp, Manns Mp (1995) Autoimmune hepatitis versus viral hepatitis C. Liver 15:225-232

Strassburg Cp, Manns Mp (2000) Autoimmune tests in primary biliary cirrhosis. Baillieres Best Pract Res Clin Gastroenterol 14:585-599

Strassburg Cp, Manns Mp (1996) Primär biliäre Zirrhose und primär sklerosierende Cholangitis. Internistische Praxis 36:57-74

Strassburg Cp, Manns Mp (2004) [Primary biliary liver cirrhosis and overlap syndrome. Diagnosis and therapy]. Internist (Berl) 45:16-26.

Strassburg Cp, Obermayer-Straub P, Alex B et al. (1996) Autoantibodies against glucuronosyltransferases differ between viral hepatitis and autoimmune hepatitis. Gastroenterology 111:1576-1586

Strassburg Cp, Obermayer-Straub P, Manns Mp (1996) Autoimmunity in hepatitis C and D virus infection. J. Viral. Hepat. 3:49-59

Strassburg Cp, Obermayer-Straub P, Manns Mp (2000) Autoimmunity in liver diseases. Clin Rev Allergy Immunol 18:127-139

Strettell Md, Thomson Lj, Donaldson Pt et al. (1997) HLA-C genes and susceptibility to type 1 autoimmune hepatitis. Hepatology 26:1023-1026.

Sudan D, Deroover A, Chinnakotla S et al. (2002) Radiochemotherapy and transplantation allow long-term survival for nonresectable hilar cholangiocarcinoma. Am J Transplant 2:774-779

Sugimura T, Obermayer-Straub P, Kayser A et al. (2002) A major CYP2D6 autoepitope in autoimmune hepatitis type 2 and chronic hepatitis C is a three-dimensional structure homologous to other cytochrome P450 autoantigens. Autoimmunity 35:501-513

Szostecki C, Krippner H, Penner E et al. (1987) Autoimmune sera recognize a 100 kD nuclear protein antigen (sp-100). Clin Exp Immunol 68:108-116

Szostecki C, Will H, Netter Hj et al. (1992) Autoantibodies to the nuclear Sp100 protein in primary biliary cirrhosis and associated diseases: epitope specificity and immunoglobulin class distribution. Scand J Immunol 36:555-564

Tan Em, Chan Ekl, Sullivan Kf et al. (1988) Antinuclear antibodies (ANAs): diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin. Immunol. Immunopathol. 47:121-141.

Terjung B, Spengler U, Sauerbruch T et al. (2000) “Atypical p-ANCA” in IBD and hepatobiliary disorders react with a 50-kilodalton nuclear envelope protein of neutrophils and myeloid cell lines. Gastroenterology 119:310-322

Tillmann Hl, Jackel E, Manns Mp (1999) Liver transplantation in autoimmune liver disease--selection of patients. Hepatogastroenterology 46:3053-3059

Tischendorf Jj, Hecker H, Kruger M et al. (2007) Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. Am J Gastroenterol 102:107-114

Tischendorf Jj, Meier Pn, Strassburg Cp et al. (2006) Characterization and clinical course of hepatobiliary carcinoma in patients with primary sclerosing cholangitis. Scand J Gastroenterol 41:1227-1234

Treichel U, Poralla T, Hess G et al. (1990) Autoantibodies to human asialoglycoprotein receptor in autoimmune-type chronic hepatitis. Hepatology 11:606-612

Tsuji K, Watanabe Y, Van De Water J et al. (1999) Familial primary biliary cirrhosis in Hiroshima. J Autoimmun 13:171-178

Tukey Rh, Strassburg Cp (2001) Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. Mol Pharmacol 59:405-414.

Tung By, Emond Mj, Haggitt Rc et al. (2001) Ursodiol use is associated with lower prevalence of colonic neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Ann Intern Med 134:89-95.

Uibo R, Salupere V (1999) The epidemiology of primary biliary cirrhosis: immunological problems. Hepatogastroenterology 46:3048-3052

Van De Water J, Gershwin Me, Leung P et al. (1988) The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. J Exp Med 167:1791-1799

Van De Water J, Shimoda S, Niho Y et al. (1997) The role of T cells in primary biliary cirrhosis. Semin Liver Dis 17:105-113

Van Hoogstraten Hj, Vleggaar Fp, Boland Gj et al. (2000) Budesonide or prednisone in combination with ursodeoxycholic acid in primary sclerosing cholangitis: a randomized double-blind pilot study. Belgian-Dutch PSC Study Group [see comments]. Am J Gastroenterol 95:2015-2022

Van Norstrand Md, Malinchoc M, Lindor Kd et al. (1997) Quantitative measurement of autoantibodies to recombinant mitochondrial antigens in patients with primary biliary cirrhosis: relationship of levels of autoantibodies to disease progression. Hepatology 25:6-11

Van Steenbergen W, Sciot R, Van Eyken P et al. (1996) Combined treatment with methotrexate and ursodeoxycholic acid in non-cirrhotic primary biliary cirrhosis. Acta Clin Belg 51:8-18.

Van Thiel Dh, Wright H, Carroll P et al. (1995) Tacrolimus: A potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. Am. J. Gastroenterol. 90:771-776.

Vento S, Cainelli F, Ferraro T et al. (1996) Autoimmune hepatitis type 1 after measles. Am. J. Gastroenterol. 91:2618-2620.

Vento S, Cainelli F, Renzini C et al. (1997) Autoimmune hepatitis type 2 induced by HCV and persisting after viral clearance. Lancet 350:1298-1299.

Vento S, Garofano T, Di Perri G et al. (1991) Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. Lancet 337:1183-1187

Vento S, Guella L, Mirandola F et al. (1995) Epstein-Barr virus as a trigger for autoimmune hepatitis in susceptible individuals. Lancet 346:608-609.

Vogel A, Heinrich E, Bahr Mj et al. (2004) Long-term outcome of liver transplantation for autoimmune hepatitis. Clin Transplant 18:62-69.

Vogel A, Liermann H, Harms A et al. (2001) Autoimmune regulator AIRE: Evidence for genetic differences between autoimmune hepatitis and hepatitis as part of the autoimmune polyglandular syndrome type 1. Hepatology 33:1047-1052.

Vogel A, Manns Mp, Strassburg Cp (2002) Autoimmunity and viruses. Clin Liver Dis 6:451-465

Vogel A, Strassburg Cp, Manns Mp (2003) 77 C/G Mutation in the Tyrosine Phosphatase CD45 and Autoimmune Hepatitis: Evidence for a Genetic Link. Genes and Immunity 4:79-81

Vogel A, Strassburg Cp, Manns Mp (2002) Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. Hepatology 35:126-131.

Volkmann M, Martin L, Baurle A et al. (2001) Soluble liver antigen: isolation of a 35-kd recombinant protein (SLA-p35) specifically recognizing sera from patients with autoimmune hepatitis. Hepatology 33:591-596

Waldenström J (1950) Leber, Blutproteine und Nahrungseiweisse. Dtsch Gesellsch Verd Stoffw 15:113-119.

Warnes Tw, Smith A, Lee Fi et al. (1987) A controlled trial of colchicine in primary biliary cirrhosis. Trial design and preliminary report. J Hepatol 5:1-7.

Weismüller Tj, Wedemeyer J, Kubicka S et al. (2008) The challenges in primary sclerosing cholangitis – Aetiopathogenesis, autoimmunity, management and malignancy. J Hepatol 48:S38-S57

Wesierska-Gadek J, Hohenuer H, Hitchman E et al. (1996) Autoantibodies against nucleoporin p62 constitute a novel marker of primary biliary cirrhosis. Gastroenterology 110:840-847

Wies I, Brunner S, Henninger J et al. (2000) Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis [see comments]. Lancet 355:1510-1515

Wiesner Rh, Grambsch Pm, Dickson Er et al. (1989) Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 10:430-436

Wiesner Rh, Ludwig J, Lindor Kd et al. (1990) A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. N Engl J Med 322:1419-1424.

Wiesner Rh, Porayko Mk, Dickson Er et al. (1992) Selection and timing of liver transplantation in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 16:1290-1299

Wolfhagen Fhj, Van Hoogstraaten Hjf, Van Buuren, H.R. (1998) Triple therapy with ursodeoxycholic acid, prednisone, and azathioprine in primary biliary cirrhosis: a 1 year randomized, placebo controlled study. J Hepatol 29:736-742

Worman Hj (1994) Primary biliary cirrhosis and the molecular cell biology of the nuclear envelope. Mt Sinai J Med 61:461-475

Wright Hl, Bou-Abboud Cf, Hassanenstein T et al. (1992) Disease recurrence and rejection following liver transplantation for autoimmune chronic active liver disease. Transplantation 53:136-139.

Zanger Um, Hauri Hp, Loeper J et al. (1988) Antibodies against human cytochrome P-450db1 in autoimmune hepatitis type 2. Proc. Natl. Acad. Sci. USA 85:8256-8260.

Zuchner D, Sternsdorf T, Szostecki C et al. (1997) Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology 26:1123-1130.

Chapter 28: Alcoholic hepatitis

Claus Niederau

Health and social problems due to alcohol abuse

Mortality due to alcohol over-consumption is high, in particular among young men (Mokdad 2000). Alcohol abuse not only increases the risk for liver disease but is also responsible for malignancies, accidents, violence, and social problems (Bellentani 1997; Vaillant 1995). Alcohol consumption in excess of 20-30 g for women and 40-60 g for men per day markedly increases the risk for liver disease (Becker 1996; Lucey 2008). However, liver cirrhosis is seen only in a minority of subjects with high alcohol consumption; less than 10% of subjects who drink more than 120 g of alcohol daily have cirrhosis (Bellentani 1997). In addition to the level of alcohol consumption, various other factors, such as sex, other genetic characteristics, and co-morbidities contribute to the risk for liver disease (Nishigushi 1991; Becker 1996; Bellentani 1997; McCollough 1998; de Alwis 2007; Lucey 2009).

Classification and natural history of alcoholic liver disease

Alcohol abuse most often causes fat accumulation of hepatocytes, called hepatic steatosis (Figure 1). Alcohol-induced steatosis is in general reversible after alcohol abstinence. Continued alcohol abuse in the presence of steatosis markedly increases the risk for development of hepatitis, fibrosis and cirrhosis (Teli 1995; Cubero 2009). Patients with alcohol-induced cirrhosis have a significantly increased risk for hepatocellular carcinoma (McCollough 1998). Patients with only fatty liver in the absence of inflammation and fibrosis have a much lower risk for development of cirrhosis than those with fatty liver plus presence of inflammation and fibrosis. The latter group of patients with alcoholic fatty liver, inflammation and fibrosis is defined as alcoholic-steato-hepatitis (ASH). The liver histology of patients with ASH is similar when compared to patients with non-alcoholic steato-hepatitis (NASH) that is often associated with obesity and diabetes (Ludwig 1980; Brunt 1999).

The diagnosis of ASH by liver biopsy thus helps to define the risk for development of cirrhosis. The histological diagnosis of ASH however should not be confused with the term “alcoholic hepatitis” that is also called “acute alcoholic hepatitis” although its course can be a rather chronic one (Lucey 2009). This overview article concentrates on “alcoholic hepatitis” which is a clinical diagnosis of a rather acute development of jaundice and liver failure associated with a high short-term mortality.

It is not exactly known which factor(s) set off the development of severe alcohol hepatitis. In general, pathogenesis and individual predisposition are governed by gene-environment interactions in all types of alcoholic liver disease (Figure 1). Based on the “second hit” or “multiple hits” hypothesis, patients are predisposed to progressive alcoholic liver disease when a specific combination of gene and environmental interaction exists (Tsukamoto 2009). A loss or gain of function genetic model has become a popular experimental approach to test the role of a gene as a second hit. Significant

interactions for progressive development of alcoholic liver disease have been proven in particular for female gender, obesity, various drugs, iron overload, and hepatitis B and C viral infections (Mueller 2009; Machado 2009; Cubero 2009). These factors may also interact in the development of hepatocellular carcinoma (HCC).

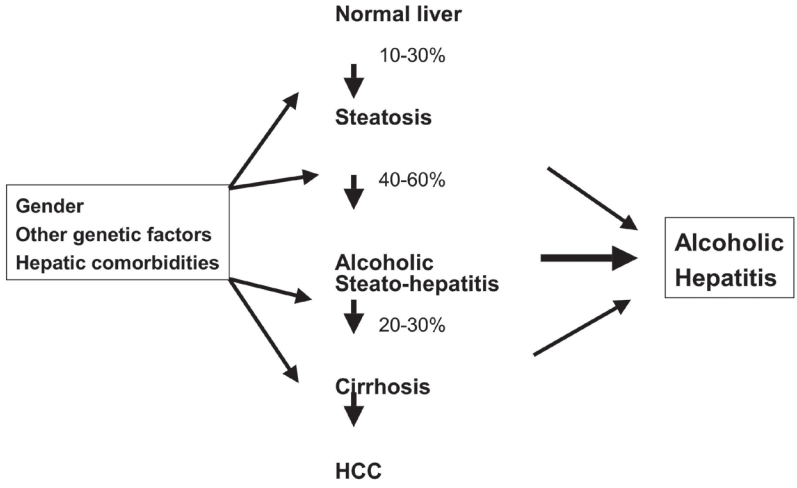


Figure 1. Effects of alcohol over-consumption on the liver.

A liver biopsy in someone with "alcoholic hepatitis" is often similar to a histological feature of ASH. Most patients with histological features of ASH however will not develop "alcoholic hepatitis". Alcohol over-consumption leads to a severe form of hepatitis and liver failure associated with a high short-term mortality only in some subjects. Such alcoholic hepatitis may be seen with or without preexisting cirrhosis.

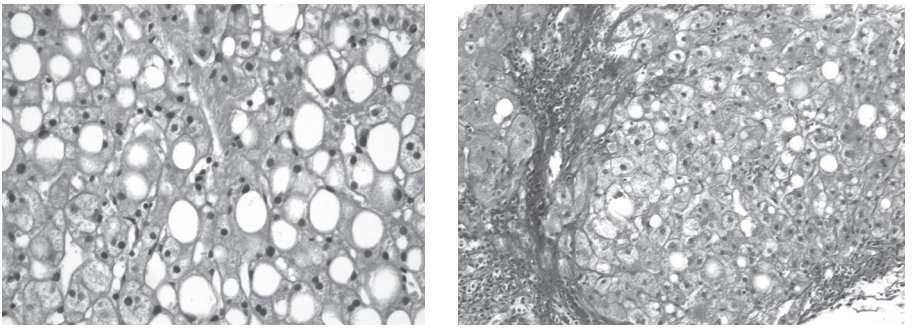
Clinical features and diagnosis of alcoholic hepatitis

Alcoholic hepatitis is a clinical diagnosis characterized by the rapid development of jaundice and liver failure most often due to long-term alcohol over-consumption (Naveau 1997; McCollough 1998; Lucey 2009). Further characteristics include fever, ascites, and in some patients hepatic encephalopathy as well. Usually the liver is enlarged and tender. Women have a higher risk for alcoholic hepatitis than men assuming that both genders drink the same amount of alcohol. The type of alcohol is not associated with the risk. Prevalence was 20% in a cohort of 1604 patients who had a history of heavy alcohol consumption and underwent a liver biopsy (Naveau 1997).

Laboratory tests show increases in serum aspartate aminotransferase (AST) to approximately twice the upper limit of normal (ULN), while the increase in alanine aminotransferase (ALT) is less pronounced. The ratio of AST to ALT is typically >2 (Cohen 1979; Matloff 1980). Other laboratory abnormalities include increases

in peripheral leukocytes, serum bilirubin, and international normalized ratio (INR) (Mathurin 2002; Orrego 1979). In the presence of an increase in serum creatinine there is a high risk for development of an often lethal hepatorenal syndrome (Multimer 1993).

The liver biopsy usually shows big fat droplets and ballooning of hepatocytes that may also include alcoholic hyaline (also called Mallory bodies); these changes are accompanied by neutrophil infiltration and intrasinusoidal fibrosis ((Figure 2 & 3) (MacSween 1986).



Figures 2 & 3. Liver biopsies of alcoholic hepatitis.

The diagnosis of alcoholic steato-hepatitis (ASH) requires the presence of fibrosis. The role of liver biopsy in defining prognosis and treatment of alcoholic hepatitis in the clinical setting remains unclear. Today, prognosis is usually not based on liver biopsy but on clinical scoring systems (Lucey 2009).

Ultrasound is routinely done to look for hepatocellular carcinoma, biliary obstruction, ascites, splenomegaly, portal vein thrombosis, and signs of portal hypertension. Ascites should be checked for spontaneous bacterial peritonitis routinely.

Differential diagnosis of alcoholic hepatitis includes severe non-alcoholic steatohepatitis (NASH), acute or chronic viral hepatitis, drug-induced injury, autoimmune hepatitis, and Wilson's disease. NASH shares the histological features of ASH except for the rapid development of jaundice and liver failure.

After discontinuation of alcohol consumption the majority of patients will recover from alcoholic hepatitis although jaundice, ascites and encephalopathy may persist for weeks or months (Alexander 1971). Even so, a considerable percentage of patients with alcoholic hepatitis still die today despite adequate treatment and abstinence (Mathurin 2002; Orrego 1979).

Course and severity

Severe alcoholic hepatitis occurs in a small fraction of patients who over-consume alcohol. The 28-day mortality is high and ranges from 30% to 50% in most cohorts (Cohen 2009). Various scores have been used to predict the prognosis of alcoholic hepatitis. Maddrey's discriminant function (Maddrey 1978) and the Model for

End-Stage Liver Disease (MELD; www.mayoclinic.org/ meld/mayomodel7.html) score may in particular help to identify patients who can benefit with corticosteriods. Most scores share some important characteristics such as serum bilirubin and prothrombin time (Srikureja 2005). Maddrey's discriminant function is calculated as [4.6x (prothrombin time–control prothrombin time, in seconds)]+serum bilirubin (mg/dL). A value of >32 indicates severe alcoholic hepatitis and consequently calls for the use of corticosteroids (Maddrey 1978). In two retrospective studies, the MELD score predicted short-term mortality in alcoholic hepatitis as well as or even better than Maddrey's discriminant function (Dunn 2005; Srikureja 2005). A MELD score >21 was associated with a 90-day mortality of 20%. The Lille score is based on pretreatment data and on the response of serum bilirubin to a 7-day treatment with corticosteroids and has been used to determine whether corticosteroids should be discontinued after 7 days because of treatment failure (Forrest 2005; Dunn 2005; Louvet 2007). Patients with Maddrey's discriminant function of <32 usually have mild disease with a short-term survival of more than 90% and will not benefit from corticosteroid treatment.

Investigators from Glasgow (Forrest 2005) reported the results of a stepwise logistic-regression identifying variables related to survival 1-4 months after hospital admission in patients with alcoholic hepatitis; by using this data the Glasgow alcoholic hepatitis score was developed (this score should not to be confused with the Glasgow coma score). The score, which includes age, peripheral leukocytes, urea nitrogen, bilirubin, and prothrombin time, may help to identify high-risk patients who should receive corticosteroids. Patients with a Maddrey's discriminant function >32 and a Glasgow alcoholic hepatitis score of >9 who were treated with corticosteroids had an 84-day survival of 59%, while untreated patients only had a 38% survival (Forrest 2007). In one study the Glasgow score indicated which subgroup of patients with a high score of Maddrey's discriminant function would benefit from corticosteroid therapy (Forrest 2007).

Child-Pugh (CP) and MELD scores have been widely used to predict survival in cirrhotic patients. Recent studies have suggested that the addition of serum sodium to MELD (MELD-Na score) may improve its prognostic accuracy. Another recent study compared the performance of CP, MELD, and MELD-Na scores in predicting 6-month mortality in patients with alcoholic cirrhosis, and developed a new prognostic score. In this study two French centres (Boursier 2009) enrolled 520 patients (mean age 56.4±10.2 years) with alcoholic cirrhosis randomly allocated into two groups. MELD, MELD-Na1, and MELD-Na2 were calculated according to UNOS recommendations. Frequencies of CP classes were: A - 29.6%, B - 25.8%, C - 44.6%. Of the 520 patients 93 died during the 6-month follow-up. In the whole population, the values of CP, MELD, MELD-Na1, and MELD-Na2 for prediction of 6-month mortality were similar. Multivariate analysis identified age, bilirubin, urea, prothrombin time, sodium, and alkaline phosphatase as independent predictors of 6-month mortality. The score combining these 6 variables was named the Prognostic Score for Alcoholic Cirrhosis (PSAC) and compared to the 4 other scores. The predictive values of PSAC were better than all other scores except for MELD-Na2. By stepwise multivariate analysis, PSAC was identified

as independently associated with 6-month mortality at the first step, and CP at the second. The new PSAC score may improve the prognostic accuracy to predict the 6-month outcome (Boursier 2009).

Another recent study analyzed the outcome of 79 patients who were admitted to an Intensive Care Unit (ICU) because of alcoholic liver disease (Rye 2009). The value of various scores was analyzed for predicting mortality including the Acute Physiology, Age and Chronic Health Evaluation (APACHE II), Sequential Organ Failure Assessment (SOFA), CP, and MELD scores. The major reason for admission was sepsis (44%). The observed mortality in the ICU was 49% and hospital mortality 68%. Compared to survivors, non-survivors had a significantly higher serum bilirubin, creatinine and prothrombin time, and lower GCS and length of ICU stay. Survival was affected by cardiac arrest pre-admission (mortality 75%) and number of organs supported (mortality 8% with no organ support, 79% ≥2 organs, 100% ≥3 organs). Renal replacement therapy was associated with 100% mortality. Mortality due to GI bleeding was only 33%. Thus, cirrhotics admitted to the ICU with cardiac arrest pre-admission, need for renal replacement therapy, or multiple organ support have a poor prognosis. The predictive accuracy of SOFA and MELD scores were superior to APACHE II and Child-Pugh scores in cirrhotic patients (Rye 2009).

A further study analyzed the mortality of 105 patients presenting with alcoholic hepatitis (Hussain 2009). Patients were evaluated by the measurement–modified discriminant function (mDF) for alcoholic liver disease, CP score, and Glasgow alcoholic hepatitis score (GAHS). Mean survival for those alive at the end of the study (n=36) was 34.6 ± 17.8 months. Mean survival for those who died (n=50) was 13.2 ± 14.4 months. The mDF, CP and GAHS scores were significant predictors of mortality in this population. Prothrombin time was also a significant predictor of mortality (Hussain 2009).

Mechanisms of alcohol-related liver injury

Alcoholic liver disease is initiated by different cell types in the liver and a number of different factors including products derived from alcohol-induced inflammation, ethanol metabolites, and indirect reactions from those metabolites, as well as genetic predisposition (Colmenero 2007). Ethanol oxidation results in the production of metabolites that have been shown to bind and form protein adducts, and to increase inflammatory, fibrotic and cirrhotic responses. Lipopolysaccharide (LPS) has many deleterious effects and plays a significant role in a number of disease processes by increasing inflammatory cytokine release. In alcoholic liver disease, LPS is thought to be derived from a breakdown in the intestinal wall enabling LPS from resident gut bacterial cell walls to leak into the blood stream. The ability of adducts and LPS to independently stimulate various cells of the liver provides for a two-hit mechanism by which various biological responses are induced and result in liver injury.

Alcohol (ethanol) can be oxidized by various enzymatic and non-enzymatic pathways (Figures 2 & 3). In hepatocytes the most important pathway is oxidation of ethanol via alcohol dehydrogenase (ADH) to acetaldehyde (Figure 4). In mitochondria, acetaldehyde is converted to acetate and in turn acetate is converted to acetyl CoA which leads the two-carbon molecule into the TCA (tricarboxylic acid cycle).

Alcohol dehydrogenase (ADH)

$$CH3CH2OH + NAD^{+} \rightarrow CH3CHO + NADH + H$$

Ethanol — Acetaldehyde

$$CH3\text{-}CHO + NAD^{+} \rightarrow CH3\text{-}COOH + NADH + H^{+}$$

Acetaldehyde — Acetate

Catalase

$$CH_3CH_2OH + H_2O_2 \_ CH_3CHO + 2\ H_2O$$

Cytochrome P-450

Microsomal Ethanol Oxidising System" (MEOS)

$$CH_3CH_2OH + NADPH + H^{+} + O_2 \rightarrow CH_3CHO + 2\ H_2O + NADP^{+}$$

Figure 4. Oxidation of ethanol to acetaldehyde by enzymatic pathways.

This oxidation generates reducing equivalents, primarily reduced nicotinamide adenine dinucleotide (NAD), i.e., NADH. The changes in the NADH–NAD+ potential in the liver inhibit both fatty acid oxidation and the TAC and may thereby increase lipogenesis (You 2004a). Ethanol has also been shown to increase lipid metabolism by inhibiting peroxisome-proliferator–activated receptor α (PPAR-α) and AMP kinase and by stimulation of sterol regulatory element-binding protein (Fischer 2003; You 2004b; Ji 2006). All these mechanisms lead to hepatic steatosis. Further enzymatic pathways of ethanol oxidation include catalase and the "Microsomal Ethanol Oxidizing System" (MEOS), a cytochrome P450 component. Oxidation of ethanol to acetaldehyde may also be due to non-enzymatic free radical pathways (Figure 5). These include strong oxidizing intermediates such as the hydroxyl radical which can abstract a hydrogen atom from ethanol, preferentially producing the 1-hydroxyethyl radical (also called the λ-hydroxyethyl radical); hypervalent iron complexes may also catalyze this reaction without involvement of •OH (Reinke 1994; Welch 2002; Qian 1999). Hydroxyethyl radicals may then react with oxygen to form a peroxy radical intermediate which can rearrange to release acetaldehyde and superoxide. Hydroxyethyl radicals can also react with proteins to produce antigenic adducts or induce mitochondrial permeability transition (Clot 1995; Sakurai 2000).

There are probably various other mechanisms by which ethanol may cause or contribute to liver disease. Ethanol increases the translocation of lipopolysaccharide (LPS) from the small and large intestines to the portal vein and on to the liver. In Kupffer cells LPS can bind to CD14 which combines with toll-like

receptor 4 (TLR4) thereby activating multiple cytokine genes (Schaffert 2009). In addition, NADPH oxidase may release reactive oxygen species (ROS) which activate cytokine genes within Kupffer cells, hepatocytes, and hepatic stellate cells. These cytokines including TNF-α may cause fever, anorexia, and weight loss. Interleukin-8 and monocyte chemotactic protein 1 (MCP-1) have been shown to attract neutrophils and macrophages. Platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β) contribute to the activation, migration, and multiplication of hepatic stellate cells thereby promoting liver fibrosis.

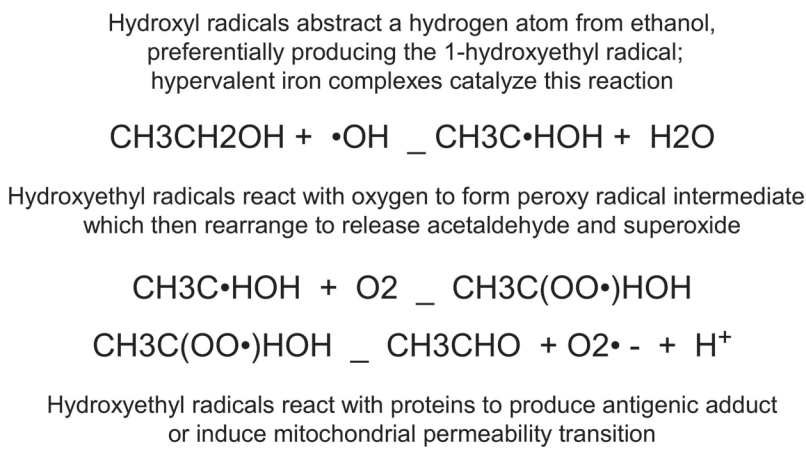


Figure 5. Oxidation of ethanol to acetaldehyde by non-enzymatic free radical pathways.

In the hepatocyte, ethanol is converted to acetaldehyde by the cytosolic enzyme alcohol dehydrogenase (ADH) and the microsomal enzyme cytochrome P-450 2E1 (CYP2E1). Acetaldehyde is converted to acetate. These reactions produce NADH and inhibit the oxidation of triglycerides and fatty acids. ROS released by CYP2E1 and mitochondria cause lipid peroxidation. Inhibition of proteosomes due to ethanol disturbes protein catabolism and may be partly responsible for the formation of Mallory bodies. Reduction in enzymes which convert homocysteine to methionine may increase homocysteine thereby injuring the endoplasmic reticulum. Decrease in binding of peroxisome-proliferator–activated receptor α (PPAR-α) to DNA reduces the expression of genes involved in fatty acid oxidation.

Glutathione transport from the cytosol into the mitochondria is reduced by ethanol. Ethanol may also activate Fas and TNF receptor 1 (TNF-R1) thereby activating caspase 8, causing mitochondrial injury and opening the mitochondrial transition pore (MTP), releasing cytochrome c, and activating caspases; all these processes contribute to apoptosis. Activation of TNF-R1 leads to nuclear factor-kappaB (NF-kB) activation (Schaffert 2009).

Gut permeability and the circulating LPS endotoxin levels component of the outer wall of gram-negative bacteria are increased in patients with alcoholic liver injury (Uesugi 2002; Bjarnson 1984; Urbaschel 2001). In various animal studies alcohol exposure promoted the transfer of LPS–endotoxin from the intestine into portal blood (West 2005). Oral treatment with antibiotics reduced such increases in LPS–endotoxin and ameliorated alcoholic liver injury in animals (Uesugi 2001; Nanji 1994; Adachi 1995). Activation of Kupffer cells by LPS–endotoxin involves CD14, toll-like receptor 4 (TLR4), and MD2 (Uesugi 2001; Akira 2001; Yin 2001). The downstream pathways of TLR4 signalling include activation of early growth response 1 (EGR1), NF-kB, and the TLR4 adapter also called toll–interleukin-1 receptor domain-containing adapter-inducing interferon-ß (TRIF) (McCuillen 2005; Zhao 2008). TRIF-dependent signalling may contribute to alcohol-induced liver damage mediated by TLR4 (Hritz 2008).

Many animal studies have shown that alcohol ingestion increases various markers of oxidative stress (Meagher 1999; Wu2009). Studies in rats and mice suggest that activated macrophages (Kupffer cells) and hepatocytes are the main sources of alcohol-induced free radicals (Bailey 1998; Kamimura 1992). Oxidative stress may mediate alcohol-induced liver injury, e.g., via cytochrome P-450 2E1 (Mansuri 1999; Lu 2008), leading to mitochondrial damage, activation of endoplasmic reticulum–dependent apoptosis, and up-regulation of lipid synthesis (Ji 2003; Yin 2001). Activated Kupffer cells will also release TNF-α; this cytokine plays an important role in the pathogenesis of alcoholic hepatitis. Circulating TNF-α concentrations are higher in patients with alcoholic hepatitis than in heavy drinkers with inactive cirrhosis, heavy drinkers who do not have liver disease and persons who do not drink alcohol and who do not have liver disease (Adachi 1994; Bird 1990). Circulating TNF-α concentrations are associated with high mortality (Bird 1990). In animal studies, knockouts of the TNF receptor 1 and the administration of the anti-TNF agent thalidomide both ameliorated alcohol-induced liver injury (Yin 1999; Imuro 1997; Enomoto 2002). Ethanol was also shown to release mitochondrial cytochrome c and to induce expression of the Fas ligand which may then cause apoptosis via the caspase-3 activation pathway (Zhou 2001). Both TNF- and Fas-mediated signals may increase the vulnerability of hepatocytes (Minagawa 2004).

Therapy

Abstinence from alcohol

After recovery from liver failure all patients with alcoholic hepatitis patients need to have psychological and social support in order to assure continued abstinence (Saitz 2007).

Supportive therapy

There is still a lack of specific therapy for patients with alcoholic hepatitis although prednisolone and pentoxyfilline may have beneficial effects in severe disease. It is, however, generally accepted that all complications and risks such as

ascites, encephalopathy, hepatorenal syndrome, and infections should be treated like in other decompensated liver diseases (Kosten 2003; Sanyal 2008; Lim 2008). The daily protein intake should be at least 1.5 g/kg. Vitamin B1 and other vitamins should be administered according to recommended references (Barr 2006).

Corticosteroids

Various studies and meta-analyses show controversial results for the use of corticosteroids in alcoholic hepatitis (Imperiale 1990; Christensen 1999; Imperiale 1999; Rambaldi 2008). In general, corticosteroids have not been shown to increase survival, in particular during longer follow-up (Rambaldi 2008). However, there is evidence that corticosteroids reduce mortality in a subgroup of patients with a Maddrey's discriminant function >32 or in those presenting with hepatic encephalopathy (Rambaldi 2008). A meta-analysis of three recent studies corroborated that corticosteroids given for 28 days increase 1-month survival by 20% in severe alcoholic hepatitis (Maddrey's discriminant function >32) (Mathurin 2002). In these studies Maddrey's discriminant function >32 resembled a MELD score of >21. In most studies prednisolone was given at 40 mg a day for 28 days. In some studies prednisolone was stopped completely at 28 days (Mathurin 2003), while the dose was gradually reduced in other studies (Imperiale 1990). Corticoids should not be given in the presence of sepsis, severe infection, hepatorenal syndrome, chronic hepatitis B, and gastrointestinal bleeding (O'Shea 2006).

The mechanisms by which corticosteroids improve short-term survival in severe alcoholic hepatitis are not fully understood. In general corticosteroids inhibit various inflammatory processes by acting on activator protein 1 and NF-kB (Barnes 1997). In some studies in patients with alcoholic hepatitis, the administration of corticosteroids was associated with a decrease in circulating levels of proinflammatory cytokines such as interleukin-8, TNF-α and others (Taieb 2000; Spahr 2001).

Most recent reviews and recommendations conclude that corticosteroids should not be given to patients with a Maddrey's discriminant function <32 or a MELD score <21 until further data can identify patients with a high short-term risk (Lucey 2009). Corticosteroids are thus ineffective in a large group of patients with alcoholic hepatitis and probably do not affect long-term outcome. There is also evidence that corticosteroids can be discontinued after 7 days when there is no obvious improvement in clinical signs and symptoms and in serum bilirubin at this time point (Maddrey 1978; Dunn 2005; Forrest 2005; Louvet 2007).

Pentoxifylline

Pentoxyfilline (400 mg TID for 28 days) reduced short-term mortality in severe alcoholic hepatitis (Maddrey's discriminant function >32) in a randomized, controlled trial; mortality was 24% in the pentoxifylline group and 46% in the placebo group ($p<0.01$) (Akrivadis 2000). This trial did not include a group on corticosteroid treatment. Although the phosphodiesterase inhibitor pentoxifylline has been suggested to act as an anti-TNF agent, TNF-α concentrations did not differ significantly between the two groups. Thus, the mechanisms by which pentoxifyl-

line may improve the prognosis in alcoholic hepatitis remains unknown. Interestingly, almost all deaths (22 of 24; 92%) in the placebo group were associated with hepatorenal syndrome while hepatorenal syndrome was considered the cause of death in only 6 of 12 patients (50%) in the pentoxifylline group. Thus, one might speculate that pentoxifylline may exert its beneficial effects by preventing the development of hepatorenal syndrome. A recent study (De BK 2009) compared the efficacy of pentoxifylline and prednisolone in the treatment of severe alcoholic hepatitis. 68 patients with severe alcoholic hepatitis (Maddrey score >32) received pentoxifylline (400 mg TID for 28 days) (n=34) or prednisolone (40 mg QD for 28 days) (n=34) for 28 days in a randomized double-blind controlled study, and subsequently in an open-label study (with a tapering dose of prednisolone) for a total of 3 months, and were followed over a period of 12 months. Twelve patients in the corticosteroid group died by the end of month 3 in contrast to five patients in the pentoxifylline group (mortality 35.3% vs 14.7%, p=0.04). Six patients in the corticosteroid group but none in the pentoxifylline group developed hepatorenal syndrome. Pentoxifylline was associated with a significantly lower MELD score at the end of 28 days of therapy when compared to corticosteroids (15.5 ± 3.6 vs 17.8 ± 4.6, p=0.04). Reduced mortality, improved risk:benefit profile and renoprotective effects of pentoxifylline compared with prednisolone suggest that pentoxifylline is superior to prednisolone for treatment of severe alcoholic hepatitis. Interestingly, another recent study showed that long-term pentoxifylline therapy effectively achieved sustained biochemical improvement and even histological improvement in non-alcoholic steatohepatitis (Satapathy 2007).

N-acetyl cysteine

A recent multicentre, randomised, controlled trial (Nguyen-Khac 2009) analysed treatment of severe acute alcoholic hepatitis via corticoids plus N-acetyl cysteine (C+NAC) versus corticoids (C) alone. The background to this approach was the hypothesis that the glutathione precursor NAC may rebuild anti-oxidant stocks in the hepatocyte. Deaths were significantly lower in the C+NAC group than in the C group at month 1 (n=7/85 (8.2%) vs. 21/89 (23.6%), p=0.005) and at month 2 (13/85 (15.3%) vs. 29/89 (32.6%), p=0.007) but not at month 3 (19/85 (22.4%) vs. 30/89 (33.7%), p=0.095) or at month 6 (23/85 (27.1%) vs. 34/89 (38.2%). Thus, NAC may improve short-term survival. This improvement, however, is lost by month 3.

Anti-TNF-α therapy

Some smaller studies have shown beneficial results using the TNF-α receptor antagonists infliximab and etanercept in patients with acute alcoholic hepatitis (Spahr 2007; Mookerjee 2003; Tilg 2003; Menin 2004). A larger randomized, controlled clinical trial compared the effects of infliximab plus prednisolone with placebo plus prednisolone in patients with severe alcoholic hepatitis (Maddrey's discriminant function >32) (Naveau 2004). The trial was stopped early by the safety monitoring board because of a significant increase in severe infections and a (nonsignificant) increase in deaths in the infliximab group. Similarly, etanercept reduced 6-month survival when compared with placebo in a randomized, placebo-

controlled trial (Boetticher 2008). Thus, TNF-α receptor antagonists should not be used for clinical therapy of alcoholic hepatitis (Lucey 2009).

Nutritional support

Many patients with alcoholic hepatitis have signs of malnutrition associated with high mortality (Mendenhall 1984; Mendenhall 1986; Stickel 2003). Parenteral and enteral nutrition has been shown to improve malnutrition in alcoholic hepatitis but has not improved survival (Mendenhall 1984). A randomised, controlled clinical trial looked at the effects of enteral nutrition of 2000 kcal/day via tube feeding versus treatment with 40 mg/day prednisolone for 28 days in severe alcoholic hepatitis. Survival in both groups was similar after one month and one year. It may be concluded that nutritional support is as effective as corticosteroids in some patients (Cabre 2000). However, corticoids in many studies failed to improve long-term survival.

Other pharmacologic treatments

The anabolic steroid oxandrolone failed to improve survival in patients with alcoholic hepatitis (Mendenhall 1984). Numerous studies have shown that alcoholic hepatitis is accompanied by oxidative stress. So far, all studies with antioxidants such as vitamin E, silymarin (milk thistle) and others have failed to improve survival in alcoholic hepatitis (Pares 1998; Mezey 2004). Older studies did show that colchicine, propylthiouracil, insulin and glucagon failed to improve survival in alcoholic hepatitis (Lucey 2009).

Liver transplantation

In current guidelines for liver transplantation, the patient needs to have at least a 6-month period of alcohol abstinence before they can be evaluated for transplantation, thus alcoholic hepatitis is usually a contraindication for liver transplantation (Lucey 1997; Everhardt 1997; Lucey 2007).

Summary

Alcoholic hepatitis is a clinical diagnosis based on a history of heavy alcohol consumption, jaundice, other signs of liver failure, and the absence of other causes of hepatitis. A liver biopsy may be helpful but is not required either to determine the diagnosis or prognosis. Abstinence from alcohol is the prerequisite for recovery. Patients with signs of malnutrition should have adequate nutritional support. Subjects with severe alcoholic hepatitis (Maddrey's discriminant function >32 or MELD score >21) who do not have sepsis or other corticosteroid contraindications may receive 40 mg prednisolone daily for 28 days (McCullough 1998; Lucey 2009). A treatment algorithm is shown in Figure 6. After 7 days of corticosteroid treatment, patients without obvious clinical benefit, without significant improvement of jaundice and with a Lille score >0.45 may have disease that will not respond to continued treatment with corticosteroids or an early switch to pentoxyfilline (Louvet 2008). In situations where administration of corticosteroids appears to be risky, pentoxifylline may be tried (Lucey 2009); this drug may in particular decrease the risk of the hepatorenal syndrome that is often lethal in alcoholic hepatitis. Patients with less severe alcoholic hepatitis

have a good short-term survival of >90% and should not be treated with corticosteroids or pentoxyfilline (Mathurin 2002).

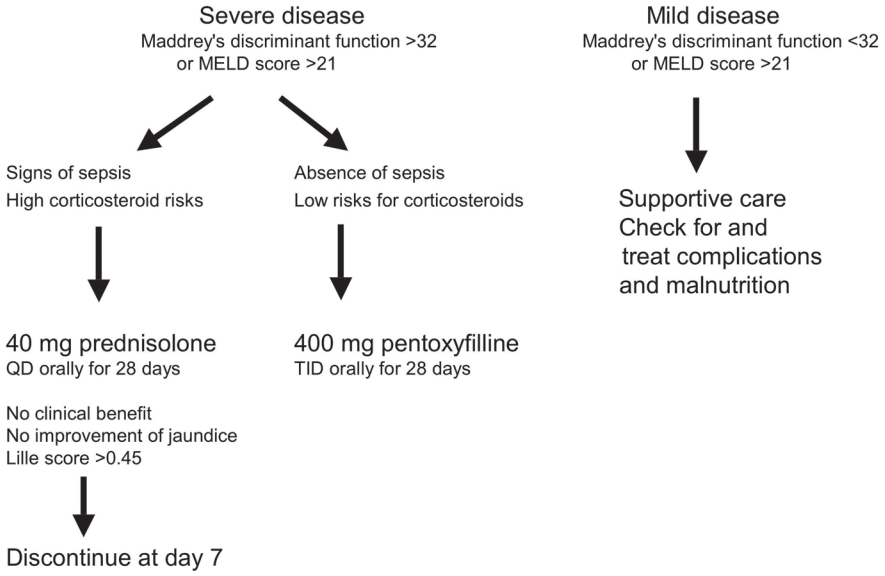


Figure 6. Treatment algorithm in alcoholic hepatitis.

References

Agarwal K, Czaja AJ, Jones De, et al. Cytotoxic T lymphocyteantigen-4 (CTLA-4) gene polymorphisms and susceptibility to type 1 autoimmune hepatitis. Hepatology 2000; 31:49-53.

Ahmed M, Mutimer D, Hathaway M, et al. Liver transplantation for autoimmune hepatitis: a 12-year experience. Transplant. Proc. 1997; 29:496

Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J. Hepatology 1999; 31:929-938.

Alvarez F, Ciocca M, Canero-Velasco C et al. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. J. Hepatol. 1999; 30:222-227.

Angulo P, Smith C, Jorgensen RA. Budesonide in the treatment of patients with primary biliary cirrhosis with suboptimal response to ursodeoxycholic acid. Hepatology 2000; 20:471-490

Bach N, Bodian C, Bodenheimer H, et al. Methotrexate therapy for primary biliary cirrhosis. Am J Gastroenterol 2003; 98:187-193.

Bandin O, Courvalin Jc, Poupon R et al. Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology 1996; 23:1020-1024

Beaune Ph, Lecoeur S, Bourdi M et al. Anti-cytochrome P450 autoantibodies in drug-induced disease. Eur J Haematol Suppl 1996; 60:89-92

Berg PA, Doniach D, Roitt IM. Mitochondrial antibodies in primary biliary cirrhosis. I. Localization of the antigen to mitochondrial membranes. J Exp Med 1967; 126:277-290

Berg PA, Klein R. Mitochondrial antigen/antibody systems in primary biliary cirrhosis: revisited. Liver 1995; 15:281-292

Berg PA, Klein R. Mitochondrial antigens and autoantibodies: from anti-M1 to anti-M9. Klin Wochenschr 1986; 64:897-909

Bergquist A, Broome U. Hepatobiliary and extra-hepatic malignancies in primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol 2001; 15:643-656.

Bergquist A, Ekbom A, Olsson R, et al. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. J Hepatol 2002; 36:321-327.

Beuers U, Rust C. Overlap syndromes. Semin Liver Dis 2005; 25:311-320

Bloch DB, Chiche JD, Orth D, et al. Structural and functional heterogeneity of nuclear bodies. Mol Cell Biol 1999; 19:4423-4430

Bluthner M, Schafer C, Schneider C, et al. Identification of major linear epitopes on the sp100 nuclear PBC autoantigen by the gene-fragment phage-display technology. Autoimmunity 1999; 29:33-42

Boberg KM, Aadland E, Jahnsen J, et al. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. Scand. J. Gastroenterol. 1998; 33:99-103

Boberg KM, Bergquist A, Mitchell S, et al. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. Scand J Gastroenterol 2002; 37:1205-1211.

Boberg KM, Fausa O, Haaland T, et al. Features of autoimmune hepatitis in primary sclerosing cholangitis: an evaluation of 114 primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. Hepatology 1996; 23:1369-1376

Bodenheimer Jr H, Schaffner F, Pezzullo J. Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 1998; 95:124-129.

Bodenheimer Jr HC, Schaffner F, Sternlieb I, et al. A prospective clinical trial of D-penicillamine in the treatment of primary biliary cirrhosis. Hepatology 1985; 5:1139-1142.

Broome U, Glaumann H, Lindstom E, et al. Natural history and outcome in 32 Swedish patients with small duct primary sclerosing cholangitis (PSC). J Hepatol 2002; 36:586-589.

Broome UO, Loof R, Bodemar L, Hultcrantz G, Danielsson R, Prytz A, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996; 38:610-615

Calmus Y, Gane P, Rouger P, et al. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990; 11:12-15

Cancado Elr, Porta G. Autoimmune hepatitis in South America. 2000; Kluwer Academic Publishers, Dordrecht, Boston, London

Chen W, Gluud C. Bile acids for primary sclerosing cholangitis. Cochrane Database Syst Rev 2003:CD003626.

Christensen E, Neuberger J, Crowe J, et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. Gastroenterology 1985; 89:1084-1091.

Crivelli O, Lavarini C, Chiaberge E, et al. Microsomal autoantibodies in chronic infection with HBsAg associated delta (delta) agent. Clin. Exp. Immunol. 1983; 54:232-238.

Czaja AJ. Frequency and nature of the variant syndromes of autoimmune liver disease. Hepatology 1998; 28:360-365.

Czaja AJ, Carpenter HA, Santrach PJ, et al. Evidence against hepatitis viruses as important causes of severe autoimmune hepatitis in the United States. J. Hepatol. 1993; 18:342-352.

Czaja AJ, Carpenter HA, Lindor KD. Ursodeoxycholic acid as adjunctive therapy for problematic type 1 autoimmune hepatitis: a randomized placebo-controlled treatment trial. Hepatology 1999; 30:1381-1386

Czaja AJ, Lindor KD. Failure of budesonide in a pilot study of treatment-dependent autoimmune hepatitis. Gastroenterology 2000; 119:1312-1316.

Dalekos GN, Wedemeyer H, Obermayer-Straub P, et al. Epitope mapping of cytochrome P4502D6 autoantigen in patients with chronic hepatitis C during alpha-interferon treatment. J Hepatol 1999; 30:366-375

Danielson A, Prytz H. Oral budesonide for treatment of autoimmune chronic hepatitis. Aliment. Pharmacol. Ther. 1994; 8:585-590.

Davis Pa, Leung P, Manns M, et al. M4 and M9 antibodies in the overlap syndrome of primary biliary cirrhosis and chronic active hepatitis: epitopes or epiphenomena? Hepatology 1992; 16:1128-1136.

Debray D, Maggiore G, Giradet JP et al. Efficacy of cyclosporin A in children with type 2 autoimmune hepatits. J. Pediatr. 1999; 135:111-114.

Devlin J, Donaldson P, Portman B, et al. Recurrence of autoimmune hepatitis following liver transplantation. Liver Transpl. Surg. 1995; 1:162-165.

Dickson ER, Murtaugh PA, Wiesner RH, et al. Primary sclerosing cholangitis: refinement and validation of survival models. Gastroenterology 1992; 103:1893-1901

Dienes HP, Popper H, Manns M, et al. Histologic features in autoimmune hepatitis. Z. Gastroenterol. 1989; 27:327-330.

Dighiero G, Lymberi P, Monot C, et al. Sera with high levels of anti-smooth muscle and anti-mitochondrial antibodies frequently bind to cytoskeleton proteins. Clin Exp Immunol 1990; 82:52-56

Doherty DG, Donaldson PT, Underhill JA, et al. Allelic sequence variation in the HLA class II genes and proteins on patients with autoimmune hepatitis. Hepatology 1994; 19:609-615

Donaldson PT. Immunogenetics in liver disease. Baillieres Clin Gastroenterol 1996; 10:533-549

Donaldson PT. TNF gene polymorphisms in primary biliary cirrhosis: a critical appraisal [editorial; comment]. J Hepatol 1999; 31:366-368

Donaldson PT, Czaja AJ. Genetic effects on susceptibility, clinical expression, and treatment outcome of type 1 autoimmune hepatitis. Clin Liver Dis 2002; 6:419-437

Donaldson PT, Doherty DG, Hayllar KM, et al. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR 4 and A1-B8-DR-3 are independent risk factors. Hepatology 1991; 13:701-706

Dubel L, Farges O, Courvalin JC, et al. Persistence of gp210 and multiple nuclear dots antibodies does not correlate with recurrence of primary biliary cirrhosis 6 years after liver transplantation. J Hepatol 1998; 28:169-170

Durazzo M, Philipp T, Van Pelt FN, et al. Heterogeneity of liver-kidney microsomal autoantibodies in chronic hepatitis C and D virus infection. Gastroenterology 1995; 108:455-462

European Liver Transplant Registry J 1996.

Farrant JM, Hayllar KM, Wilkinson MI, et al. Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology 1991; 100:1710-1717

Fernandez NF, Redeker AG, Vierling JM, et al. Cyclosporine therapy in patients with steroid resistant autoimmune hepatitis. Am. J. Gastroenterol. 1999; 94:241-248.

Frazer IH, Mackay IR, Jordan TW, et al. Reactivity of anti-mitochondrial autoantibodies in primary biliary cirrhosis: definition of two novel mitochondrial polypeptide autoantigens. J Immunol 1985; 135:1739-1745

Fujimoto M, Sato S, Ihn H, et al. Autoantibodies to pyruvate dehydrogenase complex in patients with systemic sclerosis. Possible role of anti-E1 alpha antibody as a serologic indicator for development of primary biliary cirrhosis. Arthritis Rheum 1995; 38:985-989

Geall MG, Schoenfield LJ, Summerskill WHJ. Classification and treatment of chronic active liver disease. Gastroenterology 1968; 55:724-729.

Gelpi CSE, Rodriguez-Sanchez JI. Autoantibodies against a serine tRNA-protein complex implicated in cotranslational selenocysteine insertion. Proc Natl Acad Sci US 1992; 89:9739-9743

Gershwin ME, Mackay IR. Primary biliary cirrhosis: paradigm or paradox for autoimmunity. Gastroenterology 1991; 100:822-833

Gershwin ME, Mackay IR, Sturgess A, et al. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987; 138:3525-3531

Gordon MA, Oppenheim E, Camp NJ, et al. Primary biliary cirrhosis shows association with genetic polymorphism of tumour necrosis factor alpha promoter region [see comments]. J Hepatol 1999; 31:242-247

Götz G, Neuhaus R, Bechstein WO, et al. Recurrence of autoimmune hepatitis after liver transplantation. Transplant. Proc. 1999; 31:430-431.

Goulis J, Leandro G, Burroughs AK. Randomised controlled trials of ursodeoxycholic acid therapy for primary biliary cirrhosis: a meta analysis. Lancet 1999; 354:1053-1060

Gouw AS, Haagsma EB, Manns M, et al. Is there recurrence of primary biliary cirrhosis after liver transplantation? A clinicopathologic study in long-term survivors. J Hepatol 1994; 20:500-507

Graziadei IW, Wiesner RH, Batts KP, et al. Recurrence of primary sclerosing cholangitis following liver transplantation. Hepatology 1999; 29:1050-1056

Gregorio Gv, Portman B, Reid F, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology 1997; 25:541-547

Guenguen M, Boniface O, Bernard O, et al. Identification of the main epitope on human cytochrome P450IID6 recognized by anti-liver kidney microsome antibody. J. Autoimmun. 1991; 4:607-615

Guenguen M, Meunier-Rotival M, Bernard O, et al. Anti-liver-kidney microsome antibody recognizes a cytochrome P450 from the IID subfamily. J. Exp. Med. 1988; 168:801

Haagsma EB. Clinical relevance of recurrence of primary biliary cirrhosis after liver transplantation. Eur J Gastroenterol Hepatol 1999; 11:639-642

Haagsma EB, Manns M, Klein R, et al. Subtypes of antimitochondrial antibodies in primary biliary cirrhosis before and after orthotopic liver transplantation. Hepatology 1987; 7:129-133

Harnois DM, Angulo P, Jorgensen RA, et al. High-dose ursodeoxycholic acid as a therapy for patients with primary sclerosing cholangitis. Am J Gastroenterol 2001; 96:1558-1562.

Hendrickse MT, Rigney E, Giaffer MH, et al. Low-dose methotrexate is ineffective in primary biliary cirrhosis: long-term results of a placebo-controlled trial. Gastroenterology 1999; 117:400-407.

Heneghan MA, Mcfarlane IG. Current and novel immunosuppressive therapy for autoimmune hepatitis. Hepatology 2002; 35:7-13.

Homberg JC, Abuaf N, Bernard O, et al. Chronic active hepatitis associated with anti liver/kidney microsome type 1: a second type of “autoimmune” hepatitis. Hepatology 1987; 7:1333-1339

Ikeda T, Tozuka S, Noguchi O. Effects of additional administration of colchicine in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: a prospective randomized study. j Hepatol 1996; 24:88-94

Ishii H, Saifuku K, Namihisa T. Multiplicity of mitochondrial inner membrane antigens from beef heart reacting with antimitochondrial antibodies in sera of patients with primary biliary cirrhosis. Immunol Lett 1985; 9:325-330

Johnson PJ, Mcfarlane IG. Meeting report: International autoimmune hepatitis group. Hepatology 1993; 18:998-1005

Johnson PJ, Mcfarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. N. Engl.J. Med. 1995; 333:958-963

Jones DE, Watt FE, Metcalf JV, et al. Familial primary biliary cirrhosis reassessed: a geographically-based population study. J Hepatol 1999; 30:402-407

Joplin R, Wallace LI, Johnson GD, et al. Subcellular localization of pyruvate dehydrogenase dihydrolipoamide acetyltransferase in human intrahepatic biliary epithelial cells. J Pathol 1995; 176:381-390

Joplin RE, Neuberger JM. Immunopathology of primary biliary cirrhosis. Eur J Gastroenterol Hepatol 1999; 11:587-593

Kanzler S, Gerken G, Dienes HP, et al. Cyclophosphamide as alternative immunosuppressive therapy for autoimmune hepatitis - report of three cases. Z. Gastroenterol. 1996; 35:571-578.

Kaplan MM, Alling DW, Zimmerman HJ, et al. A prospective trial of colchicine for primary biliary cirrhosis. N Engl J Med 1986; 315:1448-1454.

Kato Y, Suzuki K, Kumagai M, et al. Familial primary biliary cirrhosis. Immunological and genetic study. Am J Gastroenterol 1981; 75:188-191

Kerkar N, Hadzic N, Davies ET, et al. De-novo autoimmune hepatitis after liver transplantation. Lancet 1998; 351:409-413

Kim WR, Therneau TM, Wiesner RH, et al. A revised natural history model for primary sclerosing cholangitis. Mayo Clin Proc 2000; 75:688-694

Kirk AP, Jain S, Pocock S, et al. Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. Gut 1980; 21:7893.

Klein R, Berg, PA. Anti-M4 antibodies in primary biliary cirrhosis react with sulphite oxidase, an enzyme of the mitochondrial inter-membrane space. Clin Exp Immunol 1991; 84:445-448

Klein R, Kloppel G, Garbe W, et al. Antimitochondrial antibody profiles determined at early stages of primary biliary cirrhosis differentiate between a benign and a progressive course of the disease. A retrospective analysis of 76 patients over 6-18 years. J Hepatol 1991; 12:21-27

Klein R, Pointner H, Zilly W, et al. Antimitochondrial antibody profiles in primary biliary cirrhosis distinguish at early stages between a benign and a progressive course: a prospective study on 200 patients followed for 10 years. Liver 1997; 17:119-128

Kurki P, Miettinen A, Linder E, et al. Different types of smooth muscle antibodies in chronic active hepatitis and primary biliary cirrhosis: their diagnostic and prognostic significance. Gut 1980; 21:878-884

Lankisch TO, Strassburg CP, Debray D, et al. Detection of autoimmune regulator gene mutations in children with type 2 autoimmune hepatitis and extrahepatic immune-mediated diseases. J Pediatr 2005; 146:839-842.

Larusso NF, Shneider BL, Black D, et al. Primary sclerosing cholangitis: summary of a workshop. Hepatology 2006; 44:746-764

Lassoued K, Brenard R, Degos F, et al. Antinuclear antibodies directed to a 200-kilodalton polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patients with primary biliary cirrhosis. Gastroenterology 1990; 99:181-186

Lenzi M, Manotti P, Muratori L, et al. Liver cytosolic 1 antigen-antibody system in type 2 autoimmune hepatitis and hepatitis C virus infection. Gut 1995; 36:749-754

Leung PS, Chuang DT, Wynn RM, et al. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. Hepatology 1995; 22:505-513

Leung PS, Van De Water J, Coppel RI, et al. Molecular aspects and the pathological basis of primary biliary cirrhosis. J Autoimmun 1996; 9:119-128

Leuschner M, Guldutuna S, You T, et al. Ursodeoxycholic acid and prednisolone versus ursodeoxycholic acid and placebo in the treatment of early stages of primary biliary cirrhosis. J Hepatol 1996; 25:49-57.

Leuschner M, Holtmeier J, Ackermann H, et al. The influence of sulindac on patients with primary biliary cirrhosis that responds incompletely to ursodeoxycholic acid: a pilot study. Eur J Gastroenterol Hepatol 2002; 14:1369-1376.

Leuschner M, Maier KP, Schlichting J, et al. Oral budesonide and ursodeoxycholic acid for treatment of primary biliary cirrhosis: results of a prospective double-blind trial. Gastroenterology 1999; 117:918-925.

Lidman K, Biberfeld G, Fagraeus A, et al. Anti-actin specificity of human smooth muscle antibodies in chronic active hepatitis. Clin Exp Immunol 1976; 24:266-272

Lin F, Noyer Cm, Ye Q, et al. Autoantibodies from patients with primary biliary cirrhosis recognize a region within the nucleoplasmic domain of inner nuclear membrane protein LBR. Hepatology 1996; 23:57-61

Lindenborn-Fotinos J, Baum H, Berg PA. Mitochondrial antibodies in primary biliary cirrhosis: species and nonspecies specific determinants of M2 antigen. Hepatology 1985; 5:763-769

Lohr H, Fleischer B, Gerken G, et al. Autoreactive liver-infiltrating T cells in primary biliary cirrhosis recognize inner mitochondrial epitopes and the pyruvate dehydrogenase complex. J Hepatol 1993; 18:322-327

Lombard M, Portmann B, Neuberger J, et al. Cyclosporin A treatment in primary biliary cirrhosis: results of a long-term placebo controlled trial. Gastroenterology 1993; 104:519-526.

Luettig B, Boeker KH, Schoessler W, et al. The antinuclear autoantibodies Sp100 and gp210 persist after orthotopic liver transplantation in patients with primary biliary cirrhosis. J Hepatol 1998; 28:824-828

Mackay I, R, Taft LI, Cowling DC. Lupoid hepatitis. Lancet 1956; 2:1323-1326

Mackay IR. Primary biliary cirrhosis showing a high titer of autoantibody. N. Engl. J. Med. 1958; 258:707-713

Mackay IR, Gershwin ME. The nature of autoimmune disease. Semin Liver Dis 1997; 17:3-11

Mackay IR, Morris PJ. Association of autoimmune chronic hepatitis with HLA-A1-B8. Lancet 1972; 2:793-795

Mackay IR, Tait BD. HLA association with autoimmune type chronic active hepatitis: identification of B8-DRw3 haplotype by family studies. Gastroenterology 1980; 79:95-98

Manns M, Gerken G, Kyriatsoulis A, et al. Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. Lancet 1987; 1:292-294

Manns M, Gerken G, Trautwein C, et al. Characterization of primary biliary cirrhosis (PBC) specific mitochondrial determinants by immunoblotting. Dtsch Z Verdau Stoffwechselkr 1987; 47:59-66

Manns M, Meyer Zum Buschenfelde KH. A mitochondrial antigen-antibody system in cholestatic liver disease detected by radioimmunoassay. Hepatology 1982; 2:1-7

Manns M, Meyer Zum Büschenfelde KH, Slusarczyk J, et al. Detection of liver-kidney microsomal autoantibodies by radioimmunoassay and their relation to antimitochondrial antibodies in inflammatory liver disease. Clin. Exp. Immunol. 1984; 54:600-608

Manns MP, Bahr MJ. Recurrent autoimmune hepatitis after liver transplantation - when non-self becomes self. Hepatology 2000; 32:868-870

Manns MP, Bremm A, Schneider PM, et al. HLA DRw8 and complement C4 deficiency as risk factors in primary biliary cirrhosis. Gastroenterology 1991; 101:1367-1373.

Manns MP, Griffin KJ, Sullivan KF, et al. LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase. J. Clin. Invest. 1991; 88:1370-1378

Manns MP, Jentzsch M, Mergener K, et al. Discordant manifestation of LKM-1 antibody positive autoimmune hepatitis in identical twins. Hepatology 1990; 12:840

Manns MP, Johnson EF, Griffin KJ, et al. Major antigen of liver kidney microsomal antibodies in idiopathic autoimmune hepatitis is cytochrome P450db1. J. Clin. Invest. 1998; 83:1066-1072

Manns MP, Kruger M. Immunogenetics of chronic liver diseases. Gastroenterology 1994; 106:1676-1697.

Manns MP, Strassburg CP. Autoimmune hepatitis: clinical challenges. Gastroenterology 2001; 120:1502-1517.

Mattalia A, Luttig B, Rosina F, et al. Persistence of autoantibodies against recombinant mitochondrial and nuclear pore proteins after orthotopic liver transplantation for primary biliary cirrhosis. J Autoimmun 1997; 10:491-497

Mehal Wz, Gregory WI, Lo YM, et al. Defining the immunogenetic susceptibility to primary biliary cirrhosis. Hepatology 1994; 20:1213-1219

Milkiewicz P, Hubscher Sg, Skiba G et al. Recurrence of autoimmune hepatitis after liver transplantation. Transplantation 1999; 68:253-256

Mitchell SA, Bansi DS, Hunt N, et al. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. Gastroenterology 2001; 121:900-907

Mitchison HC, Palmer JM, Bassendine MF, et al. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol 1992; 15:336-344.

Moteki S, Leung PS, Dickson ER, et al. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. Hepatology 1996; 23:436-444

Muratori L, Cataleta M, Muratori P, et al. Detection of anti-liver cytosol antibody type 1 (anti-LC1) by immunodiffusion, counterimmunoelectrophoresis and immunoblotting: comparison of different techniques. J. Immunol. Meth. 1995; 187:259-264.

Muratori L, Sztul E, Muratori P, et al. Distinct epitopes on formiminotransferase cyclodeaminase induce autoimmune liver cytosol antibody type 1. Hepatology 2001; 34:494-501

Nakamura K, Yoneda M, Yokohama S, et al. Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis [see comments]. J Gastroenterol Hepatol 1998; 13:490-495

Neuberger J, Portmann B, Calne R, et al. Recurrence of autoimmune chronic active hepatitis following orthotopic liver grafting. Transplantation 1984; 37:363-365

Newton JI, Burt AD, Park JB, et al. Autoimmune hepatitis in older patients. Age Ageing 1997; 26:441-444

Nickowitz RE, Worman HJ. Autoantibodies from patients with primary biliary cirrhosis recognize a restricted region within the cytoplasmic tail of nuclear pore membrane glycoprotein Gp210. J Exp Med 1993; 178:2237-2242

Nickowitz RE, Wozniak RW, Schaffner F, et al. Autoantibodies against integral membrane proteins of the nuclear envelope in patients with primary biliary cirrhosis. Gastroenterology 1994; 106:193-199

Nishio A, Van De Water J, Leung PS, et al. Comparative studies of antimitochondrial autoantibodies in sera and bile in primary biliary cirrhosis. Hepatology 1997; 25:1085-1089

Nishioka M, Morshed SA, Kono K, et al. Frequency and significance of antibodies to P450IID6 protein in Japanese patients with chronic hepatiis C. J. Hepatol. 1997; 26:992-1000

Nishioka M, Morshed SA, Mcfarlane IG. Geographical variation in the frequency and characteristics of autoimmune liver diseases. Elsevier, Amsterdam. 1998.

Notghi A, Nestle U, Rittner G, et al. Chromosomal aberrations in patients with primary biliary cirrhosis. Hum Genet 1990; 85:546-550

Obermayer-Straub P, Perheentupa J, Braun S, et al. Hepatic autoantigens in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Gastroenterology 2001; 121:668-677

Okolicsanyi L, Fabris L, Viaggi S, et al. Primary sclerosing cholangitis: clinical presentation, natural history and prognostic variables: an Italian multicentre study. The Italian PSC Study Group. Eur J Gastroenterol Hepatol 1996; 8:685-691

Olsson R, Boberg KM, De Muckadell OS, et al. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. Gastroenterology 2005; 129:1464-1472

Onishi S, Sakamaki T, Maeda T, et al. DNA typing of HLA class II genes; DRB1*0803 increases the susceptibility of Japanese to primary biliary cirrhosis. J Hepatol 1994; 21:1053-1060

Ota M, Seki T, Kiyoshawa K, et al. A possible association between basic amino acids of position 13 of DRB1 chains and autoimmune hepatitis. Immunogenetics 1992; 36:40-55.

Palmer JM, Jones DE, Quinn J, et al. Characterization of the autoantibody responses to recombinant E3 binding protein (protein X) of pyruvate dehydrogenase in primary biliary cirrhosis. Hepatology 1999; 30:21-26

Palmer JM, Yeaman SJ, Bassendine MF, et al. M4 and M9 autoantigens in primary biliary cirrhosis--a negative study. J Hepatol 1993; 18:251-254

Pando M, Larriba J, Fernandez GC, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. Hepatology 1999; 30:1374-1380

Pardi DS, Loftus Jr EV, Kremers WK, et al. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. Gastroenterology 2003; 124:889-893

Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology 2002; 36:525-531.

Philipp T, Durazzo M, Trautwein C, et al. Recognition of uridine diphosphate glucuronosyl transferases by LKM-3 antibodies in chronic hepatitis D. Lancet 1994; 344:578-581

Pitkanen J, Vahamurto P, Krohn K, et al. Subcellular localization of the autoimmune regulator protein. characterization of nuclear targeting and transcriptional activation domain. J Biol Chem 2001; 276:19597-19602.

Ponsioen CY, Vrouenraets SM, Prawirodirdjo W, et al. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. Gut 2002; 51:562-566.

Poupon RE, Huet PM, Poupon R, et al. A randomized trial comparing colchicine and ursodeoxycholic acid combination to ursodeoxycholic acid in primary biliary cirrhosis. UDCA-PBC Study Group. Hepatology 1996; 24:1098-1103.

Poupon RE, Lindor KD, Cauch-Dudek K. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 1997; 113:884-890

Poupon RE, Lindor KD, Pares A, et al. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. J Hepatol 2003; 39:12-16.

Prados E, Cuervas-Mons V, De La Mata M, et al. Outcome of autoimmune hepatitis after liver transplantation. Transplantation 1998; 66:1645-1650.

Raedsch R, Stiehl A, Walker S, et al. Combined ursodeoxycholic acid plus colchicine--treatment of primary biliary cirrhosis: results of a placebo-controlled double-blind study. Z Gastroenterol 1992; 30 Suppl 1:55-57.

Ramsey C, Winqvist O, Puhakka L, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. Hum Mol Genet 2002; 11:397-409

Ratziu V, Samuel D, Sebagh M, et al. Long-term follow-up after liver transplantation for autoimmune hepatitis: evidence of recurrence of primary disease. J Hepatol 1999; 30:131-141

Rea DJ, Heimbach JK, Rosen CB, et al. Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. Ann Surg 2005; 242:451-458; discussion 458-461

Rebollo Bernardez J, Cifuentes Mimoso C, Pinar Moreno A, et al. Deflazacort for long-term maintenance of remission in type I autoimmune hepatitis. Rev Esp Enferm Dig 1999; 91:630-638.

Reynoso-Paz S, Leung PS, Van De Water J, et al. Evidence for a locally driven mucosal response and the presence of mitochondrial antigens in saliva in primary biliary cirrhosis. Hepatology 2000; 31:24-29

Richardson PD, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. J Hepatol 2000; 33:371-375

Rizzetto M, Swana G, Doniach D. Microsomal antibodies in active chronic hepatitis and other disorders. Clin. Exp. Immunol. 1973; 15:331-344

Rost D, Rudolph G, Kloeters-Plachky P, et al. Effect of high-dose ursodeoxycholic acid on its biliary enrichment in primary sclerosing cholangitis. Hepatology 2004; 40:693-698

Sanchez-Urdazpal L, Czaja AJ, Van Holk B. Prognostic features and role of liver transplantation in severe corticoid-treated autoimmune chronic active hepatitis. Hepatology 1991; 15:215-221

Schüler A, Manns MP. Treatment of autoimmune hepatitis. In: Arroyo V, Bosch J, Rodés J (eds) Treatment in Hepatology. Masson, S.A., Paris, p 375-383. 1995.

Serfaty L, De Leusse A, Rosmorduc O, et al. Ursodeoxycholic acid therapy and the risk of colorectal adenoma in patients with primary biliary cirrhosis: an observational study. Hepatology 2003; 38:203-209.

Soloway RD, Summerskill WH, Baggenstoss AH, et al. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. Gastroenterology 1972; 63:820-833

Stechemesser E, Klein R, Berg PA. Characterization and clinical relevance of liver-pancreas antibodies in autoimmune hepatitis. Hepatology 1993; 18:1-9

Sternsdorf T, Guldner HH, Szostecki C, et al. Two nuclear dot-associated proteins, PML and Sp100, are often co- autoimmunogenic in patients with primary biliary cirrhosis. Scand J Immunol 1995; 42:257-268

Strassburg CP, Alex B, Zindy F, et al. Identification of cyclin A as a molecular target of antinuclear antibodies (ANA) in hepatic and non-hepatic autoimmune diseases. J Hepatol 1996; 25:859-866

Strassburg CP, Jaeckel E, Manns MP. Anti-mitochondrial antibodies and other immunological tests in primary biliary cirrhosis. Eur J Gastroenterol Hepatol 1999; 11:595-601

Strassburg CP, Manns MP. Autoantibodies and autoantigens in autoimmune hepatitis. Semin Liver Dis 2002; 22:339-352

Strassburg CP, Manns MP. Autoimmune hepatitis versus viral hepatitis C. Liver 1995; 15:225-232

Strassburg CP, Manns MP. Autoimmune tests in primary biliary cirrhosis. Baillieres Best Pract Res Clin Gastroenterol 2000; 14:585-599

Strassburg CP, Manns MP. Primär biliäre Zirrhose und primär sklerosierende Cholangitis. Internistische Praxis 1996; 36:57-74

Strassburg CP, Manns MP. [Primary biliary liver cirrhosis and overlap syndrome. Diagnosis and therapy]. Internist (Berl) 2004; 45:16-26.

Strassburg CP, Obermayer-Straub P, Alex B, et al. Autoantibodies against glucuronosyltransferases differ between viral hepatitis and autoimmune hepatitis. Gastroenterology 1996; 111:1576-1586

Strassburg CP, Obermayer-Straub P, Manns MP. Autoimmunity in hepatitis C and D virus infection. J. Viral. Hepat. 1996; 3:49-59

Strassburg CP, Obermayer-Straub P, Manns MP. Autoimmunity in liver diseases. Clin Rev Allergy Immunol 2000; 18:127-139

Strettell MD, Thomson LJ, Donaldson PT, et al. HLA-C genes and susceptibility to type 1 autoimmune hepatitis. Hepatology 1997; 26:1023-1026.

Sudan D, Deroover A, Chinnakotla S, et al. Radiochemotherapy and transplantation allow long-term survival for nonresectable hilar cholangiocarcinoma. Am J Transplant 2002; 2:774-779

Sugimura T, Obermayer-Straub P, Kayser A, et al. A major CYP2D6 autoepitope in autoimmune hepatitis type 2 and chronic hepatitis C is a three-dimensional structure homologous to other cytochrome P450 autoantigens. Autoimmunity 2002; 35:501-513

Szostecki C, Krippner H, Penner E, et al. Autoimmune sera recognize a 100 kD nuclear protein antigen (sp-100). Clin Exp Immunol 1987; 68:108-116

Szostecki C, Will H, Netter HJ, et al. Autoantibodies to the nuclear Sp100 protein in primary biliary cirrhosis and associated diseases: epitope specificity and immunoglobulin class distribution. Scand J Immunol 1992; 36:555-564

Tan EM, Chan EKL, Sullivan KF, et al. Antinuclear antibodies (ANAs): diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin. Immunol. Immunopathol. 1998; 47:121-141.

Terjung B, Spengler U, Sauerbruch T, et al. “Atypical p-ANCA” in IBD and hepatobiliary disorders react with a 50-kilodalton nuclear envelope protein of neutrophils and myeloid cell lines. Gastroenterology 2000; 119:310-322

Tillmann HI, Jackel E, Manns MP. Liver transplantation in autoimmune liver disease--selection of patients. Hepatogastroenterology 1999; 46:3053-3059

Tischendorf JJ, Hecker H, Kruger M, et al. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. Am J Gastroenterol 2007; 102:107-114

Tischendorf JJ, Meier PN, Strassburg CP, et al. Characterization and clinical course of hepatobiliary carcinoma in patients with primary sclerosing cholangitis. Scand J Gastroenterol 2006; 41:1227-1234

Treichel U, Poralla T, Hess G, et al. Autoantibodies to human asialoglycoprotein receptor in autoimmune-type chronic hepatitis. Hepatology 1990; 11:606-612

Tsuji K, Watanabe Y, Van De Water J, et al. Familial primary biliary cirrhosis in Hiroshima. J Autoimmun 1999; 13:171-178

Tukey RH, Strassburg CP. Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. Mol Pharmacol 2001; 59:405-414.

Tung BY, Emond MJ, Haggitt RC, et al. Ursodiol use is associated with lower prevalence of colonic neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Ann Intern Med 2001; 134:89-95.

Uibo R, Salupere V. The epidemiology of primary biliary cirrhosis: immunological problems. Hepatogastroenterology 1999; 46:3048-3052

Van De Water J, Gershwin Me, Leung P et al. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. J Exp Med 1988; 167:1791-1799

Van De Water J, Shimoda S, Niho Y et al. The role of T cells in primary biliary cirrhosis. Semin Liver Dis 1997; 17:105-113

Van Hoogstraten HJ, Vleggaar FP, Boland GJ, et al. Budesonide or prednisone in combination with ursodeoxycholic acid in primary sclerosing cholangitis: a randomized double-blind pilot study. Belgian-Dutch PSC Study Group [see comments]. Am J Gastroenterol 2000; 95:2015-2022

Van Norstrand MD, Malinchoc M, Lindor KD, et al. Quantitative measurement of autoantibodies to recombinant mitochondrial antigens in patients with primary biliary cirrhosis: relationship of levels of autoantibodies to disease progression. Hepatology 1997; 25:6-11

Van Steenbergen W, Sciot R, Van Eyken P, et al. Combined treatment with methotrexate and ursodeoxycholic acid in non-cirrhotic primary biliary cirrhosis. Acta Clin Belg 1996; 51:8-18.

Van Thiel DH, Wright H, Carroll P, et al. Tacrolimus: A potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. Am. J. Gastroenterol. 1995; 90:771-776.

Vento S, Cainelli F, Ferraro T, et al. Autoimmune hepatitis type 1 after measles. Am. J. Gastroenterol. 1996; 91:2618-2620.

Vento S, Cainelli F, Renzini C, et al. Autoimmune hepatitis type 2 induced by HCV and persisting after viral clearance. Lancet 1997; 350:1298-1299.

Vento S, Garofano T, Di Perri G, et al. Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. Lancet 1991; 337:1183-1187

Vento S, Guella L, Mirandola F, et al. Epstein-Barr virus as a trigger for autoimmune hepatitis in susceptible individuals. Lancet 1995; 346:608-609.

Vogel A, Heinrich E, Bahr MJ, et al. Long-term outcome of liver transplantation for autoimmune hepatitis. Clin Transplant 2004; 18:62-69.

Vogel A, Liermann H, Harms A, et al. Autoimmune regulator AIRE: Evidence for genetic differences between autoimmune hepatitis and hepatitis as part of the autoimmune polyglandular syndrome type 1. Hepatology 2001; 33:1047-1052.

Vogel A, Manns MP, Strassburg CP. Autoimmunity and viruses. Clin Liver Dis 2002; 6:451-465

Vogel A, Strassburg CP, Manns MP. 77 C/G Mutation in the Tyrosine Phosphatase CD45 and Autoimmune Hepatitis: Evidence for a Genetic Link. Genes and Immunity 2003; 4:79-81

Vogel A, Strassburg CP, Manns MP. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. Hepatology 2002; 35:126-131.

Volkmann M, Martin L, Baurle A, et al. Soluble liver antigen: isolation of a 35-kd recombinant protein (SLA-p35) specifically recognizing sera from patients with autoimmune hepatitis. Hepatology 2001; 33:591-596

Waldenström J. Leber, Blutproteine und Nahrungseiweisse. Dtsch Gesellsch Verd Stoffw 1950; 15:113-119.

Warnes TW, Smith A, Lee FI, et al. A controlled trial of colchicine in primary biliary cirrhosis. Trial design and preliminary report. J Hepatol 1987; 5:1-7.

Weismüller TJ, Wedemeyer J, Kubicka S, et al. The challenges in primary sclerosing cholangitis – Aetiopathogenesis, autoimmunity, management and malignancy. J Hepatol 2008; 48:S38-S57

Wesierska-Gadek J, Hohenuer H, Hitchman E, et al. Autoantibodies against nucleoporin p62 constitute a novel marker of primary biliary cirrhosis. Gastroenterology 1996; 110:840-847

Wies I, Brunner S, Henninger J, et al. Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis [see comments]. Lancet 2000; 355:1510-1515

Wiesner RH, Grambsch PM, Dickson ER, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 1989; 10:430-436

Wiesner RH, Ludwig J, Lindor KD, et al. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. N Engl J Med 1990; 322:1419-1424.

Wiesner RH, Porayko MK, Dickson ER, et al. Selection and timing of liver transplantation in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1992; 16:1290-1299

Wolfhagen FHJ, Van Hoogstraaten HJF, Van Buuren HR. Triple therapy with ursodeoxycholic acid, prednisone, and azathioprine in primary biliary cirrhosis: a 1 year randomized, placebo controlled study. J Hepatol 1998; 29:736-742

Worman HJ. Primary biliary cirrhosis and the molecular cell biology of the nuclear envelope. Mt Sinai J Med 1994; 61:461-475

Wright HI, Bou-Abboud CF, Hassanenstein T, et al. Disease recurrence and rejection following liver transplantation for autoimmune chronic active liver disease. Transplantation 1992; 53:136-139.

Zanger UM, Hauri HP, Loeper J, et al. Antibodies against human cytochrome P-450db1 in autoimmune hepatitis type 2. Proc. Natl. Acad. Sci. USA 1988; 85:8256-8260.

Zuchner D, Sternsdorf T, Szostecki C, et al. Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology 1997; 26:1123-1130.

Index

A

ACH-806 212
Adefovir....114, 135, 265
Adverse drug reactions ....see Side effects
Albinterferon 180, 221
Alcohol dehydrogenase 471
Alcoholic hepatitis....467
Amantadine....179, 221
Amplicor....162
Antiretroviral treatment....271
Antisense oligonucleotides....218
Antiviral resistance testing 97
ART ..... see Antiretroviral treatment
Autoimmune haemolytic anaemia 247
Autoimmune hepatitis....421

B

BI 201335 198
BILB 1941 198
BMI-790052 198
Boceprevir 198, 207
  resistance....212

C

Celgosivir....198, 219
Cell culture
  HBV....46
Ciluprevir....198, 202, 211
Cirrhosis
  HBV/HCV coinfection 286
  Hepatitis C 24
Clevudine....151
Cobas Ampliprep....162
Coinfection
  HBV/HCV 283
  HBV/HIV....259
  HCV/HIV....269
Consensus interferon 175
Copegus 175
Cryoglobulinaemia 240
Cutaneous disorders....250
Cyclophilin B inhibitors 219

D

Dane particle....39
Debio-025 ....198, 219
Deferasirox 386
Delta hepatitis ..... see Hepatitis D
Diabetes mellitus 250

Diagnostic tests
  Hepatitis B 95
  Hepatitis C 159
Dialysis
  HCV treatment..... 185
Drug resistance
  HBV 131
  HCV 210

E

Emtricitabine..... 263
End stage liver disease..... 315
Entecavir 115, 136, 265
Epidemiology
  Hepatitis A 3
  Hepatitis B 7
  Hepatitis C 19
  Hepatitis D 145
  Hepatitis E..... 31

F

Famcyclovir 151
Ferroportin Disease..... 389
Flaviviridae 61

G

Glucosidase inhibitors 219
GS 9190 198

H

HAART..... see Antiretroviral treatment
Haemochromatosis 373
  juvenile hereditary 387
  secondary 389
  TFR2-related..... 388
HBV ..... see Hepatitis B
HBV DNA assays 96
HCC ..... see Hepatocellular carcinoma
HCV-796..... 198, 217
HCV-SuperQuant 162
HDV..... see Hepatitis D
Hepadnaviridae 37
Hepatitis A 3
  prophylaxis..... 83
  vaccination 85
Hepatitis B 7
  coinfection with HCV 283

coinfection with HIV 259
diagnostic tests.....95
genotyping 96

occult infection 284
post-exposure prophylaxis.....88
prophylaxis 83
superinfection 284
treatment 101
vaccination.....86
virology.....37
Hepatitis C 19, 159
coinfection with HBV.....283
coinfection with HIV 269
diagnostic tests.....159
extrahepatic manifestions 239
prophylaxis 84
serologic assays 160
superinfection 284
treatment 171
vaccination.....89
virology.....61
Hepatitis D
diagnostic procedures 145
prophylaxis 83
treatment 145
virology.....146
Hepatitis E 31
Hepatocellular carcinoma.....303
curative therapy 306
palliative therapy 308
HIV
coinfection with HBV.....259
coinfection with HCV.....269

I

IFN α-2bXL.....222
IMPDH 220
Infergen.....175
Interferon alpha.....152, 171
Interferons.....109
Intron 175
IRES inhibitors 218
ITMN-191.....198, 219

L

Lamivudine.....114, 131, 265
Life cycle
HCV.....68, 199
Liver cancer
prophylaxis 310
Liver fibrosis.....291
surrogate markers.....293
Liver transplantation. see Transplantation
Locteron..... 222
Lymphoproliferative disorders 240

M

MK-3281..... 198
MK-7009..... 198

N

NAFLD..... 395
NASH 395
Natural history
Hepatitis B 11
Hepatitis C 24
Nitazoxanide..... 198, 220
NS3/4A protease inhibitors..... 201
NS4A inhibitors..... 212
NS5B polymerase inhibitors..... 214
Nucleos(t)ide analogues..... 112

P

Pathogenesis
hepadnavirus infections 43
Hepatitis D 149
Pegasys 175
PEG-IFN 111, 171, 173, 273
maintenance therapy 189
PEG-Intron..... 175
Pegylated interferon.....see PEG-IFN
Penicillamine 412
PF-868554..... 198
Phlebotomy 386
Polymerase inhibitors 214, 263
Primary biliary cirrhosis 442
Primary sclerosing cholangitis..... 452
Prophylaxis
viral hepatitis..... 83
Protease inhibitors 201

R

R1626..... 198, 214
R7128..... 198, 216
R7227..... 198
RealTime HCV 162
Rebetol..... 175
Resistance .....see Drug Resistance
Ribavirin 151, 173, 273
Ribozymes 218
Roferon 175

S

Side effects..... 231
HCV treatment..... 181
IFN..... 181
ribavirin..... 182

Standard IFN.....109

T

Taribavirin 223
Telaprevir.....198, 202
Telbivudine.....115, 135, 265
Tenofovir 116, 136, 265
Tetrathiomolybdate.....414
Thrombocytopaenia 247
Thyroid disease.....249
TMC435350.....198, 210
Transient elastography.....295
Transmission
  Hepatitis A 3
  Hepatitis B 7
  Hepatitis C 19
  Hepatitis E 31
Transplantation
  HBV/HCV and HIV coinfection ...353
  HCV.....184
  HIV/HCV coinfection.....278
Treatment
  acute hepatitis B.....104
  chronic hepatitis B 104
  Hepatitis B 101
  Hepatitis C 171
  Hepatitis D 151
Treatment failure
  HCV 186
Trientine..... 413

V

Vaccination 83
  Hepatitis B 86
  Hepatitis C 89
  Hepatitis E..... 90
Valopicitabine..... 198, 215
VCH-759..... 198, 217
Versant..... 162
Virology
  HBV 37
  HCV 61
  HDV..... 146
VX-497 220

W

Wilson’s Disease 405

Z

Zinc 413

Mauss - Berg - Rockstroh - Sarrazin - Wedemeyer

HEPATOLOGY

A clinical textbook

SECOND EDITION

Hepatology is a rapidly developing area of medicine. Breakthroughs in the treatment of chronic hepatitis B, the exciting new approaches to treat chronic hepatitis C and the identification of genes associated with metabolic liver diseases are just some highlights. *Hepatology - A clinical textbook* is an up-to-date source of information for physicians, residents and advanced medical students seeking a broader understanding of liver disease. *Hepatology - A clinical textbook* is also available for free at **www.hepatologytextbook.com**.