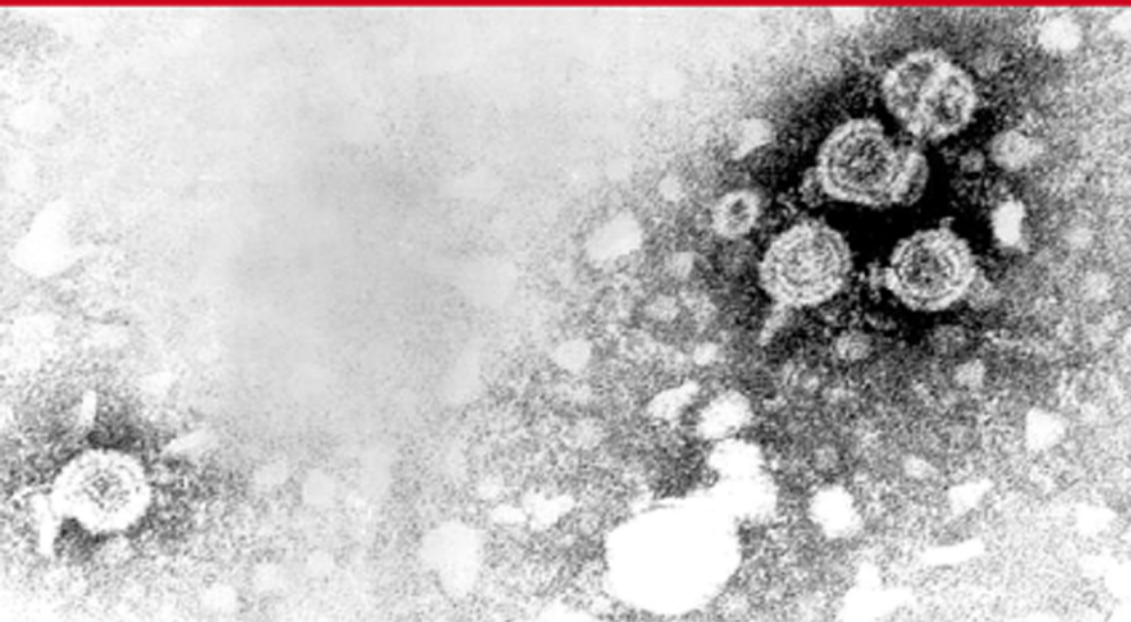


Mauss - Berg - Rockstroh - Sarrazin - Wedemeyer



# HEPATOLOGY

**A clinical textbook**

[www.HepatologyTextbook.com](http://www.HepatologyTextbook.com)

**Flying Publisher**

This textbook was made possible through  
unrestricted educational grants from  
Hoffmann–La Roche, Germany – [www.Roche.com](http://www.Roche.com)  
Gilead Sciences, Germany – [www.Gilead.com](http://www.Gilead.com)

# Hepatology 2009

[www.HepatologyTextbook.com](http://www.HepatologyTextbook.com)

Editors

Stefan Mauss

Thomas Berg

Juergen Rockstroh

Christoph Sarrazin

Heiner Wedemeyer

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.

© 2009 by Mauss, Berg, Rockstroh, Sarrazin, Wedemeyer

D-40237 Duesseldorf

Printed in Germany

ISBN: 978-3-924774-63-9

English language and style: Rob Camp

Layout: Ismael Sala Salas

## Preface

Hepatology is a rapidly evolving medical field that will continue to grow and maintain our excitement over the next few decades. Viral hepatitis is not unlike HIV was 10 or 15 years ago when the retrovirus began to be better understood and treatable. Today, hepatitis B viral replication can be suppressed by potent antiviral drugs, although there are risks regarding 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., hereditary and metabolic 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 summarise 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 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 or redundant. I am convinced that all five will remain very active in the field and will update this book regularly as the science progresses. This e-book should rapidly become an international standard.

Stefan Zeuzem

Frankfurt, 24 January 2009



## Contributing Authors

### **Fernando Agüero**

Infectious Diseases Service  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
colachito4@hotmail.com

### **Susanne Beckebaum**

Interdisciplinary Liver Transplant Unit  
University Hospital Essen  
Operatives Zentrum II  
Ebene A1  
Hufelandstr. 55  
45122 Essen, Germany  
susanne.beckebaum@uni-due.de

### **Thomas Berg**

Charité, Campus Virchow-Klinikum,  
Universitätsmedizin  
Medizinische Klinik m. S. Hepatologie  
und Gastroenterologie  
Augustenburger Platz 1  
13353 Berlin, Germany  
thomas.berg@charite.de

### **Florian van Bömmel**

Charité, Campus Virchow-Klinikum,  
Universitätsmedizin  
Medizinische Klinik m. S. Hepatologie  
und Gastroenterologie  
Augustenburger Platz 1  
13353 Berlin, Germany

### **Carlos Cervera**

Infectious Diseases Service  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
CCERVERA@clinic.ub.es

### **Vito R. Cicinnati**

Interdisciplinary Liver Transplant Unit  
University Hospital Essen  
Operatives Zentrum II  
Ebene A1  
Hufelandstr. 55  
45122 Essen, Germany  
vito.cicinnati@uni-due.de

### **Markus Cornberg**

Dept. of Gastroenterology,  
Hepatology and Endocrinology  
Medical School of Hannover  
Carl-Neuberg-Str. 1  
30625 Hannover, Germany  
cornberg.markus@mh-hannover.de

### **Juan-Carlos García-Valdecasas**

Liver Transplant Unit, Department of  
Surgery  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
JCVALDE@clinic.ub.es

### **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  
frank.gruenhage@uks.eu

### **Bernd Kupfer**

Medizinische Universitätsklinik I  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany

### **Montserrat Laguno**

Infectious Diseases Service  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
MLAGUNO@clinic.ub.es

### **Frank Lammert**

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

## 8 Contributing Authors

### **Christian Lange**

J. W. Goethe-University Hospital  
Medizinische Klinik 1  
Theodor-Stern-Kai 7  
60590 Frankfurt am Main, Germany

### **Johannes Lenz**

Medizinische Universitätsklinik I  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany

### **Jessica Lüsebrink**

Institute of Virology  
University of Bonn Medical Centre  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany

### **Michael P. Manns**

Dept. of Gastroenterology,  
Hepatology and Endocrinology  
Medical School of Hannover  
Carl-Neuberg-Str. 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  
jmmiro@ub.edu

### **Asuncion Moreno**

Infectious Diseases Service  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
AMORENO@clinic.ub.es

### **Claus Niederau**

Katholische Kliniken Oberhausen  
gGmbH, St. Josef Hospital  
Department of Internal Medicine  
Academic Teaching Hospital of the  
University  
Duisburg-Essen  
Mülheimer Str. 83  
46045 Oberhausen, Germany  
claus.niederau@st-josef.de

### **Karl-Philipp Puchner**

Charité, Campus Virchow-Klinikum,  
Universitätsmedizin  
Medizinische Klinik m. S. Hepatologie  
und Gastroenterologie  
Augustenburger Platz 1  
13353 Berlin, Germany

### **Antonio Rimola**

Liver Transplant Unit - CIBEREHD  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
ARIMOLA@clinic.ub.es

### **J. K. Rockstroh**

Medizinische Universitätsklinik I  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany  
rockstroh@uni-bonn.de

### **Christoph Sarrazin**

J. W. Goethe-University Hospital  
Medizinische Klinik 1  
Theodor-Stern-Kai 7  
60590 Frankfurt am Main, Germany  
sarrazin@em.uni-frankfurt.de

### **Martin Schäfer**

Department of Psychiatry  
and Psychotherapy  
Kliniken Essen-Mitte  
Ev. Huysenstift  
Henricistraße 92  
45136 Essen, Germany



**Carolynne Schwarze-Zander**

Immunologische Ambulanz  
Medizinische Klinik I  
Sigmund-Freud-Str. 25  
53105 Bonn  
Carolynne.Schwarze-  
Zander@ukb.uni-bonn.de

**Oliver Schildgen**

Institute of Virology  
University of Bonn Medical Centre  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany  
schildgen@virology-bonn.de

**Verena Schildgen**

Institute of Virology  
University of Bonn Medical Centre  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany

**Ulrich Spengler**

Department of Internal Medicine 1  
University Hospitals of Bonn  
University  
Sigmund-Freud-Strasse 25  
53105 Bonn, Germany

**Christian P. Strassburg**

Klinik für Gastroenterologie,  
Hepatology und Endokrinologie  
Medizinische Hochschule Hannover  
Carl-Neuberg-Str. 1  
30625 Hannover  
strassburg.christian@mh-  
hannover.de

**Montserrat Tuset**

Pharmacy Department  
Hospital Clínic - IDIBAPS  
University of Barcelona  
Villarroel, 170  
08036 - Barcelona, Spain  
mtuset@clinic.ub.es

**Jan-Christian Wasmuth**

Medizinische Klinik und Poliklinik I  
Universitätsklinikum Bonn  
Sigmund-Freud-Str. 25  
53105 Bonn, Germany  
j-c.wasmuth@uni-bonn.de

**Heiner Wedemeyer**

Dept. of Gastroenterology,  
Hepatology and Endocrinology  
Medical School of Hannover  
Carl-Neuberg-Str. 1  
30625 Hannover, Germany  
wedemeyer.heiner@mh-hannover.de

**Stefan Zeuzem**

J. W. Goethe-University Hospital  
Medizinische Klinik 1  
Theodor-Stern-Kai 7  
60590 Frankfurt am Main, Germany



# Content

## Part 1

### Basics

|   |           |
|---|-----------|
| <b>Chapter 1: Hepatitis A - Epidemiology, transmission and natural history ....</b> | <b>21</b> |
| Genomic Organisation.....   | 21        |
| Epidemiology .....  | 21        |
| Transmission.....   | 21        |
| Clinical course.....  | 21        |
| Clinical Presentation.....  | 22        |
| Extrahepatic manifestations .....   | 22        |
| Laboratory findings .....   | 23        |
| Diagnosis .....   | 23        |
| Treatment.....  | 23        |
| References .....  | 24        |
| <br>  |           |
| <b>Chapter 2: Hepatitis B - Epidemiology, transmission and natural history ....</b> | <b>25</b> |
| Introduction .....  | 25        |
| Transmission.....   | 26        |
| Natural history and clinical manifestations .....                                   | 29        |
| References .....  | 34        |
| <br>  |           |
| <b>Chapter 3: Hepatitis C - Epidemiology, transmission and natural history ....</b> | <b>37</b> |
| Epidemiology .....  | 37        |
| Transmission.....   | 37        |
| Clinical manifestations and natural history of HCV infection.....                   | 40        |
| Acute hepatitis.....  | 40        |
| Chronic hepatitis C .....   | 41        |
| Natural history .....   | 42        |
| Cirrhosis and hepatic decompensation .....  | 42        |
| References .....  | 46        |
| <br>  |           |
| <b>Chapter 4: Hepatitis E – Epidemiology, transmission and natural history....</b>  | <b>49</b> |
| Introduction .....  | 49        |
| Epidemiology and Transmission .....   | 49        |
| Clinical features.....  | 50        |
| Diagnosis .....   | 51        |
| Pregnancy .....   | 51        |
| Treatment.....  | 51        |

12 Content

**Chapter 5: HBV - Virology.....55**  
Introduction .....55  
Taxonomic classification of the *Hepadnaviridae* .....55  
Structure of virus particles and organization of the viral genome .....57  
The HBV replication cycle.....59  
Pathogenesis of hepadnavirus infections.....61  
Animal models for HBV infections .....62  
Cell culture models for *in vitro* phenotyping .....64  
References .....65

**Chapter 6: HCV - Virology .....75**  
History .....75  
Taxonomy and genotypes.....75  
Viral structure .....76  
Genome organization .....77  
Genes and proteins .....79  
Viral lifecycle .....83  
Model systems for HCV research .....87  
References .....88

**Chapter 7: Prophylaxis and vaccination of viral hepatitis.....99**  
Introduction .....99  
Prophylaxis of hepatitis viruses.....99  
Vaccination against hepatitis A .....101  
Vaccination against hepatitis B .....102  
Safety of HBV vaccines .....104  
Vaccination against hepatitis C .....105  
Vaccination against hepatitis E .....106  
References .....106

Part 2

Hepatitis B and D

**Chapter 8: Acute and chronic hepatitis B - Diagnostic tests.....113**  
Introduction .....113  
Aims of diagnostic tests in the management of HBV infection.....113  
Molecular assays in the diagnosis and management of HBV .....114  
Conclusion and future aspects.....116  
References .....116

|  |            |
|--|------------|
| <b>Chapter 9: HBV Treatment - Standard of care .....</b>   | <b>119</b> |
| Introduction .....   | 119        |
| Goals of antiviral therapy .....   | 121        |
| Indication for antiviral therapy .....   | 123        |
| Treatment indication: Summary of the German guidelines recommendations .....                                       | 125        |
| References .....   | 138        |
| <br>   |            |
| <b>Chapter 10: HBV - Resistance and implications for therapeutic strategies</b>                                    | <b>143</b> |
| Introduction .....   | 143        |
| Principles of antiviral HBV therapy – how to avoid resistance .....  | 144        |
| Treatment endpoints .....  | 144        |
| Resistance patterns of HBV polymerase inhibitors .....   | 145        |
| Combination therapy of chronic hepatitis B to enhance antiviral efficacy and delay development of resistance ..... | 148        |
| Management of drug resistance .....  | 149        |
| Special considerations in HIV/HBV-coinfected patients .....  | 149        |
| Immune escape and polymerase inhibitor resistance .....  | 150        |
| Conclusion .....   | 150        |
| References .....   | 151        |
| <br>   |            |
| <b>Chapter 11: Hepatitis D - Diagnostic procedures and therapy .....</b>   | <b>155</b> |
| Introduction .....   | 155        |
| Virology of Delta hepatitis .....  | 156        |
| Epidemiology of delta hepatitis .....  | 157        |
| Pathogenesis of HDV infection .....  | 159        |
| Clinical course of delta hepatitis .....   | 160        |
| Diagnosis of delta hepatitis .....   | 160        |
| Treatment of Delta Hepatitis .....   | 161        |
| References .....   | 164        |

Part 3  
Hepatitis C

**Chapter 12: Acute and chronic hepatitis C – Diagnostic tests .....171**

- Introduction .....171
- Serologic assays .....172
- Nucleic acid testing for HCV .....173
- Quantitative HCV RNA detection.....174
- HCV genotyping.....177
- Implications for diagnosing and managing acute and chronic hepatitis C.....179
- References .....180

**Chapter 13: Standard of care .....183**

- Management of acute hepatitis C .....183
- Standard therapy of chronic hepatitis C .....184
- Individualisation and optimisation strategies .....188
- Side effects and complications .....193
- Treatment of hepatitis C in special populations .....196
- Treatment in the future and the drug pipeline .....197
- Treatment of patients with previous antiviral treatment failure.....198

**Chapter 14: New agents for treatment.....211**

- Introduction .....211
- HCV life cycle and targets for STAT-C.....213
- Viral attachment and entry .....214
- HCV RNA translation and post-translational protein processing .....214
- HCV replication .....224
- Resistance to specific antivirals .....231
- Nucleoside inhibitors.....232
- Host proteins as targets in treating hepatitis C.....233
- Novel interferons .....235
- Albinterferon .....235
- IFN  $\alpha$ -2bXL .....236
- Novel ribavirin derivates .....237
- Outlook .....237
- References .....238

|  |            |
|--|------------|
| <b>Chapter 15: Management of adverse drug reactions .....</b>      | <b>245</b> |
| Introduction .....   | 245        |
| Flu-like symptoms, fever, arthralgia and myalgia.....              | 245        |
| Gastrointestinal disorders .....                                   | 246        |
| Weight loss .....  | 246        |
| Asthenia and fatigue.....  | 246        |
| Cough and dyspnoea .....   | 247        |
| Disorders of the thyroid gland.....                                | 247        |
| Psychiatric adverse events.....                                    | 247        |
| <br>   |            |
| <b>Chapter 16: Extrahepatic manifestations of chronic HCV.....</b> | <b>255</b> |
| Introduction .....   | 255        |
| Mixed cryoglobulinaemia.....                                       | 257        |
| Lymphoproliferative disorders.....                                 | 262        |
| HCV-related thrombocytopaenia .....                                | 263        |
| HCV-related autoimmune haemolytic anaemia .....                    | 264        |
| Endocrine manifestations .....                                     | 264        |
| Dermatological and miscellaneous manifestations .....              | 265        |
| References .....   | 267        |

## Part 4

### Coinfections

|   |            |
|---|------------|
| <b>Chapter 17: Management of HBV/HIV coinfection .....</b>                      | <b>275</b> |
| Introduction .....  | 275        |
| HBV therapy in HBV/HIV-coinfected patients without antiretroviral therapy ..... | 276        |
| ARV treatment of chronic hepatitis B in HBV/HIV coinfection.....                | 279        |
| Management of resistance to HBV polymerase inhibitors .....                     | 281        |
| Conclusion.....   | 281        |
| <br>  |            |
| <b>Chapter 18: Management of HCV/HIV coinfection .....</b>                      | <b>285</b> |
| Epidemiology of HIV/HCV coinfection .....                                       | 285        |
| Specific aspects concerning the diagnosis of HCV in HIV coinfection .....       | 286        |
| Natural course of hepatitis C in HIV-positive patients .....                    | 287        |
| Effect of hepatitis C on HIV infection .....                                    | 287        |
| Effect of HAART on hepatitis C.....   | 287        |
| Therapy.....  | 288        |
| Conclusion.....   | 294        |

**Chapter 19: Management of HBV/HCV coinfection.....297**  
Epidemiology of HBV/HCV coinfection.....297  
Screening for HBV/HCV coinfection .....297  
Viral interactions between HBV and HCV.....297  
Clinical scenarios of HBV and HCV infection .....298  
Treatment of HBV and HCV coinfection .....301  
Conclusion.....301  
References .....302

Part 5

Liver Fibrosis

**Chapter 20: Assessment of hepatic fibrosis in chronic viral hepatitis .....307**  
Introduction .....307  
Mechanisms of liver fibrosis in chronic viral hepatitis.....307  
Liver biopsy – the gold standard for staging of liver fibrosis.....308  
Surrogate markers of liver fibrosis in chronic viral hepatitis.....310  
Transient elastography .....311  
Other imaging techniques for the assessment of liver fibrosis .....312  
Clinical decision algorithms.....312  
Summary.....314  
References .....314

Part 6

Hepatocellular Carcinoma

**Chapter 21: Diagnosis, Prognosis & Therapy of Hepatocellular Carcinoma  
321**  
Classification of HCC .....321  
Epidemiology .....322  
Surveillance of patients at high risk and early HCC diagnosis.....322  
Diagnosis .....323  
Stage-adapted therapy for liver cancer.....324  
Prophylaxis of liver cancer.....328  
References .....329



## Part 7

### Transplantation

#### **Chapter 22: Management of patients before and after liver transplantation**

**335**

|   |     |
|---|-----|
| Introduction .....  | 335 |
| Timing and indications for liver transplantation .....              | 335 |
| Patient evaluation .....  | 337 |
| Pretransplant management issues .....                               | 338 |
| Living donor liver transplantation: .....                           | 341 |
| Indications, donor evaluation, and outcome .....                    | 341 |
| Long-term complications after liver transplantation .....           | 342 |
| Recurrent diseases after liver transplantation .....                | 349 |
| Outcome after liver transplantation for acute hepatic failure ..... | 359 |
| Conclusion .....  | 359 |
| References .....  | 361 |

#### **Chapter 23: Liver transplantation in hepatitis B and C and HIV coinfection**

**375**

|   |     |
|---|-----|
| Introduction .....  | 375 |
| Epidemiology .....  | 375 |
| Clinical features of coinfecting patients with ESLD ..... | 376 |
| Prognosis after decompensation .....                      | 376 |
| Management of cirrhosis complications .....               | 378 |
| Substance abuse .....                                     | 378 |
| HCV/HBV management .....                                  | 378 |
| ART .....   | 379 |
| OLT (Orthotopic liver transplantation) .....              | 380 |
| Liver disease criteria .....                              | 380 |
| HIV infection criteria .....                              | 380 |
| Clinical criteria .....                                   | 381 |
| Immunological criteria .....                              | 381 |
| Virologic criteria .....                                  | 382 |
| Other criteria .....                                      | 382 |
| Outcome of OLT in HIV-infected patients .....             | 382 |
| Conclusions .....   | 386 |
| References .....  | 386 |

Part 8

Autoimmune and Metabolic Liver Disease

**Chapter 24: Metabolic Liver Diseases: Haemochromatosis .....395**  
Definition and classification of iron overload diseases.....395  
Type 1 HFE haemochromatosis .....397  
Secondary haemochromatosis .....412  
References .....413

**Chapter 25: NAFLD and NASH.....419**  
Introduction .....419  
Prevalence.....419  
Demographics and risk factors .....419  
Pathogenesis .....420  
Natural history .....421  
Diagnosis .....422  
Diet and lifestyle recommendations .....424  
Pharmacological treatment .....424  
Surgery for obesity .....425  
Liver transplantation (LTX) for NASH.....425  
References .....425

**Chapter 26: Wilson’s Disease.....429**  
Introduction .....429  
Clinical Presentation.....429  
Diagnosis .....433  
Treatment.....436  
Monitoring of Treatment .....440  
References .....442

**Chapter 27: Autoimmune liver diseases: AIH, PBC and PSC.....447**  
Autoimmune hepatitis (AIH).....447  
Primary biliary cirrhosis .....470  
Primary sclerosing cholangitis .....481  
References .....486

## Part 1

# 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 Picornaviridae. 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 other types of sex like anilingus. 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) than in otherwise healthy adults (Hollinger 1996).

Persons in psychiatric institutions, 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 viraemia of HAV 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 fulmi-

nant 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 typically most intense 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).

Severe fulminant courses of HAV with hepatic failure are 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 old 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 (see 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 patients 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 disease. 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 reinfection.

## Treatment

As 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 supportive. 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 supportive therapy. These

## 24 Hepatitis A - Epidemiology, transmission and natural history

patients should be transferred to a centre that is capable of performing liver transplantation.

### 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.
- 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 [http://www.who.int/csr/disease/hepatitis/HepatitisA\\_who.cdscs.recd.2000\\_7.pdf](http://www.who.int/csr/disease/hepatitis/HepatitisA_who.cdscs.recd.2000_7.pdf).
- 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.[\*1]



## 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 Inoculation

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 (Wasley 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 unknown. 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%. How-

ever, 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 continuously 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  $\mu\text{g}$  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 to 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 than 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 coinfecting 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 angiomas, 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 positivity 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  $2 \times 10^3$  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 coinfecting 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).

## 34 Hepatitis B - Epidemiology, transmission and natural history

- **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, Garen 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.

## 36 Hepatitis B - Epidemiology, transmission and natural history

- 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):

## 38 Hepatitis C - Epidemiology, transmission and natural history

- 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 coinfecting 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%, al-

though 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 transmitting 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**

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 angiomas, 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 (Svirthli 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 (Hrabec 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.

#### 44 Hepatitis C - Epidemiology, transmission and natural history

- **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, although HCV usually predominates. Nevertheless, liver damage is usually worse and progression faster in patients with dual HBV/HCV infections.  
Around one third of patients coinfecting 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 dosage 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 mono-infection.

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%.

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 =  $10 \times ((0.957 \times \ln(\text{Creatinine})) + (0.378 \times \ln(\text{Bilirubin})) + (1.12 \times \ln(\text{INR}))) + 6.43$ . An online calculator and further information can be found at the website of The United Network for Organ Sharing (UNOS) (<http://www.unos.org>).

| 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.

## 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 - extrahepatic manifestations.

## 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 sero-conversion 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.
- Svrtlih 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.

## 48 Hepatitis C - Epidemiology, transmission and natural history

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 *Hepeviridae* (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 cynomolgus 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 24 subtypes of HEV have been identified by phylogenetic analysis of stored HEV sequences. Each genotype has a distinct geographical distribution and while genotype 1 and 2 appear to be confined to humans only, genotype 3 and 4 have also 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 of the 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 in this group. Furthermore, HEV was present in sewage samples collected in France, Spain and the United States (Buti 2003). One could conclude from these

findings that the incidence of HEV in industrialised countries may currently be underestimated.

Zoonotic transmission is also being discussed. People with occupational contact with swine in the United States (veterinarians and farmers) show high seroprevalence of anti-HEV-antibodies (Meng 2002; Karetnyi 1999). Also rodents may 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. Especially pregnant women are at high risk with the 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, alanin 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 the patients (Chau 2006).

A study from Japan compared the clinical features of patients infected with genotype 3 and 4 and indicated 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 alanin aminotransferase peak levels (median 3430 IU/L versus 1052 IU/L) and a lower trough prothrombin time (61 versus 84%) and that the median time in the hospital was longer (26.5 versus 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 people previously infected with HEV are protected during epidemics of the disease, indicating that immunity to HEV is induced and prevents re-infection (Bryan 1994).

Hepatitis E is widely accepted not to progress to a chronic infection. However, two recent reports describe patients that underwent organ transplant (liver, kidney, pancreas) and subsequent immunosuppressive therapy in which detectable levels of HEV RNA were found over an extended period of time. In 10 of the 16 patients

chronic hepatitis was found and attributed to hepatitis E virus (Haagsma 2008; Kamar 2008). It remains to be determined if there is a risk for immunosuppressed patients of developing chronic HEV infection.

## Diagnosis

Diagnosis of acute hepatitis E is based upon the detection of antibodies directed against 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 the 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 supportive therapy 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 transplantation.

## 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.
- 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.

## 52 Hepatitis E – Epidemiology, transmission and natural history

- 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.
- 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.
- 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.
- 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.
- 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.
- Karenyi 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.
- 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 hepatitis E virus (HEV) is highly specific for diagnosis of acute HEV infection. *J Clin Microbiol* 2005; 43: 49-56.
- 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.
- 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: HBV - Virology

Jessica Lüsebrink, Verena Schildgen and Oliver Schildgen

### Introduction

In 1965, Blumberg and colleagues - in search of tools to identify and track genetic differences in different human populations – found a novel antigen present in sera of Australian Aborigines (Blumberg 1965). This antigen was preliminarily named Australia antigen and was associated with a clinical course of hepatitis in the following years (Blumberg 1967; Blumberg 1968) and immediately thereafter to one specific type called serum hepatitis (Okochi 1968; Okochi 1970; Okochi 1993). Electron microscopy studies revealed that patients testing positive for the Australia antigen had two different types of particles in their serum that contained the Australia antigen, namely small particles of spherical and rod-like shapes with a diameter of around 22 nm, and the so-called Dane particles, of 42 nm (Dane 1970), which are the intrinsic infectious virus particles containing the viral genome and are named human hepatitis B virus (HBV) (Heermann 1984; Kaplan 1973; Robinson 1974; Robinson 1974; Robinson 1975b; Robinson 1975a; Robinson 1976b; Robinson 1976a). Meanwhile, it turns out that a number of HBV-like viruses exist, most of them displaying a very narrow host range; altogether, these viruses form the viral family *Hepadnaviridae*.

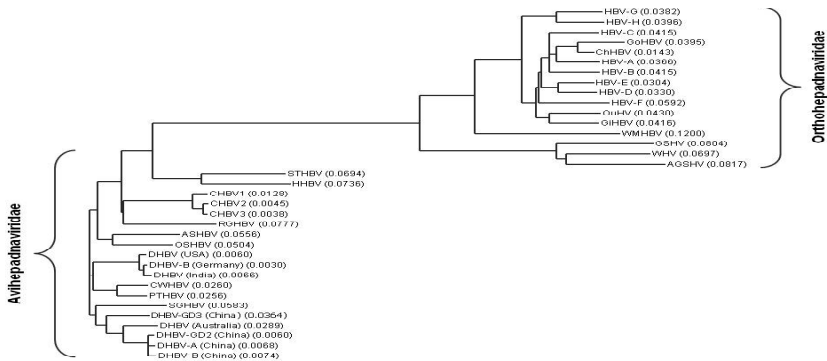
### Taxonomic classification of the *Hepadnaviridae*

The family name *Hepadnaviridae* is based on the clinical picture of infection and the target organ (liver, classical/ancient Greek: to hepar) and its nucleic acid, 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 known to date. The *Hepadnaviridae* contain one of the smallest pathogen genomes known, of just 3-3.3 kbp. The reading frames on the genome are organized in a unique and highly condensed way and overlap, which contributes to a unique replication strategy. This strategy includes a reverse transcription step that is also observed in the replication of retroviruses, but, in contrast to retroviruses, the nucleic acid packaged into hepadnaviral infectious particles is DNA, not the RNA of retroviruses.

The sub-classification into the two genera is based on the differences in the host and additionally on phylogenetic differences between mammalian and avian hepadnaviruses (Figure 1). Until now, two major species have been assigned to the avihepadnaviruses and were named after their individual hosts, namely the duck hepatitis B virus (DHBV) and the heron hepatitis B virus (HHBV). Additionally, a number of other avihepadnaviruses have been described that have not been specifically categorized (Guo 2005). On the other hand, the orthohepadnavirus genus includes the four best-known distinct species - HBV, WHV, GSHV and WMHV. The prototype species is the human hepatitis B virus (HBV) that infects humans and can be used to experimentally infect chimpanzees. WHV, the woodchuck hepatitis virus, is a well-studied orthohepadnavirus that occurs naturally in marmots and cannot be trans-

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



**Figure 1 .The figure shows 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, OSHVB=Orinoco sheldgoose HBV, OuHV=Orangutan hepadnavirus, PTHBV=puna teal HBV, RGHVB=Ross' goose HBV, SGHBV=snow goose HBV, STHBV=storck HBV, WHV=woodchuck hepatitis virus, WMHBV=woolly monkey HBV



## Structure of virus particles and organization of the viral genome

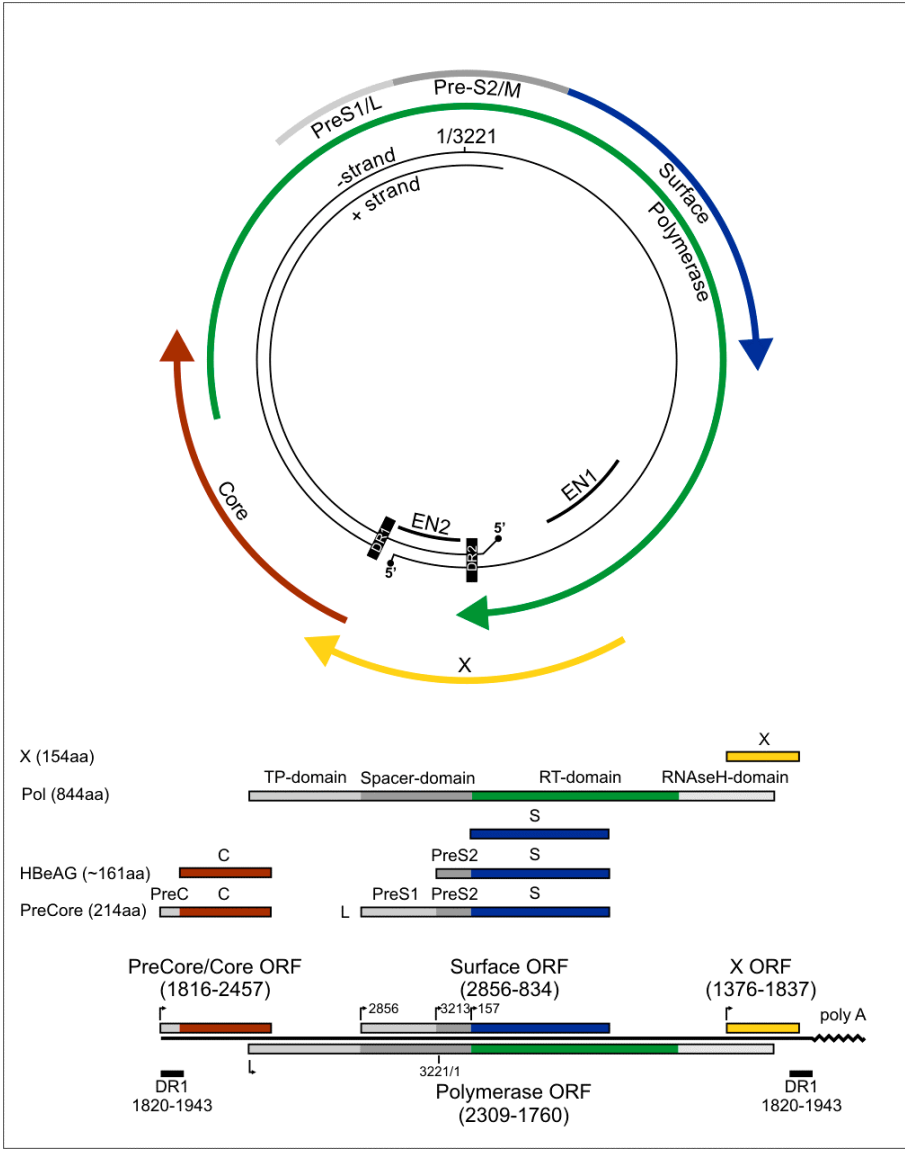
The *Hepadnaviridae* are enveloped DNA viruses with a circular partially double-stranded DNA that in concert with the core protein forms the nucleocapsid. The infectious virus, i.e., the Dane particle, displays a spherical shape with a diameter of 42–47 nm. The viral membrane that is acquired by the virus during budding or while the viral particles are transported through secretory pathways via the endoplasmic reticulum and Golgi pathways forms the surface containing three viral surface proteins. These proteins, according to their size, named HB small surface antigen (HBsAg), middle (HBMAg), or large (HBLAg), are acquired during budding into the endoplasmic reticulum (ER).

The nucleocapsid, which forms the inner part of the Dane particle, is around 28 nm in size and besides a single copy of viral genome contains the viral polymerase, which is covalently bound to the viral genome, and in turn leads to problems in the molecular diagnostics of HBV infections (see Chapter 8). As with nearly all enveloped viruses there is also evidence that the HBV particle contains proteins assumed to be of host origin (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 are in an identical orientation and overlap at least partially. Within the Dane particle the negative strand of the viral genome is present in full-length, thus carrying the whole genome. In contrast, the positive strand spans only  $\sim 2/3$  of the genome in length, whilst its 3'-end is variable in size (Lutwick 1977; Summers 1975). The viral polymerase is covalently bound to the negative strand by a phosphotyrosine bond. At the 5'-end of the positive strand a short RNA oligomer originating from the pre-genomic (pg) RNA residually remains bound covalently after the viral DNA synthesis. The negative strand, in contrast to the positive strand, contains on both the 5'-end and the 3'-end a small redundancy of 8–9 nucleotides in length, named the r-region. These redundant structures are essential for viral replication (Seeger 1986; Will 1987; Lien 1986a; Lien 1987).

The viral genome covers four open reading frames, all of them encoded by the negative 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 (Enders 1985; Cattaneo 1984). Moreover, a 0.7 kb long mRNA termed X mRNA occurs 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 the e-antigen, the polymerase, the three surface proteins, and the X protein. Whilst the core protein – that is also recognized by the immune system – is essential for the formation of nucleocapsids, the e-antigen that also contains the full core gene, is post-translationally processed and as a non-essential gene is important in the viral-host immunity interaction. E-antigen is also a marker for active viral replication and plays an important role in molecular diagnostics (Chen 2004).



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

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; the terminal protein (TP) is located at its N-terminal domain, acting as a primer in negative-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, one for M: preS2, and one for S: preS3) and overlaps with the polymerase open reading frame (Seeger 2007). So far, the role of the X protein is not fully understood, although it has been associated with the nucleus and the cytoskeleton (Doria 1995; Henkler 2001; Lara-Pezzi 2001). However, HBX (hepatitis B X protein) is required for efficient infection *in vivo* (Zhang 2001; Zoulim 1994).

## 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. Either 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 executed, or studies are limited to small series of experiments with primary permissive hepatocytes (Tuttleman 1986; Aldrich 1989; Ochiya 1989; Gripon 1993; Gripon 1988). Unfortunately, primary hepatocytes remain permissive for only a short time after being harvested from the intact liver.

Yet it is assumed that viral entry and the host range of hepadnavirus is dependent on the N-terminus of the large surface antigen (Ishikawa 1995; Chouteau 2001; Lambert 1990; Gripon 2005; Urban 2002). So far, the intrinsic HBV receptor has not been discovered, but from studies on DHBV in primary duck hepatocytes it is assumed that around  $10^4$  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 (Pugh 1989; Klingmuller 1993; Pugh 1995; Rigg 1992; Hagelstein 1997; Kock 1996). 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 core 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, acts chemically and structurally as 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 sites for liver-specific transcription factors (Schaller & Fischer 1991; Lopez-Cabrera 1991; Lopez-Cabrera 1990; Guo 1993; Courtois 1987; Raney 1995). Nevertheless, although a number of factors and interactions regulating viral transcription are known, the exact mechanisms of HBV transcription remains unclear. However, it is known that viral transcription occurs in the nucleus, and both messenger and pregenomic RNAs are transported into the cytoplasm where they are respectively translated or used as the template for progeny genome production.

In the cytoplasm, the core protein which itself can be phosphorylated by several kinases, forms the basis for the nucleocapsid. It 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 (Liao 1995; Lan 1999; Gerlich 1982; Kann 1993; Kau 1998; Daub 2002; 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 as yet 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 than in the cases of the L and M protein. All proteins have in common the S domain; the M additionally has the pre-S2 domain; the L has both the pre-S2 and the pre-S1 domain (Figure 2). The surface proteins of mammalian *Hepadnaviridae* have been shown to be N- and O-glycosylated (Schildgen 2004; Lu 2003; Block 1998; Block 1994; Schmitt 2004; Schmitt 1999). 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 (Schildgen 2004; Lu 2003; Block 1998; Block 1994; Schmitt 2004; Schmitt 1999). Moreover, the surface proteins have been demonstrated to be activators of transcription by acting individually (Kekule 1990; Caselmann 1990).

The viral polymerase, the only 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 from the polymerase domains. The terminal protein also serves as a primer for reverse transcription (Wang 1992; Weber 1994; Lanford 1997). Before or during formation of the cccDNA the terminal protein and 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 via a not fully understood mechanism, most probably dependent on cellular ligases and maybe other 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 also is the template for the pre-genomic RNA (pgRNA). This RNA is both the template for core and polymerase protein translation and is the matrix for the progeny genomes as well. The pgRNA bears a secondary structure - named  $\epsilon$ -structure - that is present at both the 5'- and the 3'-ends. The  $\epsilon$ -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 negative 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) (Wang 1992; Weber 1994; Lanford 1999; Zoulim 1994). The next few nucleotides following this initial dGMP complement a small part of the  $\epsilon$ -structure. The small terminal protein bound primer is subsequently translocated to the 3'-end by an unknown mechanism but remains covalently bound the whole time. This process is possibly a prerequisite for the correct folding of the progeny genome within the newly forming nucleocapsid. Finally, the negative strand is fully synthesized by the reverse transcription reaction while the RNA is degraded by the RNaseH activity of the enzyme. The following positive strand synthesis is initiated by an 18mer capped RNA oligo that remains from the 5'-end of the pgRNA (Lien 1986b; Loeb 1991). Nevertheless, it is assumed that, although

not actively replicating and with conflicting data on its stability, there is evidence that cccDNA may be stable in infected hepatocytes, thus contributing to chronic HBV infection. This points to another possible target, long-term therapies that support the elimination of cccDNA positive cells.

The final replication step, the assembly and release of HBV Dane particles, is not fully understood; one study on usage of glycosylation inhibitors at non-toxic doses suppressing the viremia in WHV infected woodchucks 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 vertically and horizontally via exchange of body fluids. In serum, a maximum of  $10^{10}$  to  $10^{12}$  genome copies per ml serum or body fluid can be found. In chronic infections, the viremia is subject to natural fluctuations of  $\pm$  one  $\log_{10}$  (Schildgen 2006).

The rate for chronicity, depending on the study, is  $>90\%$  in neonatal infections and approximately 10-15% in adult infections. The risk for transfusion-acquired and nosocomial infections in the past two decades has decreased due to optimized molecular diagnostics and more strict hygiene and legal regulations; however, there is still a remarkable number of such transmissions due to incautious behavior of healthcare personnel.

Once having entered the host, HBV 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, 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, and in the worst and untreated cases to liver cirrhosis (Pinzani 1995; Mathew 1996; Papatheodoridis 2005; Yoshida 2004; Rockey 2005; Liaw 2004; Rizzetto 2005; Maynard 2005).

In this context it is worthwhile noting that there is no evidence that HBV is cytotoxic for the infected hepatocyte. In contrast to other viruses that can infect the liver like herpes simplex virus (HSV), HBV is unable to induce cytopathic effects under normal infection conditions (Jilbert 1992; Kajino 1994; Wieland 2004; Thimme 2003). Liver damage (fibrosis, cirrhosis, and probably hepatocellular carcinoma) is believed to be induced by the ongoing immune reaction and a consistent inflammation of the liver.

Consequently, and confirmed by experimental data (Ando 1994; Guidotti 1994a; Guidotti 1994b; Guidotti 1996; Guidotti 1999a; Guidotti 1999b; Guidotti 2000; Kakimi 2001; Tsui 1995), it is generally assumed that massive CTL and NK-T cell action resulting in killing of infected hepatocytes is essential for elimination of the infection. It is further assumed that in those cases in which a chronicity of infection evolves, the initial cellular immune response is too weak and thus not sufficient to control the infection (Ganem 2004). Until now it has remained unclear which mechanisms are responsible for the passage from the acute phase to the chronic phase of the 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, other interferon gamma like IL-12, IL-15, etc.) is involved in suppression of transient infections (Seeger 2007).

Despite the fact that only antibodies against the S protein are neutralizing and are the major marker for immunity, it has been 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 (Schultz 1999; Pasquetto 2002; Schultz 1999). However, this does not explain why antibodies against HBsAg are present only in those patients who clear the virus; it is assumed to be a continuous control of the infection. cccDNA can be found in those patients for decades (Maynard 2005; Werle-Lapostolle 2004), whereas the above described mechanisms fail if the infection passes to the 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 viral infection. Unfortunately, due to its narrow host range this option is limited when studying HBV, because HBV refuses to replicate other than in primary hepatocytes. Consequently it has been attempted by researchers all over the world to establish animal models and cell culture systems that at least partially reproduce some stages of HBV 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 (Kim 2008; Komiya 2008; Murray 2005; Sallberg 1998; Pride 1998; Ogata 1993; Wahl 1989; Lubeck 1989; Sureau 1988; Acs 1987; Will 1983). Fortunately, for ethical, economic and scientific reasons, experiments with chimpanzees have been nearly eliminated.

### **Woodchucks and squirrels**

The woodchuck turned out to be a model for HBV infections by a lucky chance at the end of the 1970s. In the Philadelphia Zoo, where the Penrose Research Laboratory was located, it was observed that woodchucks captured in the mid-Atlantic states of the US and housed in the Philadelphia Zoo frequently suffered from hepatocellular carcinoma (Summers 1978). In contrast, in the woodchuck population trapped in New York and its countryside no hepatomas were observed. The hepatocellular carcinoma was associated with an HBV-like virus, termed woodchuck hepatitis virus (WHV). WHV surprisingly cannot infect European marmots. The woodchuck and its woodchuck hepatitis virus is an accepted model for HBV for preclinical testing for novel antiviral drugs.

In spite of a number of advantages the woodchuck model is not very widespread. Despite some successful attempts to breed woodchucks under laboratory conditions

as done at Cornell University, where a woodchuck colony is housed, most laboratories have failed to breed or only by chance have managed to breed a limited number of woodchucks. 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 permission, at least in Europe, independent of the fact that these animals may be captured as agrarian varmints, thus resulting in an overwhelming bureaucracy with customs and local legal authorities. Moreover, hibernation may influence experiments, especially if after being captured woodchucks remain in this mode. Finally, as wild captured woodchucks weigh up to 7 kg and are not willing to assist the researcher in the planned experiments, they have to be anesthetized before any manipulation is performed. However, the number of secondary reagents needed for woodchuck research, though not commercially available, is increasing and will be a useful tool in 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 (Marion 1983; Marion 1983; Marion 1986; Cullen 1996).

## Ducks

Within the genus of avihepadnaviruses, the duck hepatitis virus that infects the domestic duck was the first species 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 aspect of orthohepadnavirus infections can be studied with this model, the model has 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 chronic infection.

Moreover, up to 50% of ducks develop a liver disease unrelated to DHBV that may overlap DHBV-induced 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 (e.g., Chen 2007; Foster 2005; Le Guerhier 2003; Deres 2003; Kumar 2002; Delmas 2002; Kumar 2001; Kumar 2001; Kumar 2001; Seigner 2001; Peek 2001; Chu 1998; Xin 1998; Seifer 1998; Offensperger 1996; Hafkemeyer 1996; Lofgren 1996; Heijntink 1993) it has to be kept in mind that birds do have a biology that differs from mammalian biology in many aspects, a problem that has been lost sight of in the past.

## Mouse models

Although some important aspects of HBV infections have been investigated in transgenic mice and have led to results convincing the majority of HBV researchers, these results have to be handled with care. In transgenic mice expression of viral antigens is possible but does not necessarily reflect the situation of a natural

infection, thus some of the observed aspects may need to be considered 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-characterized *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 to hepatitis B and C viruses (Dandri 2001a; Dandri 2001b; Mercer 2001). The major advantage of this model is that primary human hepatocytes remain susceptible to HBV for a long time, but unfortunately, these models require extremely well-controlled breeding conditions that limit their broad use.

## Tupaia

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

## 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 clonal 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 or 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 that is 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 that are 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 play a role in 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). The latter methods are more laborious, but take whole genome variability into account and allow cross-resistance testing against various drugs (Delaney 2001; Zoulim 2006). Although transfection or



transduction of mutant HBV genomes allows their 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 most important to note that there is no standardization of the methods used worldwide, thus it is very difficult to compare the level of resistance caused by the individually tested mutations in a quantitative manner.

## 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 U S A* 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.
- 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.
- 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-butyldeoxyjirimycin. *Proc Natl Acad Sci U S A* 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 N Y Acad Med* 1968; 44(12):1566-1586.
- 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.
- 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 U S A* 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 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 U S A* 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.
- 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.
- 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.
- Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet* 1970; 1(7649):695-698.
- 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.
- 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.
- 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.
- 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.
- 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 U S A* 1994; 91(9):3764-3768.
- Guidotti LG, Borrow P, Brown A, McClary H, Koch R, Chisari FV. Noncytotoxic 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.
- 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.
- 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.
- Heijntink 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 well 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 U S A* 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 U S A* 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.
- 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.
- 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.
- 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 U S A* 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.
- 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.
- Liau 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.
- 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.
- 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.
- 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 U S A* 1990; 87(13):5069-5073.

## 70 HBV - Virology

- 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 U S A* 1989; 86(17):6763-6767.
- 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 U S A* 1980; 77(5):2941-2945.
- Marion PL, Robinson WS. Hepadna viruses: hepatitis B and related viruses. *Curr Top Microbiol Immunol* 1983; 105:99-121.
- 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.
- 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 U S A* 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 U S A* 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.
- 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.
- 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 U S A* 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.
- 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.
- 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.
- 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.
- Robinson WS, Lutwick LI. The virus of hepatitis, type B (first of two parts). *N Engl J Med* 1976; 295(21):1168-1175.
- Robinson WS, Lutwick LI. The virus of hepatitis, type B. (Second of two parts). *N Engl J Med* 1976; 295(22):1232-1236.

## 72 HBV - Virology

- 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.
- 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.
- 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.
- Seignerres 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.
- 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.
- 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 U S A* 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 U S A* 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-Treppo L, Tekaija 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, Ghayeb 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.
- 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 U S A* 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.
- 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.
- Werte-Lapostolle B, Bowden S, Locarnini S, Wurstthorn 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 U S A* 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.
- 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.

## 74 HBV - Virology

- 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.
- 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.
- 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 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 and classified, 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 of a separate genus distinct from the genera 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-infected individual increases significantly over time resulting in the development of different 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). The 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 the 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 alfa 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 a sufficient number of 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 Huh-7 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 infec-

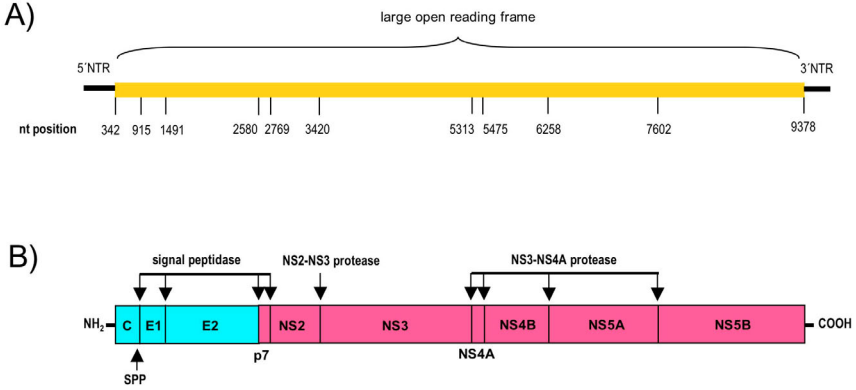
tious viral particles are produced using this otherwise powerful tool. Thus, it cannot be used for structural analysis of free virions.

Only recently have infectious HCV particles 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 which itself 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]) has been 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-infected subjects indicates that the protein is expressed during infection *in vivo* (Walewski 2001; Komurian-Pradel 2004).



**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 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 termed 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 vaccines.

The 5'NTR is approximately 341 nucleotides long and it has a complex secondary structure with 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 different 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 following 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                  | No. 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 the 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 posttranslationally (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 their 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 (SR-BI) (Scarselli 2002; Kapadia 2007), 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 exposition 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 inducing 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 for design of antiviral treatments.

The C-terminal portion of NS3 (442 aa) has ATPase/helicase activity, i.e., it catalyses 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, respectively. In addition, a nucleotide-binding motif (aa 129-134) has been identified (Einaav 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- $\alpha$  sensitivity-determining region (ISDR) that plays a significant role in the response to IFN- $\alpha$ -based therapy (Enomoto 1995; Enomoto 1996). An increasing number of mutations within the ISDR showed positive correlation with sustained virological response to IFN- $\alpha$ -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, whereas 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.

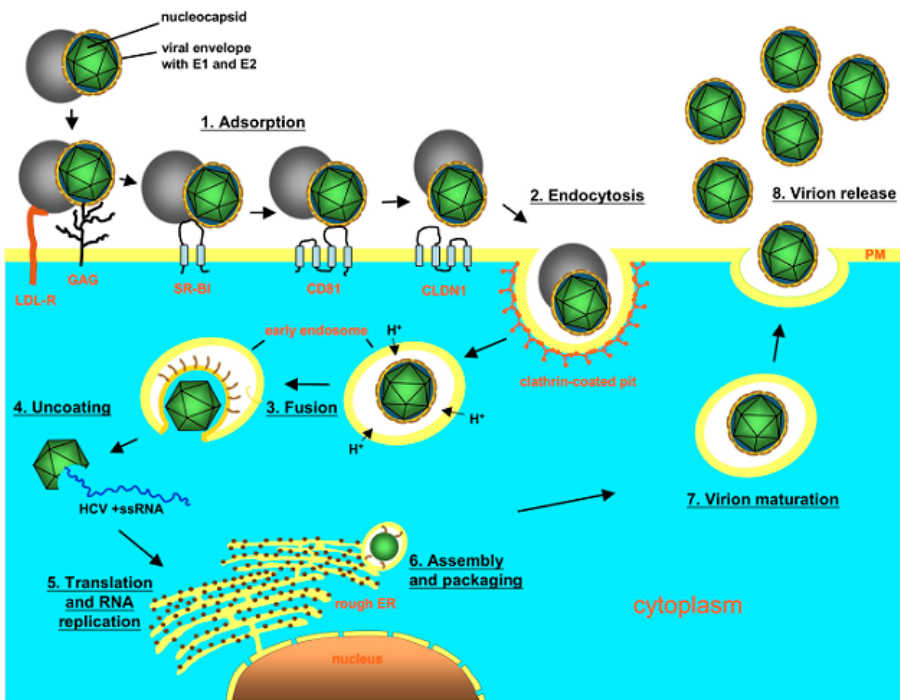
Similar to other RNA-dependent polymerases, NS5B is an error-prone enzyme that incorporates wrong ribonucleotides at a rate of approximately  $10^{-3}$  per nucleotide per generation. Unlike cellular polymerases, the viral NS5B lacks a proof-reading mechanism leading to the conservation of misincorporated ribonucleotides. These enzyme properties together with the high rate of viral replication promote 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-infected 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 red. For a detailed illustration of viral translation and RNA replication, see Pawlotsky 2007. Abbreviations: 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 to be a 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 PBMC. Experimental binding of anti-CD81 antibodies to CD81 were reported to inhibit HCV entry into Huh-7 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 were used to 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 glycosaminoglycan 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 HCV E2/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 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). Very recently, 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 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 remain to be investigated more in depth. 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).

### Translation and posttranslational processes.

As a result of the fusion of the viral envelope and the endosomal 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 build an internal ribosome entry site (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 to 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.**

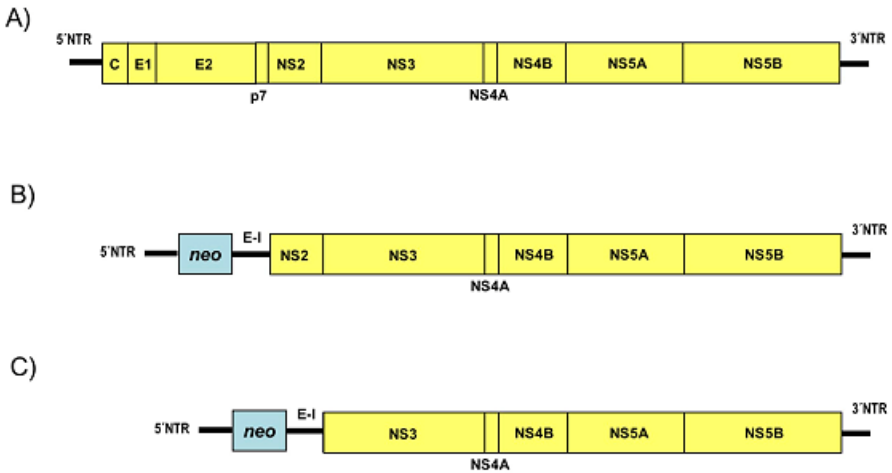
The complex process of HCV RNA replication is poorly understood. The key enzyme for viral RNA replication is NS5B an RNA-dependent RNA polymerase (RdRp) of HCV (Behrens 1996). In addition, several cellular as well as viral factors have been reported to be part of the HCV RNA replication complex. One 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. In order to facilitate synthesis of minus-strand RNA the NS3 helicase is assumed to unwind putative secondary structures of the template 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 positive-stranded RNA. The resulting sense RNA could subsequently be used 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 restricted due to a lack of small animal models and efficient cell culture systems. The development of the first HCV replicon system 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). Using total RNA derived from the explanted liver of an individual chronically infected with HCV genotype 1b, the authors amplified and cloned the entire HCV ORF sequence in two overlapping fragments. The flanking NTRs were amplified and cloned separately and all fragments were assembled to 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, the authors generated 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 (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 Huh-7 (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 Huh-7 clones can replicate replicon-specific RNA, however, 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 Huh-7 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.

In more recent years, 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 achieved 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 Huh-7, such as HepG2 or HeLa were transfected efficiently with transcripts derived from the JFH-1 replicon (Date 2004; Kato 2005).

Transfection of Huh-7 and "cured" Huh-7.5 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. 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. Huh-7 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 since it is now possible to investigate the whole viral life-cycle from virus 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, Hourieux 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 serine-type 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 co-receptors 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: physicochemical 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, Stump 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, Duvert 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.
- Duvert 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.
- Duvert 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 Natl 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, Hiragi 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 Natl 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 glycosaminoglycans 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, Stamatakis 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.
- 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 Natl 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 nonhuman 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.
- 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, 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.
- Miyazawa 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.
- Nahmias Y, 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.
- 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 Beecq 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.
- 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, Stamatakis 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.
- 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, 277:17713-17721.
- 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.
- 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 pre-penelin-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.
- Wölk B, Sansonno D, Kräusslich HG, et al. Subcellular localization, stability, and trans-cleavage 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.
- 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 (Hammit 2008). Patients with chronic liver disease do respond to vaccination but may display lower anti-HAV titers (Keefe 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 (Hammit 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](http://www.who.int); accessed Nov 12<sup>th</sup> 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, 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 ambulance services, police officers, social workers, and prison staff who have contact with drug addicts;

- 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 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 intradermal 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 profes-

sionals 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 section. 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 reinfections 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 consistently 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 frequencies 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 humoral 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 humoral 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, S.M., Hernan, M.A., Olek, M.J., Coplan, P.M., Brodovicz, K., and Walker, A.M. (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](http://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.



## 108 Prophylaxis and vaccination of viral hepatitis

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 seroconversion? *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., Patel, A.H., Meisel, H., Baumert, J., Viazov, S., Rispeter, K., Blum, H.E., Roggendorf, M., and Baumert, T.F. (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., Babudieri, S., Toti, M., Francavilla, R., Ancarani, F., Antonucci, G., Scotto, G., Di, M., V, Pastore, G., and Stroffolini, T. (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., Myint, K.S., Fourneau, M., Kuschner, R.A., Shrestha, S.K., David, M.P., Seriwatana, J., Vaughn, D.W., Safary, A., Endy, T.P., and Innis, B.L. (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., Kahn, A., Jeffries, D., Akum, A.A., Hall, A.J., Bah, E., McConkey, S.J., Hainaut, P., and Whittle, H.C. (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., Rotskams,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., Cuccia,M., Mangione,R., Marrone,F., Negrone,F.S., Parlato,A., Zamparo,E., Zotti,C., Stroffolini,T., and Mele,A. (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: Acute and chronic hepatitis B - Diagnostic tests

Oliver Schildgen

### Introduction

Over the last three decades, laboratory diagnostics of viral infections have become influenced more and more by molecular biology, the field of technology that has grown the fastest in this same 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 Chapter 6, 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 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 diagnostic tests in the management of HBV infection

The first test of an HBV infection is to diagnose whether it is acute or chronic. As a standard procedure, the patient with an 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 has a past, currently inactive HBV infection. In case of negative anti-HBcAg antibodies and the presence of anti-HBsAg antibodies the individual has been successfully vaccinated against HBV.

Based on these initial serologic diagnostics, other efforts to define the status of the infection are made. An anti-HBcAg positive but anti-HBsAg negative patient may be chronically infected with a pre-core mutant HBV or may have low level replication with a wild type HBV. 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 only expressed in case of an acute and/or ongoing infection with active replication. Unfortunately, so-called pre-core mutants exist that display active replication without expressing HBeAg while bearing a high risk for progression to cirrhosis and hepatocellular carcinoma (HCC). It is worth noting that HBeAg seroconversion occurs in up to 98% of subjects, and this is not a marker for a cure as it would be in wild type HBV, although it does act as a marker for healing.

After serologic screening is completed and a replicative HBV infection is assumed, the more expensive molecular methods such as HBV DNA are performed. This is generally to decide on whether to start treatment, to monitor treatment efficacy and treatment adherence, to identify resistant strains, and to identify pre-core 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 (Cornberg 2007; 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 to be important for deciding on initiation of treatment or changes to the drug regimen of the patient. Furthermore, sensitive methods for quantification are needed for detection of low-level viremia in patients infected with strains that are of high risk for the development of hepatocellular carcinoma such as HBeAg negative strains with the precore mutation.

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 (Cornberg 2007). Also, in HbeAg-negative chronic hepatitis B virus infections, HBV DNA is the only marker that needs to be monitored (Manesis 2003; Zacharakis 2005).

Furthermore, qualitative and quantitative measurement of viral DNA is important for monitoring another condition, occult hepatitis. This is characterized as HBV infection with measurable 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 while on HBV therapy or to monitor chronic HBV infection (de Franchis 2003; Liaw 2005).

Furthermore, the measurement of viral load after the onset of therapy is a useful and standard tool to identify 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 Tillmann 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, while not a standard procedure in the clinic, can be useful. First, viral genotype may influence success of the therapy, e.g., patients with an HBV genotype A infection have a better chance of a more favorable outcome than those infected with genotype non-A (Chen 2004; Colombo 2003; Enomoto 2006; Erhardt 2005; Flink 2006; Fung 2004; Guettouche 2005; Kao 2002; Kao 2003; Kao 2000; Kobayashi 2002; Liu 2002; Peters 2004; Sanchez-Tapias 2002; Zhang 1996).

Second, genotyping is the simplest method for identification of resistance mutations that are associated with non-response to nucleoside and nucleotide analogues. This may guide the decision on how to switch therapy, because cross-resistance between HBV polymerase inhibitors plays an important role (Schildgen 2004; Schildgen 2006; Sirma 2007).

Third, genotyping plays an important role in the identification of chains of infection in a nosocomial setting or if transmission by blood donations or blood products has occurred. Genotyping can be performed by in-house or commercial full genome sequencing. PCR followed by INNO-LiPA hybridisation has recently been developed and covers pre-identified mutations. Regular updates of the test are mandatory as new mutations are identified; newly found mutations will not be always detectable, in contrast to full genome sequencing (Hussain 2003; Hussain 2006; Osioy 2003; Osioy 2006). INNO-LiPA has the major advantage of 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 in 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 be assumed that the problem of antiviral resistance observed in HBV will become as complicated as what is happening today in HIV treatment. After genotypic analysis, 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 the virologist is when 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 contribute to therapeutic failure.

In that case, *in vitro* phenotyping procedures established in a rather small number of HBV laboratories (see Chapter 6) need to be performed. Unfortunately, known mutations can be detected and classified by commercial methods whereas novel mutations remain speculative; in phenotyping, these possibly important mutations will remain undetected or underestimated. However, it will probably be helpful to make use of novel e-learning database solutions that can help in the interpretation of sequencing results

## 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, together with anti-HBeAg antibodies and the evidence for liver injury measured by elevated liver enzymes, non-invasive fibrosis tests or histopathological findings. Assays are commercially available in formats of PCR sequencing and hybridisation, INNO-LiPA hybridisation and sequencing, as well as the Affigene HBV mutant VL19 test (Olivero 2006; Qutub 2006).

## Conclusion and future aspects

The major challenge for HBV diagnostics in the future will be the increasing number of resistance mutations and the immune escape mutants, occult hepatitis and HBeAg-negative chronic hepatitis. Novel tools such as the virtual phenotype based on database interpretation of genotype results as established for HIV resistance interpretation may help in the interpretation of laboratory results. However, a downside of the rapid increase in these sophisticated diagnostic tools are the costs for these tests which may help guide treatment decisions and avoid suboptimal HBV therapy.

## References

- 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: upgrade of the guideline. *Z Gastroenterol*. 2007; 45(6): 525-74.
- Chen, J. D., Liu, C. J., Lee, P. H., Chen, P. J., Lai, M. Y., Kao, J. H., and Chen, D. S. (2004). Hepatitis B genotypes correlate with tumor recurrence after curative resection of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2(1), 64-71.
- Colombo, M., Rumi, M. G., and Ninno, E. D. (2003). Treatment of chronic hepatitis C in Europe. *J Hepatobiliary Pancreat Surg* 10(2), 168-71.
- Conjeevaram, H. S., and Lok, A. S. (2001). Occult hepatitis B virus infection: a hidden menace? *Hepatology* 34(1), 204-6.
- de Franchis, R., Hadengue, A., Lau, G., Lavanchy, D., Lok, A., McIntyre, N., Mele, A., Paumgartner, G., Pietrangelo, A., Rodes, J., Rosenberg, W., and Valla, D. (2003). EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). *J Hepatol* 39 Suppl 1, S3-25.
- EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002: Geneva, Switzerland. Consensus statement (short version). *J Hepatol* 38(4), 533-40.
- Enomoto, M., Tamori, A., and Nishiguchi, S. (2006). Hepatitis B virus genotypes and response to antiviral therapy. *Clin Lab* 52(1-2), 43-7.
- Erhardt, A., Blondin, D., Hauck, K., Sagir, A., Kohnle, T., Heintges, T., and Haussinger, D. (2005). Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* 54(7), 1009-13.
- Flink, H. J., van Zonneveld, M., Hansen, B. E., de Man, R. A., Schalm, S. W., and Janssen, H. L. (2006). Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepati-

- tis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol* 101(2), 297-303.
- Fung, S. K., and Lok, A. S. (2004). Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology* 40(4), 790-2.
- Guettouche, T., and Hnatyszyn, H. J. (2005). Chronic hepatitis B and viral genotype: the clinical significance of determining HBV genotypes. *Antivir Ther* 10(5), 593-604.
- Hussain, M., Chu, C. J., Sablon, E., and Lok, A. S. (2003). Rapid and sensitive assays for determination of hepatitis B virus (HBV) genotypes and detection of HBV precore and core promoter variants. *J Clin Microbiol* 41(8), 3699-705.
- Hussain, M., Fung, S., Libbrecht, E., Sablon, E., Cursaro, C., Andreone, P., and Lok, A. S. (2006). Sensitive line probe assay that simultaneously detects mutations conveying resistance to lamivudine and adefovir. *J Clin Microbiol* 44(3), 1094-7.
- Kao, J. H. (2002). Hepatitis B viral genotypes: clinical relevance and molecular characteristics. *J Gastroenterol Hepatol* 17(6), 643-50.
- Kao, J. H. (2003). Hepatitis B virus genotypes and hepatocellular carcinoma in Taiwan. *Intervirol* 46(6), 400-7.
- Kao, J. H., Wu, N. H., Chen, P. J., Lai, M. Y., and Chen, D. S. (2000). Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 33(6), 998-1002.
- Keeffe, E. B., Dieterich, D. T., Han, S. H., Jacobson, I. M., Martin, P., Schiff, E. R., Tobias, H., and Wright, T. L. (2006). A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. *Clin Gastroenterol Hepatol* 4(8), 936-62.
- Kobayashi, M., Arase, Y., Ikeda, K., Tsubota, A., Suzuki, Y., Saitoh, S., Kobayashi, M., Suzuki, F., Akuta, N., Someya, T., Matsuda, M., Sato, J., Takagi, K., Miyakawa, Y., and Kumada, H. (2002). Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. *J Med Virol* 68(4), 522-8.
- Liaw, Y. F., Leung, N., Guan, R., Lau, G. K., Merican, I., McCaughan, G., Gane, E., Kao, J. H., and Omata, M. (2005). Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update. *Liver Int* 25(3), 472-89.
- Liu, C. J., Kao, J. H., Chen, P. J., Lai, M. Y., and Chen, D. S. (2002). Molecular epidemiology of hepatitis B viral serotypes and genotypes in taiwan. *J Biomed Sci* 9(2), 166-70.
- Lok, A. S., and McMahon, B. J. (2001). Chronic hepatitis B. *Hepatology* 34(6), 1225-41.
- Lok, A. S., and McMahon, B. J. (2004a). [AASLD Practice Guidelines. Chronic hepatitis B: update of therapeutic guidelines]. *Rom J Gastroenterol* 13(2), 150-4.
- Lok, A. S., and McMahon, B. J. (2004b). Chronic hepatitis B: update of recommendations. *Hepatology* 39(3), 857-61.
- Manesis, E. K., Papatheodoridis, G. V., Sevastianos, V., Cholongitas, E., Papaioannou, C., and Hadziyannis, S. J. (2003). 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* 98(10), 2261-7.
- Olivero, A., Ciancio, A., Abate, M. L., Gaia, S., Smedile, A., and Rizzetto, M. (2006). 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* 13(6), 355-62.
- Osiowy, C., and Giles, E. (2003). Evaluation of the INNO-LiPA HBV genotyping assay for determination of hepatitis B virus genotype. *J Clin Microbiol* 41(12), 5473-7.
- Osiowy, C., Villeneuve, J. P., Heathcote, E. J., Giles, E., and Borlang, J. (2006). 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* 44(6), 1994-7.

## 118 Acute and chronic hepatitis B - Diagnostic tests

- Peters, M. G., Hann Hw, H., Martin, P., Heathcote, E. J., Buggisch, P., Rubin, R., Bourliere, M., Kowdley, K., Trepo, C., Gray Df, D., Sullivan, M., Kleber, K., Ebrahimi, R., Xiong, S., and Brosgart, C. L. (2004). Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 126(1), 91-101.
- Qutub, M. O., Germer, J. J., Rebers, S. P., Mandrekar, J. N., Beld, M. G., and Yao, J. D. (2006). Simplified PCR protocols for INNO-LiPA HBV Genotyping and INNO-LiPA HBV PreCore assays. *J Clin Virol* 37(3), 218-21.
- Sanchez-Tapias, J. M., Costa, J., Mas, A., Bruguera, M., and Rodes, J. (2002). Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology* 123(6), 1848-56.
- Schildgen, O., Schewe, C. K., Vogel, M., Daumer, M., Kaiser, R., Weitner, L., Matz, B., and Rockstroh, J. K. (2004). Successful therapy of hepatitis B with tenofovir in HIV-infected patients failing previous adefovir and lamivudine treatment. *Aids* 18(17), 2325-7.
- Schildgen, O., Sirma, H., Funk, A., Olotu, C., Wend, U. C., Hartmann, H., Helm, M., Rockstroh, J. K., Willems, W. R., Will, H., and Gerlich, W. H. (2006). Variant of hepatitis B virus with primary resistance to adefovir. *N Engl J Med* 354(17), 1807-12.
- Sirma, H., Funk, A., Gerlich, W., and Schildgen, O. (2007). Does pre-treatment with lamivudine prime for adefovir resistance of hepatitis B virus infection? *J Antimicrob Chemother* 60(2), 448-9.
- Tillmann, H. L. (2007). Antiviral therapy and resistance with hepatitis B virus infection. *World J Gastroenterol* 13(1), 125-40.
- Torbenson, M., Kannangai, R., Astemborski, J., Strathdee, S. A., Vlahov, D., and Thomas, D. L. (2004). High prevalence of occult hepatitis B in Baltimore injection drug users. *Hepatology* 39(1), 51-7.
- Torbenson, M., and Thomas, D. L. (2002). Occult hepatitis B. *Lancet Infect Dis* 2(8), 479-86.
- Valsamakis, A. (2007). Molecular testing in the diagnosis and management of chronic hepatitis B. *Clin Microbiol Rev* 20(3), 426-39, table of contents.
- Volz, S., Schildgen, O., Muller, A., Tillmann, R. L., Eis-Hubinger, A. M., Kupfer, B., Bode, U., Lentze, M. L., and Simon, A. (2007). [The human bocavirus: pathogen in airway infections?]. *Dtsch Med Wochenschr* 132(28-29), 1529-33.
- Zacharakis, G. H., Koskinas, J., Kotsiou, S., Papoutselis, M., Tzara, F., Vafeiadis, N., Archimandritis, A. J., and Papoutselis, K. (2005). 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* 77(2), 173-9.
- Zhang, X., Zoulim, F., Habersetzer, F., Xiong, S., and Trepo, C. (1996). Analysis of hepatitis B virus genotypes and pre-core region variability during interferon treatment of HBe antigen negative chronic hepatitis B. *J Med Virol* 48(1), 8-16

## Chapter 9: HBV Treatment - Standard of care

Florian van Bömmel & Thomas Berg

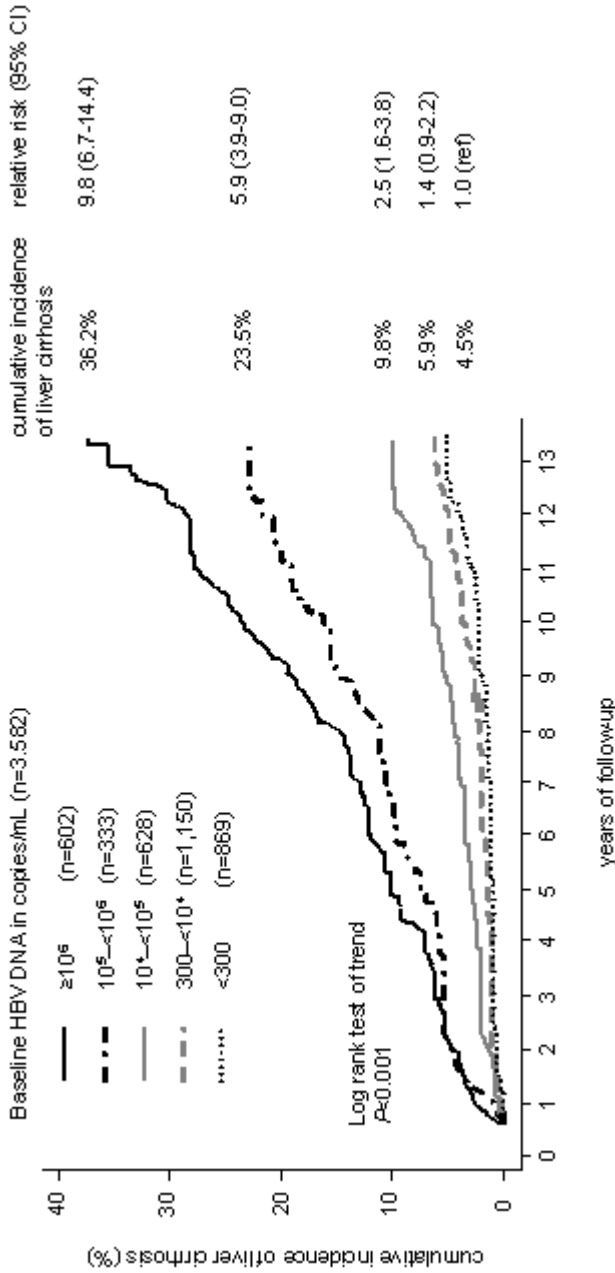
### Introduction

Despite the availability of a prophylactic vaccine, with more than 350 million chronically infected individuals worldwide, chronic hepatitis B virus (HBV) infection remains a major global health concern (EASL 2002). Chronically infected individuals carry a significantly increased risk of life-threatening liver complications such as hepatic decompensation, liver cirrhosis and hepatocellular carcinoma (HCC) (Beasley 1988). Recent studies have shown that the level of serum HBV DNA for more than a decade correlates with the risk of developing cirrhosis and HCC (Figure 1) (Iloeje 2006; Chen 2006). Therefore, suppressing the replication of HBV to levels below the limit of detection of sensitive HBV DNA diagnostic tests has become a major goal in HBV treatment.

For the treatment of chronic HBV infection two classes of agents are approved: first, antiviral nucleos(t)ide analogues directly inhibiting HBV DNA replication and second, interferon  $\alpha$ -based therapies that may modulate host immune response as well as viral replication.

The number of agents approved for the treatment of chronic HBV infection has steadily increased over recent years. In Europe seven medications for the treatment of chronic hepatitis B are available: standard interferon  $\alpha$ -2a and  $\alpha$ -2b and PEG-IFN  $\alpha$ -2a, the nucleoside analogues lamivudine, telbivudine and entecavir and the acyclic nucleotide analogues adefovir and tenofovir. More nucleos(t)ide analogues with antiviral activity against HBV such as emtricitabine, clevudine, torcitabine, amdoxovir and alamifovir are currently in clinical development.

Due to this significant expansion of therapeutic options, in most cases progression of HBV infection and prevention of complications can be managed when the infection is diagnosed in a timely manner and effectively treated. The early diagnosis of chronic hepatitis B by HBS antigen (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 the baseline HBV levels (Cornberg 2007).



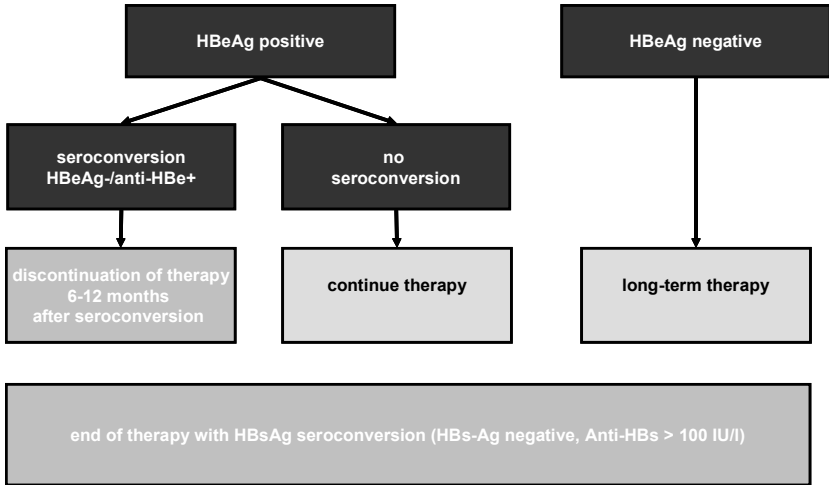
## Goals of antiviral therapy

### Treatment endpoints

The aim of chronic hepatitis B therapy is to reduce the morbidity and mortality of HBV infections such as liver failure and hepatocellular carcinoma (HCC) and increase survival. To reach this goal surrogate markers must be used during and after treatment to determine the success of therapy. This issue raises the question of which of the different parameters (e.g., transaminases, liver histology, HBeAg and HBsAg status, HBV DNA level) allows for a rapid prediction of favourable long-term outcomes with respect to prevention of HBV-related complications

In two recent studies a close correlation between baseline HBV DNA levels and progression of the disease has been demonstrated. In the REVEAL study, 3774 untreated HBV-infected individuals were followed over a mean time period of 11.4 years in Taiwan (Iloeje 2006; Chen 2006). The entry HBV DNA level was the strongest predictor of cirrhosis and HCC development (Figure 1). In multivariate models, the relative risk of cirrhosis increased when HBV DNA reached levels  $\geq 300$  copies/mL independent of whether HBeAg-negative or HBeAg-positive patients were analysed. The relative risk was 1.4 in patients with HBV DNA levels of 300 to 1000 and increased to 2.4 in patients with 1000 to 10,000, 5.4 in patients with 10,000 to 100,000 and 6.7 in patients with HBV DNA levels  $>1$  million copies/mL. In addition, individuals with HBV DNA  $\geq 10^4$  copies/mL ( $\geq 2000$  IU/ml) were found to have a 3-15 fold greater incidence of liver cancer compared to those with a HBV DNA  $<10^4$  copies/mL. One can therefore conclude that the complete and persistent suppression of HBV replication is a reliable surrogate endpoint for clinical progression of liver disease. In a meta-analysis of 26 prospective studies, treatment efficacy in patients with HBV infection revealed a statistically significant and consistent correlation between viral load level while on therapy or change in HBV DNA and histologic, biochemical and serologic response (Mommeja-Marin 2003).

In HBeAg-positive patients seroconversion from HBeAg to anti-HBe has been found to be a reliable surrogate marker for prognosis of chronic HBV leading in many cases to an inactive HBsAg carrier state (Figure 2). In these patients HBsAg remains detectable but HBV replication is controlled at low or even undetectable levels and transaminases are generally within normal ranges. Long-term observations reveal, however, that HBeAg seroconversion cannot always be taken as a guarantee for long-term remission of chronic hepatitis. A reactivation of the disease with "sero-reversion" (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. Therefore, HbeAg seroconversion should be regarded as a stable treatment endpoint only in conjunction with (durable) sustained and complete HBV DNA suppression.



**Figure 2. Possible endpoints of treatment of HBV infections.** 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 the first sign of HBeAg or HBsAg seroconversion.

In contrast, the endpoint of therapy for patients with HBeAg-negative disease is more difficult to assess and HBV DNA suppression and ALT normalization are the only practical measures of response to therapy. But still, normal ALT levels and suppression of HBV DNA levels do not guarantee sustained remission. Once therapy is stopped, durability of the response remains a concern considering the fluctuating course of HBeAg-negative chronic hepatitis B. It is well known that maintenance of undetectable HBV DNA and of normal ALT activity declines progressively the longer patients are followed after treatment.

HBsAg loss or seroconversion to anti-HBs is the ultimate goal and most desirable endpoint of antiviral therapy and signals the cure of the chronic infection. The loss of HBsAg or HBsAg-seroconversion is associated with a complete and definitive remission of chronic hepatitis B activity and an improved long-term outcome. 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 of PEG-IFN  $\alpha$  as well as nucleos(t)ide analogue-treated patients an increase of the rate of HBsAg-loss over long-term studies was demonstrated. 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 therefore seems likely that with increasing treatment durations in the future, 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), in all patients with HBV infection a cellular template of the viral genome persists in hepatocytes even decades after HbsAg seroconversion (Rehermann 1996). Therefore, it is believed that due to cccDNA persistence complete eradication of HBV infection 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 or chemotherapy, in particular with agents such as rituximab.

Criteria for response to therapy:

Virologic:

- sustained decrease of HBV DNA, to at least  $<10^4$  copies/ml ( $2 \times 10^3$  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 stage in histology or absence of progression
- reduction of inflammatory activity in histology

Potential long-term effects:

- avoidance of cirrhosis, hepatocellular carcinoma (HCC), transplantation, and death

## Indication for antiviral therapy

### In acute hepatitis B

Acute hepatitis resolves spontaneously in 95-99% of cases. The effect of antiviral therapy in acute hepatitis B has been never definitively established but due to the high rate of spontaneous remission in adults, therapy with the currently available drugs is not indicated. There is only one randomized placebo-controlled study from India showing no advantage of lamivudine therapy for acute hepatitis B over placebo (Kumar 2007). Data from Europe and the US on antiviral therapy of acute hepatitis B with compensated liver function are not available.

Several case reports, however, reveal that patients with severe and fulminant hepatitis B may benefit from early antiviral therapy with lamivudine or other nucleos(t)ide analogues by reducing the need for high-urgency liver transplantation (Tillmann 2006; Lisotti 2008). An immediate oral antiviral therapy seems justified to prevent fulminant liver failure in cases where signs of liver synthesis impairment (drop of TPZ value, increase in INR) during acute hepatitis B are present.

## In chronic hepatitis B

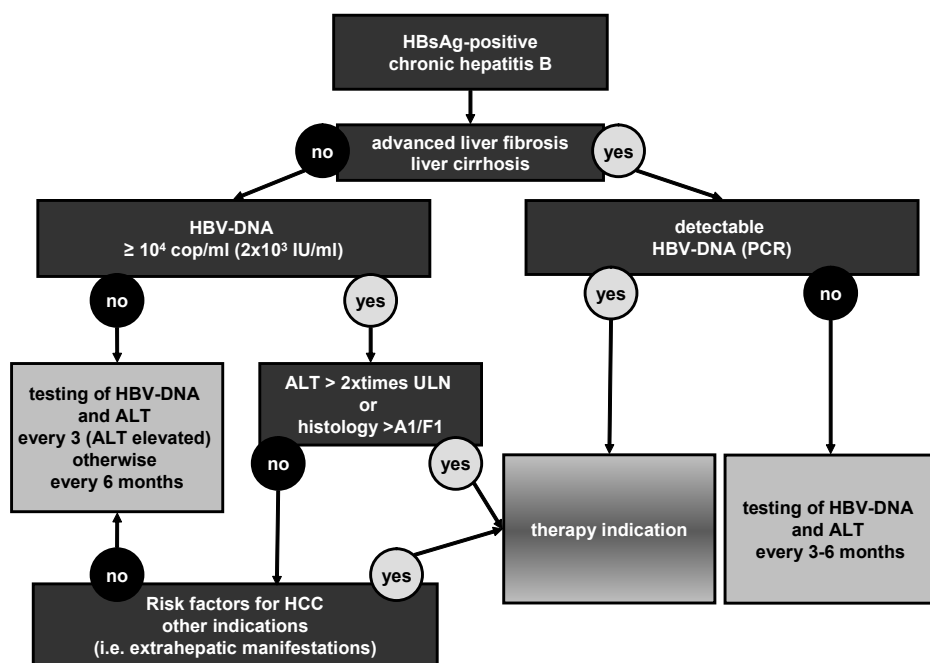
Patients with HBsAg-positive chronic hepatitis should be considered as possible candidates for antiviral therapy especially in situations when there is a significant level of HBV replication. According to current guidelines it is no longer necessary to differentiate between HBeAg-positive (wild type) and HBeAg-negative (precore mutants) chronic hepatitis B as far as concerns treatment indication. However, with respect to the choice of the proper antiviral drug(s), the HBe-Ag status may be still useful.

In Table 1 (Key recommendation guidelines for indication for antiviral treatment for HBV infection, see <http://hepatologytextbook.com/link.php?id=1>) the recommendations of the different national and international societies are shown. The views have shifted from focusing on histologically proven disease activity to the importance of evaluating HBV DNA levels as the most relevant variable for a decision to initiate therapy. Thus most of the recently published guidelines recommend antiviral treatment for patients with HBV DNA levels of >10,000 copies/mL (>2000 IU/mL), coupled with ALT levels of greater than 2 times the upper limit of normal, and significant liver fibrosis, demonstrated by liver histology greater than A1/F1.

Liver biopsy prior to the initiation of treatment can be useful for the treatment decision in some patients, as it can provide important information on disease prognosis and can help in the planning of subsequent therapeutic decisions if/when first line treatment fails. However, a liver biopsy is not mandatory to make a decision concerning treatment for the majority of patients based on the guidelines listed in Table 1 (Arkaca 2008; Buster 2008; Carosi 2008; Colle 2007; Cornberg 2007; EASL 2008; Juszczak 2008; Keefe 2007; Liaw 2008).

Patients with liver cirrhosis or high-grade liver fibrosis and any measurable HBV DNA should be considered for antiviral therapy. 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 B or C) PEG-IFN  $\alpha$  is contraindicated.

Inactive chronic HBsAg carriers, characterised by negative HBeAg status (anti-HBeAg-positivity), low HBV DNA levels (<10,000 copies/mL) together with normal serum aminotransferase levels and normal liver histology can be excluded from antiviral therapy. However, it may be difficult to differentiate between patients who are inactive HBsAg carriers and those presenting with chronic HBeAg-negative hepatitis. Elevated transaminase levels are not a reliable parameter for assessing the stage of liver fibrosis and long-term prognosis of HBV-infected patients. Even in patients with slightly elevated or even normal transaminases there is a significant risk for development of HBV-associated complications (Lok 2000; Werle-Lapostolle 2004). Therefore, control of HBV DNA levels at 3-6 months intervals is indicated, reflecting that disease activity can fluctuate. Liver biopsy may be necessary in cases where HBV DNA levels are low and ALT levels elevated.



**Figure 3.** Pathway for indication for antiviral treatment according to the German guidelines for the treatment of HBV infections.<sup>16</sup> Treatment should be considered if HBV DNA levels exceed  $10^4$  copies/mL and if a sign of ongoing hepatitis as elevated ALT > 2x upper limit of normal or liver histology > A1/F1 is present. Of note, also asymptomatic carriers with family history of HCC should receive treatment even if signs of hepatitis are absent.

HBV immunotolerant patients are mostly under 30 years of age and can be recognized by having high HBV DNA levels, by being HBeAg-positive and by normal ALT levels without any significant histological changes. According to most of the clinical practice guidelines immediate therapy is not required. However patients at higher risk for HCC - those with a positive family history, and those coming from high endemic areas like Asia or Africa - may perhaps benefit from early antiviral therapy. Studies are under way to further clarify this issue, especially to answer the question of whether early intervention by antiviral therapy can positively influence the long-term HCC risk.

## Treatment indication: Summary of the German guidelines recommendations

- All patients with chronic hepatitis B are candidates for antiviral therapy. The level of viral replication in serum (limit  $10^4$  virus copies/ml, corresponding to  $2 \times 10^3$  IU/ml), the status of inflammation and fibrosis in the biopsy, and the level of serum transaminases are primarily considered.

- Especially patients with advanced fibrosis or cirrhosis need consistent antiviral therapy after detection of viremia.
- Reactivation of hepatitis B viral 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.

### Treatment options for HBV infection

There are two classes of drug available for the treatment of HBV infections: interferon-alpha (standard or pegylated IFN  $\alpha$ ) and inhibitors of the HBV polymerase, the nucleoside and acyclic nucleotide analogues.

While IFN  $\alpha$  has been a mainstay in the treatment of chronic HBV infection for many years it is limited by its tolerability and significant side effect profile that allows its administration only for a limited period of time (6-12 months, maximum 24 months). Nucleos(t)ide analogues have a better tolerability and are therefore used in long-term therapy 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: interferon  $\alpha$ -2b and peg-interferon (PEG-IFN)  $\alpha$ -2a, lamivudine, adefovir, telbivudine, entecavir and tenofovir (Table 2).

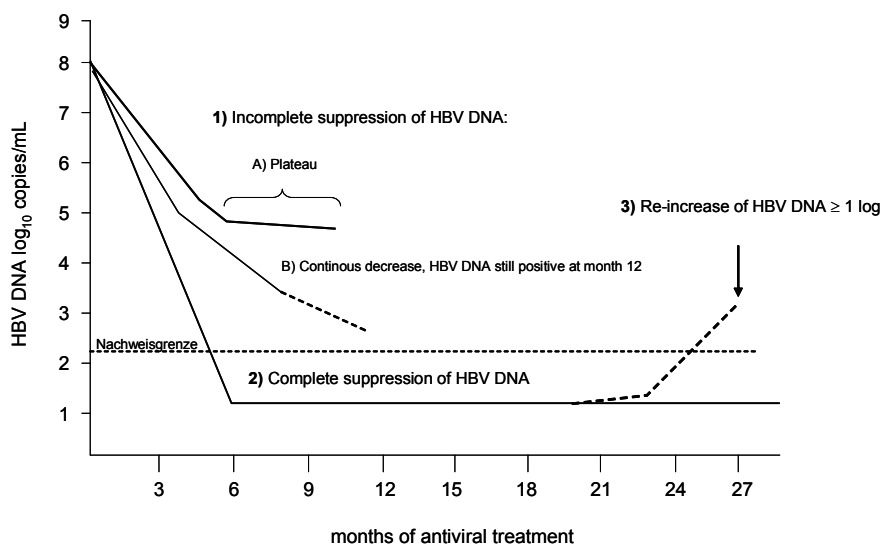
**Table 2:** Drugs currently approved for the treatment of HBV infection

| Substance                             | Name        | Dose  | Duration                 |
|---------------------------------------|-------------|---|--------------------------|
| <i>Interferon-<math>\alpha</math></i> |             |   |                          |
| Standart Interferon- $\alpha$ 2a      | Roferon ®   | 2.5-5 mio.IU/m <sup>2</sup> body surface<br>3x/week               | 4-6 months               |
| Standart Interferon- $\alpha$ 2b      | Intron A ®  | 5-10 mio. IU 3x/week  | 4-6 months               |
| Pegylated Interferon- $\alpha$ 2a     | Pegasys ®   | 180 µg/week   | 48 weeks                 |
| <i>Nucleoside analogues</i>           |             |   |                          |
| Lamivudin                             | Zeffix ®    | 100 mg/day  | long-term*               |
| Telbivudine                           | Sebivo ®    | 600 mg/day  | long-term*               |
| Entecavir                             | Baraclude ® | 0,5 mg/day<br>1 mg/day for patients with<br>lamivudine resistance | long-term*<br>long-term* |
| <i>Nucleotide analogues</i>           |             |   |                          |
| Adefovir dipivoxil                    | Hepsera ®   | 10 mg/day   | long-term*               |
| Tenofovir disoproxil fumarate         | Viread ®    | 245 mg/day**  | long-term*               |

\* see figure 6

\*\* 300 mg tenofovir disoproxil fumarate corresponds to 245 mg tenofovir disoproxil

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.



**Figure 4.** Summary of treatment efficacy of different agents for the treatment of HBV infection after one year of suppression of HBV DNA to below the limit of detection, ALT normalization and HBeAg seroconversion. Differences in results have to be interpreted with the proviso that these are not direct comparison studies.

## Interferons

IFN  $\alpha$  is a natural-occurring cytokine with immunomodulatory, antiproliferative and antiviral activity. The therapeutic efficacy of IFN  $\alpha$  can be clinically often recognised by an increase in ALT levels at least twice the baseline level. These flares often precede virologic response.

The main aim of standard or PEG-IFN  $\alpha$  treatment is to induce long-term remission via its finite treatment duration. Overall a long-term response defined by either HBeAg seroconversion or durable suppression of HBV DNA to undetectable levels can be achieved in approximately 30% of treated patients (Cooksley 2003). In these responder patients the chance for HBsAg loss in the long-term is relatively high.

### Use of Standard IFN:

Standard IFN  $\alpha$  was approved as therapy of chronic hepatitis B in 1992. IFN  $\alpha$  is applied in dosages ranging from 5 million units (MU) to 10 MU thrice weekly (or every other day). Using a meta-analysis, a significant improvement in endpoints was shown in patients with HBeAg-positive chronic hepatitis B treated with standard-interferon alpha in comparison to patients with no treatment. Complete remis-

sion of fibrotic changes was observed and was often associated with the loss of HBsAg. Furthermore, there is a trend towards reduction of hepatic decompensation, development of hepatocellular carcinoma and liver-associated deaths (Lau 2007).

A significant decrease in ALT as well as HBV DNA concentration was also shown for standard interferon alpha for the therapy of HBeAg-negative chronic hepatitis B. However, these patients relapse frequently after the end of treatment (25-89%), as evidenced by elevation of ALT levels and hepatitis B viral load (Hadziyannis 2006; Lok 2007). The relapse rate seems to be higher when treatment duration is short (16 to 24 weeks) compared to longer (12 to 24 months). A retrospective comparison of therapies lasting 5 and 12 months showed that with longer treatment the chance of a long-term response was 1.64 times as high (normalization of ALT, HBV DNA  $<1 \times 10^6$  copies/ml 1-7 years after end of therapy). The response rates were 54% overall at the end of therapy, 24% 1 year after therapy, and 18% 7 years after therapy (Hadziyannis 2006; Lok 2007).

In several studies, patients who had a long-term response to treatment demonstrated a more favorable course with respect to progression to liver cirrhosis, liver-associated death, and development of hepatocellular carcinoma than patients who were untreated, unresponsive, or who had a relapse after taking interferon alpha.

#### The use of pegylated interferon $\alpha$ (PEG-IFN)

The addition of a polyethylene glycol molecule (PEG) to IFN results in a significant increase in half-life, thereby allowing administration once weekly. In many centers standard IFN  $\alpha$  has now widely been replaced by PEG-IFN  $\alpha$ . Two types of PEG-IFN have been developed (PEG-IFN  $\alpha$ -2a and PEG-IFN  $\alpha$ -2b), of which PEG-IFN  $\alpha$ -2a has been licensed for the treatment of chronic HBV infection in a weekly dose of 180  $\mu$ g (subcutaneous) for 48 weeks in both HBeAg-positive and HBeAg-negative patients. The safety profiles of PEG-IFN and standard IFN are similar. Following therapy termination a relatively high relapse rate can be expected (>50%). The intermediate and long-term course after termination of therapy has not been sufficiently studied. The questions of optimal dose of PEG-IFN  $\alpha$ -2a (90 $\mu$ g vs. 180 $\mu$ g) and optimal duration of therapy have not been conclusively defined. However, this is currently being studied in a prospective study.

For the treatment of HBeAg-positive chronic hepatitis B using pegylated interferon alpha, four randomized controlled studies have been done. These studies compared PEG-IFN  $\alpha$  to standard-interferon, lamivudine, and/or a combination therapy of PEG-IFN alpha and lamivudine for a duration of 24, 48, 52, or 60 weeks (Lok 2007). In the phase III international approval trial a 48-week course of PEG-IFN alpha-2a (180  $\mu$ g / 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  $\alpha$ -2a alone or in combination with LAM than in patients treated with LAM alone (32% and 27% vs. 19%) (Lau 2005).

The efficacy and safety of PEG-IFN  $\alpha$ -2a (180  $\mu$ g once weekly) plus placebo, PEG-IFN  $\alpha$ -2a plus lamivudine (100 mg daily), and lamivudine alone was compared in 177, 179, and 181 patients with HBeAg-negative chronic hepatitis B, respectively (Marcellin 2004). 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  $\alpha$ -2a monotherapy (59 percent and 43 percent, respectively) and PEG-IFN  $\alpha$ -2a plus lamivudine (60% and 44%) than with lamivudine monotherapy (44% and 29%). Rates of sustained suppression of HBV DNA to below 400 copies/mL were 19% with PEG-IFN  $\alpha$ -2a monotherapy, 20% with combination therapy, and 7% with lamivudine alone. Loss of HBsAg occurred in 12 patients in the PEG-IFN groups, but not in the group of patients who received lamivudine monotherapy. There was no significant difference in the histologic response between the 3 treatment groups. In a follow up of this study the HbsAg seroconversion rate increased over time, particularly in the PEG-IFN group with up to 11% compared to 2% in the lamivudine monotherapy group 3 years after the end of the treatment period (Gaspar 2004; Marcellin 2008).

Although combination of lamivudine plus PEG-IFN failed to demonstrate any benefit when evaluated at the end of follow-up, a more pronounced on-treatment virologic response (week 48) was observed with combination therapy vs. LAM or PEG-IFN  $\alpha$  alone in all studies. 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). In summary the use of a fixed combination of lamivudine plus PEG-IFN  $\alpha$  is presently not recommended.

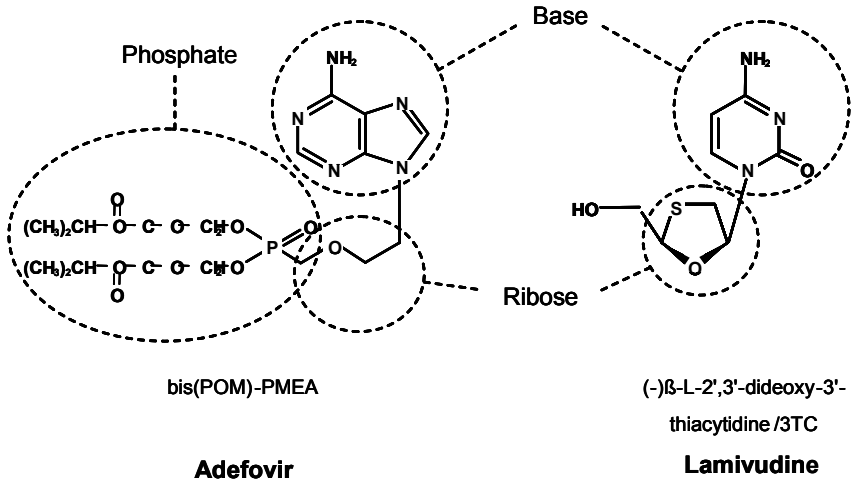
### **Nucleos(t)ide analogues**

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, their detailed mechanisms of action for inhibiting HBV DNA synthesis varies greatly from one agent to another. 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 to greater binding capacity to resistant HBV polymerase strains (Figure 7).

In contrast to interferon-based treatment strategies where a finite treatment duration of 24-48 weeks is established, treatment duration for nucleos(t)ide analogues is not well-defined and needs to be given for extended periods in order to control viral replication over the long-term. Short-term application of these agents for only 48 weeks is normally associated with prompt relapse in hepatitis B viremia.

Studies with nucleos(t)ide 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 (Wursthorn 2006; Werle-Lapostolle 2004; Lok 2000). HBeAg-seroconversion rates also increase with increasing treatment duration (Liw 2000; Lok 2000). Most importantly, effective long-term control of HBV replication by nucleos(t)ide analogues is associated with a reduction of long-term complications such as HCC and development of liver cirrhosis. There is also evidence that effective inhibition of

HBV replication can reduce HBV cccDNA, possibly running parallel to the decline in serum HBsAg level (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.



**Figure 7.** Chemical structure of the nucleoside analogue lamivudine and the acyclic nucleotide analogue adefovir dipivoxil as an example of purine- or pyrimidine-based viral transcriptase inhibitors. Lamivudine is based on cytosine while adefovir is based on adenine. In contrast to lamivudine, adefovir possesses an acyclic ribose ring that gives it a higher flexibility.

A central aspect of HBV polymerase inhibitor treatment is the prevention and the management of HBV resistance to these drugs. Resistance to nucleos(t)ide analogues can occur during suboptimal treatment and often leads to aggravation of liver disease. Therefore nucleoside-naïve 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 next-line therapy.

### Lamivudine

Lamivudine is a synthetic nucleoside analogue that was approved for the treatment of chronic hepatitis B in 1998. Lamivudine 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. Lamivudine 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 lamivudine 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 (Neuens 1997).

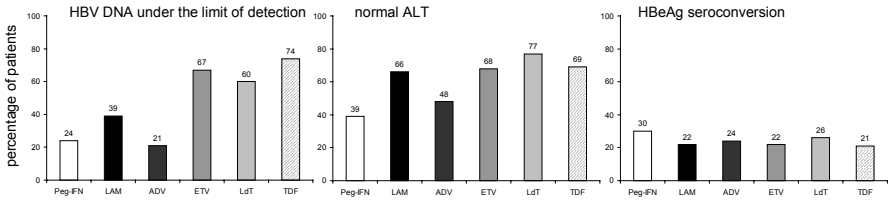
Long-term lamivudine treatment is associated with a reduced progression of liver disease in patients with advanced liver fibrosis therefore demonstrating a favourable effect on the natural course of the disease (Liaw 2004). However the use of lamivudine suffers from a steadily increasing rate of antiviral drug resistance over time, reaching approximately 70% after 5 years in patients with HBeAg-positive patients (Mauss 2007). Therefore, in many guidelines lamivudine is not any more considered a first-line agent in the treatment of chronic HBV infection. However, lamivudine 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) (Cornberg 2007). An early and complete virologic response to lamivudine within 6 months of therapy ( $<400$  copies/mL) constitutes a prerequisite for long-term control of HBV infection without the risk of development resistance.

#### Adefovir Dipivoxil

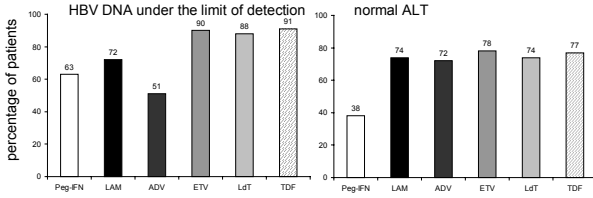
Adefovir dipivoxil was approved for treatment of chronic hepatitis B in the US in 2002 and in Europe in 2003. It is an oral diester prodrug of adefovir, a nucleotide adenosine analogue that, in its active form (adefovir diphosphate), inhibits HBV DNA polymerase. Because the acyclic nucleotide already 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 lamivudine-resistant HBV variants, and is active *in vitro* against a number of DNA viruses in addition to HBV, as well as retroviruses (i.e., HIV). The dose of 10 mg per day was derived from a study comparison with 30 mg per day, which led to stronger suppression of HBV DNA but also to an increase of creatinine levels in some patients. At higher doses adefovir caused Fanconi's syndrome in a considerable proportion of patients (Hadziyannis 2003).

Adefovir was the first acyclic nucleotide that was widely used in the treatment of lamivudine-resistant HBV infections. However, the antiviral efficacy of adefovir is rather low compared to other available antivirals and is most probably due to dose limitation, because of nephrotoxicity (Figure 5). This disadvantage makes adefovir vulnerable to HBV resistance (Hadziyannis 2006). Today, add-on therapy in cases of lamivudine resistance is the preferred strategy for adefovir, as discussed below (Lampertico 2005). With the recent approval of tenofovir, the importance of adefovir is likely to diminish.

a) HBeAg-positive patients



b) HBeAg-negative patients



**Figure 5.** Summary of treatment efficacy of agents approved for the treatment of HBV infection after one year of suppression of HBV DNA below the limit of detection, ALT normalisation and HbeAg seroconversion. Differences in the results of the studies have to be interpreted with the provision that this is a cross-study comparison and may involve differently selected study populations.

Telbivudine

Telbivudine is a thymidine analogue which is active against HBV but at least *in vitro* not active against other viruses, including HIV and HCV. It is reported to be non-mutagenic, non-carcinogenic, non-teratogenic, and to cause no mitochondrial toxicity. The favourable safety profile of telbivudine in a daily dose of 600 mg was demonstrated in previous studies. However, CK elevations were observed more than in the group treated with lamivudine and neurotoxicity may be an issue when telbivudine is administered in combination with PEG-IFN.

Telbivudine at 600 mg/day express higher antiviral activity as compared to either lamivudine at 100 mg/day or adefovir at 10 mg/day (Figure 5) and more patients achieved HBeAg loss with telbivudine compared to lamivudine (Lai 2006).

However, resistance against telbivudine has been seen in up to 21% of patients after 2 years of treatment (Lai 2006). Resistance was predominantly observed in patients who did not achieve undetectable HBV DNA after 24 weeks of treatment (Di Bisceglie 2006). Telbivudine should be used cautiously in HBeAg-positive patients with high HBV DNA levels (>10<sup>9</sup> copies/mL) because the risk of incomplete virologic response at week 24 is especially high in this patient population. Furthermore, in all patients, treatment with telbivudine should be modified when HBV DNA levels do not become undetectable after 24 weeks of treatment (therefore, switching to a more potent drug or adding in a second non-cross-resistant drug is advised). Telbivudine shows cross resistance to lamivudine and entecavir. As a consequence telbivudine should not be used in lamivudine or entecavir refractory patients.

### Entecavir

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 pregenomic messenger RNA; third, synthesis of the positive strand of HBV DNA. In comparison to all other nucleos(t)ide analogues, entecavir is more efficiently phosphorylated to its active triphosphate compound by cellular kinesis. It is a potent inhibitor of wild-type HBV but is less effective against lamivudine-resistant HBV mutants. Therefore, entecavir was approved in the dose of 0.5 mg per day for treatment naïve HBeAg-positive and HBeAg-negative patients and in the dose of 1 mg per day for patients with prior treatment with lamivudine.

Treatment-naïve HBeAg-positive patients achieved HBV DNA undetectable levels in 67% after one year and in 74% after two years (Figure 5) (Sherman 2008; Gish 2007). Long-term studies in entecavir-responding patients demonstrated that response can be maintained in nearly all patients for up to five years. So far, the rate of long-term resistance is estimated to be approximately 1% in the first years for treatment-naïve patients (Tenny 2008).

In lamivudine-resistant patients entecavir is less potent. Only 19% of these patients achieved undetectable HBV DNA after one year and 40% after two years, despite an increased entecavir dose of 1 mg/day (Sherman 2008; Gish 2007). Up to 45% of patients with lamivudine resistance were shown to develop resistance against entecavir after 5 years of treatment (Tenny 2008). Partial cross-resistance facilitates the development of entecavir resistance, as only one or two additional mutations are needed for the development of full entecavir resistance.

### Tenofovir

Tenofovir disoproxil fumarate, an ester prodrug form of tenofovir (PMPA; (R)-9-(2-phosphonylmethoxypropyl)), belongs to the group of acyclic nucleoside phosphonates (or nucleotide analogues). Tenofovir (TDF) has selective activity against retroviruses and hepadna-viruses and is currently approved for the treatment of human immunodeficiency virus (HIV) infections and for treatment of chronic hepatitis B. Tenofovir 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 lamivudine-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 lamivudine-resistant HBV infections irrespectively of the kind of mutation mediating lamivudine resistance (Van Boemmel 2008, Manns 2008). Due to possibly existing cross-resistance to adefovir, the efficacy of tenofovir may be hampered by the presence of adefovir resistance; however, a re-increase of HBV DNA during tenofovir treatment in patients with previous adefovir failure has not been observed (Berg 2008).

TDF is generally well tolerated and not associated with severe side effects to date. In large randomized clinical trials in HBV/HIV coinfecting patients the use of TDF

was associated with an excellent renal safety profile. However mild impairment of renal function due to TDF treatment up and anecdotal cases of acute renal failure and Fanconi syndrome have been reported in some HBV/HIV coinfecting patients (Gallant 2005; Verhelst 2002; Creput 2003; Karras 2003; Peyriere 2004; Coca 2002). 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 while on TDF therapy.

Two-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. No drug resistance has been observed so far. HBsAg loss was observed in 5% of the HBeAg-positive patients after 15 months of therapy with TDF (Heathcote 2008). Similar efficacy without development of resistance was also demonstrated when TDF was administered to lamivudine-refractory patients or those showing incomplete response to adefovir (Manns 2008; Van Boemmel 2008; Berg 2008).

### **Nucleos(t)ide analogue combination therapy**

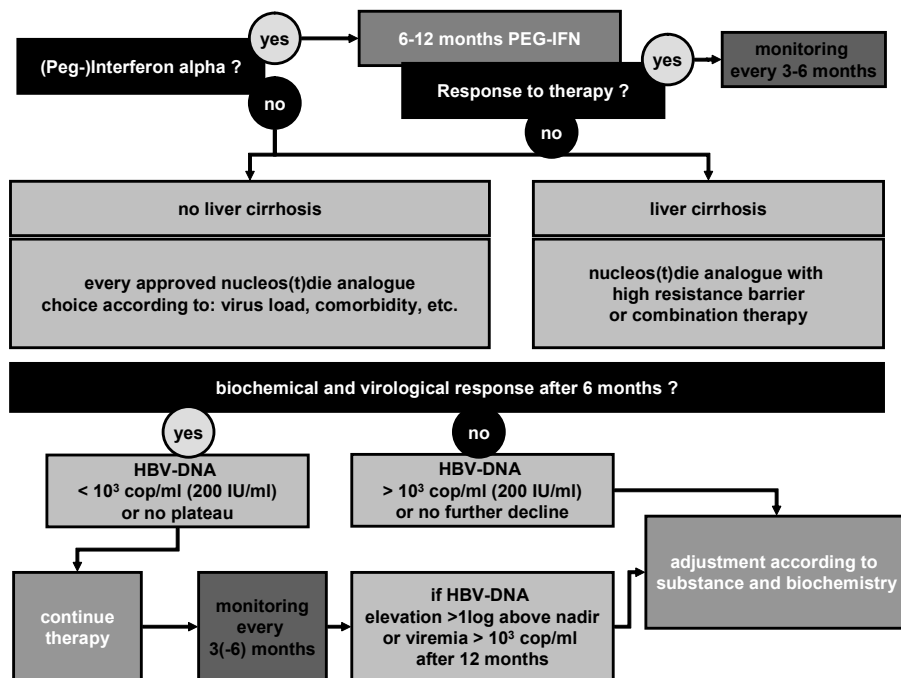
There is only one study that has compared combination therapy with lamivudine plus adefovir to lamivudine monotherapy in naive patients. There was no difference in the virologic and biochemical response between the groups. The rate of lamivudine resistance was much lower in the combination group. However, the development of resistance could not be completely avoided by adding adefovir (Sung 2003). Another study analysing the combination of lamivudine plus telbivudine showed no benefit over a median observational period of two years (Schmutz 2006). Combining telbivudine and lamivudine did not improve antiviral efficacy or protect against development of resistance (Lai 2005). No data are available on other combination therapies.

### **Prognostic factors for therapeutic success**

Several factors are positively associated with long-term remission and may help to guide treatment decisions: Pretreatment factors predictive of HbeAg seroconversion are low HBV DNA, high ALT levels (greater than 2-5 times ULN) and high inflammatory activity scores on liver biopsy. These general baseline predictors are relevant especially for treatment regimes with PEG-IFN  $\alpha$  but may also be relevant for nucleos(t)ide analogues (Fried 2008). In contrast HBV genotype, another baseline predictor, has been shown to be associated only with differences in viral response to interferon alpha. Thus patients with HBV genotype A, prevalent in northern Europe and USA, show a much better rate of HBeAg-seroconversion (47-52%) than patients with HBV genotype D (22-25%), prevalent in the south of Europe, or HBV genotypes B or C, prevalent in Asia (30-40% resp. 28-30%) (Erhardt 2005; Flink 2006).

## How to treat

Basically two different treatment strategies can be chosen: One is to treat with (pegylated) interferon alpha in order to induce long-term control via limited treatment. The other concept is long-term inhibition of viral replication by nucleoside and nucleotide analogues that inhibit HBV replication (Figure 6).



**Figure 6.** Suggestion for an algorithm for treatment of HBV infection with PEG-IFN according to the German guidelines (Cornberg 2007).

First, the possibility of an interferon therapy should be evaluated. However when a patient does not fulfill the criteria for PEG-IFN  $\alpha$  or has contraindications or is intolerant to IFN  $\alpha$ , long-term therapy with nucleos(t)ides analogues is recommended. When an oral drug is chosen several parameters have to be considered - 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 lamivudine, 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 those drugs with a high genetic barrier for resistance should be used (i.e., entecavir and/or tenofovir).

**Monitoring of patients before and during antiviral therapy**

Before therapy, HBV DNA levels should be measured with a highly sensitive assay and these results should be confirmed 1-2 months after starting therapy. ALT levels reflecting the inflammatory activity as well as creatinine levels should be measured. 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 nucleos(t)ide analogue. 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 at 4 to 6 weeks and then every 3 months. The early identification of viral resistance and, thus, an early adjustment of therapy is crucial. Patients with suppression of HBV replication to <300 copies/ml (60 IU/ml) for at least 2 years may perhaps be monitored every 6 months. However, no studies have been done yet to support this recommendation.

In HBeAg-positive patients, HBeAg and anti-HBe as well as HBsAg and anti-HBs should also be measured when HBV DNA levels become undetectable, to identify seroconversion as an endpoint (Table 3).

**Table 3:** Recommendation for laboratory tests for monitoring of antiviral therapy

| Tests before antiviral treatment   | Interval  |
|--|---|
| Quantitative HBV DNA<br>HBe-Ag, anti HBe<br>HBV genotype<br>ALT level<br>Creatinine level<br>Other chemistry tests | If IFN-based treatment is planned   |
| Tests during antiviral treatment   | Interval  |
| Quantitative HBV DNA   | After 4-6 weeks, after 12 weeks, then every 3-6 months  |
| HBe-Ag, anti-HBe   | 3-6 months, if HBV DNA is undetectable  |
| HBs-Ag, 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 pretreatment history is not known, but first check for treatment adherence! |
| ALT level  | Initially every month, than every 3-6 months  |
| Creatinine level*  | 3-6 months  |
| Other chemistry tests  | 3-6 months  |

\* Patients treated with TDF should initially be checked every 4 weeks to exclude decrease in kidney function



### **The level of HBV DNA as a parameter for response to antiviral therapy**

During antiviral therapy, the decrease of HBV DNA from baseline is the most important tool in monitoring treatment efficacy. Complete response to antiviral therapy is defined as suppression of HBV DNA below the limit of detection of a sensitive real time PCR assay (Figure 4). Incomplete suppression is characterized by persistent HBV replication despite antiviral therapy. Ongoing HBV replication should be avoided, to prevent selection of resistant HBV strains by replication of the virus in the presence of drug in the so called “plateau phase”. A re-increase of HBV DNA despite continuous antiviral therapy is often caused by viral resistance. Adherence to therapy is also important in preventing drug resistance.

In this respect, measuring 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.

In case of incomplete virologic response an appropriate rescue therapy should be initiated. Timing and type of the rescue strategies differs with respect to the nucleos(t)ide analogue which was previously used. For instance, selection of telbivudine and lamivudine resistance can occur within a relatively short treatment period of 6 months if complete suppression of viremia is not induced – leading to the recommendation that therapy should be adapted in the case of incomplete HBV DNA suppression at month 6. In contrast, entecavir and tenofovir and to a lesser extent adefovir have not been shown to be associated with a significant risk for resistance within the first two years of therapy in treatment-naïve patients. In case of incomplete viral suppression at week 48 continuation of monotherapy is advisable for entecavir and tenofovir, as long as HBV DNA levels continue to decrease.

In treatment-naïve patients with incomplete response to first line lamivudine, telbivudine or adefovir, a switch to tenofovir has been shown to be quite successful. The debate, however, is still ongoing on whether to switch or add in tenofovir as the optimal management.

No definite therapeutic strategies have been evaluated yet for either tenofovir- or entecavir-treated patients who show still ongoing viral replication even after 1-2 years. An add-on therapy with a non cross-resistant agent is recommended.

### **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 occurs, antiviral therapy should be continued for at least 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  $\alpha$  reached HBeAg seroconversion after 48 weeks, studies have been conducted to predict the probability of seroconversion in relationship to viral kinetics. Fried and co-workers showed in a retrospective analysis that early prediction of stable seroconversion was possible as early as week 12 on therapy provided HBV DNA concentration had reached levels below 5 log / ml in this time (Fried 2008). In 53% of these patients HBeAg seroconversion was observed while patients with HBV DNA levels between 5 and 9 log

copies / mL or levels above 9 log / ml achieved HBeAg seroconversion only in 17% and 14%, respectively. Thus, individualized PEG-IFN  $\alpha$  strategies will certainly be an interesting option in the future.

Criteria for optimal treatment duration are still lacking in patients with HBeAg-negative chronic hepatitis B. PEG-IFN  $\alpha$  should be given for 48 weeks and a possibly unlimited long-term use of nucleos(t)ide analogues is recommended. The effect of stopping therapy after 4 to 5 years of complete viral suppression in HBeAg-negative patients on adefovir therapy was recently evaluated by Hadziyannis and coworkers in a small preliminary study (Hadziyannis 2006). Despite all patients suffering a slight virologic relapse within 3 months of stopping therapy, most patients maintained clinical remission over the following 4 years without any therapy, and 28% of them lost HBsAg. In patients with liver cirrhosis, however, oral antiviral treatment should not be discontinued because of the risk of liver decompensation during a virologic rebound and should be administered life-long or until HbsAg loss/seroconversion is achieved.

## References

- Akarca US. Chronic hepatitis B a guideline to diagnosis, approach, management, and follow-up 2007 Turkish association for the study of liver. *Turk J Gastroenterol.* 2008, 19(4):207-30.
- 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, 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).
- Buster EH, van Erpecum KJ, Schalm SW, Zaaijer HL, Brouwer JT, Gelderblom HC, de Knegt RJ, Minke Bakker C, Reesink HW, Janssen HL; Netherlands Association of Gastroenterologists and Hepatologists. Treatment of chronic hepatitis B virus infection - Dutch national guidelines. *Neth J Med.* 2008, 66(7):292-306.
- Carosi G, Rizzetto M. Treatment of chronic hepatitis B: recommendations from an Italian workshop. *Dig Liver Dis.* 2008, 40(8):603-17.
- 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.
- Colle I, Adler M, Brenard R, et al. Management and treatment of chronic hepatitis B virus: Belgian Association for the Study of the Liver (BASL) 2007 guidelines. *Acta Gastroenterol Belg.* 2007;70:389-420.
- Cooksley WG, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, Chang WY, Zahm FE, Pluck N. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. *J Viral Hepat.* 2003, 10(4):298-305.
- 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(12):1281-328.
- Di Bisceglie A, Lai C, Gaines E, More A, Authors M. Telbivudine GLOBE trial: maximal early HBV suppression is predictive of optimal two-year efficacy in nucleoside-treated hepatitis B patients [abstract]. *Hepatology* 2006; 44: 230A-1A.

- 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 Dec 21;333(25):1657-61.
- EASL Clinical Practice Guidelines: Management of chronic hepatitis B. European Association for the Study of the Liver. *J Hepatol*. 2008 Oct 29. [Epub ahead of print]
- EASL Jury: EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002: Geneva, Switzerland. Consensus statement (short version). *J Hepatol* 2003, 38:533-540.
- 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.
- Fried MW, Piratvisuth T, Lau GK, Marcellin P, Chow WC, Cooksley G, Luo KX, Paik SW, Liaw YF, Button P, Popescu M. HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. *Hepatology*. 2008, 47(2):428-34.
- Gaspar G, Monereo A, Garcia-Reyena A, de Guzman M. Fanconi syndrome and acute renal failure in a patient treated with tenofovir: a call to action. *AIDS* 2004; 18:351-2.
- 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.
- Juszczak J, Boroń-Kaczmarska A, Cianciara J, et al. Polish Experts Group on HBV. Therapeutic recommendations on 2008 year (antiretroviral treatment of chronic hepatitis B). *Przegl Epidemiol*. 2008;62(2):379-81.
- Hadziyannis SJ, Tassopoulos N, Heathcote EJ, Chang T-T, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Wulfsohn MS 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, Sevastianos V, Rapti IN, Tassopoulos N. Sustained biochemical and virological remission after discontinuation of 4 to 5 years of adefovir dipivoxil (ADV) treatment in HbeAg-negative chronic hepatitis B. 57th Annual Meeting of the American Association for the study of Liver Diseases 2006, abstract 114.
- Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B up to 5 years. *Gastroenterology* 2006; 131: 1743–51.
- Hadziyannis SJ, Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: natural history and treatment. *Semin Liver Dis* 2006; 26: 130–41.
- Heathcote J, George J, Gordon S et al. Tenofovir disoproxil fumarate (TDF) for the treatment of HBeAg positive chronic hepatitis B: week 72 TDF data and week 24 adefovirdipivoxil switch data (study 103). 43rd Annual Meeting of the European Association for the Study of the Liver 2008, abstract 1593.
- 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: 678–86.
- Keeffe EM, Zeuzem S, Koff RS, et al. *Clin Gastroenterol Hepatol*. 2007, 5:890-97.
- 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 Jan;45(1):97-101

## 140 HBV Treatment - Standard of care

- 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(2):528-36.
- Lai CL, Gane E, Hsu C, et al. Two-year results from GLOBE trial in patients with hepatitis B: greater clinical and antiviral efficacy for telbivudine (LdT) vs lamivudine [abstract]. *Hepatology* 2006; 44: 222A.
- Lampertico P, Vigano M, Manenti E, Iavarone M, Lunghi G, Colombo M. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. *Hepatology* 2005; 42:1414–9.
- Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N; 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; 352(26):2682-95.
- Lau GK. Does treatment with interferon-based therapy improve the natural history of chronic hepatitis B infection? *J Hepatol*. 2007; 46(1):6-8.
- Liaw Y-F, Suh DJ, Omata M. Available at: [www.apasl.info/guidelinesHBV.html](http://www.apasl.info/guidelinesHBV.html).
- Liaw YF, Sung JJ, Chow WC et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *New Engl J Med* 2004; 351:1521-1531.
- Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Chien RN, Dent J, Roman L, Edmundson S, Lai CL. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *Gastroenterology*. 2000; 119(1):172-80.
- Lisotti A, Azzaroli F, Buonfiglioli F, Montagnani M, Alessandrelli F, Mazzella G. Lamivudine treatment for severe acute HBV hepatitis. *Int J Med Sci*. 2008;5(6):309-12.
- Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–39.
- 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(1):172-80.
- Manns M, Jeffers L, G. Dalekos G et al. The antiviral response to tenofovir disoproxil fumarate (TDF) is comparable in lamivudine (LAM)-naive and LAM-experienced subjects treated for chronic Hepatitis B (CHB). 43rd Annual Meeting of the European Association for the Study of the Liver 2008, abstract 1587.
- Marcellin P, Jacobson I, Habersetzer F, Senturk H, Andreone P, Moyes C, Horban A, Teuber G, Sorbel J Anderson J et al.:Tenofovir disoproxil fumarate (TDF) for the treatment of HBeAg-negative chronic hepatitis B: week 72 TDF data and week 24 adefovir dipivoxil switch data (study 102). 43rd Annual Meeting of the European Association for the Study of the Liver 2008, abstract 1602.
- Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N; Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group. *N Engl J Med*. 2004; 351(12):1206-17.
- 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(6):1309-19.

- Nevens F, Main J, Honkoop P, Tyrrell DL, Barber J, Sullivan MT, Fevery J, De Man RA, Thomas HC. Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. *Gastroenterology*. 1997; 113(4):1258-63.
- 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(10):1104-8.
- Schmutz G, Nelson M, Lutz T, Sheldon J, Bruno R, von Boemmel F, Hoffmann C, Rockstroh J, Stoehr A, Wolf E, Soriano V, Berger F, Berg T, Carlebach A, Schwarze-Zander C, Schurmann D, Jaeger H, Mauss S. Combination of tenofovir and lamivudine versus tenofovir after lamivudine failure for therapy of hepatitis B in HIV-coinfection. *AIDS*. 2006; 20(15):1951-4.
- Sherman M, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, Sette H, Tsai N, Tenney DJ, Vaughan J et al.: Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. *Hepatology* 2008, 48:99-108.
- Sung JJ, Lai JY, Zeuzem S et al. A randomised double-blind phase II study of lamivudine compared to lamivudine plus adefovir dipivoxil for treatment naive patients with chronic hepatitis B: week 52 analysis. *J Hepatol* 2003; 38 (suppl 2):25-6.
- Tenny DJ, Pokornowsky KA, Rose RE, et al. Entecavir at five years shows long-term maintenance of high genetic barrier to hepatitis B virus resistance [abstract]. *Hepatol Int* 2008; 2: A88-9.
- Tillmann HL, Hadem J, Leifeld L, Zachou K, Canbay A, Eisenbach C, Graziadei I, Encke J, Schmidt H, Vogel W, Schneider A, Spengler U, Gerken G, Dalekos GN, Wedemeyer H,
- Van Bömmel 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. 43rd Annual Meeting of the European Association for the Study of the Liver 2008, abstract 1594.
- Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C, Marcelin P, Goodman Z, Delaney WE 4th, Xiong S, Brosgart CL, Chen SS, Gibbs CS, Zoulim F. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology*. 2004; 126(7):1750-8.
- Wursthorn K, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, Longerich T, Schirmacher P, Metzler F, Zankel M, Fischer C, Currie G, Brosgart C, Petersen J. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. *Hepatology*. 2006; 44(3):675-84.



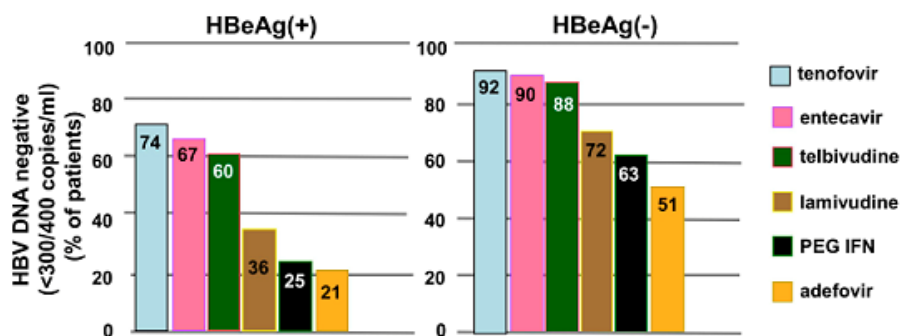
## Chapter 10: HBV - Resistance and implications for therapeutic strategies

By Stefan Mauss and Heiner Wedemeyer

### Introduction

Interferon monotherapy has been the standard of care for chronic hepatitis B since the mid-90s. Primary resistance to interferon present as HBe- or HBs-antigen loss or 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 so far.

Since the introduction of lamivudine, the 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 the 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, particularly in HBe-antigen negative patients harbouring the precore-mutant, a long-term suppression of HBV is needed due to the high relapse rate after discontinuation of antiviral therapy in the absence of HBs-antigen seroconversion, a rare event during 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.

The development of resistance and cross-resistance of HBV polymerase inhibitors is a relevant issue for 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 in patients with few effective treatment options. In addition, some HBV polymerase variants may interact with immunological relevant epitopes of the envelope resulting in immune escape mutants. These mutants may be able to infect successfully vaccinated individuals. Although this finding is still only an *in vitro* observation, the confirmation of this phenomenon in pa-

tients 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**

Treating patients for longer periods with HBV polymerase inhibitors can result in the development of viral resistance - particularly in patients without strong suppression of viral replication (Lai 2006). As a consequence of this, therapy with HBV polymerase inhibitors should fully suppress viral replication (HBV DNA 300 or less copies/ml). If this goal is not achieved with HBV polymerase monotherapy within 6-12 months, treatment should be revisited and combination therapy with non-cross resistant HBV polymerase inhibitors considered. Testing of HBV DNA is recommended after the first 4-6 weeks of therapy and then every 3-6 months while on therapy. Resistance to nucleoside HBV polymerase inhibitors eliminates or markedly reduces antiviral efficacy of all other nucleosides and may affect even nucleotide HBV 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 study (Liaw 2004) and even with a lower overall survival in another 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 or even to start out with combination therapy in cirrhotic individuals.

## **Treatment endpoints**

In HBe-antigen-positive patients infected with wild-type HBV strains HBe-antigen seroconversion has been shown to be associated with a reduction in liver-associated morbidity and increased survival (Niederer 1996). Thus, HBe-antigen seroconversion is considered a clinical endpoint in this patient population and discontinuation of HBV polymerase inhibitors is recommended about 6-12 months after HBe-antigen seroconversion in patients who do not have liver cirrhosis (Cornberg 2007). HBe-antigen loss is reported in up to 50% of patients treated with HBV polymerase inhibitors after prolonged periods of therapy for several years (Hadziyannis 2006). Treatment with pegylated interferon alfa for 48 weeks results in HBe-antigen seroconversion in about a third of patients (Lau 2005).

Discontinuation of HBV polymerase inhibitor therapy in patients without HBe-antigen seroconversion usually results in relapse of chronic hepatitis B. With interferon alfa the situation may be more complex and at least partially dependent on the HBV-genotype in addition to the HBe-antigen status (Erhardt 2005).

Treatment endpoints in HBeAg-negative hepatitis B are obviously in most cases restricted to sustained normalisation of ALT levels and suppression of HBV DNA as HBs-antigen seroconversion is a rare event with the 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 prolonged treatment of 2-5

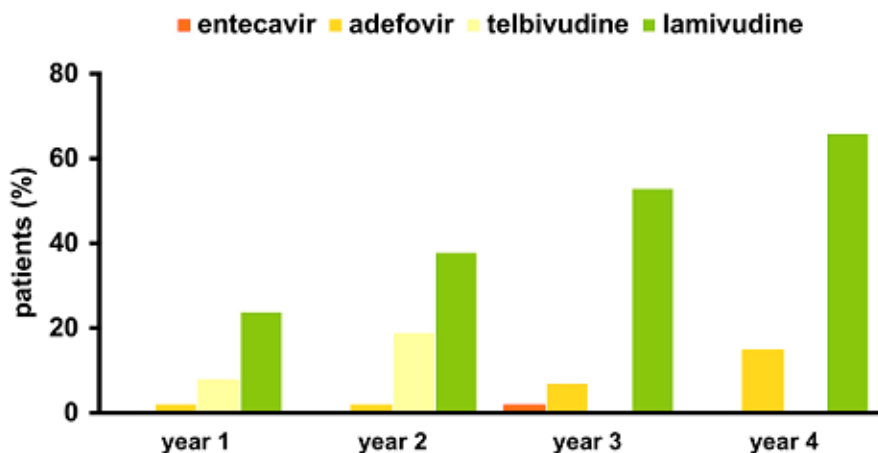


years. Most guidelines therefore recommend indefinite treatment of patients with HBeAg-negative chronic hepatitis B. PEG-Interferon alpha-2a has been studied also in HBeAg-negative hepatitis B leading to a 6-month off-treatment response (HBV-DNA <400 copies/ml) in up to 20% of patients (Marcellin 2004).

HBs-antigen seroconversion is induced in about 5% of patients after a year of treatment with pegylated interferon. In addition, about 20% patients reach a low replicative status of their chronic hepatitis B, at least temporarily, after discontinuation of interferon (Bonino 2007). For HBV polymerase inhibitors HBs-antigen seroconversion has been reported in less than 5% of HBe-antigen negative patients in published prospective studies.

## Resistance patterns of HBV polymerase inhibitors

Lamivudine was the first approved HBV polymerase inhibitor. It is characterised by good clinical tolerability, moderate antiviral efficacy and rather quick development of resistance in cases of not fully suppressive antiviral therapy (Figure 2).



1. Qi YL et al. EASL 2005. Abstract 57. 2. Borroto-Esoda K. EASL 2006. Abstract 483. 3. Colonno R.J. ISVHLD 2006. 4. Lai et al. AASLD 2006

Figure 2. Cumulative incidence of resistance to HBV polymerase inhibitors.

These numbers are average estimates based on numerous studies. Resistance rates differ between trials and cohorts. Overall, resistance has been higher in HBeAg-patients than in HBeAg-negative patients. Long-term data for adefovir has only been reported for HBeAg-negative patients; resistance rates may be higher for HBeAg-positive individuals. Data for entecavir is biased since 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 after one year of treatment.

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 and about 70-80% of patients without HBe-antigen seroconversion develop lamivudine-resistant variants after four or more years (Figure 3).

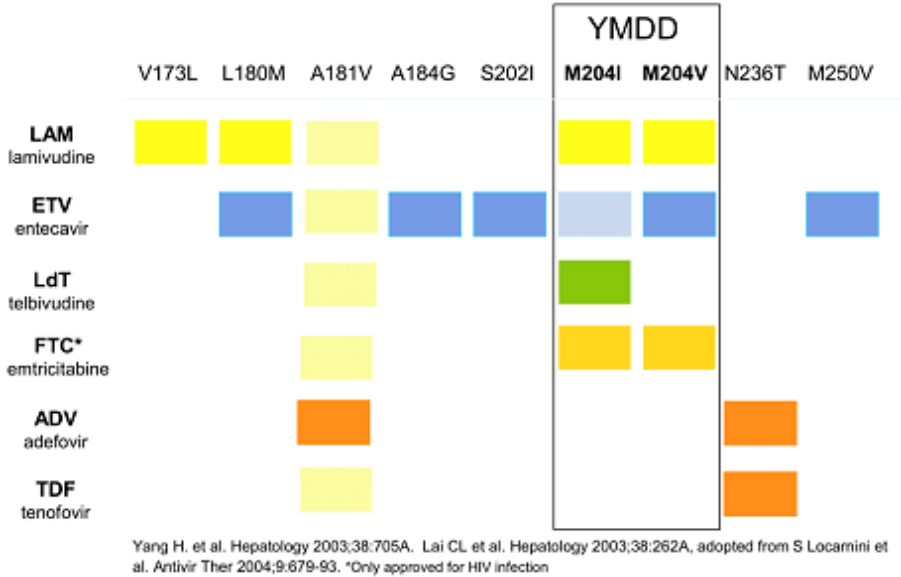


Figure 3. Resistance patterns of different antiviral drugs used for the treatment of chronic hepatitis B.

The numbers indicate the respective amino acid position in the HBV polymerase gene. For entecavir resistance, the 204/180 variant 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 not only *in vitro* but also *in vivo*.

Lamivudine mutations may confer cross-resistance to telbivudine, emtricitabine and entecavir. Preliminary data indicate that development of multiple lamivudine-associated mutations may reduce the efficacy of tenofovir therapy (Lada 2008).

Emtricitabine has comparable antiviral properties and a similar resistance profile to lamivudine (Lim 2006). However, it is approved as an antiretroviral 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 HBe-antigen positive and negative patients. However, resistance development is considerable in naïve patients and the resistance pattern is essentially the same as for lamivudine (Lai 2006), resulting in complete cross-resistance of the two compounds (Figure 4). 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).

| <b>Resistance against nucleoside analogues</b>  |   | <b>Recommended Therapeutic Option<br/>"add-on"</b>          |
|---|---|---|
| lamivudine                                      | → | adefovir, tenofovir<br>(switch to entecavir)                |
| telbivudine                                     | → | adefovir, tenofovir<br>(switch to entecavir)                |
| entecavir                                       | → | adefovir, tenofovir   |
| <b>Resistance against nucleotide analogues</b>  |   |   |
| adefovir (LAM-naive)                            | → | entecavir, telbivudine, lamivudine<br>(switch to tenofovir) |
| adefovir (LAM-resist.)                          | → | entecavir + tenofovir                                       |
| tenofovir<br>(no <i>in vivo</i> data available) | → | entecavir, telbivudine, lamivudine                          |

Figure 4. 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 nephrotoxicity. Due to tubular damage of the kidney the approved dose was limited to 10 mg/day, although 30 mg/day has shown 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 the registration 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).

Adding adefovir to 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 a nucleoside HBV polymerase inhibitor with good antiviral efficacy and a slow development of resistance in treatment naïve patients (Chang 2006; Lai 2006). This is due to the fact that more than one mutation in the HBV polymerase gene is required to confer resistance to it. However, entecavir shares some resistance mutations with lamivudine and telbivudine (Figure 4). 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 the 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; Colonna 2007).

According to preliminary data entecavir may have a reduced efficacy in patients with adefovir resistance (Reijnders 2007).

Tenofovir is approved for treatment of HIV and HBV. Early data from HBV/HIV coinfecting patients showed a strong antiviral potency and slow development of resistance (Núñez 2002; Nelson 2003; van Bommel 2004). In the pivotal registration trials tenofovir was superior to adefovir resulting in substantially higher rates of full viral suppression in HBe-antigen positive (tenofovir 69% vs. adefovir 9%, HBV DNA <40 IU/ml) and HBe-antigen negative patients (tenofovir 91% vs. adefovir 56%, HBV DNA <40 IU/ml) at 52 weeks of therapy (Heathcote 2007; Marcellin 2007). In HIV-positive patients anecdotal cases of renotubular dysfunction have been reported. Otherwise tenofovir is well tolerated. It is active in lamivudine-resistant patients, although an accumulation of a high number of lamivudine-induced mutations may partially decrease the antiviral efficacy of tenofovir (Schmutz 2006; Lada 2008). Anecdotal data suggest reduced efficacy in the presence of adefovir resistance, which was not confirmed in a recently presented cohort study (van Bommel 2006; Berg 2008).

## **Combination therapy of chronic hepatitis B to enhance antiviral efficacy and delay development of resistance**

Combination therapy is thought to be superior to monotherapy, particularly in patients with highly replicative hepatitis B (HBV DNA >10<sup>9</sup> 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 2003). Trials that will provide more evidence on how to best use the current antiviral options are being designed. However, these trials may require large patient numbers and long 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 presently no strategic trials assessing these two different therapeutic approaches are being conducted. However, it has to 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 that have a high resistance barrier, even treatment-naïve patients with high levels of HBV replication may be initially treated with monotherapy. The key issue, however, is to adapt therapy 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 as a reduction of HBV DNA to at least  $<10^3$  copies/ml (200 IU/ml) or by a continuous drop of HBV DNA up to month 12. In contrast, if a rise in HBV DNA by at least one log is observed while on continuous antiviral therapy, a secondary resistance is highly likely to be present. HBV resistance usually arises several months before biochemical relapse with elevation of transaminases and 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.

However, up to 30% of all viral breakthroughs are the result of adherence problems. 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 cross-resistance (Locarnini 2004). Knowledge of the antiviral efficacy, the resistance barrier and the resistance profile of each available oral antiviral drug should be 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, nucleoside addition to ongoing nucleotide treatment should be performed if adefovir or tenofovir treatment failure occurs (Figure 5). An additional switch from adefovir to tenofovir can be assessed as a second measure.

Historically most data have been generated for patients having experienced lamivudine resistance. In this setting the advantage of adding adefovir rather than switching to adefovir has been 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 and before a biochemical relapse has occurred.

## Special considerations in HIV/HBV-coinfected patients

In patients with chronic hepatitis B and HIV the first question to ask is whether patients have an indication for antiretroviral therapy. In patients without such an indication ( $>350$  CD4-positive cells/ $\mu$ l) interferon or an HBV polymerase inhibitor without HIV activity are an option. The initially recommended monotherapy with entecavir is now considered obsolete as anti-HIV activity of entecavir was recently described that led to the emergence of relevant HIV mutations (M184V) in anecdotal cases (MacMahon 2007). Currently adefovir and telbivudine are recommended based on limited *in vivo* data for adefovir or exclusively on *in vitro* data for telbivudine (Delaugerre 2002; Sheldon 2005). 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 coinfecting 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 an antiretroviral regimen in HBV/HIV-coinfecting 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 in particular of lamivudine resistance is the induction of conformational changes in the HBs-antigen due to an overlapping reading frame in the genetic sequence of the HBV polymerase and the HBs-antigen (Figure 5, see <http://hepatologytextbook.com/link.php?id=3>). Because of this, mutations in the HBV polymerase may induce changes in the envelope of the virus resulting in altered immunogenicity. This may result in vaccine escape mutants. *In vitro* and *ex vivo* studies support this hypothesis which could have important public health implications (Mathews 2006; Sheldon 2007). 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 vaccine escape mutants of HBV.

The YMDD-motif is also part of an MHC-class I-restricted CTL epitope. YMDD-specific 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 more 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, differences in potency to control the development of resistance and differences in antiviral efficacy (Figure 5). In consequence, resistance due to suboptimal treatment by one compound eliminates or reduces the effect of the following agents due to partial or complete cross-resistance. This is well documented for lamivudine, telbivudine and entecavir (Figure 3). There are some data indicating the possibility of partial cross-resistance between entecavir and tenofovir. The potential of adefovir to induce resistance to tenofovir is not well studied and currently under discussion.

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 when rapid suppression of HBV replication is achieved.

The choice of the first treatment strategy will determine any future treatment options and may, in cases of suboptimal therapeutic approaches, result in a rapid exhaustion of options within only a few years.

## References

- Benhamou Y, Bochet M, Thibault V, Di Martino V, Caumes E, Bricaire F, Opolon P, Katlama C, Poynard T. 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, 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).
- Bonino F, Marcellin P, Lau GK, Hadziyannis S, Jin R, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Brunetto MR, Farci P, Popescu M, McCloud P; 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.
- 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.
- 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, Walsh A, Fang J, Hsu M, Mazzucco C, Eggers B, Zhang S, Plym M, Kleszczewski K, Tenney DJ. 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, Jilg W, Erhardt A, Wirth S, Schirmacher P, Fleig WE, Manns MP. 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, 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.
- Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, Rizzetto M, Craxi A; 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 in 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

## 152 HBV - Resistance and implications for therapeutic strategies

- hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol.* 2006;101(2):297-303.
- Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Brosgart CL, Borroto-Esoda K, Arterburn S, Chuck SL; 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 EJ, Gane E, DeMan R, Lee S, Flisiak R, Manns MP, Tchernev K, Kaldas O, Shiffman ML, Sorbel J, Anderson J, Mondou E, Rousseau F. 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.
- Lada O, Gervais A, Branger M, Peytavin G, Colin G, Fraqueiro G, Males S, Martinot-Peignoux M, Matheron S, Marcellin P. 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, 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(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, Viganò 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.
- Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N; 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, Chung YH, Lee YS, Yoo W, Kim SO. 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, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J; 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.
- Lim SG, Ng TM, Kung N, Krastev Z, Volfova M, Husa P, Lee SS, Chan S, Shiffman ML, Washington MK, Rigney A, Anderson J, Mondou E, Snow A, Sorbel J, Guan R, Rousseau F; 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, Pawlotsky JM, Zoulim F. Management of antiviral resistance in patients with chronic hepatitis B. *Antivir Ther*. 2004; 9 (5): 679-93.
- Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, Jeffers L, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart CL; 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, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N; 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, Buti M, Krastev Z, Germanidis G, Kaita KD, Kotzev I, Buggisch P, Weillert F, Trinh HN, Sorbel J, Anderson J, Mondou E, Rousseau F. 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, 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.
- 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.
- 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, Hoffmann C, Rockstroh J, Stoehr A, Wolf E, Soriano V, Berger F, Berg T, Carlebach A, Schwarze-Zander C, Schurmann D, Jaeger H, Mauss S. 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-naïve 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.
- Sung JJ, Lai JY, Zeuzem S et al. A randomised double-blind phase II study of lamivudine compared to lamivudine plus adefovir dipivoxil for treatment naïve patients with chronic hepatitis B: week 52 analysis. *J Hepatol* 2003; 38 (suppl 2):25-6.

## 154 HBV - Resistance and implications for therapeutic strategies

- Tenney DJ, Rose RE, Baldick CJ, Levine SM, Pokornowski KA, Walsh AW, Fang J, Yu CF, Zhang S, Mazzucco CE, Eggers B, Hsu M, Plym MJ, Poundstone P, Yang J, Colonno RJ. 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 Bommel F, Zoellner B, Moeller B et al. Is tenofovir effective in treatment of adefovir resistant hepatitis B virus infections? *Hepatology*, 2006, 44, 567A.
- Westland C, Delaney W 4th, Yang H, Chen SS, Marcellin P, Hadziyannis S, Gish R, Fry J, Brosgart C, Gibbs C, Miller M, Xiong S. Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. *Gastroenterology.* 2003;125(1):107-16.

# 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.

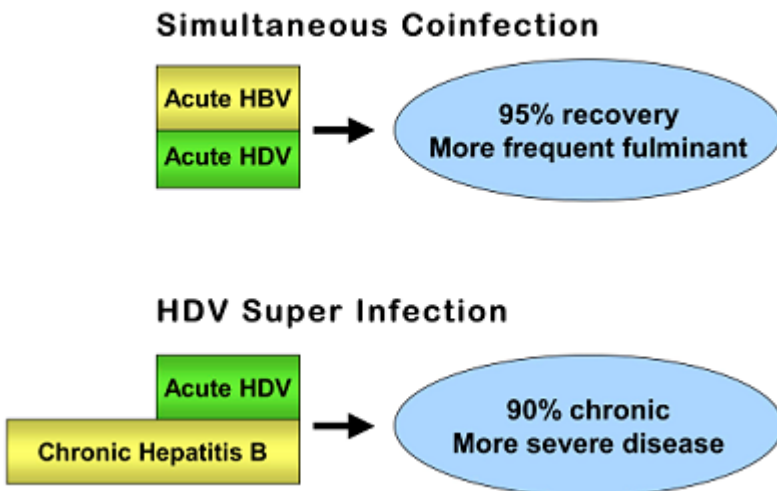


Figure 1. Courses of delta hepatitis.

## 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 HIDA-T-I treatment trial in Germany, Turkey and Greece were proven to be infected with HDV genotype I (Zachou 2006).

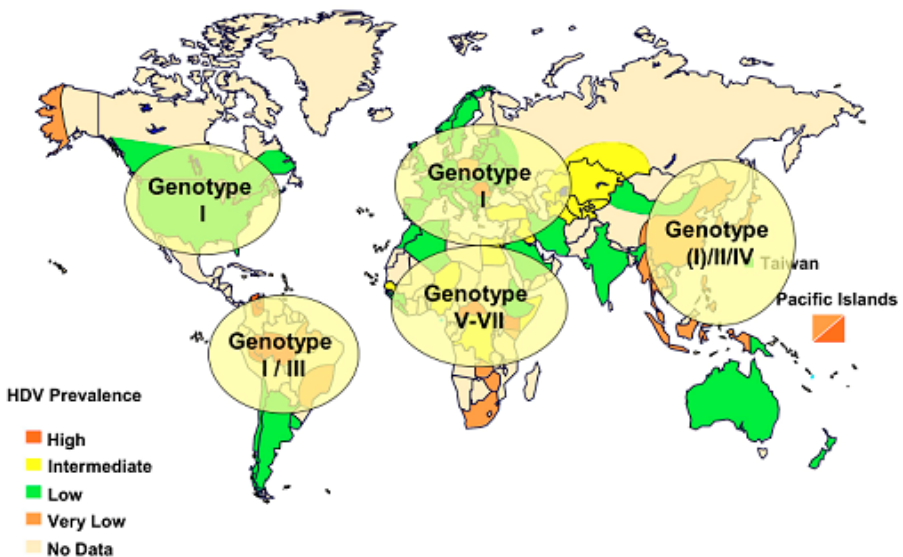


Figure 2. Prevalence of HDV genotypes.

## Epidemiology of delta hepatitis

Hepatitis delta 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 prevalences 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).

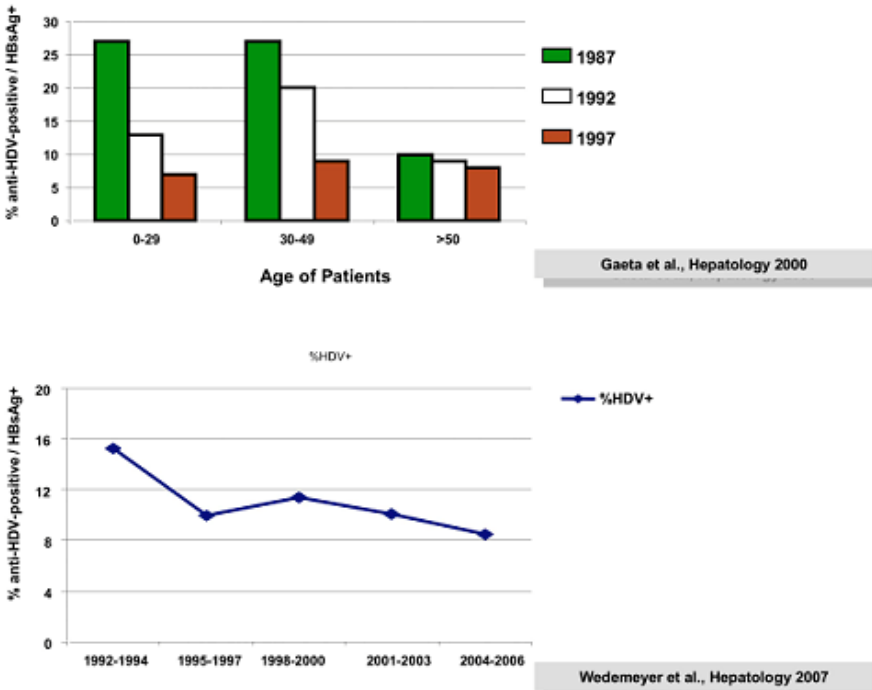


Figure 3. Prevalence of hepatitis D virus in Italy and Germany.

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).

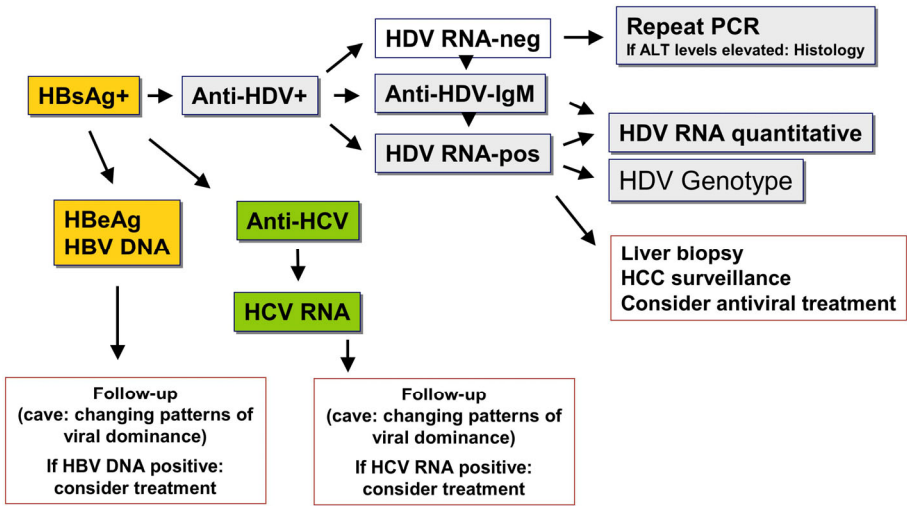


Figure 4. Diagnostic steps in delta hepatitis.

| Origin of patients     | HDV diagnosis 1992-1996<br>N = 43 | HDV diagnosis 1997-2006<br>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 [%] | 11.6 (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. Anti-viral 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  $\alpha$ -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 antivi-



ral treatment 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  $\alpha$ -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 (Figure 5).

## 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; Figure 6). 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<sub>10</sub> to 5.8 log<sub>10</sub> 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).

|  |  |
|--|--|
| <b><u>Nucleos(t)ide analogues</u></b>  |  |
| • Famciclovir ineffective  | <i>Yurdaydin et al., J Hepatol 2002</i>  |
| • Lamivudine ineffective   | <i>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</i> |
| • Ribavirin ineffective  | <i>Niro et al., Hepatology 2006</i>  |
| <i>Garripoli et al., Liver 1994</i>  | <i>Gunsar et al., Antiv Therapy 2005</i>   |
| <b><u>Interferon alpha</u></b>   |  |
| • Sustained biochemical responses in 0-36% of patients<br>Few studies with virologic endpoints<br>Treatment for >12 months may be required | <i>Farci et al., NEJM 1994<br/>Di Marco et al., J Viral Hepatitis 1996<br/>Niro et al., J Viral Hepatitis 2005; Yurdaydin et al., J Viral Hepatitis 2008</i>                                 |
| • Higher IFN doses were associated with better survival in small cohort study  | <i>Farci et al., Gastroenterology 2004</i>   |

**Table 2. Treatment options in delta hepatitis.**

## Recombinant interferon alpha

Interferon alpha 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) (Figure 7).

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% (Castelnaud 2006; Niro 2006; Erhardt 2006) (Table 3).

|  |   |
|--|---|
| <p><b>Castelnaud, Gault et al. Hepatology 2006</b><br/>14 patients, 12 months of pegIFN <math>\alpha</math>-2b</p>   | <p><b>SVR in 6 patients (43%)</b></p>   |
| <p><b>Niro, Rizzetto et al. Hepatology 2006</b><br/>38 patients, 72 weeks pegIFN <math>\alpha</math>-2b<br/>16 patients monotherapy<br/>22 patients + ribavirin (first 48 weeks)</p> | <p><b>SVR: 8 patients (21%)</b><br/><b>Ribavirin had no additional effect</b></p> |
| <p><b>Erhardt, Häussinger et al. Liver Int 2006</b><br/>12 patients, 48 weeks of pegIFN <math>\alpha</math>-2b</p>   | <p><b>SVR in 2 patients (17%)</b></p>   |

**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  $\mu$ g PEG-IFN  $\alpha$ -2a qw plus 10 mg adefovir dipivoxil qd (group A, N = 31), 180  $\mu$ g PEG-IFN  $\alpha$ -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  $\alpha$ -2a alone or adefovir monotherapy had similar mean HBsAg levels at week 0 and week 48, the PEG-IFN  $\alpha$ -2a/adefovur 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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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.



- 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 pre-core 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, Agelopoulos 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.

## 166 Hepatitis D - Diagnostic procedures and therapy

- 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 associated 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.
- Uzunlimoglu 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 adefovir dipivoxil vs. pegylated interferon-alfa-2a plus placebo vs. adefovir 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: Acute and chronic hepatitis C – Diagnostic tests

Christian Lange and 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.

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 3<sup>rd</sup> generation assays. Positive serologic results require HCV RNA measurement 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 have a very high sensitivity 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 therefore essential in treatment monitoring. Qualitative and quantitative HCV RNA assays are now being widely replaced by real-time PCR-based assays that 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 directed against multiple HCV epitopes are detected by commercially available 2<sup>nd</sup> and 3<sup>rd</sup> 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. 2<sup>nd</sup> generation EIAs additionally detect antibodies directed 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 3<sup>rd</sup> 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 2<sup>nd</sup> generation assays. Anti-HCV-IgM measurement can narrow the diagnostic window in only a minority of patients. Moreover, anti-HCV-IgM detection is 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 with production of 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 development of the more 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 false-negatively-classifying 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.

## 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 antiviral therapy in patients with chronic hepatitis C, the World Health Organization (WHO) has 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).

| Name of assay                                | Distributor                           | Technology    | Marked status |
|--|---------------------------------------|---------------|---------------|
| <b>Qualitative HCV RNA detection assays</b>  |                                       |               |               |
| Amplicor™ HCV 2.0                            | Roche Molecular systems               | PCR           | FDA, CE       |
| Versant™ HCV                                 | Siemens Medical Solutions Diagnostics | TMA           | FDA, CE       |
| <b>Quantitative HCV RNA detection assays</b> |                                       |               |               |
| 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       |
| 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. In addition the cost of a qualitative assay is lower compared to a quantitative assay. For this 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 an 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 is characterised by 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 which is 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 and 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 HCV RNA amount. The dynamic range of the CE-labeled Amplicor™ HCV-Monitor 2.0 assay is 600 to approximately 500,000 IU/ml with a specificity of almost 100%, independent from the HCV genotype (Lee 2000; Konnick 2002). For higher HCV RNA concentrations pre-dilution of the original sample is required.

### **Branched DNA-Hybridizing Assay (Versant™ HCV RNA Assay 3.0, Siemens)**

Branched DNA-Hybridizing 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 in a range of 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.

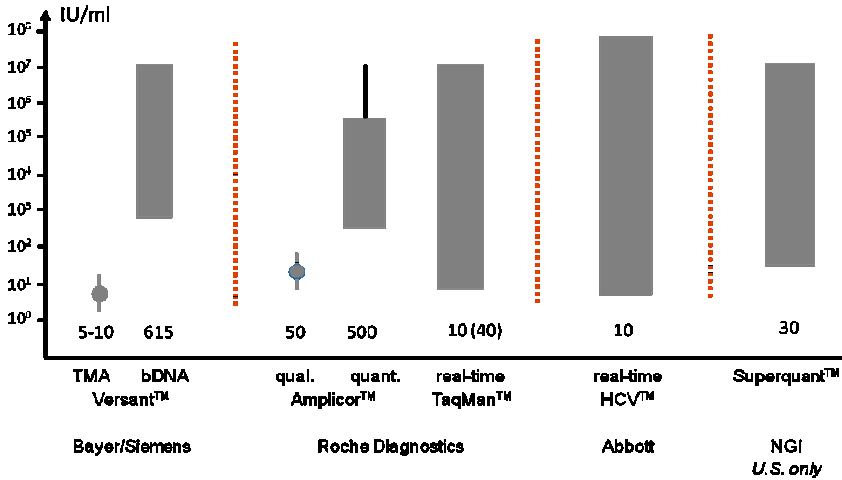


Figure 1. Detection limits and linear dynamic ranges of commercially available HCV RNA detection assays.

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 (USA) and Abbott Laboratories (USA). 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 2009.

All commercially available HCV RNA assays are calibrated to the WHO standard that is based on the HCV genotype 1. Significant differences between different RT-PCR assays and other quantitative HCV RNA tests have been reported which in 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 assay (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. HCV genotype 2-5 samples 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 underquantification 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 within one system and complete automation.

## Abbott RealTime HCV Test (North Chicago, IL, USA)

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 *Curcubita pepo*, which is used as an internal standard. This internal standard is amplified with different primers than those of the HCV RNA, which might also 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, which has developed by the rapid replication cycle with the production of  $10^{12}$  virions per day and the low fidelity of the HCV RNA polymerase. So far, six genotypes (1-6) and multiple subtypes (a, b, c...) have been characterised. Different genotypes vary in at least 30% of their RNA sequence with a median variability of approximately 33%. HCV subtypes are defined by differences in their RNA sequence of between 10 and 29%. 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 geno-

typing 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, however, 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 to identify 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 genotype 1 subtypes. In a recent 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 HCV genotype and subtype by direct analysis of the nucleotide sequence of the 5'UTR region. Incorrect genotyping rarely occurs by using 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).

## 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 after several weeks 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 does not help in this constellation 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 2<sup>nd</sup> or 3<sup>rd</sup> generation EIAs is adequate because their sensitivity is over 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. Serologic 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 by commercially used 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 resistances. Low HCV RNA concentration (<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) infection 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  $2_{\log}$  decline of HCV RNA concentration at week 12 but still detectable HCV RNA levels and undetectable HCV RNA until 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 genotype 2 and 3 infections generally allow treatment durations 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. Therefore HCV RNA is quantified before and 12 weeks after treatment initiation and antiviral therapy should be discontinued when a decrease of less than  $2_{\log_{10}}$  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. Finally, SVR, which is 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 PS, Griffith 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 Microbiol* 32(4): 289-93.
- Konnick EQ, Erali M, Ashwood ER, Hillyard 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 ER, Hillyard 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 SJ, Layden 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 H, Cornberg 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.
- 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, Babel 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 F, Zeuzem 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.

## 182 Acute and chronic hepatitis C – Diagnostic tests

- 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 JD, Gretsch 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.
- Vermeiren 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: Standard of care

By Markus Cornberg, Michael P. Manns and Heiner Wedemeyer

### Management of acute hepatitis C

The aim 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, the early treatment of acute HCV infection with interferon alpha (IFN) is the only option to prevent persistent HCV infection; however, the diagnosis of acute primary HCV infection may be difficult and distinction from the exacerbation of an underlying unrecognized chronic HCV infection may be difficult (Pawlotsky 2002).

The immediate treatment of patients with symptomatic acute hepatitis C with recombinant IFN or PEG-IFN monotherapy for 24 weeks can prevent the development of chronic hepatitis C in approximately 90% of cases (Broers 2005; Jaeckel 2001; Santantonio 2005; Vogel 1996; Wiegand 2006). However, compliance and adherence to therapy are prerequisites for this (Wiegand 2006) (Table 1). A combination with ribavirin does not seem to be necessary. Symptomatic patients also have a good chance of clearing HCV spontaneously (Gerlach 2003; Hofer 2003). This usually occurs within 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 be considered for immediate treatment since these patients have a higher risk for chronic evolution (Flieg 2004).

However, the overall impact of early treatment of acute HCV infection to prevent chronic disease has limitations. One problem is that primary HCV infection is usually asymptomatic and most patients are not identified in this early stage of disease. Another reason is that a number of patients have medical contraindications for treatment with interferon (IFN) or otherwise may not be suitable for therapy due to social issues like being active drug users. There are two concerns to treating active drug users with IFN: In the case of successful therapy there is a risk of re-infection with HCV (Davis 2001). Another reason is the side effect profile of IFN, especially the neuropsychiatric problems during therapy that may result in worsening of addictive behaviour (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 open questions on the treatment of acute hepatitis C (Table 2) that may be answered in ongoing clinical studies. For example, a study coordinated by the German Competence Network for Viral Hepatitis (Hep-Net) (Manns 2003) is under way to test if a wait-and-see strategy is as effective as immediate treatment ([www.kompetenznetz-hepatitis.de/study\\_house/hcv\\_III\\_studie.htm](http://www.kompetenznetz-hepatitis.de/study_house/hcv_III_studie.htm)). 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 re-cruited in 24 centers | IFN $\alpha$ -2b (4 weeks 5 MU daily, 20 weeks 5 MU tiw) | 89 days after infection (range 30-112 days)   | 24 weeks | 43/44 (98%)                                     |
| (Santantonio 2005) | 28                          | PEG-IFN $\alpha$ -2b (1.5 $\mu$ g/kg/week)               | 12 weeks after onset of disease (17/28 chronic, 16 treated)   | 24 weeks | 15/16 (94%)                                     |
| (Broers 2005)      | 27 (22 IDU)                 | PEG-IFN $\alpha$ -2b (1.5 $\mu$ g/kg/week)               | 100 $\pm$ 82 days after onset of symptoms, 63 $\pm$ 82 days after diagnosis (asymptomatic) (22/27 chronic, 14 were treated) | 24 weeks | 8/14 (57%)<br><br>7/8 (88%) of adherent pts.    |
| (Wiegand 2006)     | 89 re-cruited in 53 centers | PEG-IFN $\alpha$ -2b (1.5 $\mu$ g/kg/week)               | 76 days after infection (range 14-150 days), 27 days after onset of symptoms (range 5-131)                                  | 24 weeks | 63/89 (71%)<br><br>58/65 (89%) of adherent pts. |

**Table 1. Pivotal studies investigating early PEG-IFN therapy in patients with acute HCV infection.**

| Question                          | Answer   |
|-----------------------------------|--|
| Which patients should be treated? | Asymptomatic >Symptomatic?<br>HCV genotype 1 >HCV genotype 2/3<br>IDU patients?  |
| When to start treatment?          | Immediately in asymptomatic patients<br>Symptomatic patients: Immediate therapy versus wait-and-see?   |
| Which drugs?                      | Interferon alpha (5 MU daily first 4 weeks)<br>or PEG-Interferon alpha (standard dose for chronic hepatitis C)<br>Future: New enzyme inhibitors? |
| Treatment duration?               | <24 weeks?   |

**Table 2. Questions and best possible answers regarding therapy of acute HCV infection.**

## Standard therapy of chronic hepatitis C

### Aim of antiviral therapies

The importance of an effective treatment against hepatitis C is reflected by the 130 million people that are chronically infected with the virus. Despite the implementation of blood-donor screening in the early 90s, there will be an anticipated increase of HCV-related cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) over the next 10-20 years (Davis 2003). The aim of antiviral therapy is the cure of hepatitis C by sustained elimination of the virus. Long-term benefits are the reduction of HCV-related morbidity and mortality (Veldt 2007). A sustained elimi-



nation of HCV is achieved if the HCV RNA is negative 6 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 are also HCV RNA negative 5 years later and no signs of hepatitis have been documented (Manns 2008; McHutchison 2006; Swain 2007).

Several extrahepatic manifestations, such as cryoglobulinemia, non-Hodgkin's 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 SVR. However, antiviral therapy may worsen extrahepatic manifestations (Mazzaro 2005; Missiani 1994; Pischke 2008; Zignego 2007).

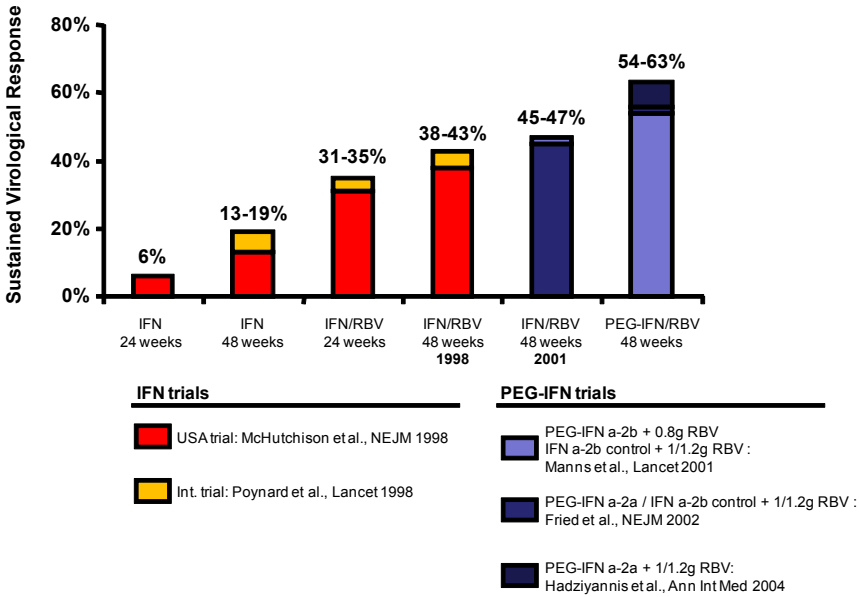
## Basic therapeutic concepts and treatment

Before the identification of HCV as the infectious agent for non-A, non-B hepatitis (Choo 1989) it turned out that IFN led to a normalization of transaminases and an improvement in liver histology (Hoofnagle 1986). Due to the identification of HCV it became possible to measure the success of therapy as a long-lasting disappearance of HCV RNA from serum, the so-called sustained virological response (SVR). Since the mid-80s, the SVR rate has increased from 5-20% with IFN monotherapy and up to 40-50% with the combination of IFN and ribavirin (Manns 2001; McHutchison 1998; McHutchison 2002; Poynard 1998). The development of pegylated interferon alpha (PEG-IFN) added a new milestone to the treatment of chronic hepatitis C (Cornberg 2002). Two PEG-IFNs are available; PEG-IFN  $\alpha$ -2b (PEG-Intron<sup>TM</sup>, Schering-Plough) and PEG-IFN  $\alpha$ -2a (PEGASYS<sup>TM</sup>, Hofmann La-Roche). Pegylation of the IFN allows a once weekly administration due to an improved pharmacokinetic profile. PEG-IFN/ribavirin combination therapy improved the overall SVR to 54-63% (Fried 2002; Hadziyannis 2004; Manns 2001) (Table 3).

There seems to be no difference between the two PEG-IFNs in combination with ribavirin regarding SVR (Mauss 2005). However, the PEG-IFNs do have different pharmacokinetic profiles due to their different polyethylene glycol moieties. PEG-IFN  $\alpha$ -2b is bound to a single linear 12 kDa polyethylene glycol molecule, whereas PEG-IFN  $\alpha$ -2a is covalently attached to a 40 kDa branched chain polyethylene glycol moiety. The distinct sizes of the PEG-IFNs influence the volume of distribution. PEG-IFN  $\alpha$ -2b is given adjusted for body weight (1.5  $\mu$ g/kg once weekly), while the larger PEG-IFN  $\alpha$ -2a is given in a fixed dose of 180  $\mu$ g once weekly [reviewed in (Cornberg 2002; Pedder 2003)] (Table 4).

Ribavirin should be administered according to the bodyweight of the patient. A retrospective analysis of the PEG-IFN  $\alpha$ -2b/ribavirin pivotal trial revealed that the optimal ribavirin (Rebetol<sup>TM</sup>, Schering-Plough) dose is at least 11 mg/kg (Manns 2001). A prospective, multicenter, open-label, investigator-initiated study confirmed that PEG-IFN  $\alpha$ -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  $\alpha$ -2a, a ribavirin (Copegus<sup>TM</sup>, Hofmann La-Roche) dose of 1000 mg if <75 kg or 1,200 mg if  $\geq$ 75 kg is recommended for HCV genotype 1 patients, while a dose of 800 mg riba-

virin is suggested for patients with HCV genotypes 2 and 3 (Table 4). The benefit of higher ribavirin doses has not been observed for genotype 2/3 patients in combination with PEG-IFN alpha-2a (Hadziyannis 2004). However, when reducing the treatment duration, i.e., to 16 weeks, a higher ribavirin dose may be important (Shiffman 2007). The Hadziyannis study (Hadziyannis 2004) also confirmed the 24 week schedule for HCV genotype 2/3 patients whereas patients with HCV genotype 1 require 48 weeks of therapy (Table 3). The 24-week regimen for patients with HCV genotypes 2 and 3 has also been confirmed for the combination of PEG-IFN  $\alpha$ -2b and ribavirin (Cornberg 2003; Zeuzem 2004) (Table 3).



**Table 1. Development of chronic hepatitis C therapy.**

The sustained virologic response rates have improved from around 5% with interferon mono-therapy in the early 90s to >60% currently with an optimized standard therapy of PEG-IFN plus ribavirin.

Early HCV RNA kinetics predict outcome and success of the treatment. Patients with HCV genotype 1 who do not show an HCV RNA decline of more than 2 log<sub>10</sub> or who have serum concentrations of more than 30,000 IU/ml HCV RNA after 12 weeks of therapy (TW12), have no chance of achieving a SVR (Berg 2003; Davis 2003). Thus, therapy should be discontinued in these patients.

The main challenge for the future is to improve the success rates for the difficult-to-treat and non-responsive HCV genotype 1 patients. While patients with HCV genotypes 2 and 3 can be cured in more than 75% of cases, the 40-50% SVR for patients with HCV genotype 1 is still unsatisfactory.

| Study             | Treatment   | HCV genotype | Duration | SVR                    |
|-------------------|---|--------------|----------|------------------------|
| (Manns 2001)      | 1.5µg/kg PEG-IFN $\alpha$ -2b<br>800 mg ribavirin       | HCV-1        | 48 weeks | 42%                    |
|                   |   | HCV-2/3      | 48 weeks | 82%                    |
|                   | 1.5µg/kg PEG-IFN $\alpha$ -2b<br>>10.6 mg/kg ribavirin  | HCV-1        | 48 weeks | 48%<br>(retrospective) |
|                   |   | HCV-2/3      | 48 weeks | 88%<br>(retrospective) |
| (Fried 2002)      | 180µg PEG-IFN $\alpha$ -2a                              | HCV-1        | 48 weeks | 46%                    |
|                   | 1000/1200 mg ribavirin                                  | HCV-2/3      | 48 weeks | 76%                    |
| (Hadziyanis 2004) | 180µg PEG-IFN $\alpha$ -2a<br>800 mg ribavirin          | HCV-1        | 24 weeks | 29%                    |
|                   |   | HCV-2/3      | 48 weeks | 40%                    |
|                   |   |              | 24 weeks | 78%                    |
|                   | 180µg PEG-IFN $\alpha$ -2a<br>1000/1200 mg ribavirin    | HCV-1        | 24 weeks | 73%                    |
|                   |   | HCV-2/3      | 48 weeks | 41%                    |
|                   |   |              | 24 weeks | 51%                    |
| (Zeuzem 2004)     | 1.5µg/kg PEG-IFN $\alpha$ -2b<br>800-1400 mg ribavirin  | HCV-2        | 24 weeks | 78%                    |
|                   |   | HCV-3        | 48 weeks | 77%                    |
| (Kamal 2005)      | 1.5µg/kg PEG-IFN $\alpha$ -2b<br>1000/1200 mg ribavirin | HCV-4        | 24 weeks | 93%                    |
|                   |   | HCV-3        | 36 weeks | 29%                    |
|                   |   |              | 48 weeks | 66%                    |
|                   |   |              | 48 weeks | 69%                    |

**Table 3. 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.**

|   | Dosing                               |
|---|--------------------------------------|
| <b>Type-I Interferons</b>                   |                                      |
| Pegylated Interferon alpha-2a (Pegasys®)    | 180 µg once weekly                   |
| Pegylated Interferon alpha-2b (PEG-Intron®) | 1.5 µg/kg once weekly                |
| Interferon alpha-2a (Roferon®)              | 3 – 4.5 Mill I.U. three times weekly |
| Interferon alfa-2b (Intron A®)              | 3 Mill I.U. three times weekly       |
| Consensus Interferon (Infergen®)            | 9 µg three times weekly              |
| <b>Ribavirin</b>                            |                                      |
| Ribavirin (Copegus®)                        | 800 – 1200 mg daily                  |
| Ribavirin (Rebetol®)                        | 600 – 1400 mg daily                  |

**Table 4. Approved drugs for the treatment of chronic hepatitis C (2008).**

## Individualisation and optimisation strategies

### Adherence to therapy

Adherence to therapy is one of the most important factors associated with the success of therapy (McHutchison 2002). The definition of adherence used here is the 80/80/80 rule, where patients 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. One of the first studies investigating the effect of adherence demonstrated that patients who fulfilled the 80/80/80 rule had 63% sustained response compared to 52% of those with less than 80% adherence (McHutchison 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

An optimal treatment duration may also improve the management of chronic hepatitis C. There are two different concepts to optimise the treatment duration. While some patients with HCV genotype 1 may need longer treatment to improve the response, patients with HCV genotypes 2 and 3 may be treated for a shorter period of time to reduce costs and side-effects.

Many studies are investigating reductions in treatment duration for HCV genotypes 2 and 3 to 16, 14, or even 12 weeks. The first reported results are very promising, but it turns out that we have to consider individual factors when treating patients for less than 24 weeks. The rapid virological response (RVR) after 4 weeks of therapy (HCV RNA negative in the serum at TW4) is one of the critical factors associated with the success of this shorter therapy. Only patients who show a rapid virological response (RVR) at week 4 had high SVR rates after 16 weeks (von Wagner 2005) (Table 5), 14 weeks (Dalgard 2004), or 12 weeks of therapy (Mangia 2005), whereas those without an RVR had low response rates, even with the 24-week schedule. However, 12 weeks seem to be the limit for some patients, since the relapse rates after 12 weeks were higher compared to the standard 24 week schedule (Mangia 2005).

In addition to the RVR, other factors are associated with the response in patients with HCV genotypes 2 and 3. These were the HCV genotype and the baseline viral load. Patients with HCV genotypes 2 and 3 should be analysed separately because patients with HCV genotype 2 respond better to PEG-IFN and ribavirin therapy than those infected with HCV genotype 3 (Mangia 2005; Zeuzem 2004) (Table 3). Furthermore, the shorter treatment schedules revealed that HCV genotype 3 patients with low baseline viremia (HCV RNA <400-800,000 IU/ml) had a much better chance of responding than those with high viral load (HCV RNA >400-800,000 IU/ml) (Dalgard 2004; Shiffman 2007; von Wagner 2005). In conclusion, patients with HCV genotype 2 and patients with HCV genotype 3 and low viral load who have an RVR after 4 weeks of therapy can be treated for less than 24 weeks and patients without an early virological response (EVR) (especially HCV genotype 3

and high viral load) may be treated for longer than 24 weeks (i.e., 48 weeks) (Figure 1B). Also, patients with liver cirrhosis should not be considered for a shorter treatment duration (Aghemo 2006). Tailoring treatments for patients with HCV genotype 2 and 3 will reduce costs, side effects and further optimize the response rates.

| Study                        | Treatment   | Subgroups                | Therapy weeks | SVR                                   |
|------------------------------|---|--------------------------|---------------|---------------------------------------|
| (Mangia 2005)<br>N = 283     | 1.0µg/kg PEG-IFN<br>α-2b<br>1000-1200 mg<br>ribavirin | Standard group           | 24            | 76%                                   |
|                              |   | Standard group           | 24            | 91% if TW4 HCV RNA<br><50 IU/ml       |
|                              |   | >50 IU/ml TW4 (no RVR)   | 24            | 64%                                   |
|                              |   | <50 IU/ml TW4 (RVR)      | 12            | 85%                                   |
| (Dalgard 2008)<br>N = 428    | 1.5µg/kg PEG-IFN<br>α-2b<br>800-1400 mg<br>ribavirin  | <50 IU/ml TW4 (RVR)      | 24            | 91% ITT, 93% with F24<br>HCV RNA test |
|                              |   | <50 IU/ml TW4 (RVR)      | 14            | 81% ITT, 86% with F24<br>HCV RNA test |
|                              |   | >50 IU/ml TW4 (no-RVR)   | 24            | 55% ITT, 59% with F24<br>HCV RNA test |
| (von Wagner 2005)<br>N = 153 | 180 µg PEG-IFN<br>α-2a<br>800-1200 mg<br>ribavirin    | >600 IU/ml TW4           | 24            | 36%                                   |
|                              |   | <600 IU/ml TW4           | 24            | 80%, 87% if HCV RNA<br><800,000 IU/ml |
|                              |   | <600 IU/ml TW4           | 16            | 82%, 94% if HCV RNA<br><800,000 IU/ml |
| (Shiffman 2007)<br>N = 1469  | 180 µg PEG-IFN<br>α-2a<br>800 mg ribavirin            | All patients             | 24            | 70%                                   |
|                              |   | All patients             | 16            | 62%                                   |
|                              |   | <50 IU/ml TW4 (RVR)      | 24            | 85%                                   |
|                              |   | <50 IU/ml TW4 (RVR)      | 16            | 79%                                   |
|                              |   | <400,000 IU/ml TW0 (LVL) | 24            | 82%                                   |
| <400,000 IU/ml TW0 (LVL)     | 16  | 81%                      |               |                                       |

RVR= rapid virologic response, LVL= low viral load

**Table 5. Optimization of treatment duration in patients with HCV genotypes 2 and 3.**

We face the opposite problem in patients with HCV genotype 1. Extending treatment duration beyond 48 weeks is one strategy that may improve response rates in some of these difficult-to-treat patients. The rationale is to extend the time of HCV RNA negativity, especially in patients with a slow viral decline (first time HCV RNA negative between TW12 and TW24) to reduce the relapse rate in these so-called “slow responder” patients. Several studies have investigated the efficacy and safety of 48 weeks versus 72 weeks of treatment with PEG-IFN plus ribavirin in patients with chronic hepatitis C (Berg 2006; Mangia 2008; Pearlman 2007; Sanchez-Tapias 2006) (Table 6). Sanchez-Tapias and colleagues report a benefit of extended therapy in patients who were HCV RNA positive at treatment week 4. The relapse rate after 72 weeks of therapy was significantly reduced in these patients (Sanchez-Tapias 2004). However, treatment duration beyond one year may lead to higher drop out rates (Table 6), which may result in lower intent-to-treat responses (Berg 2006; Sanchez-Tapias 2004). Another study by Berg 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

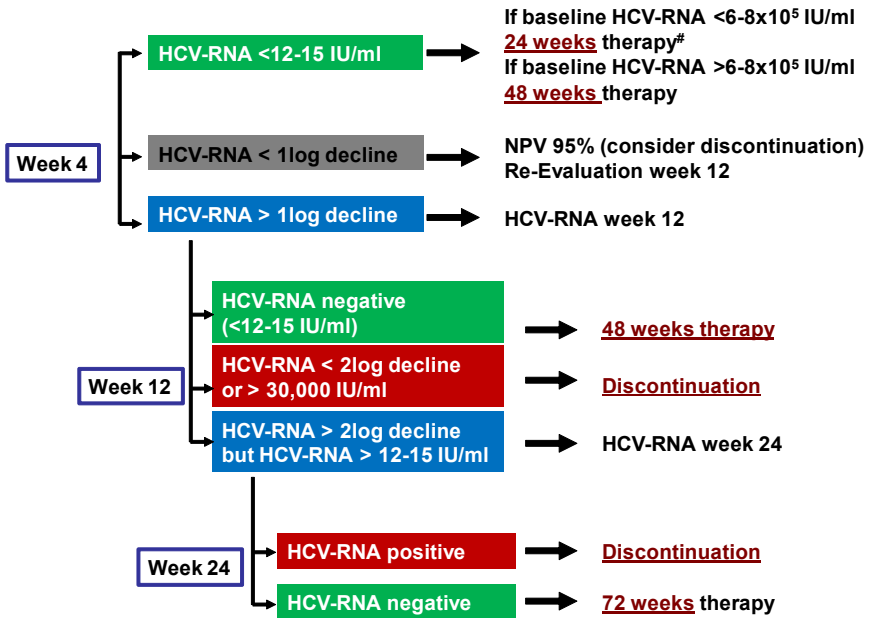
treated for 72 instead of 48 weeks (29% vs 17%, p = 0.04). A particular benefit from extended treatment duration was seen in patients with low-level viraemia (<6000 IU/ml) at week 12 (Berg 2006). In conclusion, extension of therapy to 72 weeks will improve response rates for patients with a slow viral response (>2log<sub>10</sub> decline but >50 IU/ml at TW12 (Figure 1A)) but high motivation and compliance of the patient is mandatory.

On the other hand, it is possible to reduce the treatment duration to 24 weeks in patients with HCV genotype 1 who have low viral load (LVL) at baseline and a rapid virological response (RVR) after 4 weeks of therapy (Ferenci 2008; Jensen 2006; Zeuzem 2006) (Table 6; Figure 1A).

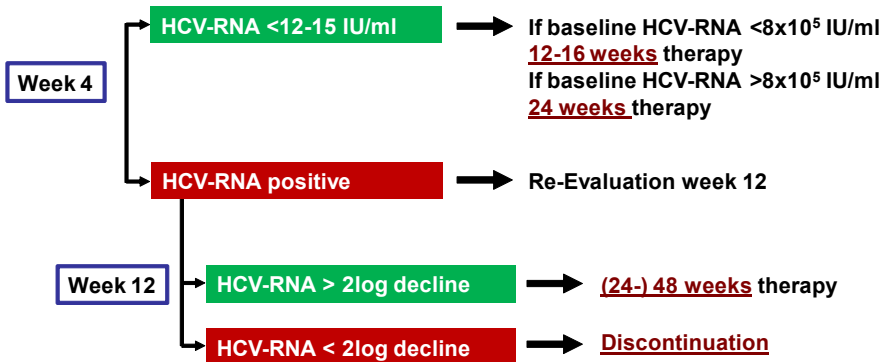
| Study                 | Treatment  | Subgroups (Slow responder)  | Therapy weeks | SVR, (relapse), discontinuation of therapy |
|-----------------------|--|---|---------------|--|
| (Sanchez-Tapias 2006) | 180 µg PEG-IFN α-2a<br>800 mg ribavirin                                    | >50 IU/ml TW4 (no RVR)  | 48            | 28% (53% relapse), 18% discontinuation     |
|                       |  |   | 72            | 44% (17% relapse), 36% discontinuation     |
| (Berg 2006)           | 180 µg PEG-IFN α-2a<br>800 mg ribavirin                                    | >50 IU/ml TW12  | 48            | 17%, 24% discontinuation                   |
|                       |  |   | 72            | 29%, 41% discontinuation                   |
| (Mangia 2008)         | 180 µg PEG-IFN α-2a<br>or 1.5 µg/kg PEG-IFN α-2b<br>1000-1200 mg ribavirin | >600 IU/ml TW8 &<br><600 IU/ml TW12                               | 48            | 38%, (43% relapse)                         |
|                       |  |   | 72            | 63%, (15% relapse)                         |
| (Pearlman 2007)       | 1.5 µg/kg PEG-IFN α-2b<br>800-1400 mg ribavirin                            | ≥2log decline TW12 &<br>>10 IU/ml TW12                            | 48            | 18%, (59% relapse), 14% discontinuation    |
|                       |  |   | 72            | 38%, (20% relapse), 15% discontinuation    |
| Study                 | Treatment  | Subgroups (fast responder)  | Therapy weeks | SVR, (relapse), discontinuation of therapy |
| (Zeuzem 2006)         | 1.5µg/kg PEG-IFN α-2b<br>800-1400 mg ribavirin                             | <600,000 IU/ml TW0<br><600,000 IU/ml TW0 &<br><29 IU/ml TW4 (RVR) | 24            | 50%  |
|                       |  |   | 24            | 89%  |
| (Jensen 2006)         | 180 µg PEG-IFN α-2a<br>or<br>800mg or 1000-1200 mg ribavirin               | <50 IU/ml TW 4 (RVR)  | 24            | 89%  |
| (Ferenci 2008)        | 180 µg PEG-IFN α-2a<br>or<br>1000-1200 mg ribavirin                        | <50 IU/ml TW 4 (RVR)  | 24            | 74% ITT, 79% PP (N = 120 HCV-G1)           |

RVR= rapid virologic response

**Table 6. Optimization of treatment duration in patients with HCV genotype 1.**



**Figure 1A. Recommendation for treatment algorithm for patients with HCV genotype 1.** We also recommend this algorithm for patients with HCV genotypes 4-6 because of limited data for those patient groups. Sensitive HCV RNA assays (limit of detection 12-15 IU/ml or 50 IU/ml) at weeks 4, 12, and 24, may determine treatment duration. Reducing treatment duration is not recommended in patients with liver cirrhosis, insulin resistance or hepatic steatosis.



**Figure 1B. Recommendation for 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.

## Amantadine

Another strategy to enhance the success of therapy in patients with HCV genotype 1 may be the additional use of amantadine (Smith 1997). Brillanti and colleagues were among the first who demonstrated a promising SVR with the triple therapy of IFN/ribavirin and amantadine in previous IFN non-responders (Brillanti 2000). The dilemma of the many small studies conducted was that the results varied from study to study. 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 robust placebo-controlled study with more than 700 patients in cooperation with Hep-Net testing the addition of amantadine to PEG-IFN/ribavirin therapy in treatment naïve patients finally gave the answer and demonstrated that triple therapy with amantadine has no additional benefit (von Wagner 2007).

## Other interferons

There are other type 1 interferons in development. Albinterferon alfa-2b (Alb-IFN) (Human Genome Sciences, Novartis), which is an 85.7 kD protein consisting of interferon alpha-2b genetically fused to human serum albumin, further extends the half-life of IFN to approximately 148 hours. The pharmacokinetic profile of albuferon allows dosing at intervals of 2-4 weeks compared to one week with the PEG-IFNs. Results of a phase-II trial testing multiple doses of Alb-IFN versus PEG-IFN  $\alpha$ -2a in HCV genotype 1-patients demonstrated comparable antiviral efficacy of Alb-IFN (Zeuzem 2008). These data led to the initiation of phase-III clinical trials evaluating the efficacy of Albuferon in combination with Ribavirin.

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, a head-to-head study comparing CIFN and standard IFN monotherapy revealed only minor differences in efficacy. These results suggest that patients with HCV genotype 1 may have a small advantage with CIFN (Tong 1997). A recent study reported better SVR in naïve patients with chronic hepatitis C when treated with CIFN in combination with ribavirin compared to standard IFN plus ribavirin (Sjogren 2005). Some studies investigating the effect of high and daily dosing of CIFN, in combination with ribavirin in naïve, as well as in non-responder patients, demonstrate a promising SVR (Cornberg 2006; Jaeckel 2001). 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 optimal response. Both IFN and ribavirin induce side effects that can impact the management of patients with chronic hepatitis C (Table 7). IFN-related side effects can be divided into IFN-induced bone marrow suppression, flu-like symptoms, neuropsychiatric disorders, and autoimmune syndromes. The main adverse event of ribavirin is hemolytic anemia. Overall, side effects result in 10-20% premature withdrawals from therapy and an additional 20-30% of patients require dose modifications. These numbers are lower in recent studies, suggesting an improved understanding and management of adverse events (Fried 2002), which potentially may lead to higher SVR. However, these percentages were recorded from registration trials using 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 at baseline. 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 HCV patients, especially those with advanced liver disease. A promising novel agent is the oral thrombopoietin-receptor agonist eltrombopag that has already been tested in patients with chronic hepatitis C and liver cirrhosis (McHutchison 2007). Eltrombopag was able to increase platelet levels in 75-95% of patients depending on the dose and antiviral therapy could then be initiated. Twelve weeks of antiviral therapy were completed by 36-65% of patients receiving 30-75 mg of eltrombopag, vs only 6% of patients in the placebo group (McHutchison 2007). Neutropenia is another of the most common reasons for dose modifications. Granulocyte macrophage colony-stimulating factor (GM-CSF) could potentially be used to stabilize neutrophil counts during IFN therapy (Shiffman 1998; Van Thiel 1995). 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 the severity declines over time. These symptoms include fever, chills, headache, arthralgia, and myalgia (Table 7). Antipyretic drugs such as paracetamol can help prevent or reduce these side effects.

Neuropsychiatric side effects such as irritability, severe fatigue, and apathy are frequent (Table 7) and pose a great problem for many patients, also affecting other family members. Severe depression can occur and even 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 therapy and the response rates (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 (Kraus 2008). SSRI treatment is highly effective in HCV patients during IFN-based therapies, when starting early after the onset of clinically relevant depression. A preemptive anti-depressive therapy may not be necessary.

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 deteriorate 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, and frequently results in ribavirin dose reduction or even discontinuation, which may significantly affect the overall SVR, especially in patients with HCV genotype 1 (Manns 2001).

Treatment with erythropoietin can effectively reverse ribavirin associated anaemia and allow full adherence to ribavirin therapy (Afdhal 2004). Although the use of erythropoietin can reduce the incidence and severity of ribavirin induced anaemia, it remains controversial whether this will affect SVR. A prospective, randomized, controlled trial has evaluated the effect of erythropoietin on SVR. Patients receiving PEG-IFN  $\alpha$ -2b plus 13.3 mg/kg/day ribavirin were compared to patients receiving PEG-IFN  $\alpha$ -2b, 13.3 mg/kg/day ribavirin and 40,000 U/week erythropoietin. Although ribavirin dose reductions were significantly fewer in those patients who took erythropoietin, no improvement in SVR was documented. A third group receiving a higher starting dose of 15.2 mg/kg/day in combination with erythropoietin showed significantly higher SVR, however there was no control group (Shiffman 2007). Overall, erythropoietin may improve quality of life and in some individuals it may also improve the chance to achieve SVR, but the treatment is expensive and off-label in many countries. This problem 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 immunomodulatory 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) or VX-497 have been studied (Sintchak 2000), but with limited effects (Cornberg 2002). An-

other 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 anemia (Watson 2002). First results of a phase II study demonstrated that viramidine in combination with PEG-IFN  $\alpha$ -2a led to significantly less anemia compared to ribavirin plus PEG-IFN (Gish 2005). However, phase-III studies with both PEG-IFNs in combination with fixed doses of viramidine were inferior to combination with ribavirin (Benhamou 2006; Marcellin 2007).

Meanwhile, 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 2001).

| Side effects                | Incidence with PEG-IFN $\alpha$ and ribavirin<br>(Fried 2002; Hadziyannis 2004; Manns 2001) |
|-----------------------------|---|
| Headache                    | 47-62%  |
| Pyrexia                     | 40-46%  |
| Myalgia                     | 37-56%  |
| Rigor                       | 24-48%  |
| Arthralgia                  | 24-34%  |
| 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 7. Common side effects (>20% of patients) recorded in the major PEG-IFN/ribavirin trials (Fried 2002; Hadziyannis 2004; Manns 2001).**

The incidence of side effects among different studies is difficult to compare since the studies had significant differences in genetic and socioeconomic backgrounds. Furthermore, there were methodological differences in assessing side effects. 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 questionable. 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 in the past (reviewed in Tassopoulos 1999). Third, the efficacy of therapy may be lower, since patients with elevated transaminases seem to respond better (Zeuzem 2000). However, up to one third of patients with normal ALT can present with significant liver fibrosis necessitating an effective treatment (Bacon 2002; Zeuzem 2004). Zeuzem demonstrated that 48 weeks of PEG-IFN  $\alpha$ -2a plus ribavirin led 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 (Zeuzem 2004). 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 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 course post-transplant in recent years (Berenguer 2005) with progression to 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 clear.

Antiviral therapy of HCV may be begun 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 and ribavirin is only poorly tolerated in decompensated cirrhosis and thus this approach will be feasible only in 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 plus ribavirin combination therapy (Chalasani 2005; Terrault 2003). 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 several 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 solved yet. Patients with established graft hepatitis should be treated with PEG-IFN and ribavirin. Whether re-infection can be prevented in the future either by the new direct antivirals inhibiting HCV replication or by a combination of anti-HCV antibodies with neutralizing properties will have to be addressed in 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 in patients on dialysis were better than in patients not undergoing dialysis with SVR between 21-64%. Data on combination therapies with ribavirin are limited since ribavirin has traditionally been considered to be contraindicated in this setting. However, ribavirin can be given at lower doses in dialysis patients, from 200-400 mg daily (Bruchfeld 2001). Several trials on the use of PEG-IFN plus ribavirin in dialysis patients are ongoing and final data are not available. However, it has to be considered that there might be significant differences between the two pegylated interferons in the setting of dialysis since PEG-IFN  $\alpha$ -2a is eliminated mainly by the liver while PEG-IFN  $\alpha$ -2b is cleared via the kidney (Cornberg 2002). Thus, only PEG-IFN  $\alpha$ -2a has been approved in this setting. Future studies need to evaluate the potential of viremagine in particular for this special patient population.

## Treatment in 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). The first drug that demonstrated proof-of-concept in humans for an HCV protease inhibitor was BILN-2061 (Boehringer-Ingelheim). BILN-2061 given twice daily as monotherapy for 2 days reduced HCV RNA by 2-3 log<sub>10</sub> in most patients infected with HCV genotype 1 (Hinrichsen 2004). Unfortunately, further clinical trials are on hold due to preclinical cardiac toxicity issues. Other promising HCV protease inhibitors (Reesink 2005; Zeuzem 2005a; Zeuzem 2005b) as well as HCV polymerase inhibitors (Afdhal 2005; O'Brien 2005) are under investigation. Drug resistance may become a problem with these new compounds (Sarrazin 2005) and combination therapies may be unavoidable. Phase II/III trials investigating these new drugs in combination with PEG-IFN have begun. The majority of these new compounds were developed using an *in vitro* replicon system (Lohmann 1995) derived from patients with HCV genotype 1.

Therefore, it is understandable that, e.g., BILN-2061, was less effective in patients with HCV genotypes 2 and 3 (Reiser 2005). It is an unmet need to develop other *in vitro* replicon systems derived from patients with non-genotype 1. Recently, a new *in vitro* culture-generated HCV clone derived from a patient infected with HCV genotype 2a was established and for the first time this was shown to be infectious in a chimpanzee (Wakita 2005).

Another approach to 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 (Rehermann 2005; Wedemeyer 2002). The aim of a therapeutic vaccination is to stimulate the hepatitis C-specific immune responses to control viral replication. The first therapeutic vaccines are currently being tested in phase I/II studies (Manns 2004; Nevens 2003).

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 (see Chapter 14).

## Treatment of patients with previous 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 several years from approval and recent data indicate that a STAT-C drug plus PEG-IFN and ribavirin may not be sufficient to achieve SVR in many nonresponders. 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 may have varied between patients. Most importantly, HCV RNA kinetics and the response profile during earlier therapy have to be taken into account before starting a new treatment. Thus 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 to clear HCV RNA at any point during treatment. Definitions used for trials assessing novel therapy approaches, i.e., STAT-C (see Chapter 14) have generally defined non-response as the failure to achieve a  $\geq 2 \log_{10}$  reduction in HCV RNA after 12 weeks. Classifications of non-response include null response, which is used as a  $< 2 \log_{10}$  decline in HCV RNA at any time. A partial virologic response is defined as a  $\geq 2 \log_{10}$  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 2).

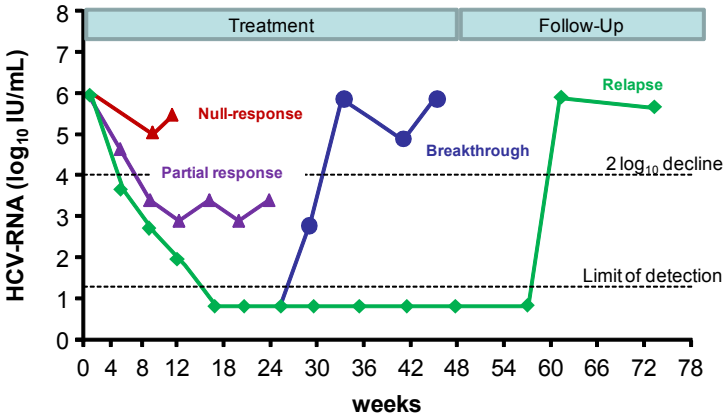


Figure 2. Different scenarios of treatment failure to antiviral therapy in chronic hepatitis C.

### Re-treatment of patients who 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 8). Patients with HCV genotype 1 and higher fibrosis scores have lower chances of achieving SVR (Jacobson 2005; Poynard 2008). Patients after relapse should be treated for 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 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 (patients)                 | Patient population                           | Treatment regimen   | SVR |
|----------------------------------|--|---|-----|
| EPIC <sup>3</sup> (Poynard 2008) | Relapse after PEG-IFN $\alpha$ -2a/ribavirin | 48 weeks<br>1.5 $\mu$ g/kg PEG-IFN $\alpha$ -2b<br>+ 800-1400 mg ribavirin  | 34% |
| EPIC <sup>3</sup> (Poynard 2008) | Relapse after PEG-IFN $\alpha$ -2b/ribavirin | 48 weeks<br>1.5 $\mu$ g/kg PEG-IFN $\alpha$ -2b<br>+ 800-1400 mg ribavirin  | 32% |
| EPIC <sup>3</sup> (Poynard 2008) | Relapse after IFN/ribavirin                  | 48 weeks<br>1.5 $\mu$ g/kg PEG-IFN $\alpha$ -2b<br>+ 800-1400 mg ribavirin  | 43% |
| (Jacobson 2005)                  | Relapse after IFN/ribavirin                  | 48 weeks<br>1.5 $\mu$ g/kg PEG-IFN $\alpha$ -2b<br>+ 800 mg ribavirin       | 50% |
| (Jacobson 2005)                  | Relapse after IFN/ribavirin                  | 48 weeks<br>1.0 $\mu$ g/kg PEG-IFN $\alpha$ -2b<br>+ 1000-1200 mg ribavirin | 32% |

**Table 8. SVR of IFN/ribavirin or PEG-IFN/ribavirin relapse patients.**

## Re-treatment of non-responders to current standard therapy

Patients who are non-responders to the standard PEG-IFN/ribavirin therapy have demonstrated SVR ranging from 2 to 12% with a standard PEG-IFN/ribavirin re-treatment (Jacobson 2005; Marcellin 2008; Poynard 2008; Schiff 2008; Shiffman 2004) (Table 9). Thus, indication for re-treatment is limited. Retreatment is justified if adherence was a major problem during the earlier treatment regimen. Previous HCV RNA reduction predicts the likelihood of SVR. Thus, patients with previous partial response may benefit from re-treatment with an optimized treatment regimen, i.e., extended treatment duration.

If a patient is a previous non-responder to IFN-based or PEG-IFN-based therapy and they have detectable HCV RNA at week 12, treatment should be discontinued. The EPIC<sup>3</sup> trial demonstrated that any HCV RNA level >750 IU/mL was associated with a 0% chance of subsequent SVR (Poynard 2008). 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 9). 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 (Marcellin 2008) (Table 9), confirming older data (Cornberg 2006).



| Study (patients)  | Patient population                                    | Treatment regimen  | SVR |
|---|---|--|-----|
| Bocepravir<br>Non-responder<br>control arm<br>(Schiff 2008) | Nonresponder (null-responder)<br>to PEG-IFN/ribavirin | 48 weeks<br>1.5 µg/kg PEG-IFN $\alpha$ -2b<br>+ 800-1400 mg ribavirin              | 2%  |
| EPIC <sup>3</sup><br>(Poynard 2008)                         | Nonresponder to<br>PEG-IFN $\alpha$ -2a/ribavirin     | 48 weeks<br>1.5 µg/kg PEG-IFN $\alpha$ -2b<br>+ 800-1400 mg ribavirin              | 6%  |
| EPIC <sup>3</sup><br>(Poynard 2008)                         | Nonresponder to<br>PEG-IFN $\alpha$ -2b/ribavirin     | 48 weeks<br>1.5 µg/kg PEG-IFN $\alpha$ -2b<br>+ 800-1400 mg ribavirin              | 7%  |
| REPEAT<br>(Marcellin<br>2008)                               | Nonresponder to<br>PEG-IFN $\alpha$ -2b/ribavirin     | 48 weeks<br>180 µg PEG-IFN $\alpha$ -2a<br>+ 1000/1200 mg ribavirin                | 9%  |
| REPEAT<br>(Marcellin<br>2008)                               | Nonresponder to<br>PEG-IFN $\alpha$ -2b/ribavirin     | 72 weeks<br>180 µg PEG-IFN $\alpha$ -2a<br>+ 1000/1200 mg ribavirin                | 14% |
| REPEAT<br>(Marcellin<br>2008)                               | Nonresponder to<br>PEG-IFN $\alpha$ -2b/ribavirin     | 48 weeks (Induction)<br>360/180µg PEG-IFN $\alpha$ -2a<br>+ 1000/1200 mg ribavirin | 7%  |
| REPEAT<br>(Marcellin<br>2008)                               | Nonresponder to<br>PEG-IFN $\alpha$ -2b/ribavirin     | 72 weeks (Induction)<br>360/180µg PEG-IFN $\alpha$ -2a<br>+ 1000/1200 mg ribavirin | 16% |
| (Jacobson<br>2005)  | Nonresponder to<br>IFN/ribavirin                      | 48 weeks<br>1.5 µg/kg PEG-IFN $\alpha$ -2b<br>+ 800 mg ribavirin                   | 10% |
| (Jacobson<br>2005)  | Nonresponder to<br>IFN/ribavirin                      | 48 weeks<br>1.0 µg/kg PEG-IFN $\alpha$ -2b<br>+ 1000-1200 mg ribavirin             | 6%  |
| HALT-C<br>(Shiffman<br>2004)                                | Nonresponder to<br>IFN/ribavirin                      | 48 weeks<br>180µg PEG-IFN $\alpha$ -2a<br>+ 1000-1200 mg ribavirin                 | 12% |

**Table 9. SVR of PEG-IFN/ribavirin non-responders.**

## PEG-IFN maintenance therapy

There has been much interest concerning the use of low-dose PEG-IFN maintenance therapy; data has suggested that IFN may halt the progression of liver disease (Nishiguchi 1995). There are two major trials that have analysed if maintenance treatment with IFN may alter the natural course of chronic hepatitis C. The Colchicine vs. PEG-interferon alfa-2b Long-term (COPILOT) study conducted by Afdhal and colleagues compared low-dose 0.5 µg/kg PEG-IFN  $\alpha$ -2b to colchicine in non-responders to previous standard IFN-based or PEG-IFN-based 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 dif-

ference. However, an 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 (Afdhal 2008). The authors conclude that maintenance therapy may have a role in patients with portal hypertension. However, analyses of other issues such as the extent of beta-blocker use have to be 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, a 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 2007). In conclusion, long-term treatment with low-dose PEG-IFN cannot be recommended for non-responder patients.

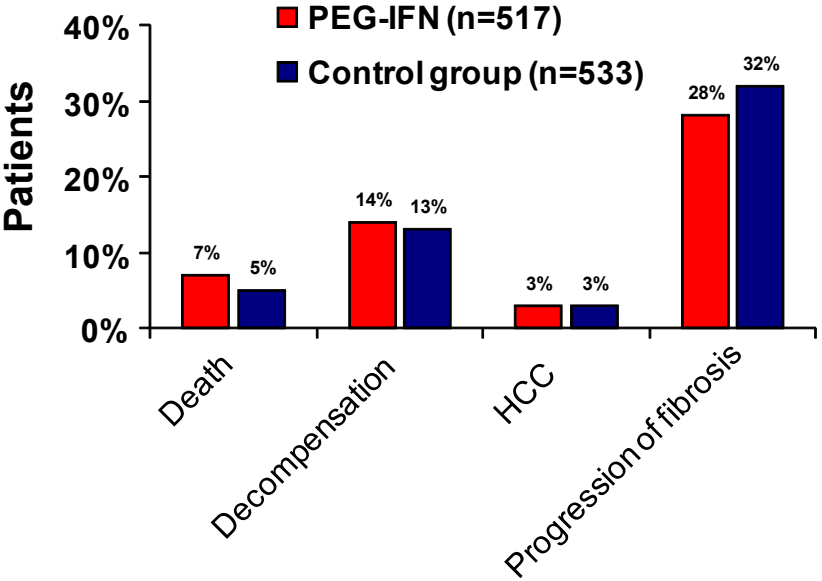


Figure 3. Results of the HALT-C study. Efficacy of low-dose (90 µg/week) PEG-IFN alpha-2a long-term (3.5 years) treatment in non-responders (Di Bisceglie 2007).

## References

- Afdhal N, Rodriguez-Torres M, Lawitz E *et al*. Enhanced antiviral efficacy for valopicitabine (NM283) plus PEG-Interferon in hepatitis C patients with HCV genotype-1 infection: Results of a phase IIa multicenter trial. *J Hepatol* 2005;42:39-40.
- Afdhal NH, Dieterich DT, Pockros PJ *et al*. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology* 2004;126(5):1302-11.
- Afdhal NH, Levine R, Brown R *et al*. Colchicine versus PEG-interferon alfa 2b long term therapy: Results of the 4 year copilot trial. *Journal of Hepatology* 2008;48:S4.
- Aghemo A, Rumi MG, Soffredini R *et al*. Impaired response to interferon-alpha2b plus ribavirin in cirrhotic patients with genotype 3a hepatitis C virus infection. *Antivir Ther* 2006;11(6):797-802.
- Bacon BR. Treatment of patients with hepatitis C and normal serum aminotransferase levels. *Hepatology* 2002;36(5 Suppl 1):S179-S184.
- Benhamou Y, Pockros P, Rodriguez-Torres M *et al*. The safety and efficacy of Viramidine((R)) plus pegylated interferon alpha-2B versus ribavirin plus pegylated interferon alpha-2B in therapy-naive patients infected with HCV: Phase 3 results. *Journal of Hepatology* 2006;44:S273.
- Berenguer M. What determines the natural history of recurrent hepatitis C after liver transplantation? *J Hepatol* 2005;42(4):448-56.
- Berg T, Kronenberger B, Hinrichsen H *et al*. Triple therapy with amantadine in treatment-naive patients with chronic hepatitis C: a placebo-controlled trial. *Hepatology* 2003;37(6):1359-67.
- Berg T, Sarrazin C, Herrmann E *et al*. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003;37(3):600-9.
- Berg T, von Wagner M, Nasser S *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(4):1086-97.
- Blatt LM, Davis JM, Klein SB *et al*. The biologic activity and molecular characterization of a novel synthetic interferon-alpha species, consensus interferon. *J Interferon Cytokine Res* 1996;16(7):489-99.
- Boker KH, Dalley G, Bahr MJ *et al*. Long-term outcome of hepatitis C virus infection after liver transplantation. *Hepatology* 1997;25(1):203-10.
- Brillanti S, Levantesi F, Masi L *et al*. Triple antiviral therapy as a new option for patients with interferon nonresponsive chronic hepatitis C. *Hepatology* 2000;32(3):630-4.
- Broers B, Helbling B, Francois A *et al*. Barriers to interferon-alpha therapy are higher in intravenous drug users than in other patients with acute hepatitis C. *J Hepatol* 2005;42(3):323-8.
- Bruchfeld A, Lindahl K, Schvarcz R *et al*. Dosage of ribavirin in patients with hepatitis C should be based on renal function: a population pharmacokinetic analysis. *Ther Drug Monit* 2002;24(6):701-8.
- Bruchfeld A, Stahle L, Andersson J *et al*. Ribavirin treatment in dialysis patients with chronic hepatitis C virus infection--a pilot study. *J Viral Hepat* 2001;8(4):287-92.
- Chalasani N, Manzarbeitia C, Ferenci P *et al*. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. *Hepatology* 2005;41(2):289-98.
- 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(4902):359-62.

- Cornberg M, Hadem J, Herrmann E *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(2):291-301.
- Cornberg M, Hinrichsen H, Teuber G *et al.* Mycophenolate mofetil in combination with recombinant interferon alfa-2a in interferon-nonresponder patients with chronic hepatitis C. *J Hepatol* 2002;37(6):843-7.
- Cornberg M, Huppe D, Wiegand J *et al.* [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(6):517-22.
- Cornberg M, Wedemeyer H, Manns MP. Treatment of chronic hepatitis C with PEGylated interferon and ribavirin. *Curr Gastroenterol Rep* 2002;4(1):23-30.
- 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(3):366-75.
- Dalgard O, Bjoro K, Hellum KB *et al.* Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* 2004;40(6):1260-5.
- Dalgard O, Bjoro K, Ring-Larsen H *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(1):35-42.
- Davis GL, Albright JE, Cook SF *et al.* Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl* 2003;9(4):331-8.
- Davis GL, Rodrigue JR. Treatment of chronic hepatitis C in active drug users. *N Engl J Med* 2001;345(3):215-7.
- Davis GL, Wong JB, McHutchison JG *et al.* Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003;38(3):645-52.
- Di Bisceglie AM, Shiffman ML, Everson GT *et al.* Prolonged Antiviral Therapy With Peginterferon to Prevent Complications of Advanced Liver Disease Associated With Hepatitis C: Results of the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial. *Hepatology* 2007;46(4):80A.
- Dumortier J, Scoazec JY, Chevallier P *et al.* Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. *J Hepatol* 2004;40(4):669-74.
- Everson GT. Treatment of chronic hepatitis C in patients with decompensated cirrhosis. *Rev Gastroenterol Disord* 2004;4 Suppl 1:S31-S38.
- Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. *Hepatology* 2002;36(1):3-10.
- Ferenci P, Laferl H, Scherzer TM *et al.* Peginterferon alfa-2a and ribavirin for 24 weeks in hepatitis C type 1 and 4 patients with rapid virological response. *Gastroenterology* 2008;135(2):451-8.
- Fleig WE, Krummenerl P, Lesske J *et al.* [Diagnosis, progression and therapy of hepatitis C virus infection as well as viral infection in children and adolescents--results of an evidenced based consensus conference of the German Society for Alimentary Metabolic Disorders and in cooperation with the Hepatitis Competence Network]. *Z Gastroenterol* 2004;42(8):703-4.
- Forns X, Garcia-Retortillo M, Serrano T *et al.* Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. *J Hepatol* 2003;39(3):389-96.
- Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;36(5 Suppl 1):S237-S244.

- 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(13):975-82.
- Gerlach JT, Diepolder HM, Zachoval R *et al.* Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. *Gastroenterology* 2003;125(1):80-8.
- Gish RG, Nelson D, Arora S *et al.* Virological response and safety outcomes in therapy-naive patients treated for chronic hepatitis C with viramidine in combination with pegylated interferon alfa-2a. *J Hepatol* 2005;42:39.
- Hadziyannis SJ, Sette H, Jr., Morgan TR *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(5):346-55.
- Hinrichsen H, Benhamou Y, Wedemeyer H *et al.* Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients. *Gastroenterology* 2004;127(5):1347-55.
- Hofer H, Watkins-Riedel T, Janata O *et al.* Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. *Hepatology* 2003;37(1):60-4.
- Hoofnagle JH, Mullen KD, Jones DB *et al.* Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. *N Engl J Med* 1986;315(25):1575-8.
- Jacobson IM, Brown RS, Jr., Freilich B *et al.* Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007;46(4):971-81.
- Jacobson IM, Gonzalez SA, Ahmed F *et al.* A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. *Am J Gastroenterol* 2005;100(11):2453-62.
- Jaeckel E, Cornberg M, Wedemeyer H *et al.* Treatment of acute hepatitis C with interferon alfa-2b. *N Engl J Med* 2001;345(20):1452-7.
- Janssen HL, Brouwer JT, van der Mast RC *et al.* Suicide associated with alfa-interferon therapy for chronic viral hepatitis. *J Hepatol* 1994;21(2):241-3.
- Jensen DM, Morgan TR, Marcellin P *et al.* Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* 2006;43(5):954-60.
- Kaiser S, Hass HG, Gregor M. Successful retreatment of interferon/ribavirin nonresponders with daily dosing of consensus interferon. *J Hepatol* 2005;42 (Suppl. 2):207-8.
- Kamal SM, El Tawil AA, Nakano T *et al.* Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. *Gut* 2005;54(6):858-66.
- Kraus MR, Schafer A, Schotker K *et al.* Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. *Gut* 2008;57(4):531-6.
- Lau JY, Tam RC, Liang TJ *et al.* Mechanism of action of ribavirin in the combination treatment of chronic HCV infection. *Hepatology* 2002;35(5):1002-9.
- Lisker-Melman M, Di Bisceglie AM, Usala SJ *et al.* Development of thyroid disease during therapy of chronic viral hepatitis with interferon alfa. *Gastroenterology* 1992;102(6):2155-60.
- Lohmann V, Korner F, Koch J *et al.* Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 1999;285(5424):110-3.
- Mangia A, Minerva N, Bacca D *et al.* Individualized treatment duration for hepatitis C genotype 1 patients: A randomized controlled trial. *Hepatology* 2008;47(1):43-50.

- Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005;352(25):2609-17.
- Manns M, Lindsay KL, Gordon SC *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.
- Manns MP, Berg T, Wedemeyer H *et al.* Immunization with the therapeutic hepatitis C virus (HCV) peptide vaccine IC41 in 66 chronic hepatitis C non-responder patients. *Hepatology* 2004;40(4):251A.
- Manns MP, McHutchison 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(9286):958-65.
- Manns MP, Meyer S, Wedemeyer H. The German network of excellence for viral hepatitis (Hep-Net). *Hepatology* 2003;38(3):543-4.
- 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. *Clin Endocrinol (Oxf)* 1996;44(6):635-42.
- Marcellin P, Freilich B, Andreone P *et al.* HCV-RNA status at week 12 of treatment with peginterferon alfa-2a/RBV predicts SVR in patients with prior non-response to pegylated interferon alfa-2b/RBV: Results from repeat study. *Journal of Hepatology* 2008;48:S301.
- Marcellin P, Lurie Y, Rodrigues-Torres M *et al.* The safety and efficacy of taribavirin plus pegylated interferon alfa-2a versus ribavirin plus pegylated interferon alfa-2a in therapy-naive patients infected with hcv: Phase 3 results. *Journal of Hepatology* 2007;46:S7.
- Mauss S, Berger F, Felten G *et al.* Peginterferon alfa-2a versus peginterferon alfa-2b in the treatment of chronic hepatitis C. *J Hepatol* 2005;42:213.
- Mazzaro C, Zorat F, Caizzi M *et al.* Treatment with peg-interferon alfa-2b and ribavirin of hepatitis C virus-associated mixed cryoglobulinemia: a pilot study. *J Hepatol* 2005;42(5):632-8.
- McHutchison JG, Dusheiko G, Shiffman ML *et al.* Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. *N Engl J Med* 2007;357(22):2227-36.
- McHutchison JG, Gordon SC, Schiff ER *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(21):1485-92.
- McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002;123(4):1061-9.
- McHutchison JG, Shiffman ML, Gordon SC *et al.* Sustained virologic response (SVR) to interferon-alpha-2b +/- ribavirin therapy at 6 months reliably predicts long-term clearance of HCV at 5-year follow-up. *Journal of Hepatology* 2006;44:S275.
- Misiani R, Bellavita P, Fenili D *et al.* Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. *N Engl J Med* 1994;330(11):751-6.
- Musselman DL, Lawson DH, Gumnick JF *et al.* Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med* 2001;344(13):961-6.
- 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(9):1303-7.
- Nevens F, Roskams T, Van Vlierberghe H *et al.* A pilot study of therapeutic vaccination with envelope protein E1 in 35 patients with chronic hepatitis C. *Hepatology* 2003;38(5):1289-96.

- Nishiguchi S, Kuroki T, Nakatani 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(8982):1051-5.
- O'Brien C, Godofsky E, Rodriguez-Torres M *et al.* Randomized trial of valopicitabine (NM283), alone or with peg-interferon, vs. retreatment with peg-interferon plus ribavirin (pegifn/RBV) in hepatitis C patients with previous non-response to pegifn/RBV: First interim results. *Hepatology* 2005;42(4):234A.
- Ozes ON, Reiter Z, Klein S *et al.* A comparison of interferon-Con1 with natural recombinant interferons-alpha: antiviral, antiproliferative, and natural killer-inducing activities. *J Interferon Res* 1992;12(1):55-9.
- Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. *Hepatology* 2002;36(5 Suppl 1):S65-S73.
- 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(6):1688-94.
- 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(4):553-5.
- Pischke S, Cornberg M, Manns MP. [Hepatitis associated cryoglobulinemia.]. *Internist (Berl)* 2008;49(3):297-304.
- Poynard T, Marcellin P, Lee SS *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(9138):1426-32.
- Poynard T, Schiff E, Terg R *et al.* Sustained viral response (SVR) is dependent on baseline characteristics in the retreatment of previous alfa interferon/ribavirin (I/R) nonresponders (NR): Final results from the epic3 program. *Journal of Hepatology* 2008;48:S369.
- Reddy KR, Shiffman ML, Morgan TR *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(1):124-9.
- Reesink HW, Zeuzem S, van Vliet A *et al.* Initial results of a phase 1B, multiple-dose study of VX-950, a hepatitis C virus protease inhibitor. *Gastroenterology* 2005;128 (Suppl.2):A-697.
- Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005;5(3):215-29.
- Reiser M, Hinrichsen H, Benhamou Y *et al.* Antiviral efficacy of NS3-serine protease inhibitor BILN-2061 in patients with chronic genotype 2 and 3 hepatitis C. *Hepatology* 2005;41(4):832-5.
- Rodriguez-Luna H, Khatib A, Sharma P *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(2):190-4.
- Sanchez-Tapias JM, Diago M, Escartin P *et al.* Longer treatment duration with peginterferon alfa-2A (40kd) (Pegasys (R)) and ribavirin (Copegus (R)) in naive patients with chronic hepatitis C and detectable HCV RNA by week 4 of therapy: Final results of the randomized, multicenter TeraViC-4 study. *Hepatology* 2004;40(4):218A.
- Sanchez-Tapias JM, Diago M, Escartin P *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(2):451-60.

- Santantonio T, Fasano M, Sinisi E *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(3):329-33.
- Sarrazin C, Kieffer T, Bartels D *et al.* Characterization of viral variants in the HCV NS3 protease domain of genotype 1 patients that are selected during 14 days of dosing with VX-950. *Hepatology* 2005;42(4):751A.
- Schaefer M, Schwaiger M, Garkisch AS *et al.* Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. *J Hepatol* 2005;42(6):793-8.
- Schiff E, Poordad E, Jacobson I *et al.* Boceprevir (B) combination therapy in null responders (NR): Response dependent on interferon responsiveness. *Journal of Hepatology* 2008;48:S46.
- Shiffman ML, Di Bisceglie AM, Lindsay KL *et al.* Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004;126(4):1015-23.
- Shiffman ML, Hofmann CM, Luketic VA *et al.* 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(3):382-9.
- Shiffman ML, Salvatore J, Hubbard S *et al.* Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007;46(2):371-9.
- Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007;357(2):124-34.
- Sintchak MD, Nimmesgern E. The structure of inosine 5'-monophosphate dehydrogenase and the design of novel inhibitors. *Immunopharmacology* 2000;47(2-3):163-84.
- Sjogren MH, Sjogren R, Holtzmuller K *et al.* Interferon alfacon-1 and ribavirin versus interferon alpha-2b and ribavirin in the treatment of chronic hepatitis C. *Dig Dis Sci* 2005;50(4):727-32.
- Smith JP. Treatment of chronic hepatitis C with amantadine. *Dig Dis Sci* 1997;42(8):1681-7.
- Snoeck E, Wade JR, Duff F *et al.* 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(6):699-709.
- Soza A, Everhart JE, Ghany MG *et al.* Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2002;36(5):1273-9.
- Svensson JO, Bruchfeld A, Schvarcz R *et al.* Determination of ribavirin in serum using highly selective solid-phase extraction and high-performance liquid chromatography. *Ther Drug Monit* 2000;22(2):215-8.
- Swain MG, Lai MY, Shiffman ML *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.
- Tassopoulos NC. Treatment of patients with chronic hepatitis C and normal ALT levels. *J Hepatol* 1999;31 Suppl 1:193-6.
- Terrault NA. Prophylactic and preemptive therapies for hepatitis C virus-infected patients undergoing liver transplantation. *Liver Transpl* 2003;9(11):S95-S100.
- Todros L, Saracco G, Durazzo M *et al.* Efficacy and safety of interferon alfa therapy in chronic hepatitis C with autoantibodies to liver-kidney microsomes. *Hepatology* 1995;22(5):1374-8.
- Tong MJ, Reddy KR, Lee WM *et al.* Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial. Consensus Interferon Study Group. *Hepatology* 1997;26(3):747-54.



- Van Thiel DH, Faruki H, Friedlander L *et al.* Combination treatment of advanced HCV associated liver disease with interferon and G-CSF. *Hepatology* 1995;42(6):907-12.
- Veldt BJ, Heathcote EJ, Wedemeyer H *et al.* Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007;147(10):677-84.
- Vogel W, Graziadei I, Umlauf F *et al.* High-dose interferon-alpha2b treatment prevents chronicity in acute hepatitis C: a pilot study. *Dig Dis Sci* 1996;41(12 Suppl):81S-5S.
- von Wagner M, Hofmann WP, Teuber G *et al.* Randomized, double-blind, placebo-controlled trial of peginterferon alfa-2a (40KD) and ribavirin with and without 400 mg amantadine-sulphate for 48 weeks in treatment naive HCV genotype 1-infected patients. *Hepatology* 2007;46(4):342A-3A.
- von Wagner M, Huber M, Berg 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(2):522-7.
- 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(7):791-6.
- Watson J. Prospects for hepatitis C virus therapeutics: levovirin and viremagine as improved derivatives of ribavirin. *Curr Opin Investig Drugs* 2002;3(5):680-3.
- Wedemeyer H, He XS, Nascimbeni M *et al.* Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. *J Immunol* 2002;169(6):3447-58.
- Wesche B, Jaeckel E, Trautwein C *et al.* Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C. *Gut* 2001;48(3):378-83.
- Wiegand J, Buggisch P, Boecher W *et al.* Early monotherapy with pegylated interferon alfa-2b for acute hepatitis C infection: The HEP-NET Acute-HCV-II Study. *Hepatology* 2006;43(2):250-6.
- Zeuzem S, Buti M, Ferenci P *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(1):97-103.
- Zeuzem S, Diago M, Gane E *et al.* Peginterferon alfa-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. *Gastroenterology* 2004;127(6):1724-32.
- Zeuzem S, Feinman SV, Rasenack J *et al.* Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000;343(23):1666-72.
- Zeuzem S, Hultcrantz R, Bourliere M *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(6):993-9.
- Zeuzem S, Sarrazin C, Rouzier R *et al.* Anti-viral activity of SCH 503034, a HCV protease inhibitor, administered as monotherapy in hepatitis C genotype-1 (HCV-1) patients refractory to pegylated interferon (PEG-IFN-alpha). *Hepatology* 2005;42(4):233A-4A.
- Zeuzem S, Sarrazin C, Wagner F *et al.* Combination therapy with the HCV protease inhibitor, SCH 503034, plus peg-intron in hepatitis C genotype-1 peg-intron non-responders: Phase IB results. *Hepatology* 2005;42(4):276A-7A.
- Zeuzem S, Yoshida EM, Benhamou Y *et al.* Albinterferon alfa-2b dosed every two or four weeks in interferon-naive patients with genotype 1 chronic hepatitis C. *Hepatology* 2008;48(2):407-17.
- Zignego AL, Ferri C, Pileri SA *et al.* Extrahepatic manifestations of Hepatitis C Virus infection: a general overview and guidelines for a clinical approach. *Dig Liver Dis* 2007;39(1):2-17.



## Chapter 14: New agents for treatment

Christian Lange and Christoph Sarrazin

### Introduction

Hepatitis C therapy has steadily advanced since the hepatitis C virus (HCV) was first isolated in 1989. From the introduction of interferon (IFN)- $\alpha$  monotherapy to the current standard of care, combination therapy with pegylated (peg) interferon- $\alpha$  plus ribavirin, the efficacy of achieving a sustained virologic response (SVR), consisting of HCV RNA undetectable 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- $\alpha$  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 circumstances (Fried 2002).

The exploding knowledge of the HCV life cycle and of structural features of the HCV proteins, obtained by crystallographic analysis and replicative cell culture systems, has spurred the development of many promising “specifically targeted antiviral therapy for hepatitis C” (STAT-C) compounds (Kim 1996; Lohmann 1999; Lindenbach 2005; Wakita 2005) (Figure 1; Table 1).

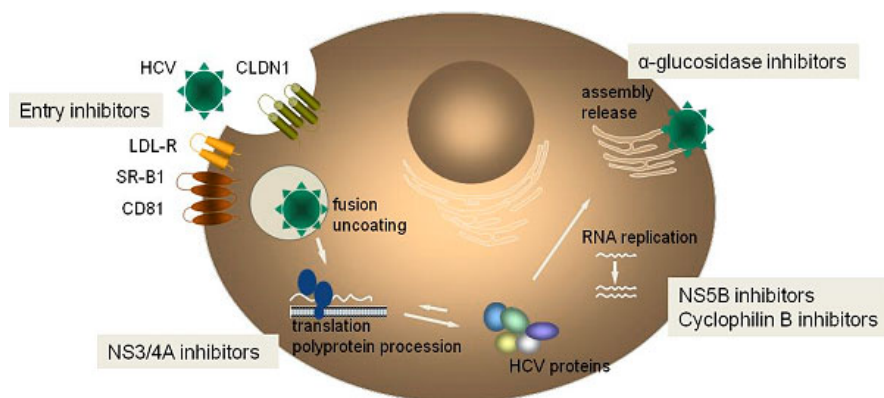


Figure 1. HCV life cycle and targets for STAT-C.

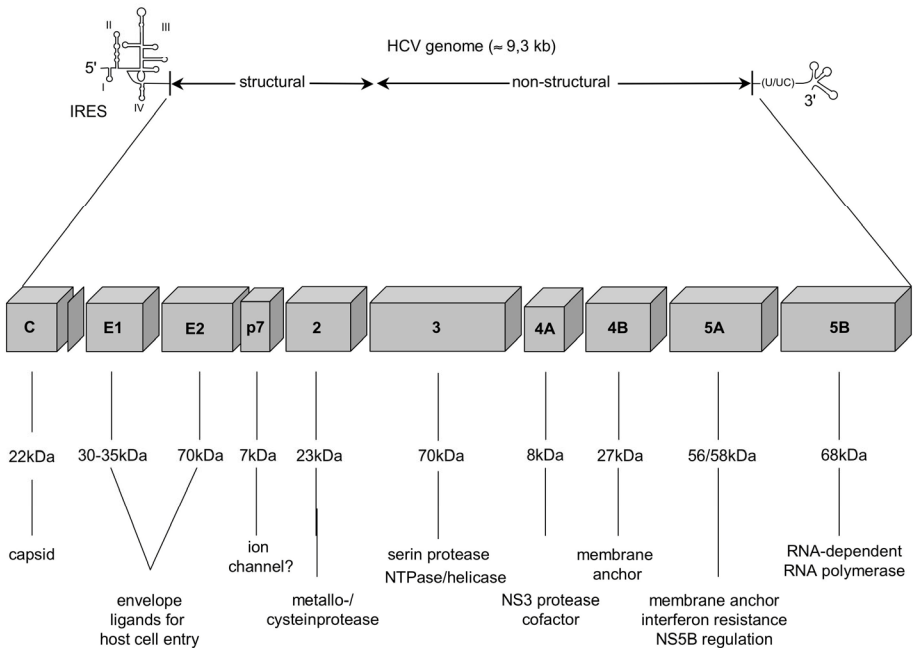
| Drug name  | Company              | Study Phase |
|--|----------------------|-------------|
| <b>NS3/4A protease inhibitors</b>                    |                      |             |
| Ciluprevir (BILN 2061)                               | Boehringer Ingelheim | Stopped     |
| Telaprevir (VX-950)                                  | Vertex               | Phase III   |
| Boceprevir (SCH503034)                               | Schering-Plough      | Phase III   |
| TMC435350  | Tibotec & Medivir    | Phase II    |
| ITMN-191 (R7227)                                     | InterMune, Roche     | Phase II    |
| VX-500   | Vertex               | Phase II    |
| MK-7009  | Merck                | Phase II    |
| BILN 12202   | Boehringer Ingelheim | Phase II    |
| <b>Nucleoside analoge NS5B polymerase inhibitors</b> |                      |             |
| Valopicitabine (NM283)                               | Idenix/Novartis      | Stopped     |
| R7128 (prodrug) / PSI-6130                           | Roche /Pharmaset     | Phase I     |
| MK-0608  | Merck                | Phase I     |
| R1626 (prodrug) / R1479                              | Roche                | Stopped     |
| <b>Non-nucleoside NS5B polymerase inhibitors</b>     |                      |             |
| HCV-796  | ViroPharma/Wyeth     | Phase II    |
| BILB 1941  | Boehringer Ingelheim | Stopped     |
| PF-868554  | Pfizer               | Phase I     |
| GS 9190  | Gilead               | Phase I     |
| GSK625433  | GlaxoSmithKline      | Phase I     |
| VCH-759  | ViroChem Pharma      | Phase I     |
| <b>NS5A inhibitors</b>                               |                      |             |
| BMI-790052   | BMI                  | Phase I     |
| <b>Host protein inhibitors</b>                       |                      |             |
| Celgosivir ( $\alpha$ -glucosidase inhibitor)        | Migenix              | Phase II    |
| Debio-025 (cyclophilin B inhibitor)                  | Debiopharm           | Phase II    |
| Nitazoxanide (unknown mechanism)                     | Romark Laboratories  | 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- $\alpha$  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; Pawlowsky 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 organization 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, 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 (Civacir) (Davis 2005) or monoclonal (HCV-AB 68) (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 (VanCompernelle 2003).

## **HCV RNA translation and post-translational 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  $\beta$ -barrels and four  $\alpha$ -helices. The serine protease catalytic triad – histidine 57, asparagine 81 and serine 139 – is located in a small groove between the two  $\beta$ -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- 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.

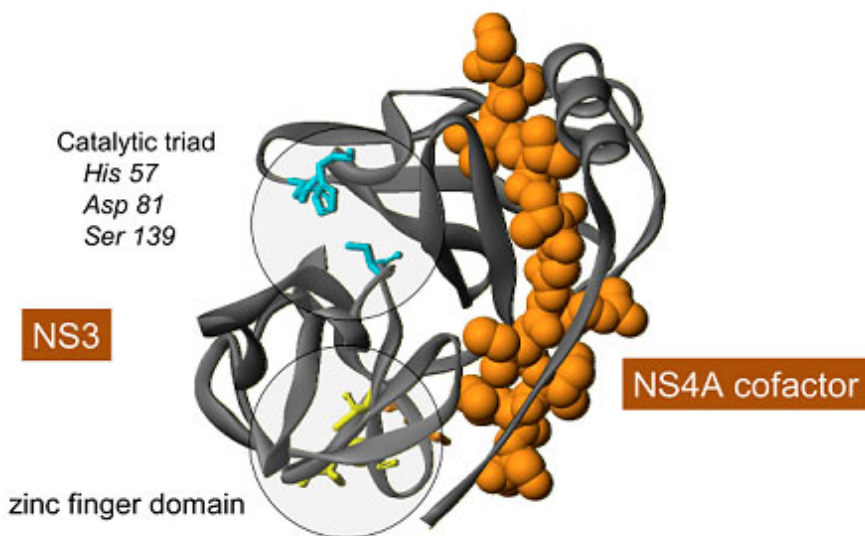


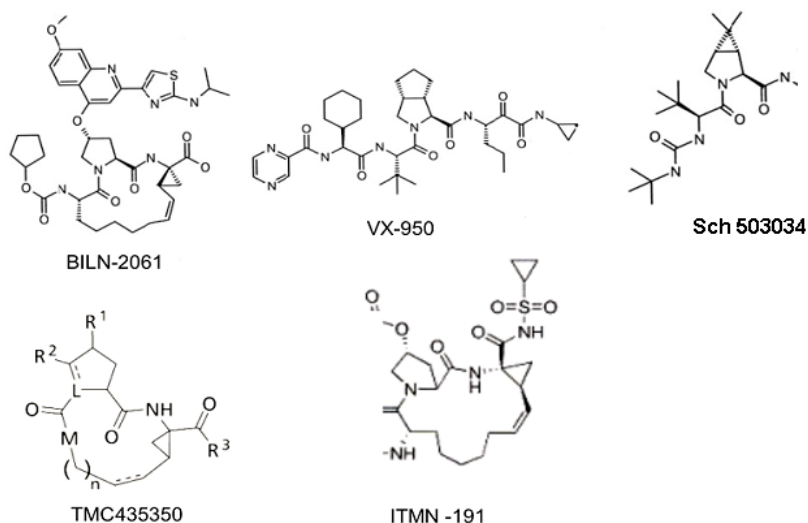
Figure 3. Molecular structure of the HCV NS3/4A protease.

## 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  $\beta$ -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 chemical classes, the macrocyclic inhibitors and the  $\alpha$ -ketoamid 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 have currently progressed to phase III studies (Figure 4).



**Figure 4. Molecular structure of NS3/4A inhibitors.**

### 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 for 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 a  $\alpha$ -ketoamid derivative binding the enzyme covalently but reversibly, with a half-life of 58 minutes of the enzyme-inhibitor complex (Lin 2006). Telaprevir is currently in phase III evaluation.

**Telaprevir monotherapy phase I study**

A double-blind, randomized placebo-controlled phase Ib clinical trial evaluating telaprevir monotherapy over 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.

**Telaprevir / peg Interferon  $\alpha$ -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  $\alpha$ -2a in treatment-naïve genotype 1 patients (Forestier 2007). Telaprevir was administered 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  $\alpha$ -2a in comparison to PEG-IFN  $\alpha$ -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  $\alpha$ -2a/placebo group, of 3.99  $\log_{10}$  in the telaprevir/placebo group and of 5.49  $\log_{10}$  in the telaprevir/PEG-IFN  $\alpha$ -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  $\alpha$ -2a, no viral breakthrough was seen during the combination therapy of the 14-day treatment period.

**Telaprevir / PEG-IFN  $\alpha$ -2a and ribavirin triple therapy phase I study**

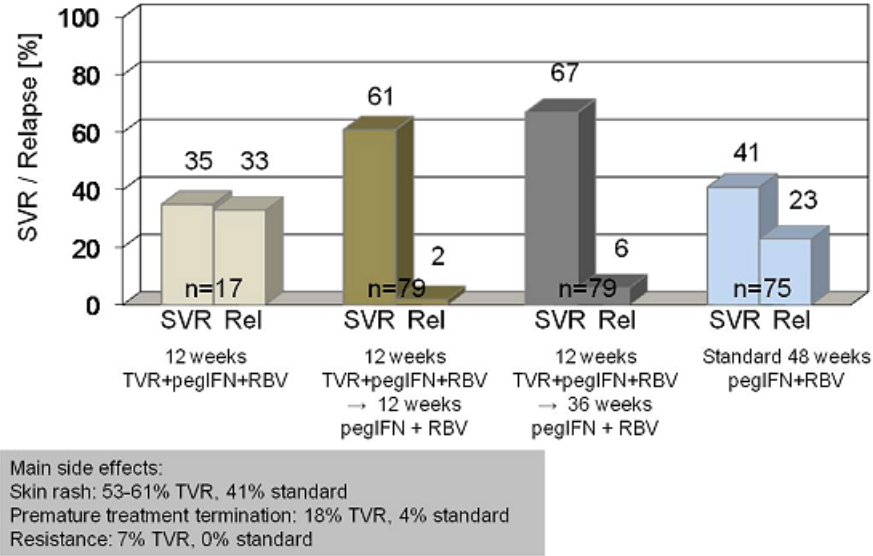
A parallel study evaluated the safety and efficacy of telaprevir (750 mg every 8 hours) in combination with PEG-IFN  $\alpha$ -2a and weight-based ribavirin in treatment-naïve genotype 1 patients for 28 days (Lawitz 2008). At the end of the 28-day treatment period, all patients had undetectable HCV RNA serum levels and 8 of 12 patients achieved an SVR.

**Telaprevir and peg Interferon with and without ribavirin, phase II studies**

**Treatment naïve phase II studies (PROVE 1 and 2)**

To answer the question whether with the addition of telaprevir to PEG-IFN  $\alpha$ -2a and ribavirin both treatment duration can be reduced and SVR rates can be improved in treatment naïve genotype 1 patients, larger phase II clinical trials (PROVE 1 & 2) were initiated (see Table 2 for study design) (McHutchison 2008).

In PROVE 1, telaprevir, PEG-IFN  $\alpha$ -2a and ribavirin were administered for 12 weeks in combination, followed by PEG-IFN  $\alpha$ -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, anaemia and gastrointestinal disorders.



**Figure 5. Results of PROVE 1 (USA).**

Combination therapy of telaprevir (TVR) and PEG-IFN  $\alpha$ -2a +/- ribavirin (RBV) in treatment-naïve genotype 1 patients.

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 show 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 fol-

lowed 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 (Zeuzem 2008).

**Treatment-naïve genotype 1 patients**

TVR 3x750mg/day  
pegIFN-<sub>2a</sub> 180µg/week  
Ribavirin 1000/1200mg/day

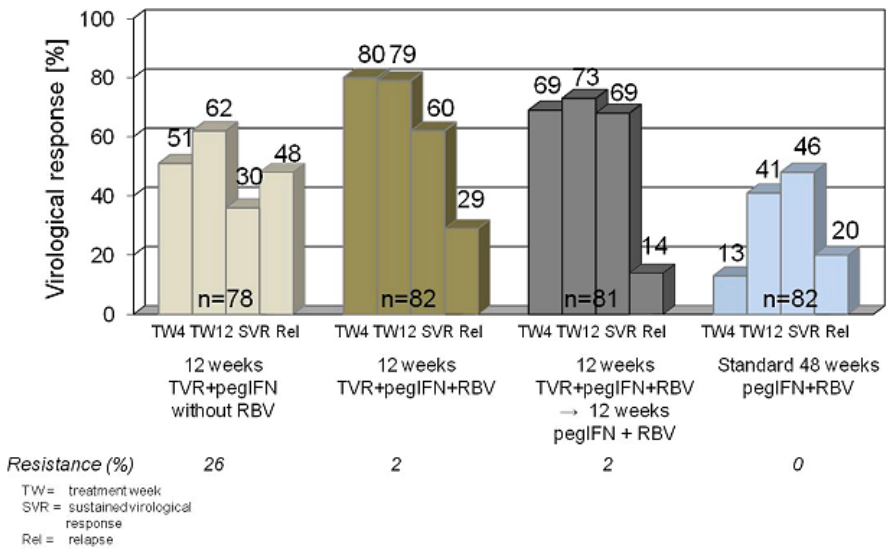
**Rules to stop treatment after 12/24 weeks**

PROVE 1: Stop only if RVR\* was achieved  
PROVE 2: Stop independently from viral response  
\*RVR: HCV RNA undetectable after 4 wks of treatment

| Study arm                                      | Treatment  | Number of patients |                  |
|--|--|--------------------|------------------|
|  |  | PROVE 1 (USA)      | PROVE 2 (Europe) |
| 12 weeks triple                                | 12 weeks: TVR + pegIFN- <sub>2a</sub> + RBV  | 17                 | 80               |
| 12 weeks combi                                 | 12 weeks: TVR + pegIFN- <sub>2a</sub>  | 0                  | 80               |
| 24 weeks (12 weeks triple + 12 weeks standard) | 12 weeks: TVR + pegIFN- <sub>2a</sub> + RBV<br>12 weeks: pegIFN- <sub>2a</sub> + RBV | 80                 | 80               |
| 48 weeks (12 weeks triple + 36 weeks standard) | 12 weeks: TVR + pegIFN- <sub>2a</sub> + RBV<br>36 weeks: pegIFN- <sub>2a</sub> + RBV | 82                 | 0                |
| Standard treatment                             | 48 weeks: pegIFN- <sub>2a</sub> + RBV  | 81                 | 80               |

**Table 2. Study design PROVE-1 & -2.**

Combination therapy of telaprevir (TVR) and PEG-IFN<sub>2a</sub> +/- Ribavirin in treatment-naïve genotype 1 patients.



**Figure 6. Interim results PROVE 2 (Europe).**

Combination therapy of telaprevir (TVR) and PEG-IFN  $\alpha$ -2a +/- ribavirin (RBV) in treatment-naïve genotype 1 patients.

In conclusion, PROVE 1 and 2 show that 12 weeks of triple therapy is too short because of the 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 probably be used for the determination of more individualized treatment duration.

**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  $\alpha$ -2a and ribavirin was administered for 12 to 24 weeks followed by PEG-IFN  $\alpha$ -2a and ribavirin alone for 0 to 24 weeks. Retreatment of non-responders with 12 weeks of triple therapy followed by 12 weeks of standard therapy led to a week 12 SVR rate of 52% (73% relapser, 41% non-responder) which is significantly higher than the current rate of on-treatment response in the standard treatment arm (30%). 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 2008). A phase III clinical trial (REALIZE) evaluating telaprevir in non-responders has recently begun.

Genotype 1 patients: non-responders, relapsers

TVR 3x750mg/day  
 pegIFN-<sub>2a</sub> 180µg/week  
 Ribavirin 1000/1200mg/day

| Study arm                                      | Treatment  | Number of patients |
|--|--|--------------------|
| 24 weeks combi                                 | 24 weeks: TVR + pegIFN- <sub>2a</sub>  | 110                |
| 24 weeks (12 weeks triple + 12 weeks standard) | 12 weeks: TVR + pegIFN- <sub>2a</sub> + RBV<br>12 weeks: pegIFN- <sub>2a</sub> + RBV | 110                |
| 48 weeks (24 weeks triple + 24 weeks standard) | 24 weeks: TVR + pegIFN- <sub>2a</sub> + RBV<br>24 weeks: pegIFN- <sub>2a</sub> + RBV | 110                |
| Standard treatment                             | 48 weeks: pegIFN- <sub>2a</sub> + RBV  | 110                |

**Table 3. Study design of PROVE 3.** Combination therapy of telaprevir (TVR) and PEG-IFN  $\alpha$ -2a +/- ribavirin (RBV) in genotype 1 non-responders and relapsers.

## **Boceprevir (SCH 503034)**

Boceprevir is another novel peptidomimetic orally bioavailable  $\alpha$ -ketoamid HCV protease inhibitor that forms a covalent but reversible complex with the NS3 protein (Malcolm 2006) (Figure 5). 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 occurred in some patients, especially in those treated with lower doses.

### **Boceprevir & PEG-IFN $\alpha$ -2b combination phase I study**

A subsequent phase Ib study evaluated the combination of boceprevir and PEG-IFN  $\alpha$ -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  $\alpha$ -2b for 14 days in comparison to 14 days of PEG-IFN  $\alpha$ -2b monotherapy (Figure 7). 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  $\alpha$ -2b and PEG-IFN  $\alpha$ -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  $\alpha$ -2b, 1.08 and 1.61  $\log_{10}$  for boceprevir monotherapy and 1.08 and 1.26  $\log_{10}$  for PEG-IFN  $\alpha$ -2b monotherapy. Boceprevir was well-tolerated alone and in combination with PEG-IFN  $\alpha$ -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.

## **Boceprevir and peg Interferon with and without ribavirin, phase II studies**

### **Treatment naïve phase II study (SPRINT-1)**

The SPRINT 1 trial investigated safety, tolerability and antiviral efficacy of boceprevir (800 mg three times a day) in combination with PEG-IFN  $\alpha$ -2b and ribavirin in treatment-naïve genotype 1 patients (Table 4) (Kwo 2008).

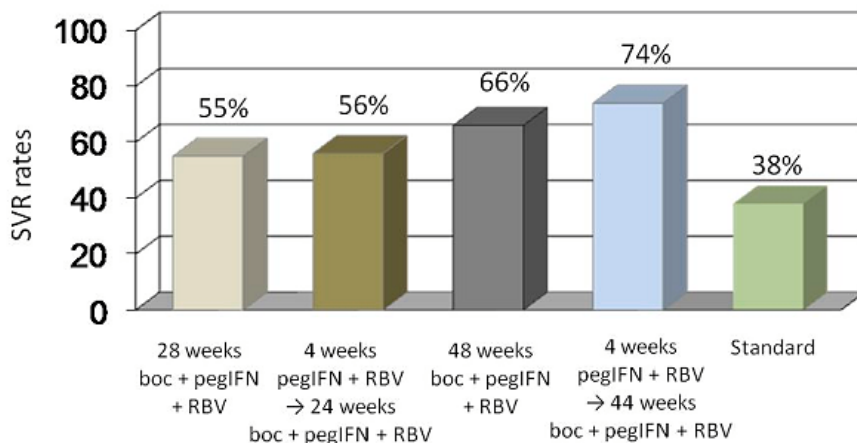
Treatment-naive genotype 1 patients  
 Boceprevir 3 x 800 mg/day  
 pegIFN- $\alpha$ 2b 1,5  $\mu$ g/kgKG  
 Ribavirin 800/1200 mg/day

| Study arm                               | Treatment  | Number of patients |
|---|--|--------------------|
| 28 weeks triple                         | 28 weeks: Boceprevir + pegIFN- $\alpha$ 2b + RBV                                       | 80                 |
| 4 weeks +<br>24 weeks triple            | 4 weeks: pegIFN- $\alpha$ 2b + RBV<br>24 weeks: Boceprevir + pegIFN- $\alpha$ 2b + RBV | 80                 |
| 4 weeks +<br>44 weeks triple            | 4 weeks: pegIFN- $\alpha$ 2b + RBV<br>44 weeks: Boceprevir + pegIFN- $\alpha$ 2b + RBV | 80                 |
| 48 weeks triple                         | 48 weeks: Boceprevir + pegIFN- $\alpha$ 2b + RBV                                       | 80                 |
| 48 weeks triple (low dose<br>Ribavirin) | 48 weeks: Boceprevir + pegIFN- $\alpha$ 2b<br>+ RBV (400 – 1000 mg/day)                | 80                 |
| Standard treatment                      | 48 weeks: pegIFN- $\alpha$ 2b + RBV  | 80                 |

**Table 4: Study design of SPRINT-1 (USA, Canada, Europe).**

Combination therapy of boceprevir and PEG-IFN  $\alpha$ -2a +/- ribavirin (RBV) in treatment-naive genotype 1 patients.

Treatment with boceprevir in combination with PEG-IFN  $\alpha$ -2b and ribavirin was either performed continuously for 28 or 48 weeks or for 24 or 44 weeks after a previous 4-week treatment period with PEG-IFN  $\alpha$ -2b and ribavirin alone. This study design was chosen to determine whether a lead-in treatment with PEG-IFN  $\alpha$ -2 plus ribavirin has beneficial effects in avoiding the development of resistance and on antiviral efficacy. The control group was treated with PEG-IFN  $\alpha$ -2b and ribavirin for 48 weeks. SVR rates after 28 weeks of triple therapy were 55% vs. 56% of 4 weeks lead-in plus 24 weeks of triple therapy. SVR rates after 48 weeks of triple therapy were 66% vs. 74% in the 4 weeks lead-in plus 44 weeks of triple therapy (Figure 8, see <http://hepatologytextbook.com/link.php?id=4>). After 4 weeks of triple therapy with boceprevir, PEG-IFN and ribavirin, 39% of patients achieved a rapid virologic response. The most common side-effects related to boceprevir were fatigue, nausea, depression, anemia and neutropenia. In general, SPRINT-1 has revealed a higher antiviral efficacy of combination therapies with boceprevir in comparison to the standard of care with slightly better results in the 4 week lead-in phase especially for the longer treatment duration of 48 weeks.



Premature treatment termination: 28% boceprevir, 14% standard  
Resistance: 5-11% BOC, 0% standard

**Figure 8. Interim results of SPRINT-1.**

Combination therapy of boceprevir and PEG-IFN  $\alpha$ -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  $\alpha$ -2b and ribavirin resulted in only slightly increased SVR rates compared to standard treatment (14% versus 2%) (Schiff 2008).

### TMC435350

TMC435350 is a novel macrocyclic HCV serine protease inhibitor with pharmacokinetics allowing once daily administration (Figure 4). In healthy volunteers, tolerability of a 600 mg single dose and of daily 400 mg doses for five days was excellent (Reesink 2008). TMC435350 monotherapy (200 mg) for five days was evaluated in a phase Ib study in six genotype 1 non-responders (Reesink 2008). Mean maximal reductions in HCV RNA load were 3.9 log<sub>10</sub>. No viral breakthrough was seen on this short-term treatment period although sequencing analyses did identify quasispecies less sensitive to TMC435350. Currently, TMC435350 is in phase II evaluation (OPERA-1 trial) with RVR rates of up to 89% after 28 days of triple therapy (Manns 2008).

### ITMN-191 (R7227)

ITMN-191 is a newly developed macrocyclic orally bioavailable NS3/4A inhibitor binding the enzyme's active site non-covalently (Courcambeck 2008) (Figure 4). In healthy volunteers, ITMN-191 was safe and well tolerated. Currently, antiviral efficacy, safety and tolerability of ITMN-191 200 to 600 mg monotherapy and in com-

combination with PEG-IFN  $\alpha$ -2a and ribavirin is being assessed in genotype 1 patients with chronic hepatitis C. Preliminary data indicate that ITMN-191 monotherapy is capable of reducing HCV RNA viral loads up to 4 log<sub>10</sub> in treatment-naïve patients after 2 weeks of treatment (Forestier 2008). Phase II studies evaluating ITMN-191 in treatment-naïve patients and in non-responders are ongoing. Moreover, clinical trials evaluating ITMN-191 in combination with an HCV polymerase inhibitor (R7128) are ongoing (Roche press release).

### 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 inhibition was proven. Importantly, no cross-resistance between ACH-806 and peptidomimetic NS3/4A protease inhibitors was observed in vitro (Wyles 2008; Yang 2008).

### 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 9).

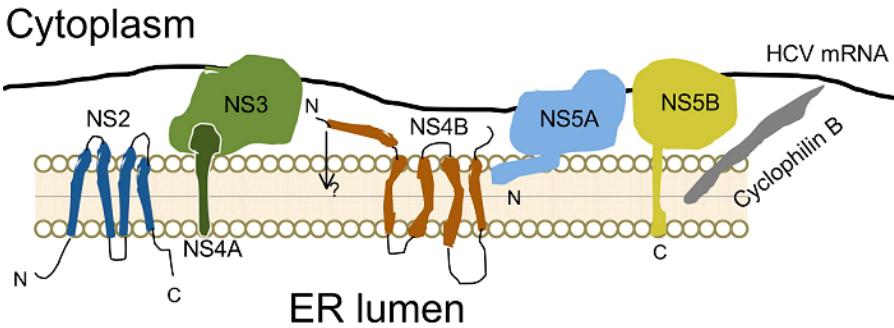
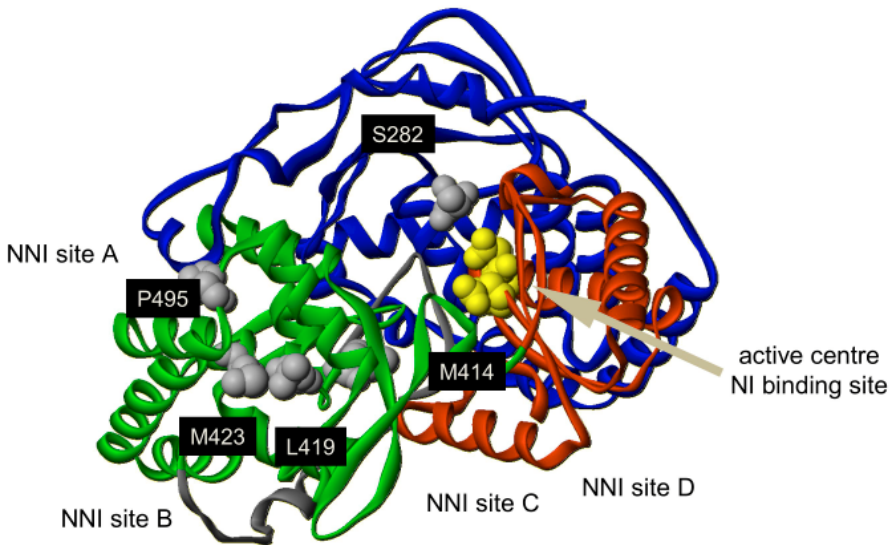


Figure 9. The HCV replication complex.



NS5B is an RNA-dependent RNA-polymerase which catalyzes the synthesis of a complementary negative-strand RNA by using the positive-strand RNA genome as a template (Lesburg 1999; Bartenschlager 2004) (Figure 10). 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 poly-protein translation. Because of its 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 will be generated each day (Weiner 1991).



**Figure 10. Structure of the HCV NS5B RNA polymerase and binding sites.**

NS5A seems to play a manifold role in HCV replication (Lindenbach 2005). It was shown that NS5A is involved in the early formation of the replication complex by interacting with intracellular lipid membranes (Lindenbach 2005; Appel 2008). Moreover, it was 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). Furthermore, mutations within the NS5A protein have been clinically associated with resistance / sensitivity to IFN-based antiviral therapy (Pascu 2004; Wohnsland 2007). A first NS5A inhibitor proof of principle study in patients with chronic hepatitis C was recently presented and showed a sharp decline of HCV RNA concentrations after treatment for only one day (Nettles 2008).

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 which supports 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 exerts antiviral impact against HCV mediated by cyclophilin B inhibition *in vitro* has led to current developments of non-immunosuppressive cyclophilin B inhibitors.

## Compounds targeting HCV replication

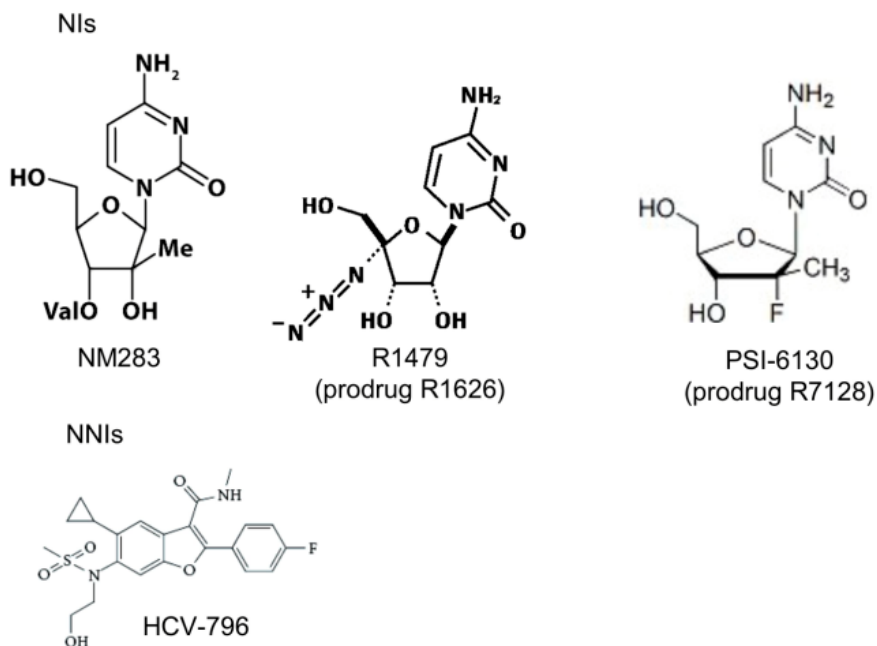
### NS5B polymerase inhibitors

NS5B RNA polymerase inhibitors can be divided into two distinct categories. Nucleoside analog inhibitors (NIs) like valopicitabine (NM283), R7128 or R1626 mimic the natural substrates of the polymerase and are incorporated into the growing RNA chain, thus causing direct chain termination by tackling 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) like HCV-796 achieves NS5B inhibition by binding to the allosteric enzyme sites, which results in conformational protein change before the elongation complex is formed (Beaulieu 2007; Wohnsland 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 10). 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.

### Valopicitabine (NM283)

Valopicitabine is a cytidine analogue and was the first polymerase inhibitor advanced to clinical trials (Pierra 2005) (Figure 11). In an initial monotherapy study, 800 mg valopicitabine administered daily resulted in a decrease of more than 1 log<sub>10</sub> in HCV RNA load after 15 days of treatment in genotype 1 patients that had previously failed to respond to standard treatment.



**Figure 11. Molecular structure of NS5B polymerase inhibitors.**

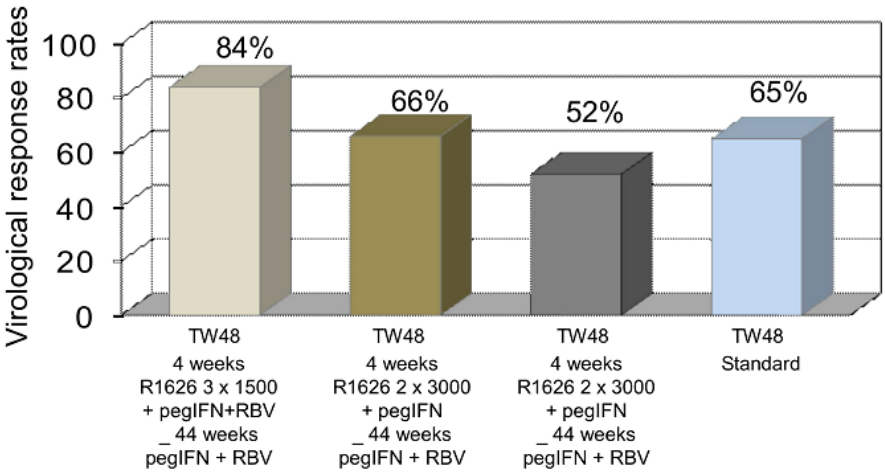
Combination therapy of valopicitabine with PEG-IFN  $\alpha$ -2a was evaluated in two phase II clinical trials and yielded disappointing results. In treatment-naïve genotype 1 patients, 200-800 mg valopicitabine plus PEG-IFN  $\alpha$ -2a was administered for 48 weeks compared with PEG-IFN  $\alpha$ -2a monotherapy for four weeks with the addition of valopicitabine from week 5 onwards. At week four, all individuals treated with combination therapy demonstrated greater reductions in HCV RNA than those treated with PEG-IFN  $\alpha$ -2a monotherapy (3 to 3.8  $\log_{10}$  versus 1.8  $\log_{10}$  IU/ml). End-of-treatment data suggests that valopicitabine retains antiviral activity throughout the 48-week treatment period. However, only lower doses of valopicitabine (200 mg) were well-tolerated and SVR rates were not significantly higher than after standard treatment. The most common side effects were diarrhea, nausea and vomiting. The second trial evaluated combination therapy of valopicitabine and PEG-IFN  $\alpha$ -2a in genotype 1 non-responders to standard therapy, but no benefit compared to retreatment with PEG-IFN  $\alpha$ -2a and ribavirin was achieved (Chang 1994). Treatment with ribavirin plus valopicitabine and pegylated interferon results in reduced antiviral efficacy due to biological interactions between valopicitabine and ribavirin (Coelmont 2006).

The development of valopicitabine was stopped because of its insufficient risk/benefit profile according to an overall evaluation of the US Food and Drug Administration (FDA).

**R1626**

R1626, the oral prodrug of R1479, is another nucleoside (cytidine) analogue inhibitor (Figure 11). In untreated genotype 1 patients, R1626 monotherapy led to 3.7, 2.6 and 1.2 log<sub>10</sub> reductions of HCV RNA load at doses of 4500 mg, 3000 mg and 1500 mg twice a day, respectively (Roberts 2008). No resistant mutants emerged during the treatment period of 14 days.

A subsequent phase IIa clinical trial assessed the combination of R1626 (1500mg three times daily, 1500 mg twice daily or 3000 mg twice daily) with PEG-IFN  $\alpha$ -2a alone or with PEG-IFN  $\alpha$ -2a plus ribavirin for four weeks followed by 44 weeks of PEG-IFN  $\alpha$ -2a plus ribavirin in treatment naïve genotype 1 patients. The control group was treated for 48 weeks of PEG-IFN  $\alpha$ -2a plus ribavirin (Pockros 2008). End-of-treatment response rates at week 48 (HCV RNA <15 IU/ml) were 84% (3 x 1500 mg), 66% (2 x 3000 mg) and 52% (2 x 1500 mg) compared to 65% in the control group (Figure 12 for details). R1626 caused reversible grade 3 neutropenia in 40–70% of patients which is not acceptable for a standard of care. Recently, a phase IIb clinical trial was initiated evaluating 24 weeks of combination therapy with lower doses of R1626 or PEG-IFN  $\alpha$ -2a together with ribavirin followed by 24 weeks of PEG-IFN  $\alpha$ -2a plus ribavirin in treatment-naïve genotype 1 patients. However, due to infectious severe adverse events the further development of R1626 was stopped.



Reversible grade 3 neutropenia (<500/nl) in 40-70%

**Figure 12. Antiviral efficacy and safety of R1626 in combination with PEG-IFN  $\alpha$ -2a and ribavirin (RBV) in treatment-naive genotype 1 patients.**

**R7128**

R7128, a prodrug of PS-I6130, is a newly developed nucleoside (cytidine) analog NS5B inhibitor (Klumpp 2006) (Figure 11). R7128 monotherapy at 1500 mg for 14 days in genotype 1 non-responders to standard treatment was safe and caused a mean maximal HCV RNA reduction of 2.7 log<sub>10</sub> IU/ml. No viral rebound was observed during the treatment period.

Preliminary results of a study investigating the combination of R7128 (500 or 1500 mg once daily), PEG-IFN  $\alpha$ -2a and ribavirin over 28 days in treatment-naïve genotype 1 patients have been presented. The mean HCV RNA decline was 2.6 log<sub>10</sub> at day 14 and 4.0 log<sub>10</sub> at day 28. R7128 was safe, but some of the treated patients suffered from headache, insomnia or elevated serum triglycerides (Lalezari 2008). Importantly, R7128 was highly effective in genotype 2 and 3 non-responders as well (Gane 2008).

**HCV-796**

HCV-796 is an orally bioavailable, non-nucleoside NS5B inhibitor belonging to the benzofuran family (Figure 11). HCV-796 monotherapy (50 to 1500 mg twice daily) for 14 days in treatment-naïve HCV-infected patients led to a mean reduction of HCV RNA load of 1.4 log<sub>10</sub> at day 4, followed by viral breakthrough in most patients indicating a rapid development of resistance against HCV-796 (Chandra 2006).

A second trial evaluated the combination of HCV-796 with PEG-IFN  $\alpha$ -2a in treatment-naïve patients with chronic hepatitis C (Villano 2007). HCV-796 was administered for 14 days at doses from 100 to 1500 mg twice daily. After 14 days of treatment, mean HCV RNA log<sub>10</sub> reductions were 3.3 to 3.5 in patients treated with HCV-796 and PEG-IFN  $\alpha$ -2a compared to 1.7 in patients treated with PEG-IFN  $\alpha$ -2a alone. HCV-796 was well tolerated and no viral breakthrough occurred during combination therapy. Thus, the combination of NNIs with pegylated interferons may be sufficient to overcome this rapid induction of resistance. However, the development of HCV-796 is on hold due to unusually high elevations of serum transaminases.

**VCH-759**

VCH-759 is a novel orally bioavailable, thiophene non-nucleoside NS5B inhibitor. A multiple dose-ascending study was performed to investigate antiviral efficacy, tolerability, safety and pharmacokinetics of VCH-759 monotherapy at doses of 400 mg three times daily, 800 mg three times daily, or 800 mg twice daily for 10 days in treatment-naïve genotype 1 patients. The mean maximal decline in HCV RNA serum levels was between 1.9 and 2.5 log<sub>10</sub> in a dose-dependant manner. In general, VCH-759 was well tolerated, with the most frequent adverse events being gastrointestinal disorders (Cooper 2007).

## **Drugs specifically 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 insufficient and the development of VGX-410C has been 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 not clear at the moment whether ribozymes will be move forward in 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 initiate 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,  $\alpha$ -glucosidases, enzymes necessary in the processing of some glycoproteins, are important for the correct folding and assembly of HCV envelope proteins. These  $\alpha$ -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, only drugs targeting host proteins that disturb viral assembly and release have been developed. These are summarized below.

## Resistance to specific antivirals

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 preexisting drug-resistant variants have a fitness advantage and can be selected to become the dominant viral quasi-species. Many of these resistant mutants exhibit an attenuated replication with the consequence that, after termination of exposure to specific antivirals, the wild-type may displace the resistant variants (Tong 2006; Sarrazin 2007). During both protease and polymerase inhibitor administration viral breakthrough of resistant mutants has been observed. Thus, drug resistance is a highly relevant issue in any successful STAT-C programme. Importantly, no cross-resistance between protease inhibitors, polymerase inhibitors and host enzyme inhibitors like debio-025 or celgosivir has been observed to date (Koev 2008). Next, we will look at the known mutations in the protease and polymerase genes.

## Resistance to NS3/4A inhibitors

**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. The A156T 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, see <http://hepatologytextbook.com/link.php?id=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  $\alpha$ -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 A156/T were uncovered

by wild-type reduction and became dominant after day 8. These single mutation 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  $\alpha$ -2a was sufficient to inhibit the breakthrough of resistant mutations in a 14-day study (Forestier 2007).

**Boceprevir.** In the replicon system, mutations at three positions conferring boceprevir resistance were discovered (Table 5). T54A, A156S and V170A confer low level resistance to boceprevir whereas A156T, which also confers telaprevir and ciluprevir resistance, exhibited 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 2008).

**ITMN-191.** D168A/E/V/S mutations were observed to confer ITMN-191 resistance *in vitro*. ITMN-191 has a modified molecular structure of ciluprevir, which explains the D168A/V cross-resistance of both compounds (Courcambeck 2008). No clinical data evaluating ITMN-191 resistance are available so far.

## Resistance to NS5B inhibitors

In general, there seems to be a high genetic barrier for the development of resistance to nucleoside analog inhibitors (NIs), which can be explained by the fact that single-point mutations alter the active site, which results in a strongly reduced activity of the polymerase. In contrast, resistance to NNIs has been rapidly observed and does not necessarily attenuate the replication cycle.

## Nucleoside inhibitors

**Valopicitabine.** Resistance against valopicitabine was investigated in the replicon system. Variants carrying the S282T mutation confer high-level resistance, but are strongly reduced in their replicatory fitness compared to wild-type (Ludmerer 2005; Mo 2005). Consequently, during valopicitabine monotherapy no viral breakthrough was observed.

**R1626 (prodrug of R1479).** In the HCV replicon assay, S96T has been described to confer resistance against R1626 (Le Pogam 2006). For R1626 so far no clinical resistance with break-through of HCV RNA concentrations on therapy has been described and no resistant mutants have been detected by clonal analysis of viral variants in patients during antiviral therapy (Le Pogam 2008).

**R7128 (prodrug of PSI-6 130):** S282T has been revealed in the replicon assay to confer resistance against R7128. As for R1626 no clinical resistance has been observed in patients treated with R7128 so far (Le Pogam 2008).



## Non-nucleoside inhibitors

**HCV-796.** During HCV-796 monotherapy, rapid viral breakthrough was observed in most patients. Sequencing analyses of the NS5B gene in the replicon assay revealed resistance mutations at positions L314, C316, I363 and S365A (Howe 2006).

For the four different classes of non-nucleoside inhibitors, studies in the HCV replicon system reveal specific resistance mutations with partially overlapping resistance profiles (Table 6, see <http://hepatologytextbook.com/link.php?id=6>). For example, in chimpanzees treated with the non-nucleoside benzothiadiazine site polymerase inhibitor A-837093, Y448, G554, D559 and C316 variants have been described in association with viral resistance. Interestingly, several mutations were still detectable 2 weeks after termination of therapy, indicating that the fitness levels of these resistance mutation-containing isolates are similar to those of the wild-type virus (Chen 2007).

## Host proteins as targets in treating hepatitis C

### Cyclophilin B inhibitors (Debio-025)

Cyclophilin B is a ubiquitously-expressed cis-trans isomerase involved in the folding of many proteins. It has been shown that cyclophilin B enhances NS5B activity and thus facilitates HCV replication. 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 during the 14 days of treatment.

Combination therapy of Debio-025 200 mg, 600 mg or 1000 mg and PEG-IFN  $\alpha$ -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 performed 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  $\alpha$ -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 of the patients treated with 1000 mg Debio-025 daily (Flisiak 2008). Studies determining SVR rates of combination therapy with Debio-025 and PEG-IFN  $\alpha$ -2a are ongoing.

### Glucosidase inhibitors (Celgosivir (MX-3253))

Celgosivir is an orally bioavailable prodrug of the  $\alpha$ -glucosidase inhibitor castanospermine. To evaluate celgosivir monotherapy, treatment-naïve 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 only in a minority of patients.

To assess whether celgosivir in addition to PEG-IFN  $\alpha$ -2b and ribavirin is able to enhance virologic responses a study was performed in genotype 1 patients who previously failed to respond to standard treatment. 400 mg celgosivir was administered once daily over a treatment period of 12 weeks. Early virologic response rates (defined as 2 log<sub>10</sub> 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  $\alpha$ -2b and ribavirin alone. Mean log<sub>10</sub> 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.

Two clinical trials evaluated nitazoxanide monotherapy as lead-in treatment, followed by the combination of nitazoxanide plus PEG-IFN  $\alpha$ -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 weeks of nitazoxanide plus PEG-IFN  $\alpha$ -2 and ribavirin or by 36 weeks of nitazoxanide plus PEG-IFN  $\alpha$ -2a alone. The control group was treated for 48 weeks with PEG-IFN  $\alpha$ -2a and ribavirin. SVR rates were 79% for nitazoxanide, followed by additional PEG-IFN  $\alpha$ -2a and ribavirin, and 64% for nitazoxanide, followed by additional PEG-IFN  $\alpha$ -2a, compared to 45% in the control group (Rossignol 2008). 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 weeks of nitazoxanide alone showed no significant differences in rapid viral, early viral and end-of-treatment responses compared to the 12 weeks lead-in (Rossignol 2008). SVR rates reached up to 80% with best results seen in those groups with a 4 week lead-in phase. In general, nitazoxanide was well tolerated and no serious adverse events were reported. Studies in patients infected with HCV genotypes other than genotype 4 have been initiated.

## 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 400 mg 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  $\alpha$  with amantadine did not increase SVR rates compared to IFN- $\alpha$  alone (Zeuzem 2000). The combination of amantadine with PEG-IFN  $\alpha$  and 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-naïve HCV genotype 1 infected patients in Germany. In this study, no significant difference of triple therapy including amantadine 200mg twice daily in comparison to the standard treatment of PEG-IFN  $\alpha$ -2a plus ribavirin was observed (von Wagner 2008).

## Novel interferons

Both current forms of pegylated IFN  $\alpha$  have to be injected once weekly. For some patients less frequent injections are likely to be associated with fewer side-effects, higher treatment comfort and better treatment adherence. Therefore, novel interferons are under development which are designed to ensure a 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 alfa to increase virologic response rates especially in patients nonresponsive to the current interferon  $\alpha$ -2a/b based regimens. Another approach is the generation of chemically modified interferons to achieve oral bio-availability. The most promising developments in this field are summarized below.

## Albinterferon

Albinterferon is a novel recombinant interferon comprised of IFN  $\alpha$ -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 900mg 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 (Balan 2006).

Another study including 115 genotype 1 non-responders to standard treatment evaluated the combination of ribavirin plus 900 mg to 1200 mg albinterferon every other 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 (Zeuzem 2008).

The efficacy of albinterferon in achieving SVR rates in patients infected with genotypes 2 or 3 is equivalent to that of standard therapy, according to another phase II clinical trial.

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 ongoing ACHIEVE trials (phase III). Generally, pneumonitis has also been observed upon administration of interferons and pegylated interferons, representing a class effect of interferon  $\alpha$ . In a recent press release the first results of the phase III study on genotype 2/3 infected patients were announced. Overall, study arms with albinterferon showed equal SVR rates in comparison with the standard of care.

## **IFN $\alpha$ -2bXL**

IFN  $\alpha$ -2bXL is a formulation of recombinant IFN  $\alpha$ -2b providing a sustained release of active IFN  $\alpha$  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  $\alpha$ -2bXL or PEG-IFN  $\alpha$ -2b for 14 days. IFN  $\alpha$ -2bXL showed an almost significant superior antiviral activity at the end of the dosing period. Importantly, IFN  $\alpha$ -2bXL led less frequently to treatment-related adverse events (Trepo 2007). Extended clinical trials evaluating IFN  $\alpha$ -2bXL in combination with ribavirin are ongoing.

## **Controlled-release recombinant interferon alpha-2b (Locteron)**

Locteron is a novel recombinant IFN  $\alpha$ -2b formulation that provides sustained IFN- $\alpha$  plasma levels when injected every other week. The SELECT-1 trial (phase II) evaluated locteron for twelve weeks at doses from 160  $\mu$ g to 640  $\mu$ g 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 of standard interferons. Side-effects were equivalent to standard interferons but the administration was more convenient for patients (Herrmann 2008). A phase IIb study has recently started.

## Belerofon

Belerofon is the most advanced orally bioavailable interferon and has currently progressed to phase II evaluation. Data evaluating antiviral activity and safety are expected to be published in late 2008.

## Novel ribavirin derivatives

### 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  $\alpha$ -2b compared to weight-adapted ribavirin. Rates of anemia were strongly reduced in the taribavirin group (5% versus 24%, defined as Hb<10g/dl). 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  $\alpha$ -2a in an equivalent study design (Poordad 2008). Rates of anemia and SVR were comparable to those obtained from VISER 1. Because a retrospective study analysis of VISER 1 and 2 indicated that higher plasma levels of taribavirin are associated with higher SVR rates, a subsequent trial evaluating weight-adapted taribavirin doses has recently been initiated. Interim results from this study reveal comparable antiviral efficacy of taribavirin and ribavirin (Gish 2007).

## Outlook

There is an urgent need for improvement in the treatment of hepatitis C as only about 40-50% of all patients infected with HCV can currently be cured. Moreover, interferon- $\alpha$ -based treatment regimes frequently cause severe side-effects and require long treatment durations, challenging patient adherence. It can be anticipated that the tremendous progress in developing specific 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 inhibitors of the HCV protease 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 and ribavirin has been shown to be suitable for successful reduction of resistance development against specific antivirals. Based on phase II study results such 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 are 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 with specific antivirals remain to be evaluated. In particular, it needs to be determined whether interferon-free combinations of specific antivirals can be successfully established.

## References

- 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 non-structural protein 5A for hepatitis C virus infectious particle assembly." *PLoS Pathog* 4(3): e1000035.
- 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.
- 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 J, Cosset 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.
- 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 E, Ghali Pal. e (2007). "Antiviral activity of the non-nucleoside polymerase inhibitor, VCH-759, in chronic hepatitis C patients: Results from a randomized, double-blind, placebo-controlled, ascending multiple dose study." *Hepatology* 46(Suppl. 4): 864A.
- 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, Pruetz 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 CM, Glenn 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.
- Evans MJ, von Hahn T, Tschernie 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-naïve patients with chronic hepatitis C genotype 1 infection." *J Hepatol* 44(2): 275-82.
- Flisiak R, Dumont J, Crabbe R (2007). "Cyclophilin inhibitors in hepatitis C viral infection." *Expert Opin Investig Drugs* 16(9): 1345-54.
- Flisiak R, Feinman V, Jablkowski M, Horban A, et al. (2008). "Efficacy and safety of increasing doses of the cyclophilin inhibitor DEBIO-025 in combination with pegylated interferon alpha-2a in treatment naïve chronic HCV patients." *J Hepatol* 48(Suppl. 2): 62.
- Flisiak R, Horban A, Gally P, Bobardt M, et al. (2008). "The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfecting 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.
- Foster G (2004). "Past, present, and future hepatitis C treatments." *Semin Liver Dis* 24(Suppl. 2): 97-104.
- Fried MW (2002). "Side effects of therapy of hepatitis C and their management." *Hepatology* 36(5 Suppl 1): S237-44.
- Gallego J, Varani 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. 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..
- Gish RG, Arora S, Rajender Reddy K, Nelson DR, et al. (2007). "Virological response and safety outcomes in therapy-naïve patients treated for chronic hepatitis C with tariba-

## 240 New agents for treatment

- virin 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.
- 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." *Virology* 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.
- 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.
- Kwo P, Lawitz EJ, McCone J, et al. (2008). HCV SPRINT-1: Boceprevir plus Peginterferon alfa-2b/Ribavirin for Treatment of Genotype 1 Chronic Hepatitis C in Previously Untreated Patients. *Hepatology.* 48(Suppl 4): 1024A.
- 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 Hepatol* 49: 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 quasiespecies may contribute to high barrier to R1626 resistance in vivo." *J Hepatol* 48(Suppl. 2): 10A.
- 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, 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.
- McHutchison J, Everson G, Gordon S, Jacobson I, et al. (2008). "PROVE1: Results from a phase 2 study of telaprevir with peginterferon alfa-2a and ribavirin in treatment-naïve subjects with hepatitis C." *J. Hepatol.* 48(Suppl. 2): 4.

## 242 New agents for treatment

- McHutchison JG, Shiffman ML, Terrault N, et al. (2008). A Phase 2b Study of Telaprevir with Peginterferon-Alfa-2a and Ribavirin in Hepatitis C Genotype 1 Null and Partial Responders and Relapsers Following a Prior Course of Peginterferon-Alfa-2a/b and Ribavirin Therapy: PROVE3 Interim Results. *Hepatology*. 48(Suppl 4): 431A.
- 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.
- 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 S, McHutchison 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, Chun E, Hammond Je, et al. (2008). "Treatment week 12 results of weight-based taribavirin versus weight-based ribavirin, both with peginterferon alfa-2b, in naïve chronic hepatitis C, genotype 1 patients." *J Hepatol* 48(Suppl. 2): 373.
- 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 H, Verloes R, Abou Farha K, Van Vliet A, et al. (2008). "Safety of the HCV protease inhibitor TMC435350 in healthy volunteers and safety and activity in chronic hepatitis c infected individuals: a phase 1 study." *J Hepatol* 48(Suppl. 2): 28.
- 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(2): 398-406.
- Rossignol J, Elfert A, Keeffe E (2008). "Evaluation of a 4 week lead-in phase with nitazoxanide prior to nitazoxanide+peginterferon in treating chronic hepatitis C." *J Hepatol* 48(Suppl. 2): 311.
- Rossignol J, Kabil S, El-Gohary Y, Keeffe E (2008). "Randomized controlled trial of nitazoxanide-peginterferon-ribavirin, nitazoxanide-peginterferon and peginterferon-ribavirin in the treatment of patients with chronic hepatitis c genotype 4." *J. Hepatol.* 48(Suppl. 2): 30.
- Rossignol J, Elfert A, Keeffe EB, et al. (2008). Evaluation of a 4 Week Lead-In Phase with Nitazoxanide (NTZ) Prior to Peginterferon (PegIFN) Plus NTZ for Treatment of Chronic Hepatitis C: Final Report. *Hepatology.* 48(Suppl 4): 344A.
- 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.
- 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.
- 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, Kwok 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.
- Susser S, Welker M, Zettler M, Wohnsland A, et al. (2008). "Clonal analysis of mutations selected in the HCV NS3 protease domain of genotype 1 non-responders treated with boceprevir." *J Hepatol* 48(Suppl. 2): 29.
- 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.
- 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.
- VanCompernelle 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.
- 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.

## 244 New agents for treatment

- 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 W, Sarrazin C (2007). "Viral determinants of resistance to treatment in patients with hepatitis C." *Clin Microbiol Rev* 20(1): 23-38.
- Wyles D, Kaihara K, Schooley 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* 52(5): 1862-4.
- Zeuzem S, Sarrazin C, Wagner F et al. (2005). "Antiviral activity of SCH 503034, a HCV protease inhibitor, administered 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.
- Zeuzem S, Hezode C, Ferenci P, et al. (2008). "Telaprevir in Combination with Peginterferon-Alfa-2a with or without Ribavirin in the Treatment of Chronic Hepatitis C: Final Results of the PROVE2 Study." *Hepatology* 48(Suppl 4): 418A.
- 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 drug reactions

Martin Schaefer and 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 and ribavirin will experience adverse events that can threaten good adherence. Therefore, proactive clinical management is crucial to avoid suboptimal therapy and treatment discontinuations.

The most common adverse events in patients on treatment with pegylated interferon plus ribavirin are flu-like symptoms, myalgia, sleep disturbances, asthenia, gastrointestinal disorders and depressive episodes (Table 1).

| Psychiatric side effects  | Incidence |
|---|-----------|
| Fatigue   | 70-80%    |
| Sleep disturbances  | 45-65%    |
| Irritability  | 60-85%    |
| Cognitive disturbances with impairments of concentration and memory | 45-60%    |
| Depressive episodes   | 50-60%    |
| Mild  | 20-40%    |
| Moderate  | 15-30%    |
| Severe  | 1-5%      |
| Delirium, psychosis   | 1-6%      |
| Suicidal syndrome   | <1%       |

**Table 1. Incidence of most reported IFN  $\alpha$ -induced psychiatric side-effects.** Data from Outpatient Department, Essen-Mitte Clinics, Essen.

For most adverse events, clinical trials with dose adjustment have not been done, and because of this, recommendations in this review 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 PEG-IFN 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 over the first weeks of treatment (Figure 1).

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.

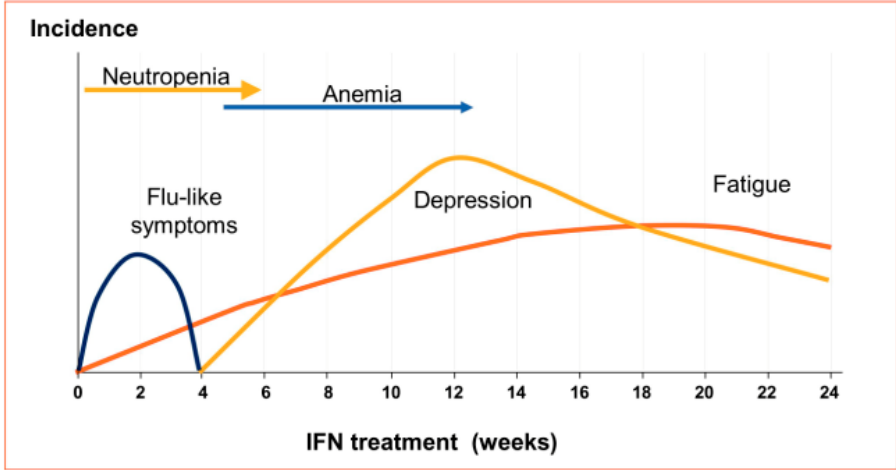


Figure 1. Time course of interferon-associated adverse events.

## Gastrointestinal disorders

Nausea can be mitigated by using prokinetic agents such as metoclopramide or domperidone taken before the ribavirin. This may also positively influence the frequently observed loss of appetite.

Dry mouth has been reported as a result of inhibition of saliva production, a frequent complication of ribavirin, which may continue after discontinuation of 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 of patients 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, reduction of ribavirin 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, *in press*). A first prospective

randomised controlled trial showed superior effects of the 5-HT-3 receptor antagonist ondansetron compared to placebo (Piche 2005). However, currently available data does not offer 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 mucosa swelling, anaemia and asthenia.

## Disorders of the thyroid gland

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 clinical symptomatic hyperthyroidism may be treated with  $\beta$ -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 $\alpha$ -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 hepatological 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); otherwise, patients are monitored by structured interviews utilising the Hamilton Depression Scale (HAMDS) or Montgomery Asperg Depression Scale (MADRS), rating depressive symptoms and any changes in scores not fulfilling DSM-IV criteria for major depression. Regarding 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 ap-

pear 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 of interferon are selective serotonin re-uptake inhibitors (SSRI) such as citalopram, paroxetine or tricyclic antidepressants such as doxepin. The introduction of SSRI and other current antidepressants has markedly reduced the adverse event 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 (Table 2). In patients with predominantly agitation and aggression, other strategies, e.g., modern antipsychotics, may be added.

| First choice   |             | Second choice* |               |
|----------------|-------------|----------------|---------------|
| lacking drive  | fearful     | lacking drive  | fearful       |
| self-conscious | agitated    | self-conscious | agitated      |
| Paroxetine     | Mirtazapine | Clomipramine   | Doxepin       |
| Citalopram     |             | Imipramine     | Amitriptyline |
| S-Citalopram   |             |                |               |
| Sertraline     |             |                |               |

\*increased risk of adverse events

**Table 2. Antidepressants of choice to treat interferon  $\alpha$ -associated depressive symptoms.**

The efficacy of antidepressants for the treatment of interferon  $\alpha$ -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 most appropriate agents for the treatment of interferon  $\alpha$ -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 days 8-14 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.

In the 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 use.

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-infected patients 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  $\alpha$  (Kraus 2005). Finally, a recent trial confirmed a protective effect of preemptive initiation of treatment with antidepressants before the start of interferon-based therapy in cases of elevated depression scores (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 zopiclone 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 to add current antidepressants with sedative effects, such as mirtazapine, as needed.

## Haematological 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 of 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.

G-CSF increases neutrophils in patients treated with interferon-based therapies. However G-CSF has not been proven to have a clinical benefit 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 inhibiting 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. Erythropoietin can be successfully used to correct the 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 hepatitis C therapy in patients who take erythropoietin (Afdahl 2004; Pockros 2004; Shiffman 2007). At present erythropoietin is not approved for correction of ribavirin-induced anaemia in hepatitis C therapy.

Mild to moderate thrombocytopenia is a frequent observation in patients with advanced liver fibrosis and may complicate interferon-based therapy. Reduction of interferon dosing may be indicated to reverse severe thrombocytopenia. In studies eltrombopag has been used successfully to increase platelet count in patients with hepatitis C associated thrombocytopenia (McHutchison 2007).

## Skin disorders and hair loss

Some skin disorders such as lichen ruber planus, necrotizing vasculitis or porphyria cutanea tarda are associated with hepatitis C infection. The effects of hepatitis C therapy are often not well-studied and based only on cohorts (Berk 2007).

Interferon and ribavirin therapy may have an effect on the skin itself including dry skin, itching, eczema and new or exacerbation of 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 dose 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 and ribavirin (Fried 2002; Manns 2001).

## Conclusion

In summary, the toxicity of interferon-based therapy in combination with ribavirin is considerable and requires active management and profound knowledge, particularly about the management of 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 the efficacy of therapy, in particular in HCV genotype 1 patients. Current studies indicate that most agents will have a substantial adverse event profile, i.e., increasing haematological or dermatological problems while on therapy. Early assessment of and therapy for 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, Wright T, Younossi Z, Goon BL, Tang KL, Bowers PJ; Proactive Study Group. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology*. 2004 May;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, Craxi 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.

## 252 Management of adverse drug reactions

- 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, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM; PEGASYS International Study Group: 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 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, Berg T, Gordon SC, Campbell FM, Theodore D, Blackman N, Jenkins J, Afdhal NH and the TPL102357 Study Group. 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, Carpiello 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, Wright TL, Mody SH, Tang KL, Goon BL, Bowers PJ, Leitz G, Afdhal NH; PROACTIVE Study Group. 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, Weinreb 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.
- Sammur 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, 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 Bommel 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; *in press*.
- 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 of chronic HCV

Karl-Philipp Puchner and Thomas Berg

### Introduction

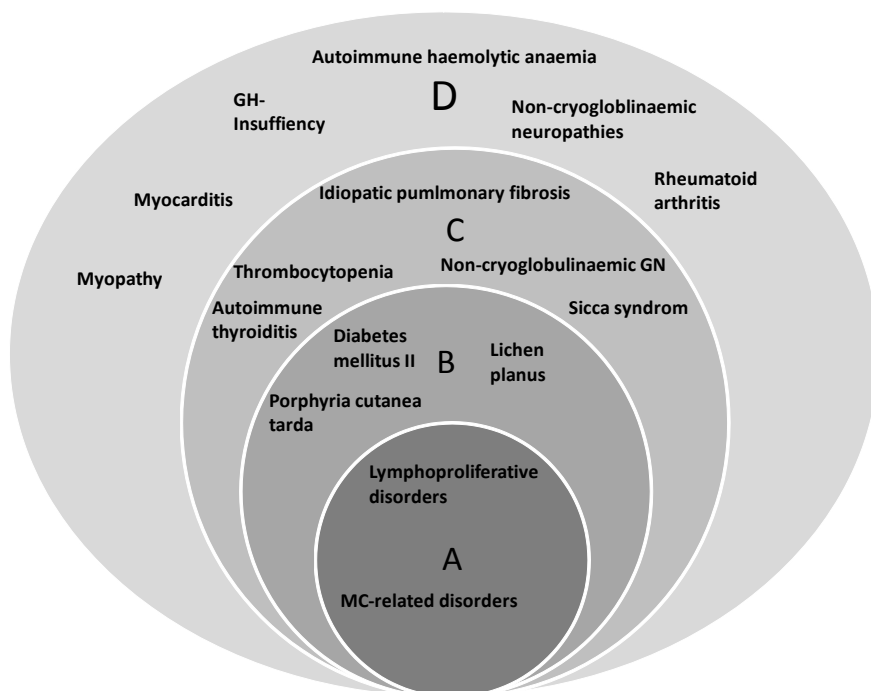
Patients with chronic hepatitis C virus (HCV) infection may develop a great number of extrahepatic manifestations (EHM) (Table 1).

Anywhere from 40-76% of patients infected with HCV develop at least one EHM during the course of the disease (Cacoub 2000; Cacoub 1999). The pathogenesis of these EHM is still not fully understood, although most studies suggest that the presence of mixed cryoglobulinaemia (MC), particular lymphotropism of the virus, molecular mimicry and non-MC autoimmune phenomena constitute the major pathogenic factors (Ferri 2007). Depending on the pathogenic and epidemiological evidence provided by different studies (Zignego 2007) the HCV EHM can be classified into four categories: (A) Associations that rest upon strong epidemiological prevalence and clear pathogenic mechanisms; (B) Disorders for which substantial data demonstrate a higher prevalence in HCV populations than in control populations, but exact pathogenic mechanisms still need to be determined; (C) Syndromes which require a more detailed characterisation or for which it is still unclear whether the higher prevalence noticed in HCV populations is in fact due to HCV infection or other confounding etiologies (interferon therapy side effects, liver cirrhosis, etc.); (D) Anecdotal observations (Figure 1).

| Organ/ System involved | Manifestation                          | Suggested pathomechanism                 |
|------------------------|--|--|
| <b>Endocrine</b>       | Thyroid dysfunction                    | Non-MC autoimmune phenomena              |
|                        | Thyroid autoantibodies                 | Lymphotropism                            |
|                        | Hashimoto's thyroiditis                | Non-MC autoimmunity                      |
|                        | Insulin resistance / diabetes mellitus | Unknown-eventually upregulation of SOC-3 |
|                        | GH-insufficiency                       | Unknown                                  |
| <b>Blood vessels</b>   | Small vessel vasculitis                | MC                                       |
|                        | Polyarteriitis nodosa                  | MC and Non-MC autoimmune phenomena       |
|                        | Peripheral neuropathy                  | MC and Non-MC autoimmune phenomena       |







**Figure 1. Schematic representation of EHM categories.**

A) Associations that rest upon strong epidemiological evidence and clear pathogenic mechanisms; B) Associations that rest upon high prevalence, but still unclear pathogenic mechanisms; C) Associations for which the high prevalence in HCV populations could be due to HCV infection and/or confounding factors; D) Anecdotal observations.

Evidence of HCV infection should always be actively sought in patients with rare pathologies such as porphyria cutanea tarda, non-organ specific autoantibodies, sicca-syndrome, idiopathic pulmonary fibrosis, lichen planus or mixed cryoglobulinaemia with or without related disorders.

## Mixed cryoglobulinaemia

Cryoglobulinaemia refers to the presence of abnormal immunoglobulins in the serum, which have the unusual property of precipitating at temperatures below 37°C and re-dissolving at higher temperatures. Cryoglobulins (CGs) are classified, on the basis of their clonality, into three types (Table 2). Type II CGs and type III CGs (mixed cryoglobulinaemia) are highly prevalent in patients with chronic HCV infection, while type I CGs are mostly found in patients with lymphoproliferative disorders (multiple myeloma, B-cell lymphoma, Waldenström macroglobulinaemia).

| Type     | 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. Types of cryoglobulinaemia.**

## Epidemiology

Mixed cryoglobulinaemia (MC) is the best-known and most studied EHM of chronic HCV infection. MC can be found in 19-50% of patients with HCV infection according to different studies. At the same time, only a small fraction of these patients (less than 15%) have symptomatic disease. Still, asymptomatic cryoglobulinaemic patients may develop CG-related symptoms in the course of the disease. 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).

## Aetiology and pathogenesis

MC derives from poly- or monoclonal expansion of small B-lymphocytes, preferentially located in the bone marrow and liver. This condition is associated with Bcl2 proto-oncogene activation, which is able to consequently inhibit apoptosis, leading to extended cell survival. In 8-10% of cases, MC composed of type II CGs can evolve, after a long period of time, into frank B-cell non-Hodgkin's lymphoma (NHL) (Zignego 2007; Sansonno 1998; Ferri 1994).

## Diagnosis

Detection of CGs is carried out by keeping patient serum at 4° for up to 7 days. When cryoprecipitate is visible, CGs can be purified and characterised using immunofixation electrophoresis. In case of evidence of MC in HCV positive patients, presence of MC-syndrome must be sought out. Vigilant monitoring is required, as asymptomatic MC patients may develop MC-related disorders in the course of the disease. The diagnosis of MC-syndrome rests upon serologic (C4 reduction, mixed CGs, rheumatoid factor positivity, anti-HCV), pathologic (leukocytoclastic vasculitis, clonal B-cell infiltrates) and clinical (purpura, membranous proliferative glomerulonephritis, peripheral neuropathy) criteria. In presence of mixed CGs, low C4 counts, leukocytoclastic vasculitis and purpura, a definite symptomatic MC can be diagnosed (Ferri 2002).

## Clinical features

Typical symptomatic MC-related disorders are:

**Systemic vasculitis:** MC can be associated with a systemic vasculitis characterized by the deposition of immunocomplexes containing CGs, complement and large amounts of HCV antigens in small and medium-sized blood vessels. The most common symptoms of MC-vasculitis are weakness, arthralgias and purpura (Meltzer and Franklin triad). MC-vasculitis may also lead to Raynaud's Disease and sicca syndrome, glomerulonephritis and peripheral neuropathy.

**Renal impairment:** The predominant renal impairment associated with MC is the membranous proliferative glomerulonephritis (MPGN), characterised in most cases by proteinuria, mild haematuria and mild renal insufficiency. Less common are other renal manifestations, such as membranous nephropathy, rapidly progressive GN and exudative-proliferative GN, which have also been observed in the absence of MC (Daghestani 1999). 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).

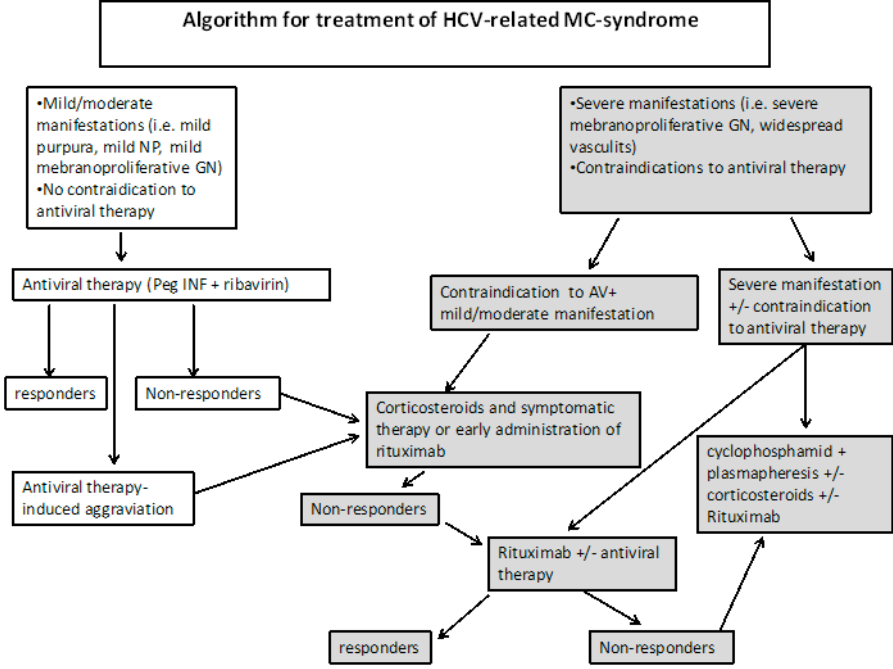
**Peripheral neuropathy:** Peripheral neuropathy, on the basis of endoneural microangiopathy, constitutes a further common complication of MC. MC-related neuropathy, clinically present as mononeuropathy or polyneuropathy, is mostly sensory and is characterized by numbness, burning skin crawling and pruritus, predominantly in the hands and feet (Tembl 1999; Lidove 2001).

**Cirrhosis:** The causal association between CGs and progression of liver fibrosis suggested in the past by numerous authors was not confirmed in a recently published 10-year prospective study. It demonstrates that the 10-year rates of progression to cirrhosis are similar in cryoglobulinaemic and non-cryoglobulinaemic HCV-infected patients (Vigano 2007). With respect to recent data, it is rather unlikely that CGs are an independent risk factor for the progression of liver fibrosis.

## Treatment

While asymptomatic MC per se constitutes no indication for treatment, symptomatic MC should be always treated. Given the fact of the causal correlation between HCV infection and MC, the therapeutic approach of symptomatic MC should concentrate on the eradication of the virus. Indeed, improvement of clinical MC is reported in 50-70% of patients receiving antiviral therapy based on interferon alpha (IFN $\alpha$ ) and ribavirin and correlates with the reduction of HCV RNA concentrations (Calleja 1999; Zuckerman 2000). Thus, antiviral therapy should be considered the first line therapeutic approach in HCV-infected patients with MC-related disorders. However, with multiorgan involvement, antiviral therapy may be limited due to the severity of a specific MC-related disorder, treatment failure, side effects or contra-

indications. In such cases other therapeutic strategies, such as cytostatic-immunosuppressive and/or plasmapheresis should be considered (Figure 2; Table 4).



**Figure 2. Algorithm for the treatment of HCV-related MC syndrome.** In the absence of contraindications to antiviral therapy, based on PEG-IFN  $\alpha$  plus ribavirin and mild/moderate manifestations, antiviral therapy constitutes first line treatment. Nevertheless cautious monitoring is required, as antiviral therapy may lead to aggravation of certain EHM (i.e., neuropathy, cytopenias). In case of contraindications to antiviral therapy (i.e., uncontrolled depression, untreated thyroid disorders, gestation, severe cytopenias, etc.) and mild/moderate EHM, symptomatic and antiphlogistic therapy with corticosteroids is indicated. Low-dose or short-term corticosteroid pulses are recommended because high-dose corticosteroids and prolonged application may cause substantial increase in levels of viraemia. Alternatively to corticosteroids, rituximab can be administered. Severe or acute conditions require primarily an immunosuppressive therapeutic approach, as quick amelioration is required. Rituximab constitutes a very attractive new first line therapeutic option. In the absence of contraindications, a combination of rituximab and antiviral therapy can further optimize the treatment. Refractory or fulminant disease makes the application of cyclophosphamide, cycles of plasma exchange, eventually in combination with corticosteroids and rituximab, mandatory.

---

|   |   |
|---|---|
| Suggested HCV-specific causative factors                          | Confounding factors   |
| Immuno-complexes, CGs   | Hypersplenism   |
| Specific autoantibodies against platelet glycoproteins            | Low serum thrombopoietin due to impaired liver function   |
| Direct viral cytopathic effect in thrombocytes and megakaryocytes | IFN $\alpha$ -induced suppression of megakaryopoiesis and IFN $\alpha$ /ribavirin-induced autoimmune thrombocytopenia |

---

**Table 4. Multifactorial aetiology of thrombocytopenic conditions in HCV patients.**

**Systemic vasculitis:** For HCV-associated cryoglobulinaemic vasculitis type II antiviral strategies such as PEG-IFN  $\alpha$  plus ribavirin aiming at the eradication of the virus constitute the current standard therapy (Ferri 2006). However, controversy remains concerning the effectiveness of anti-HCV treatment in patients with active cryoglobulinaemic vasculitis, reported by persistence of cryoglobulinaemic vasculitis following successful antiviral treatment (Levine 2005). When antiviral therapy is contraindicated the therapeutic approach should rest upon mild dosage corticosteroids, as steroids can increase viral replication, and low antigen-content diet. In moderate-severe cases of skin vasculitis or widespread vasculitis an initial therapy with rituximab, a monoclonal chimeric antibody against CD 20 B-cell specific antigen, is suggested. Efficacy and safety have been demonstrated in patients with symptomatic MC resistant to IFN  $\alpha$  therapy. HCV RNA in this study increased to approximately twice the baseline levels in the responders (Sansonno 2003). A combined application of rituximab with PEG-IFN  $\alpha$  plus ribavirin in cases of severe MC-related vasculitis resistant to antiviral therapy seems to be the optimal therapeutic strategy, achieving in responders amelioration of MC-related symptoms and complete eradication of HCV (Craxi 2008). In severe rituximab-refractory MC-related vasculitis or acute manifestations, cycles of plasma exchange plus corticosteroids and/or immunosuppressive drugs (i.e., cyclophosphamide) are indicated (Saadoun 2008).

**Renal impairment:** The application of antiviral therapy based on PEG-IFN  $\alpha$  plus ribavirin as first line therapy in patients with MC-related renal impairment is subject to certain limitations. Ribavirin clearance is reduced in patients with renal failure and dialysis does not eliminate the drug. Ribavirin levels should be cautiously monitored in patients with reduced creatinine clearance, for accumulation of ribavirin may induce severe haemolytic anaemias. Concentration-controlled low dosage ribavirin (200 mg-800 mg/d) treatment in combination with IFN  $\alpha$  or PEG-IFN  $\alpha$  was in most cases well tolerated by HCV patients with significantly impaired glomerular filtration rate (GFR) due to HCV-related glomerulonephritis and led to sustained viral response and significant improvement of GFR (Bruchfeld 2003). Still, during the active phase of renal disease antiviral therapy is generally not recommended. Steroids and immunosuppressive drugs (usually cyclophosphamide)

and on occasion plasmapheresis are recommended in these cases (Garini 2007). However, these therapeutic approaches may cause a substantial increase in the levels of viraemia (Margin 1994). In cases of simultaneous bone marrow B-cell infiltration, resistance or intolerance of conventional therapy, the application of rituximab is indicated (Roccatello 2004). Consequently, in severe manifestations or acute phases of MC-related renal disease, rituximab seems to be a highly efficient first line therapeutic tool (Roccatello 2008). Symptomatic treatment such as angiotensin converting enzyme inhibitors and/or angiotensin receptor antagonists should always accompany the antiviral or immunosuppressive treatment (Kamar 2006).

**Peripheral neuropathy:** Antiviral therapy should be applied cautiously in the presence of neuropathy. While cases of HCV-related peripheral neuropathy responsive to antiviral therapy with IFN  $\alpha$  and ribavirin have been described (Koskinas 2007), several authors report on aggravation of preexisting MC-related neuropathy or even *de novo* occurrence of demyelinating polyneuropathy during IFN  $\alpha$  or PEG-IFN  $\alpha$  treatment (Boonyapisit 2002; Khiani 2008). Therefore, it is presumable that genetic susceptibility and other idiosyncratic factors may influence the response of the HCV-associated neuropathy to IFN  $\alpha$ , making close monitoring of the course of this EHM during IFN  $\alpha$  treatment indispensable.

## Lymphoproliferative disorders

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 recognized for many decades. A causative association between HCV and NHL was postulated relatively recently and has been the subject of intense investigation. HCV-associated lymphoproliferative disorders (LPDs) are mainly observed during the course of MC.

8-10% of MC-II evolves into B-cell NHL, after long-term infection. However, a remarkably high prevalence of B-cell NHL was also found in HCV patients without MC (Silvestri 1999).

## Aetiology and pathogenesis

Regarding the pathogenesis of the HCV-associated LPDs, the majority of studies pinpoint two presumable mechanisms: 1) an indirect one, relying on the chronic nature of HCV infection, and 2) a direct one, relying on the particular lymphotropism of HCV, hence on the very invasion of B-cells by HCV. In the first mechanism, the key point seems to be sustained B-cell activation and proliferation, noticed during chronic HCV infection. 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. The resulting sustained B-cell proliferation consequently favors the occurrence of t(14;18) translocations with consecutive Bcl-2 rearrangement and other genetic aberrations, which in turn promote the malignant transformation of B-cells (Pileri 1998; Zignego 2000). The second mechanism suggests that Bcl-2 rearrangement and consecutive inhibition of B-cell apoptosis arise directly from viral mutagenic properties (Machina 2004). Thus, both

mechanisms lead simultaneously through complex, multistep, pathogenic pathways to the manifestation of an LPD.

## Epidemiology

A large-scale retrospective cohort study conducted by the US Veterans Administration suggests that HCV infection confers a 20-30% increased risk of NHL overall (Giordano 2007). The most prevalent HCV-associated LPDs 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.

## Treatment

It has been demonstrated that antiviral treatment can lead to the regression of clonal proliferation (Giannelli 2003). Interestingly, the haematologic response to antiviral therapy was noticed only in correlation with the decrease or disappearance of viraemia. Thus the confounding role of interferon as antiproliferative agent can be ruled out. Whereas antiviral therapy seems to be appropriate to treat low-grade HCV-positive NHL regardless of histological subtype, in intermediate and high-grade NHL, chemotherapy is expected to be necessary and antiviral treatment may serve as maintenance therapy after chemotherapy completion (Vallisa 2005). As chemotherapy may lead to a substantial increase in the levels of viraemia, vigilant monitoring of the HCV RNA levels and transaminases is indicated (Ennishi 2008). Thus a consecutive exacerbation of the infection, making discontinuation of chemotherapy mandatory, is not unlikely to occur. Furthermore, regular monitoring of transaminases during treatment is essential, for short-term hepatic toxicity of chemotherapy seems to be increased among HCV-positive patients.

## HCV-related thrombocytopaenia

Thrombocytopaenic conditions (platelet counts below  $150 \times 10^3/\mu\text{L}$ ) are often observed in patients with chronic hepatitis C infection (Wang 2004). Due to the multitude of confounding factors (i.e., hypersplenism, inadequate production of thrombopoietin, severe hepatic impairment, iatrogenic factors) favoring thrombocytopaenic conditions in patients with chronic HCV infection, it is uncertain whether thrombocytopaenia should be considered as a definite EHM of HCV infection (Table 4). Nevertheless the detection of HCV in platelets (Takehara 1994) and megakaryocytes (Bordin 1995) makes a direct involvement of HCV in HCV-related thrombocytopenia probable. Recently, a controlled prospective study conducted in 106 HCV-infected patients with and without thrombocytopaenia demonstrated a clearly higher detection rate of HCV RNA in platelets of thrombocytopaenic patients compared to non-thrombocytopaenic patients (De Almeida 2004).

Furthermore, it has been suggested that exposure to HCV may be a causative factor for the production of platelet-associated immunoglobulin G, inducing thrombocytopaenia through a similar immunological mechanism to what is operating in immune thrombocytopaenic purpura (ITP). Interestingly, serologic evidence of HCV

infection was found in 30% of patients with ITP. In non-cirrhotic thrombocytopenic patients with chronic HCV infection, disappearance of HCV RNA after IFN  $\alpha$  therapy correlates with an improvement of thrombocytopenia (Iga 2005; Rajan 2005). Thus HCV may also be directly involved in the process that leads to thrombocytopenia. There is no consensus regarding the optimal treatment of HCV-related thrombocytopenia. Along with classical therapeutic approaches such as corticosteroids, intravenous immunoglobulins and splenectomy, antiviral therapy constitutes a further option. Nevertheless, caution is recommended in thrombocytopenic patients treated with PEG-IFN  $\alpha$  and ribavirin as significant aggravation of HCV thrombocytopenia (platelet counts below  $50 \times 10^3/\mu\text{L}$ ) may occur while on this regimen. A new orally active thrombopoietin receptor agonist, eltrombopag, may in the future be applied in thrombocytopenic HCV-infected patients. Application of eltrombopag, before and during antiviral therapy in cirrhotic thrombocytopenic HCV patients, leads to a sustained increase of platelet counts, thereby permitting the initiation and/or continuation of antiviral therapy (McHutchison 2007). A promising therapeutic approach in HCV-thrombocytopenia appears to be rituximab and should be taken into consideration, especially in case of refractory disease or aggravation during the course of antiviral therapy (Weitz 2005).

## HCV-related autoimmune haemolytic anaemia

Occasional studies have seen chronic HCV infection in association with Coombs' positive/negative autoimmune haemolytic anaemia (AHA). AHA has been frequently observed in HCV patients treated with IFN  $\alpha$  and or ribavirin and consequently recognized as a possible side effect of antiviral treatment (De la Serna-Higuera 1999; Nomura 2004). Interestingly, a small number of case reports present cases of AHA in treatment-naïve HCV patients (Cha 2001; Fernandez 2006; Srivivasan 2001). In all case reports extensive work up for established infectious causes, autoimmune markers including CGs in two cases, drug induced haemolysis and other possible confounding factors, was negative. Though hypersplenism was present in two cases, the gravity of the conditions described and complete clinical response to CS makes a non-cryoglobulinaemic HCV-related autoimmune phenomenon as causative entity presumable. Large-scale studies focusing on the prevalence and incidence of AHA in treatment-naïve HCV patients are required to confirm these anecdotal observations.

## Endocrine manifestations

Thyroid disease is more commonly found in patients with chronic HCV infection than in the 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  $\alpha$  therapy may induce thyroid disease or unmask autoimmune syndromes (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  $\alpha$  therapy (Marazuela 1996). It is still unclear whether the development of thyroid disorders in chronic hepatitis C patients is due to the infection with HCV or to "cooperation" between IFN  $\alpha$  and HCV. Presence of thyroid autoantibodies with or without clini-



cal manifestations significantly increases the risk of developing an overt thyroiditis during antiviral therapy. Therefore, thyroid function should be monitored during treatment.

The relationship between chronic HCV infection and diabetes mellitus type II and/or insulin resistance has been analysed in many studies. Most of them indicate an independent higher prevalence of diabetes mellitus II and/or insulin resistance in chronic HCV patients compared to control groups (Knobler 2000; Mason 1999; Hui 2003), while in others this association was only confirmed in the presence of confounding factors (Mehta 2003). However, the fact that viral eradication after antiviral treatment may lead to an improvement of insulin resistance is a strong argument in favor of a causal relationship between HCV infection and the presence of diabetes mellitus II (Imazeki 2008). Recently, it has been suggested that the appearance of insulin resistance correlates with certain genotypes of HCV and that an HCV-dependent up-regulation of SOC-3 may be responsible for the induction of cell resistance towards insulin. Furthermore, insulin resistance should be regarded as an independent risk factor for the progression of liver fibrosis in patients with chronic HCV infection (Moucari 2008; Kawaguchi 2004). On the other hand, therapy with IFN  $\alpha$  has been rarely associated with the development of anti-pancreas autoimmunity and the appearance of diabetes mellitus type I (Betterle 2000). With respect to available data, monitoring of glycaemia and treatment of hyperglycaemic conditions is recommended in patients with chronic HCV infection.

Finally, the link between HCV infection, growth hormone (GH) insufficiency and low insulin-like growth factor (IGF-1) needs to be further investigated. Reduced GH secretion could be due to a direct inhibitory effect of HCV infection at the level of the pituitary (Plöckinger 2007).

## **Dermatological and miscellaneous manifestations**

There is a multitude of cutaneous disorders that may be sporadically associated with chronic HCV infection. Epidemiologic studies have confirmed the existence of a strong correlation between the sporadic form of porphyria cutanea tarda (PTC) and HCV. Given that HCV markers are found in over 50% of patients with PTC from southern Europe, PTC is recognized as a definite EHM of chronic HCV infection (Fargion 1992). The pathogenesis of PTC in HCV patients seems to be multifactorial, with excessive iron deposition in the liver due to chronic HCV infection and genetic predisposition playing the key role in the development of this HCV EHM.

Strong evidence of a close association of HCV infection with lichen planus was provided in studies performed in Japan and southern Europe (Nagao 1995; Carrozzo 1995). The high prevalence (27%) of HCV infection in patients with lichen planus could not be confirmed in other populations suggesting again a genetic predisposition and HLA-DR6 as the major pathogenic factor (Ingafou 1998).

A pathogenic link between HCV infection and idiopathic pulmonary fibrosis (IPF) has been suggested by the higher frequency of HCV markers in patients with IPF than in controls (Ueda 1992). Moreover the evidence of an increase in lymphocyte and neutrophil number in bronchoalveolar lavage of patients with HCV chronic infection indicates that HCV can induce alveolitis (Yamaguchi 1997). Chronic

hepatitis C is linked to pulmonary fibrosis directly or indirectly through underlying cryoglobulinaemia and vasculitis (Ferri 1997).

Finally, a causal relationship with HCV infection has been suspected for several myocardial impairments, including dilated cardiomyopathy, hypertrophic cardiomyopathy and chronic myocarditis. The pathogenesis of these HCV-associated myocardial impairments probably relies on autoimmune phenomena with the particular involvement of the human major histocompatibility (MHC) class II antigen (Matsumori 2000).

| Author     | Patients   | Treatment  | Result  |
|------------|--|--|---|
| Zuckerman  | N = 9<br>symptomatic-MC<br>non-responders to IFN $\alpha$ monotherapy                          | IFN $\alpha$ x 3 /week<br>plus ribavirin<br>15mg/kg/d  | CGs undetectable within 6 weeks in 7/9 patients; clinical improvement in 9/9 within 10 weeks                                      |
| Sansonno   | N = 20<br>MC-vasculitis and peripheral neuropathy resistant to IFN $\alpha$ monotherapy        | Rituximab 375 mg/m <sup>2</sup> / week x 4   | 16 patients complete clinical response; 12 sustained response throughout follow-up. Viraemia increase in responders.              |
| Saadoun    | N = 16<br>MC-vasculitis in relapsers or non-responders to IFN $\alpha$ /PEG-IFN $\alpha$ + RBV | Rituximab 375 mg/m <sup>2</sup> / week x 4;<br>pegINF $\alpha$ 1.5 ug/kg/ week plus RBV (600mg-1200mg/d) for 12 months | 10/16 complete clinical response; CGs and RNA HCV undetectable in responders  |
| Bruchfeld  | N = 7<br>HCV-related renal manifestations (2/7 MC-related)                                     | IFN $\alpha$ plus low-dose ribavirin (200-600mg) or pegINF $\alpha$ plus low-dose ribavirin                            | Improvement of GRF and proteinuria in 4/7 patients and sustained viral response in 5/7.   |
| Roccatello | N = 6<br>MC-systematic manifestations predominantly renal (5/6)                                | rituximab 375mg/m <sup>2</sup> /week x 4 plus rituximab 375mg/m <sup>2</sup> 1 month and 2 months later                | Decrease of cryocrit and proteinuria at month 2, 6, 12.   |
| Koskinas   | N = 4<br>MC patients with severe sensory-motor polyneuropathy                                  | INF $\alpha$ -2b 1.5ug/kg/week plus ribavirin 10.6mg/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 3. Treatment of MC-related disorders in patients with chronic HCV infection.**

## References

- Antonelli A, Ferri C, Pampana A, et al. Thyroid disorders in chronic hepatitis C. *Am J Med* 2004; 117: 10-13.
- 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, Bertocelli 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.
- Betterle C, Fabris P, Zanchetta R, Pedini B, Tositti G, Bosi E, et al. Autoimmunity against pancreatic islets and other tissues before and after interferon -alpha therapy in patients with hepatitis C virus chronic infection. *Diabetes Care* 2000; 23: 1177-81.
- 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.
- 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'Hepatitis C. *Medicine* 2000; 79: 47-56.
- Cacoub P, Poynard T, Ghillani P, et al. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC group. *Multidepartment Virus C. Arthritis Rheum* 1999; 42: 2204-2212.
- 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.
- 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.
- 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.
- Craxi A, Giacomo L, Zignego AL. Hepatitis C virus (HCV) infection: A systemic disease. *Mol Aspects Med* 2008; 29 (1-2): 85-95.
- 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.
- Ennishi D, Terui Y, Yokoyama M, Mishima Y, Takahashi S, Takeuchi K, et al. Monitoring serum hepatitis C virus (HCV) RNA in patients with HCV-infected CD20-positive B-cell lymphoma undergoing rituximab combination chemotherapy. *Am J Hematol*. 2008; 83: 59-62.
- 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.
- Fernández 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: 360-5.
- Ferri C, Monti M, La Civita L, Careccia G, Mazzaro C, Longombardo G, Lombardini F, Greco F, Pasero G, Bombardieri S, Zignego A.L. Hepatitis C virus infection in non-Hodgkin's

## 268 Extrahepatic manifestations of chronic HCV

- lymphoma complicating mixed cryoglobulinaemia. *Eur J Clin Invest* 1994; 24: 781-784.
- Ferri C, Zignego AL, Pileri SA. Cryoglobulins. *J Clin Pathol* 2002; 55: 4-13.
- Daghestani L, Pomeroy C. Renal manifestations of hepatitis C infection. *Am J Med* 1999; 106: 347-354.
- Ferri C, Sebastiani M, Guiggiolo D, Cazzato M, Longombardo G, Antonelli A, Puccini R, Michelassi C, Zignego AL. Mixed cryoglobulinaemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum* 2004; 33: 355-374.
- Ferri C, Mascia MT. Cryoglobulinemic vasculitis. *Curr Opin Rheumatol* 2006; 18:54-63.
- Ferri C, Antonelli A, Mascia M.T, et al. B cells and mixed cryoglobulinemia. *Autoimmun Rev* 2007; 7: 114-20.
- Garini G, Allegri L, Lannuzzella F, Vaglio A, Buzio C. HCV-related cryoglobulinemic glomerulonephritis: implications of antiviral and immunosuppressive therapies. *Acta Biomed*. 2007;78:51-9.
- 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, et al. Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. *JAMA* 2007;297:2010-7.
- 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- $\alpha$  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, Ide T, Taniguchi E, et al. Clearance of HCV improves insulin resistance,  $\beta$ -cell function, and hepatic expression of insulin receptor substrates 1 and 2. *Am J Gastroenterol* 2007; 102: 1-7
- 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.
- Khiani V, Kelly T, Adeel S, Jensen D, Mohanty SR. Acute inflammatory demyelinating polyneuropathy associated with pegylated interferon  $\alpha$  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; 27:414-20.
- Levine JW, Gota C, Fessler BJ, *et al.* Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. *J Rheumatol* 2005; 32:1164-7.
- Lidove O, Cacoub P, Maisonneuve 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: 290-2.
- 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; 101:4262-67.
- 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
- 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.
- Mason AL, Lau JY, Hoang N, *et al.* Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; 29: 328-333.
- 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.
- 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; 357:2227-36.
- 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: 910-4.
- Nomura H, Tanimoto H, Kajiwara E, Shimono J, Maruyama T, Yamashita N, *et al.* Factors contributing to ribavirin-induced anemia. *J Gastroenterol Hepatol.* 2004 Nov;19(11):1312-7.
- Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998; 282: 938-941.
- 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: 547-51.
- 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.
- 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(12):3054-61

## 270 Extrahepatic manifestations of chronic HCV

- 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(1):111-117.
- Srinivasan R. Autoimmune hemolytic anemia in treatment naive chronic hepatitis C infection. *J Clin Gastroenterol* 2001;32: 245–247.
- 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.
- 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.
- 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.
- Sansonno D, De Vita S, Iacobelli A.R, Cornacchiulo V, Boiocchi M, Dammacco F. Clonal analysis of intrahepatic B cells from HCV-infected patients with and without mixed cryoglobulinemia. *J. Immunol* 1998; 160: 3594-3601.
- 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 Jan 4 [Epub ahead of print]
- 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: 618-23.
- 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: 266-8.
- 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.
- 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.
- 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.
- 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, Gianelli F, Marrocchi ME, Mazzocca A, Ferri C, Giannini C, Monti M, et al. T(14;18) translocation in chronic hepatitis C virus infection. *Hepatology* 2000; 31: 474-79.
- 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* 2007; 39: 2-17.
- Zuckerman E, Keren D, Slobodin G, et al. Treatment of refractory, symptomatic, hepatitis C virus related mixed cryoglobulinaemia with ribavirin and interferon- alpha. *J Rheumatol* 2000; 27: 2172-78.





## Part 4

# Coinfections



# Chapter 17: Management of HBV/HIV coinfection

Stefan Mauss and Jürgen Rockstroh

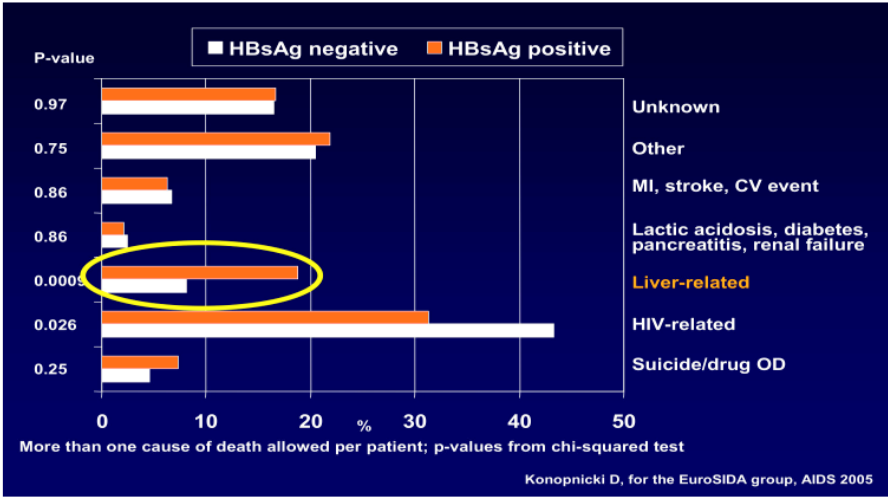
## Introduction

The prevalence and transmission route of HBV coinfection in the HIV population varies substantially geographically (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% have 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 after the introduction of vaccination on a nationwide level (Shepard 2006). In Europe, vaccination of children and members of risk groups is reimbursed by the 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). From 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 from 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) (Figure 1). In the Multicentre AIDS Cohort Study, an 8-fold increased risk of liver-related mortality was seen among HBV/HIV-coinfected people compared to HIV-monoinfected individuals, particularly in those with low CD4 nadir counts (Thio 2002). An independent observation from a large cohort confirming this association in a different way 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**

These two large cohort studies and data from HBV-monoinfection studies showing a reduction in morbidity and mortality justify treatment of hepatitis B in HBV/HIV-coinfected patients. In the case of antiretroviral therapy, HBV is often treated simultaneously as some nucleoside and nucleotide reverse transcriptase inhibitors are active as HBV polymerase inhibitors as well. Therefore, antiretroviral therapy should be adjusted according to the status of the HBV infection 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 without antiretroviral therapy. Treatment with interferon is one possible therapeutic option in this situation. The main limitation of HBV polymerase inhibitors may be an induction of HIV resistance by most anti-HBV agents as they act simultaneously with HIV reverse transcriptase inhibitors.

### **HBV therapy in HBV/HIV-coinfected patients without antiretroviral therapy**

The recommendations of the European AIDS Clinical Society (EACS) for the treatment of chronic hepatitis B in HIV-coinfected patients without antiretroviral therapy are shown in Figure 2 (Rockstroh 2008).

For the decision to start hepatitis B therapy the degree of liver fibrosis and the HBV DNA level are important. The introduction of the level of HBV replication as a 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).

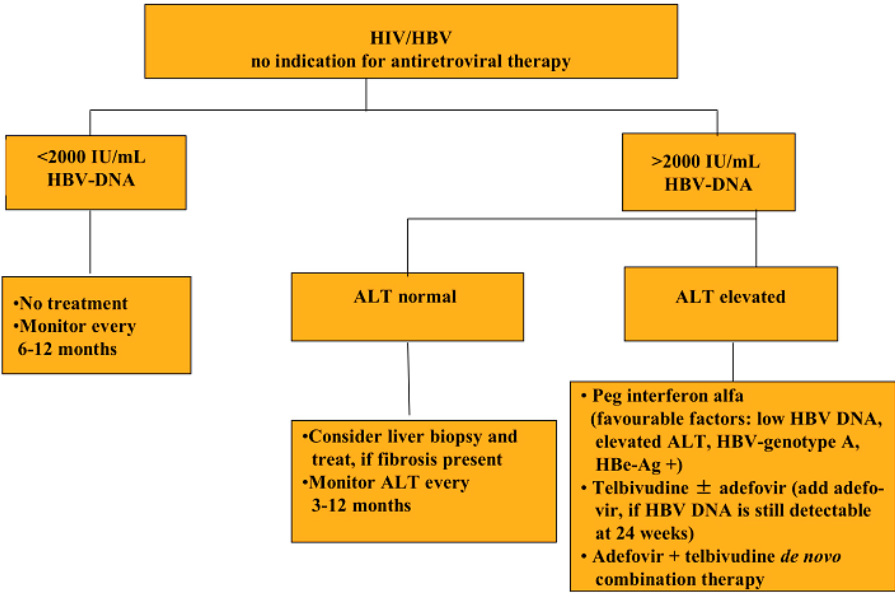
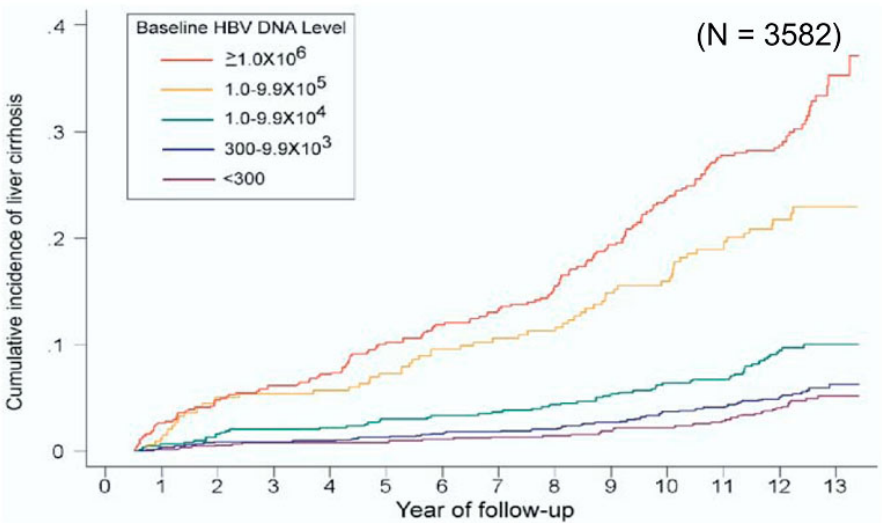


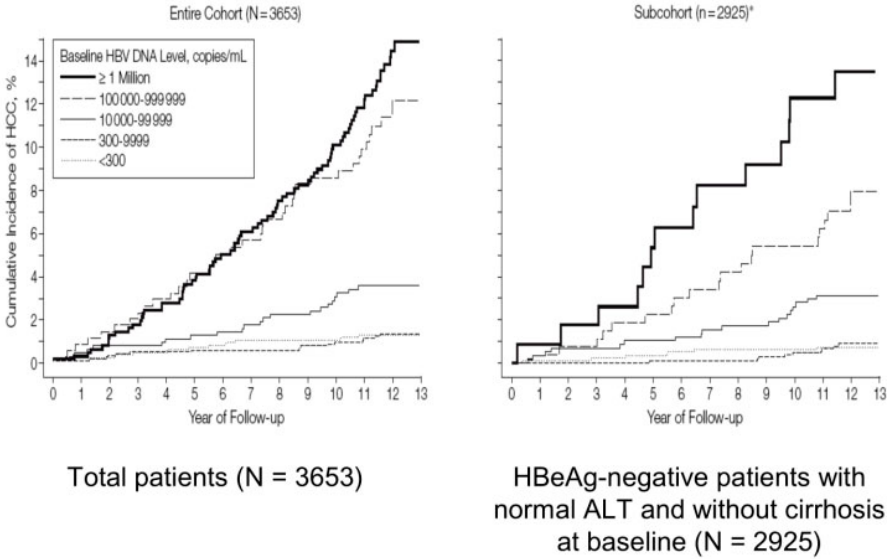
Figure 2. Treatment algorithm for therapy of HBV only in HIV-coinfected patients (EACS 2008).



Iloeje UH et al. Gastroenterology 2006; 130: 678

Figure 3. The REVEAL Study: The association of HBV DNA levels and liver cirrhosis.

This association was even observed in patients with normal ALT levels (Chen 2006) (Figure 4). It should be mentioned that this cohort consists of Asian patients without HIV coinfection predominantly infected at birth or in early childhood. Even so, the results are considered too important to not form part of the management of HIV-coinfected patients.



Chen CJ et al. JAMA 2006; 295: 65

**Figure 4. The REVEAL Study: The association of HBV DNA and the development of hepatocellular carcinoma.**

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, particularly 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 an 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. At a level of liver fibrosis of METAVIR F2 or more treatment of hepatitis B should be initiated.

For patients with HBV DNA >2000 IU/ml and increased ALT, treatment for HBV is an option, in particular in the presence of relevant liver fibrosis.

In patients not on antiretroviral therapy PEG-IFN  $\alpha$ -2a or -2b seems to be 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 or prospective, controlled studies are available. Favourable factors for treatment success with interferon are low HBV DNA, increased ALT, HBV genotype A or infection with HBV wild type.

Alternatively patients can be treated with HBV polymerase inhibitors. However, due to their antiretroviral activity, tenofovir, emtricitabine and lamivudine are contraindicated in the absence of an 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 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 (Idenix data on file). No clinical data exist yet for treatment of coinfecting patients.

Because of its greater antiviral efficacy, telbivudine is preferred by most experts over 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.

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 allowing the use of tenofovir plus lamivudine/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.

## **ARV treatment of chronic hepatitis B in HBV/HIV coinfection**

For patients on antiretroviral therapy a wider choice of HBV polymerase inhibitors is available. In principle the treatment algorithm in Figure 5 is based on the same principles as outlined above (Rockstroh 2008).

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 the strategy of choice remains the same. The recommendation to continue lamivudine/emtricitabine is based on the delay of resistance to adefovir seen when doing so (Lampertico 2007).

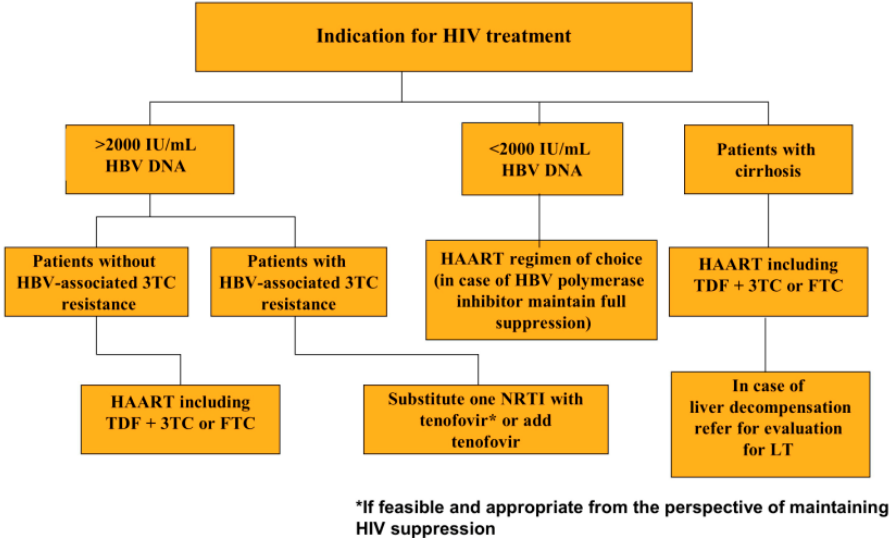


Figure 5. Treatment algorithm for HBV therapy in patients with antiretroviral therapy (EACS 2008).

For patients with liver cirrhosis a maximally active and stable 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 therapy results are not fully suppressive, adding entecavir should be considered. For patients with hepatic decompensation and full treatment options for HBV and stable HIV infection, liver transplantation should be considered, as life expectancy seems not to be different from HBV-monoinfected patients (Coffin 2007). 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 considered the standard therapy 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). 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 like with any other antiviral. In prospective, controlled studies tenofovir was clearly superior to adefovir for treat-



ment of HBe-antigen positive and HBe-antigen negative patients (Heathcote 2007; Marcellin 2007).

The acquisition of adefovir resistance mutations and multiple lamivudine resistance mutations may impair the activity of tenofovir (Fung 2005; Lada 2008; van Bömmel 2006), although even in these situations tenofovir retains activity against HBV (Berg 2008).

In lamivudine-resistant HBV the antiviral efficacy of entecavir in HIV-coinfected patients is reduced as in HBV-monoinfection (Shermann 2008). Because of this and the property of tenofovir as an approved antiretroviral it is the preferred choice in treatment naïve HIV-coinfected patients with an antiretroviral treatment indication.

The use of entecavir, telbivudine or adefovir as 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. This strategy is left to the individual doctor/patient decision.

It is a general belief that combination therapy with tenofovir plus lamivudine/ emtricitabine is superior to monotherapy, in particular in patients with high replicative HBV infection. However to date no conclusive studies supporting this are available (Schmutz 2006).

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 flares of necroinflammatory activity, resulting 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 however the choice is still limited to two mostly non-cross-resistant classes, the nucleotide and nucleoside compounds. In HIV-coinfected patients not on antiretroviral therapy the choice is even 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.

For HBV/HIV-coinfected patients the treatment of choice for antiretroviral therapy 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/emtricitabine as a primary combination therapy has theoretical advantages, but studies supporting this concept do not exist.

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.
- 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 coinfecting 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-coinfecting 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.
- 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.

- 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 Euro-SIDA cohort. *AIDS* 2005; 19(6):593-601.
- Lada O, Gervais A, Branger M, Peytavin G, Colin G, Fraqueiro G, Males S, Martinot-Peignoux M, Matheron S, Marcellin P. 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.
- 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.
- 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.
- 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.
- Núñez M, Soriano V. Management of patients co-infected with hepatitis B virus and HIV. *Lancet Infect Dis* 2005; 5:374-382.
- 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, Puoti M, Soriano V, Tural C; 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.
- 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, Sette H, Tsai N, Tenney DJ, Vaughan J, Kreter B, Hinds R; A1463026 Benefits of Entecavir for Hepatitis B Liver Disease (BEHoLD) Study Group. 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.
- 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.

## 284 Management of HBV/HIV coinfection

- 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 Boemmel F, Zoellner B, Moeller B et al. Is tenofovir effective in treatment of adefovir resistant hepatitis B virus infections? *Hepatology*, 2006, 44, 567A.
- Vibert E, Adam R, Tralhao JG, Azoulay D, Castaing D, Samuel D, Duclos-Vallee JC. Evolution after listing and results of liver transplantation for hepatocellular carcinoma in HIV/HCV and HIV/HBV coinfecting patients. *J Hepatol* 2008; 48 (Suppl. 2): S80 (abstract 191).

## Chapter 18: Management of HCV/HIV coinfection

Jürgen Kurt Rockstroh and Stefan Mauss

### Epidemiology of HIV/HCV coinfection

HIV and HCV share transmission pathways, which explains the high rate of coinfection with both viruses. Of the 33 million HIV-infected persons worldwide in 2007 it is estimated that 4-5 million of them have concomitant hepatitis C virus infection. Whereas both viruses are transmitted with high efficacy via direct blood-to-blood contact, HCV is less easily transmitted via the sexual route. Thus, the prevalence of hepatitis C coinfection within different countries, regions and populations is closely related to the prevalence of blood-borne (mainly intravenous drug use) HIV infection. Among all HIV-infected patients in Europe, Australia and the US, at least one out of four is also infected with hepatitis C (Rockstroh 2004). Particularly high hepatitis C coinfection rates are observed in Eastern European countries like Byelorussia and the Ukraine where intravenous drug use is the main route of HIV transmission, with hepatitis C coinfection rates as high as 70%. On the contrary, 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). An additional at-risk population is prison inmates. 65-70% of HIV-positive prisoners in the US are coinfecting with hepatitis C, whereas within the general US HIV-positive population, the coinfection rate is estimated to be 18-25% (Weinbaum 2005). In Asia, coinfection rates among Chinese plasma donors show coinfection rates of 85% whereas rates 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, 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 upon regular STD screening (Gotz 2005; Danta 2007).

HCV is detected in 4-8% of infants born to HCV-infected mothers. Dual HCV/HIV infection increases the risk for transmission of both viruses and high levels of HCV viraemia in the mother increases the risk of perinatal HCV transmission (Zanetti 1995). However, in HIV/HCV-coinfecting 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 world population where the global burden of hepatitis C is estimated to be roughly around 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, both viruses are transmitted with different efficacy depending on 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               |

\* On sexual contact the sexual risk confers to cumulative exposure

**Table 1. Average estimated risk of transmission for HIV, HCV and HCV/HIV coinfection.**

## Specific aspects concerning the diagnosis of HCV in HIV coinfection

The presence of HCV can be confirmed serologically by the detection of antibodies to the virus with an ELISA test. 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. In more than 80% of HIV-infected 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). In observations from hemophiliac patients mean concentrations of HCV RNA increase by 1 log over the first two years after HIV seroconversion (Eyster 1994). The levels of HCV viraemia 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 viraemia have been reported in patients who subsequently developed liver failure.

Interestingly, however, spontaneous clearance of HCV RNA can also be observed in some HIV/HCV-coinfected patients experiencing significant immune reconstitution following HAART initiation (Fialaire 1999). In contrast, patients with positive HCV antibodies and negative HCV RNA have been reported, 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 coinfecting patients.

The distribution of HCV genotypes in HIV-infected 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).

## Natural course 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, recent studies of the European epidemic of sexually transmitted acute hepatitis C infection in HIV-infected individuals suggest that despite underlying HIV infections spontaneous resolution of HCV may occur in up to 20-30% of newly infected patients.

Once chronic hepatitis C is established numerous large cohort studies have demonstrated a faster clinical HCV progression in the presence of HIV. 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 observation period, no case of liver failure was observed in HCV-positive HIV-negative hemophiliacs. Subsequently, several studies confirm the unfavorable course of hepatitis C in HIV-coinfected hemophiliacs, particularly in the setting of progressive immunodeficiency and CD4 counts  $<100/\mu\text{l}$  (Rockstroh 1996).

In addition, the time interval between HCV exposition and development of cirrhosis was found to be shortened in coinfecting 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, in coinfecting hemophiliacs mortality due to advanced liver disease increases ten years earlier than the same increase in HIV-negative hemophiliacs with hepatitis C (Darby 1997).

## 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 any significant differences with regard to CD4 cell count recovery between HCV-positive and HCV-negative HIV patients (Kaufmann 2003). Subsequent studies have indeed found that after adjusting for use of HAART, no difference in the CD4 cell count recovery can be observed (Sulkowski 2002). Updated information from a recent analysis in 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).

## 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 seem to happen during 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. Taking into account that liver disease progresses, especially in patients whose CD4 count drops below 200/ $\mu$ l, 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, 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).

A recent paper also addressed the amount of immune reconstitution achieved by 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 the 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 have recommended earlier initiation of antiretroviral therapy in HIV patients with HCV coinfection.

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).

## 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). Most recently, several studies have demonstrated 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 a negative HCV RNA six months after the completion of HCV therapy. Negative HCV RNA at the end of the treatment period is described as an end-of-treatment response (ETR). 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 plus ribavirin is regarded as standard therapy in coinfecting patients. Table 2 summarizes the main results from randomized clinical trials investigating the efficacy of pegylated interferon plus ribavirin in HIV/HCV-coinfecting individuals.

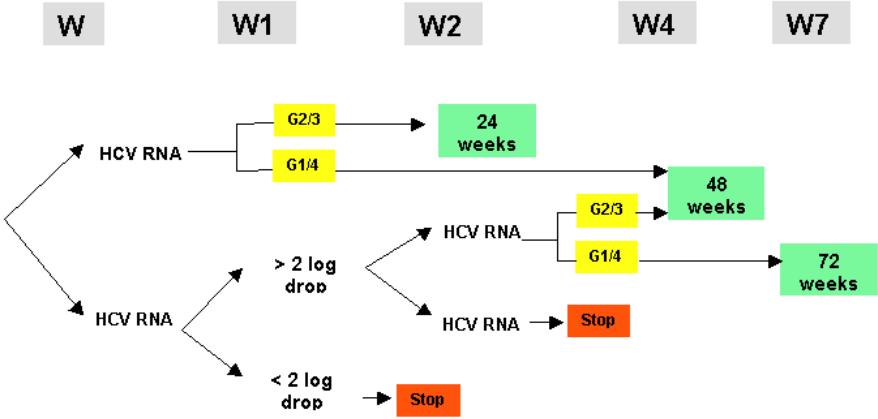
|   | ACTG5071 | APRICOT | RIBAVIC     | Laguno | PRESCO      |
|---|----------|---------|-------------|--------|-------------|
| Number of Patients                        | 66       | 289     | 194         | 52     | 389         |
| PEG-INF $\alpha$                          | 2a       | 2a      | 2b          | 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 analysis, \*\*\*sustained virological response, intent-to-treat

**Table 2. Results from randomized clinical trials investigating the efficacy of pegylated interferon plus ribavirin in HIV/HCV-coinfecting individuals.**

Overall, SVR rates of up to 50% can be achieved (Torriani 2004; Nunez 2007). The difference in SVR between the various studies can be explained mainly by differences in ribavirin dosages used. In the initial HCV treatment trials in HIV-coinfecting individuals, due to the fear of interactions between ribavirin and commonly used NRTIs for HIV treatment, an 800 mg daily dosage of ribavirin was chosen for most patients independent of the prevailing genotype. This led to suboptimal SVR rates. However, in the most recent trial (PRESCO), 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. Therefore, currently daily administration of ribavirin 1000 mg (<70 kg body weight) and 1200 mg ( $\geq$ 70 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  $\alpha$ -2a is 180  $\mu$ g/kg body weight once weekly and for PEG-IFN  $\alpha$ -2b it is 1.5  $\mu$ g/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 (Figure 1). Indeed the PRESCO trial indicates that at least some patients may benefit from a longer duration of HCV combination therapy, of up to 72 weeks (Figure 1).



\*In patients with low baseline viral load (<400 000 IU/l) and minimal liver fibrosis.

**Figure 1. Algorithm for management of hepatitis C in HIV coinfection:** Proposed optimal duration of hepatitis C virus therapy in HIV/HCV coinfecting patients (w: week; G: genotype; \*in patients with baseline HCV viral load <400,000 U/l and minimal liver fibrosis) (modified according to Rockstroh 2008).

As HCV treatment offers the possibility of eradicating HCV within defined treatment periods and this clearly appears to be potentially advantageous for the subsequent management of the patient’s HIV infection, every patient should be considered for 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 coinfecting patients. However, a liver biopsy is not mandatory for decisions on treatment of chronic HCV infection. Current therapy is particularly recommended in 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).

More recently, insulin resistance (which can be determined using the homeostasis model assessment at insulin resistance (HOMA-IR) score) has been reported as a

negative predictor of achieving SVR and therefore may also be considered during pretreatment evaluation. When liver biopsy or non-invasive tests for assessing hepatic fibrosis (e.g., elastometry by fibroscan, Echosense, France) have demonstrated lower grades of liver fibrosis (F0-F1) regardless of HCV genotype, treatment can be deferred. It is especially important to perform a liver disease stage assessment in patients with a low likelihood of achieving SVR.

|   |
|---|
| <b>Diagnosis of hepatitis C</b>   |
| HCV-Ab (positive 1-5 months after infection, may rarely be lost with immunosuppression)<br>HCV RNA levels* (while not prognostic for progression, it is for response to treatment)  |
| <b>Status of liver damage</b>   |
| Grading of fibrosis (e.g., Fibroscan, liver biopsy, serum fibromarkers**)<br>Hepatic synthetic function (e.g., coagulation, protein, albumin, CHE)<br>Ultrasound and AFP every 6 months in cirrhotics (gastroscopy upon diagnosis of cirrhosis and every 1-2 years thereafter)        |
| <b>Before HCV treatment</b>   |
| HCV genotype and serum HCV RNA<br>Auto-antibodies (ANA, SMA, ANCA and LKM1)<br>TSH, thyroid autoantibodies if applicable  |
| <b>Monitoring of HCV treatment</b>  |
| Differential blood count and liver enzymes every 2-4 weeks<br>HCV RNA at week 4 (to evaluate rapid virological response), week 12, 24, 48, (72 if applicable) and 24 weeks after stopping HCV therapy<br>CD4 count every 12 weeks<br>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, Fibrotest, Forns and other indexes   |

**Table 3. Diagnostic procedures for hepatitis C in HIV-coinfection (adapted from Rockstroh 2008).**

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 coinfecting patient has severe immune deficiency (CD4 count <200 cells/ $\mu$ l), the CD4 count should be improved using HAART prior to commencing anti-HCV treatment. Patients with a CD4 relative percentage >25 % are more likely to achieve SVR than those with lower CD4 percentages (Opravić 2007). If an early virologic response of at least 2 log<sub>10</sub> reduction 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-interferon plus ribavirin for HIV/HCV-coinfected patients are shown in Figure 1. The procedures for diag-

nosis 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. Use of didanosine (ddI) 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. Therefore, ddI use is now 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 when possible, as increased toxicity can be expected. RBV therapy plus AZT is associated with enhanced anaemia while RBV plus d4T is associated with increased mitochondrial toxicity and weight loss and a high potential to worsen pre-existing lipotrophy. 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-treated patients and other nucleosides in achieving SVR in HIV/HCV-coinfected patients receiving PEG-IFN/ribavirin therapy and concomitant HAART.

**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 HCV treatment optimizing the dose and duration as well as the best supportive therapy. Table 4 summarizes possible interventions for HCV/HIV-coinfected non-responders and relapsers to previous interferon-based therapies (Rockstroh 2008).

| Category  | Recommended intervention  |
|---|---|
| Suboptimal prior treatment schedules<br>Interferon (monotherapy or with ribavirin)<br>Low doses of ribavirin<br>Short length of therapy | Re-treatment using combination therapy with peg-interferon $\alpha$ plus weight-based ribavirin doses |
| Limiting toxicities and poor adherence  | Optimal support (SSRI, paracetamol/NSAID, pharmacists, use of haematopoietic growth factors [14])     |
| Virologic failure   | Wait for new antivirals to come to market   |

NSAID, non-steroidal anti-inflammatory drugs; PEG, polyethylene glycol; SSRI, selective serotonin reuptake inhibitors.

**Table 4. Classification of and interventions for HCV/HIV-coinfected patients who are non-responders/relapsers to prior interferon-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 (2 tests 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. Unfortunately clear guidance is difficult at this point due to the lack of controlled data. 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/ribavirin for 24-48 weeks. Currently the NEAT initiative is organizing a large multicenter trial in acute HCV infection that will assess whether PEG-IFN can be given alone or whether combination therapy with ribavirin is needed. The duration of therapy will also be evaluated.

## Liver transplantation in HIV/HCV coinfecting patients

In general, HIV/HCV coinfecting 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-coinfecting patients at an earlier age and the course is more aggressive with a shorter survival compared to HCV-monoinfecting 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 recent 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-coinfecting individuals the CD4+ count has to be at least above 100 cells/ $\mu$ l. Additionally, the patient has to have either undetectable HIV viraemia 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 use, HCC metastasis in other organs, a second malignant disease, cardiopulmonary disease or older age with an elevated risk of mortality related to the operation. At least 50 HIV-positive patients are reported to have received liver transplants because of end-stage liver disease due to hepatitis C infection to date. Approximately 75% of those have remained alive for up to 2 years after the transplant (Rockstroh 2004; Ragni 2003). In the largest cohort, survival at 1 year and 3 years was similar to that for HIV-negative transplant recipients. Regardless of additional immunosuppression by immunosuppressive drugs, the risk of opportunistic diseases remains low in the post-transplant period as long as HIV replication is successfully suppressed with HAART.

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 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 when boosted 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, a combination therapy with pegylated interferon plus ribavirin seems to currently be the best management option 1-3 months after liver transplantation and after re-infection with hepatitis C virus is detected (more detail available in Chapters 22 and 23).

## 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 on 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 pegylated interferon plus ribavirin combination therapy as the current gold standard of therapy allowing SVR rates of almost 50% in HIV/HCV-coinfected individuals under optimized management conditions (ribavirin 1000-1200mg 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 impatiently awaited as new treatment strategies for HIV/HCV-coinfected patients are urgently needed.

## 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.
- Bani-Sadr F, Denoed 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 coinfectd patients. *Impact of protease inhibitor therapy. Hepatology* 2001; 34: 283-287
- Cribier B, Rey D, Schmitt C et al: High hepatitis C viremia and impaired antibody response in patients coinfectd 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.
- 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, Goedert 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
- 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
- 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
- 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.
- 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 peg-interferon- $\alpha$  2a (40 kd) + ribavirin in patients with HIV-HCV coinfection. *J Acquir Immune Def Syndr* 2008; 47: 36-49
- Pineda JA, García-García JA, Aguilar-Guisado M, et al. Grupo para el Estudio de las Hepatitis Viricas 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.
- 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
- Pol S, Thiers V, Nousbaum J, et al. Changing distribution of HCV genotypes in Europe in the last decades. *J Hepatol* 1994; 21: S13
- 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
- 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 coinfecting 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-coinfecting hemophiliacs. *AIDS* 1998; 12: 829-830

## 296 Management of HCV/HIV coinfection

- 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
- 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.
- Torriani FJ, Rodriguez-Torres M, Rockstroh JK et al: peg-interferon- $\alpha$  2a + ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *New Engl J Med* 2004; 358: 438-450
- 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
- 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 and 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 worldwide. 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 coinfecting 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 in acute and chronic 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-coinfecting 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 coinfecting patients (Zarski 1998). Furthermore, coinfecting 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; Cacciola 1999; Sagnelli 2001).

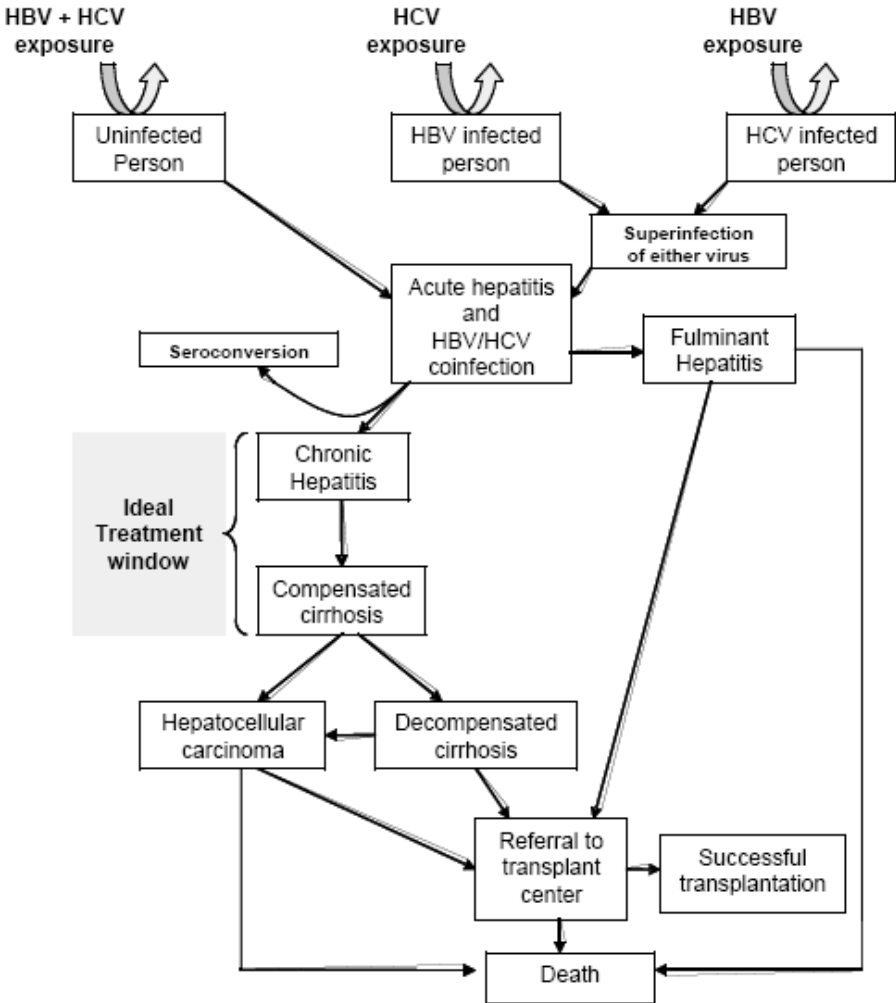


Figure 1. Clinical scenarios of HBV/HCV coinfection (modified after Crockett & Keeffe 2005).

### Chronic hepatitis in HBV/HCV coinfection

Various immune profiles are found in patients with chronic HBV/HCV hepatitis (Table 1).

|          | 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 coinfecting patients with chronic hepatitis.**

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 mono-infection. 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.

### Cirrhosis

Higher rates of cirrhosis have been demonstrated in HBV/HCV-coinfecting patients. In comparison to patients with HBV mono-infection higher rates of cirrhosis (44% vs. 21%) and decompensated liver disease (24% vs. 6%) were demonstrated in coinfecting patients (Fong 1991). Compared to HCV mono-infecting 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-coinfecting 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-coinfecting patients compared to 2.0 in HBV and 3.7 in patients with HCV mono-infection. The cumulative risk of developing HCC after 10 years was 45% in HBV/HCV-coinfecting patients compared with 16% in HBV and 28% in HCV mono-infecting patients (Chiaromonte 1999). HBV/HCV-coinfecting 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 coinfecting patients. As with HBV and HCV mono-infection, treatment of coinfecting 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 mono-infection are met (see chapter 9 on therapy of HBV and chapter 13 on therapy of HCV).

In coinfecting 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-coinfecting patients will be carried out using pegylated IFN. A recent prospective multicenter study including 19 co-infected patients found the combination of PEG-IFN  $\alpha$ -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 coinfecting 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-coinfecting 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 coinfecting patients, as flares of the untreated virus may occur.

## References

- Alberti A, Pontisso P, Chemello L, et al. The interaction between hepatitis B virus and hepatitis C virus in acute and chronic liver disease. *J Hepatol* 1995;22(1 Suppl):38.
- Aroldi A, Lampertico P, Montagnino G, et al. Natural history of hepatitis B and C in renal allograft recipients. *Transplantation* 2005;79(9):1132.
- Cacciola I, Pollicino T, Squadrito G, et al. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N Engl J Med* 1999;341(1):22.
- Chiaromonte M, Stroffolini T, Vian A, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. *Cancer* 1999;85(10):2132.
- Chu CJ, Lee SD. Hepatitis B virus/hepatitis C virus coinfection: epidemiology, clinical features, viral interactions and treatment. *J Gastroenterol Hepatol* 2008;23(4):512.
- Chu CM, Sheen IS, Liaw YF. The role of hepatitis C virus in fulminant viral hepatitis in an area with endemic hepatitis A and B. *Gastroenterology* 1994;107(1):189.
- Chu CM, Yeh CT, Liaw YF. Low-level viremia and intracellular expression of hepatitis B surface antigen (HBsAg) in HBsAg carriers with concurrent hepatitis C virus infection. *J Clin Microbiol* 1998;36(7):2084.
- Crockett SD, Keeffe EB. Natural history and treatment of hepatitis B virus and hepatitis C virus coinfection. *Ann Clin Microbiol Antimicrob* 2005;4:13.
- Fong TL, Di Bisceglie AM, Waggoner JG, et al. The significance of antibody to hepatitis C virus in patients with chronic hepatitis B. *Hepatology* 1991;14(1):64.
- 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.
- Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. *Hepatology* 1995;22(4 Pt 1):1101.
- Liaw YF. Concurrent hepatitis B and C virus infection: Is hepatitis C virus stronger? *J Gastroenterol Hepatol* 2001;16(6):597.
- Liaw YF. Hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. *J Gastroenterol* 2002;37 Suppl 13:65.
- Liaw YF, Chen YC, Sheen IS, et al. Impact of acute hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. *Gastroenterology* 2004;126(4):1024.
- Liaw YF, Lin SM, Sheen IS, et al. Acute hepatitis C virus superinfection followed by spontaneous HBeAg seroconversion and HBsAg elimination. *Infection* 1991;19(4):250.
- Liaw YF, Yeh CT, Tsai SL. Impact of acute hepatitis B virus superinfection on chronic hepatitis C virus infection. *Am J Gastroenterol* 2000;95(10):2978.
- Mimms LT, Mosley JW, Hollinger FB, et al. Effect of concurrent acute infection with hepatitis C virus on acute hepatitis B virus infection. *Bmj* 1993;307(6912):1095.
- Mohamed Ael S, al Karawi MA, Mesa GA. Dual infection with hepatitis C and B viruses: clinical and histological study in Saudi patients. *Hepatogastroenterology* 1997;44(17):1404.
- Pallas JR, Farinas-Alvarez C, Prieto D, et al. Coinfections by HIV, hepatitis B and hepatitis C in imprisoned injecting drug users. *Eur J Epidemiol* 1999;15(8):699.
- Pothhoff A, Wedemeyer H, Boecher WO, et al. The HEP-NET B/C co-infection trial: A prospective multicenter study to investigate the efficacy of pegylated interferon-alpha2b and ribavirin in patients with HBV/HCV co-infection. *J Hepatol* 2008.
- Reddy GA, Dakshinamurthy KV, Neelaprasad P, et al. Prevalence of HBV and HCV dual infection in patients on haemodialysis. *Indian J Med Microbiol* 2005;23(1):41.
- Sagnelli E, Coppola N, Messina V, et al. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002;36(5):1285.

- Sagnelli E, Coppola N, Scolastico C, et al. HCV genotype and "silent" HBV coinfection: two main risk factors for a more severe liver disease. *J Med Virol* 2001;64(3):350.
- Sato S, Fujiyama S, Tanaka M, et al. Coinfection of hepatitis C virus in patients with chronic hepatitis B infection. *J Hepatol* 1994;21(2):159.
- Wietzke P, Schott P, Braun F, et al. Clearance of HCV RNA in a chronic hepatitis C virus-infected patient during acute hepatitis B virus superinfection. *Liver* 1999;19(4):348.
- Yalcin K, Degertekin H, Yildiz F, et al. A severe hepatitis flare in an HBV-HCV coinfecting patient during combination therapy with alpha-interferon and ribavirin. *J Gastroenterol* 2003;38(8):796.
- Zarski JP, Bohn B, Bastie A, et al. Characteristics of patients with dual infection by hepatitis B and C viruses. *J Hepatol* 1998;28(1):27.
- Zhou J, Dore GJ, Zhang F, et al. Hepatitis B and C virus coinfection in The TREAT Asia HIV Observational Database. *J Gastroenterol Hepatol* 2007;22(9):1510.





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 rather than invasive liver biopsy are increasingly being utilized because of low patient acceptance and the low but ever-present morbidity of liver biopsies. Yet, despite recent advances in the prediction of liver fibrosis by 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 to 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  $\geq$  F2) fibrosis, because in patients with no or minor fibrosis antiviral treatment may be delayed, while in patients with more significant fibrosis treatment should be initiated. However, non-invasive markers should be able to reliably predict liver cirrhosis, as these patients may need special dose adjustments or surveillance while on antiviral treatment.

### Mechanisms of liver fibrosis in chronic viral hepatitis

Liver fibrosis in chronic viral hepatitis 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). End-stage liver fibrosis (cirrhosis) may be complicated by 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 viral hepatitis such as 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 beta (TGF- $\beta$ ). The activation of hepatic stellate 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 deg-

radation. 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, which can also be performed in patients with severe coagulation deficiencies. This technique is resource-intensive and also 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; Skripnova 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 inter-observer 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 in chronic viral hepatitis

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 markers 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, see <http://hepatologytextbook.com/link.php?id=7>). 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.

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 coinfecting 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 2007(a); Shaheen 2007(b)). 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 2007(b); Poynard 2007). Of note, the reliability for the detection of earlier fibrosis stages appears to be relatively low (Shaheen 2007(b); 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 2007(b); Poynard 2007).

In summary, surrogate markers may support the clinical decision making process, but a single surrogate marker or score is now unable to 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 (see Table 2, <http://hepatologytextbook.com/link.php?id=7>).

## Transient elastography

Transient elastography (TE) is a non-invasive technique for the assessment of 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. Commonly 10 successful measurements are necessary for the assessment of liver stiffness. Usually these measurements can be made in less than 5 minutes. At present TE machines are exclusively available by echosense / France (FibroScan®). The 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).

The evaluation of liver stiffness in subjects without apparent liver disease showed that liver stiffness results are influenced by gender and body mass index (BMI). In general, liver stiffness is higher in men than in women ( $5.81 \pm 1.54$  vs.  $5.23 \pm 1.59$  kPa) (Roulot 2008). It is important to note that the applicability of TE is limited to relatively lean patients (BMI  $\leq 28$  kg/m<sup>2</sup>), patients without ascites, and “cooperative” patients. In addition, the assessment of liver fibrosis by TE is hampered in patients with acute liver injury such as acute viral or alcoholic hepatitis, or flares of a chronic viral hepatitis, all of which may lead to an overestimation of liver fibrosis (Arena 2008; Coco 2007; Sagir 2008). In contrast to 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 who are 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 the high acceptance by patients, it can easily be used during follow-up 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 recurrence after liver 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 co-infected patients (e.g., German Guidelines for the Management of Patients with Chronic Hepatitis C Viral Infection, in press).

Recent studies comparing TE with liver biopsy demonstrated 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  $\geq 2$  compared to more advanced stages of liver fibrosis with a sensitivity ranging from 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 these patients. Vice versa, if TE predicts significant fibrosis a biopsy may not be necessary. One drawback for clinical practice is that the different TE studies have suggested 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 a reliable technique to predict the stage of liver cirrhosis is still a matter of debate and needs to be evaluated in further studies (Foucher 2006).

## **Other imaging techniques for the assessment of liver fibrosis**

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 definite endpoints such as mortality have yet to be performed. These studies 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 calling for 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 a reduction of liver biopsies 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.



| Study                          | Population         | Cut off (kPa) |     |           |          |           |
|--------------------------------|--------------------|---------------|-----|-----------|----------|-----------|
|                                |                    | F=0           | F≥1 | F≥2       | F≥3      | F=4       |
| Castera et al.<br>[59]         | HCV<br>N=183       | n.d           | n.d | 7.1       | 9.5      | 12.5      |
|                                |                    |               |     | Se: 0.67  | Se: 0.73 | Se: 0.87  |
|                                |                    |               |     | Sp: 0.95  | Sp: 0.91 | Sp: 0.91  |
|                                |                    |               |     | PPV: .95  | PPV: .87 | PPV: .77  |
|                                |                    |               |     | NPV: .48  | NPV: .81 | NPV: .97  |
| Ziol et al.<br>[60]            | HCV<br>N=327       | n.d           | n.d | 8.8       | 9.6      | 14.6      |
|                                |                    | n.d           | n.d | Se: 0.56  | Se: 0.86 | Se: 0.86  |
|                                |                    |               |     | Sp: 0.91  | Sp: 0.85 | Sp: 0.96  |
|                                |                    |               |     | PPV: .88  | PPV: .71 | PPV: .78  |
|                                |                    |               |     | NPV: .56  | NPV: .93 | NPV: .97  |
| Foucher et al.<br>[61]         | HCV / HBV<br>N=711 | n.d           | n.d | 7.2       | 12.5     | 17.6      |
|                                |                    |               |     | Se: 0.64  | Se: 0.65 | Se: 0.77  |
|                                |                    |               |     | Sp: 0.85  | Sp: 0.95 | Sp: 0.97  |
|                                |                    |               |     | PPV: .90  | PPV: .90 | PPV: .91  |
|                                |                    |               |     | NPV: .52  | NPV: .80 | NPV: .92  |
| Ogawa et al.<br>[62]           | HCV / HBV<br>N=229 | 3.5           | 6.4 | 9.5       | 11.4     | 15.4      |
|                                |                    |               |     | Se: 0.67  | Se: 0.67 | Se: 0.67  |
|                                |                    |               |     | Sp: 0.95  | Sp: 0.95 | Sp: 0.95  |
|                                |                    |               |     | PPV: .95  | PPV: .95 | PPV: .95  |
|                                |                    |               |     | NPV: .48  | NPV: .48 | NPV: .48  |
|                                |                    | 6.3           | 6.7 | 9.1       | 13.7     | 26.4      |
|                                |                    |               |     | Se: 0.67  | Se: 0.67 | Se: 0.67  |
|                                |                    |               |     | Sp: 0.95  | Sp: 0.95 | Sp: 0.95  |
|                                |                    |               |     | PPV: .95  | PPV: .95 | PPV: .95  |
|                                |                    |               |     | NPV: .48  | NPV: .48 | NPV: .48  |
| Arena et AL:<br>[63]           | HCV<br>N=150       |               |     | 7.8       | 10.8     | 14.8      |
|                                |                    |               |     | Se: 0.83  | Se: 0.91 | Se: 0.94  |
|                                |                    |               |     | Sp: 0.82  | Sp: 0.94 | Sp: 0.92  |
|                                |                    |               |     | PPV: .83  | PPV: .89 | PPV: .73  |
|                                |                    |               |     | NPV: .79  | NPV: .95 | NPV: .98  |
| De Ledinghen<br>et al.<br>[52] | HIV/HCV<br>N=72    | n.d           | n.d | 4.5       | n.d      | 11.8      |
|                                |                    |               |     | Se: 0.93  |          | Se: 1.0   |
|                                |                    |               |     | Sp: 0.18  |          | Sp: 0.93  |
|                                |                    |               |     | PPV: n.d. |          | PPV: n.d. |
|                                |                    |               |     | NPV: n.d. |          | NPV: n.d. |

**Table 3. Cut-off values for transient elastography in different study populations**

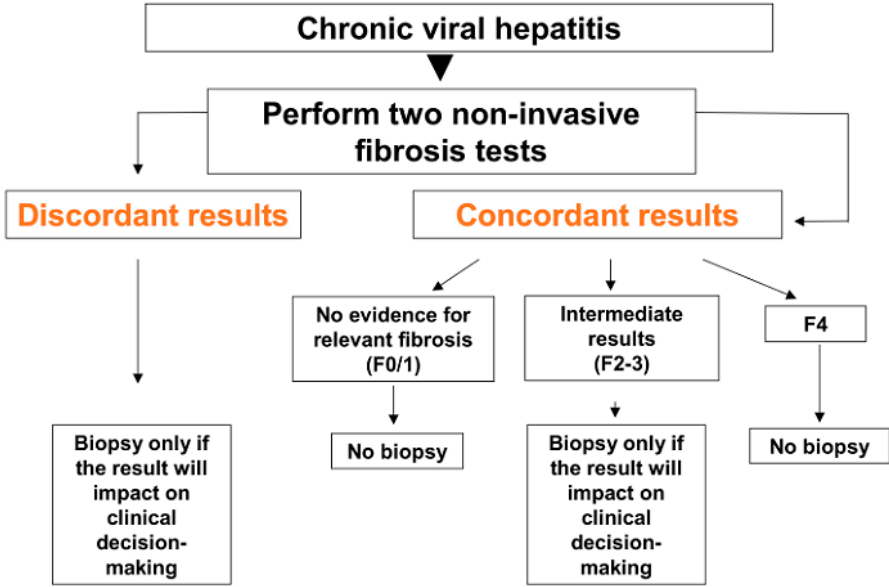


Figure 1. Potential clinical decision algorithm for safer liver biopsies in patients with chronic viral hepatitis

## Summary

Non-invasive tests will not replace liver biopsies, but smart combinations of both options may save many patients from the invasive procedure. Whatever the current standard of care, the patient should be informed about non-invasive tests, their applicability and their limitations, and the decision to biopsy the liver should ultimately be made together with the informed patient.

## 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, Gilibert 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, Leandro G, Dhillon AP, Burroughs AK. 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, Castera L, Sandrin L, Merrouche W, Lavrand F, Lamireau T. 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, Dhumeaux D, Beaugrand M. 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, Galle PR, Dienes HP, Lohse AW. 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, Bertet J, Couzigou P, de Ledinghen V. 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.

## 316 Assessment of hepatic fibrosis in chronic viral hepatitis

- Gronbaek K, Christensen PB, Hamilton-Dutoit S, Federspiel BH, Hage E, Jensen OJ, Vyberg M. 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, Denk H, Desmet V, Korb G, MacSween RN, 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 coinfecting 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, Murata M, Sawayama Y, Hayashi J. 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, Naveau S, Thabut D, Lebrech D, Zoulim F, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. 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 hemophilic 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.

### 318 Assessment of hepatic fibrosis in chronic viral hepatitis

- 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 of Hepatocellular Carcinoma

Ulrich Spengler

## Classification of HCC

Tumours are classified in order to stratify patients with respect to their survival prognosis, 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.

**Table 1: „Barcelona Clinic Liver Cancer“ (BCLC)-Classification**

| <b>Tumor Stage</b>    | <b>General State of Health</b> | <b>Tumour-Characteristics</b>                           | <b>Child-Stage</b> |
|-----------------------|--------------------------------|---|--------------------|
| <b>0 Very early</b>   | <b>good</b>                    | <b>single nodule &lt; 2cm</b>                           | <b>A &amp; B</b>   |
| <b>A Early</b>        | <b>good</b>                    | <b>single nodule &lt; 5 cm,<br/>3 nodules &lt; 3 cm</b> | <b>A &amp; B</b>   |
| <b>B Intermediate</b> | <b>good</b>                    | <b>large, multiple nodules</b>                          | <b>A &amp; B</b>   |
| <b>C Advanced</b>     | <b>reduced</b>                 | <b>vascular invasion,<br/>extrahepatic secondaries</b>  | <b>A &amp; B</b>   |
| <b>D Terminal</b>     | <b>severely reduced</b>        | <b>any form</b>   | <b>C</b>           |

Sala et al. Sem Liver 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. Recent 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 hyper-perfusion 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.

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 algorithm 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.

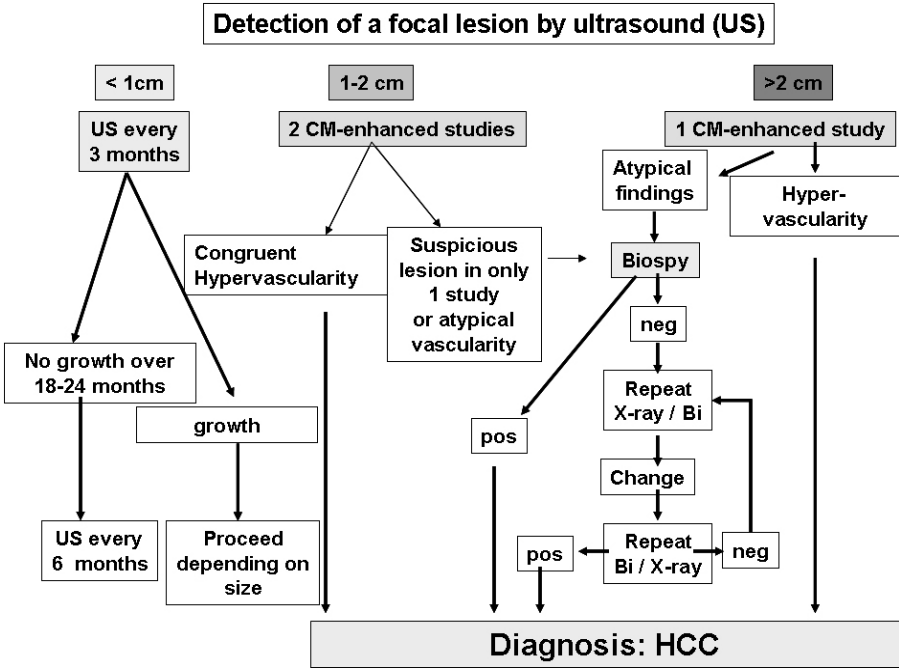


Figure 1. Diagnostic algorithm for the diagnosis of hepatocellular carcinoma depending on tumour size.

## 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/ $\mu$ l) 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 mid-term, 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 1D). 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 have 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 <sup>90m</sup>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 <sup>90m</sup>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 palliative 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/ $\beta$ -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.

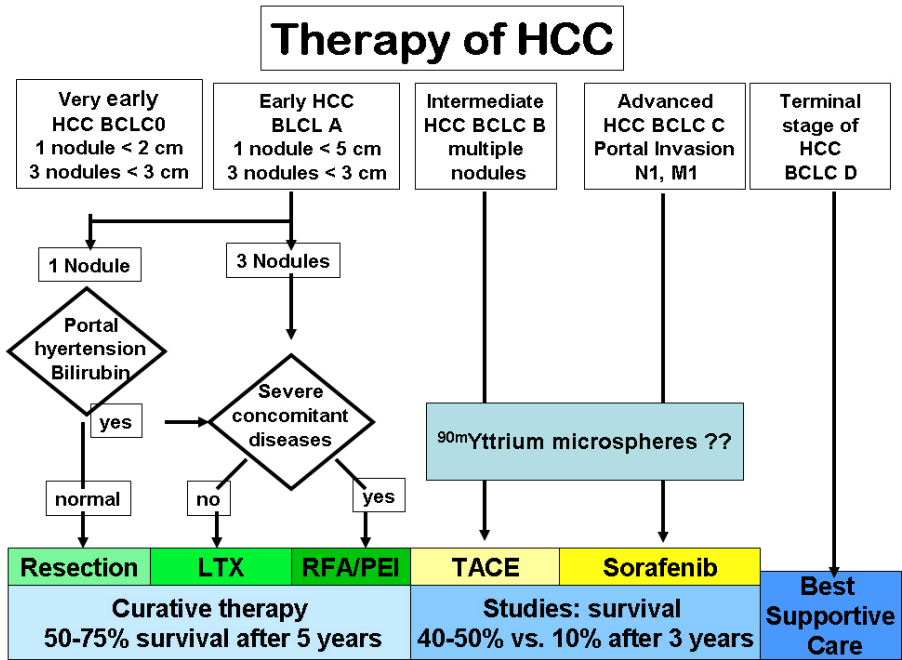


Figure 2. Overview of stage-adapted therapy of liver cancer in relationship to the BLCL criteria.

## 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; Papatheoridis 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 fac-



tors 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, Valla D, Belghiti J, Degott C, Terris B. 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, Recarli A, Trevisi P, Ribero ML, Martelli C, Porru S, Nardi G. 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, Annunziata A, Perrone F; CLIP (Cancer of the Liver Italian Programme) Investigators. 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, Cremaschini F, Franceschi S, Ribero ML, Garte S, Nardi G, Donadon V, Donato F. 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, Piralì F, Tagger A, Ribero ML, Trevisi P, Martelli C, Nardi G, Donato F; Brescia HCC Study Group. 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, Tatsch K, Lubiensky A, Reiser MF, Helmlinger T. 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.

### 330 Diagnosis, Prognosis & Therapy of Hepatocellular Carcinoma

- Lampertico P, Del Ninno E, Vigano M, Romeo R, Donato MF, Sablon E, Morabito A, Colombo M. 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, Milne N, Nguyen TH, Ramsinghani NS, Tokita KM, Tsai FY, Vajgrt DJ, Imagawa DK. 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, Ayuso C, Sala M, Muchart J, Sola R, Rodes J, Bruix J; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation 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, Montalto F, Ammatuna M, Morabito A, Gennari L. 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, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. 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 survival 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 Abstract book International Liver Cancer Association 2007.
- van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, Schalm SW, Janssen HL. 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, Castillo I, Weiland O, Nevens F, Hansen BE, Schalm SW. 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, Naito M, Nishio K, Suzuki H, Yoshimura T, Tamakoshi A; JACC Study Group. 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, Lee LP, Lin ZY, Hsieh MY, Wang LY, Chuang WL, Liaw YF. 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, Wong WM, Wong BC. 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

## Transplantation



## Chapter 22: Management of patients before and after liver transplantation

S. Beckebaum, G. Gerken and V.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 next 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, pre-transplant evaluation, management while on the waiting list, living donor liver transplantation (LDLT), and management of early and long term complications post-LT.

### 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) (Table 1) to prioritize patients on the waiting list. In the Eurotransplant countries, the 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).

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.

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 previous malignancy must be carefully considered and likelihood of recurrence estimated.

A major issue in patients transplanted for alcoholic liver disease is the likelihood of relapse (Neuberger 2007). It is policy 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).





## Pretransplant 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.

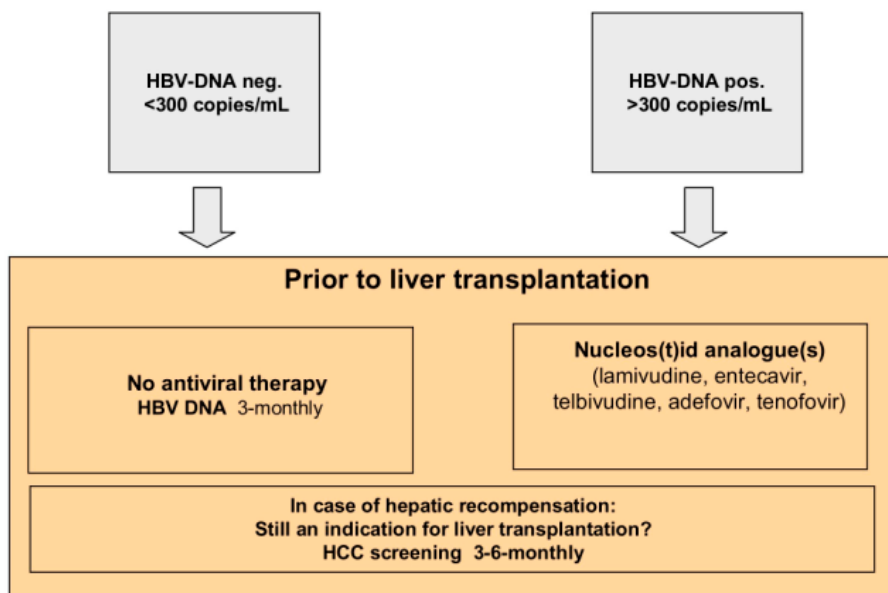
Special attention regarding specific, disease-related therapy prior to surgery should be given to transplant candidates undergoing LT for HCC or virally-related liver diseases.

| 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).



**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 transplant. The German Guidelines propose monitoring for HBV DNA at least every three months (Cornberg 2007). Neg., negative, pos., positive, HCC, hepatocellular carcinoma.

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 2008(a); Ono 2001; Mutimer 2000; Seehofer 2001). LAM has been proposed to be downgraded from first-line to second-line therapy (Tan 2007). In patients with end-stage liver disease, a more potent nucleos(t)ide analogue (entecavir [ETV] or tenofovir [TDF]) is preferred because development of resistance to LAM could result in clinical decompensation.

In patients with LAM resistance, recent results have shown that adefovir dipivoxil (ADV) and LAM combination therapy is superior to ADV monotherapy (Vassiliadis 2007; Lampertico 2007). An open-label study in HBV patients with LAM resistance who were wait-listed (N = 226) or post-LT (N = 241), ADV was added on at some time in almost all patients; however, the exact time period of combined treatment was not clearly documented (Schiff 2007). 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 with high viral loads. In contrast its antiviral efficacy is much lower in patients with lamivudine resistant

HBV, as due to partial cross-resistance, development of full entecavir resistance is a one step procedure (Colonna 2006; Chang 2006).

### **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 on 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 decompensated cirrhosis and with MELD score  $\geq 18$  should be restricted to exceptional cases and monitored by a transplant center. Although pre-transplantation IFN-alpha therapy has been 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 HCC transplant candidates**

Although LT has been recognized as the most effective means of treating HCC patients, success is limited by long waiting times for transplant, 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 chemoembolisation or radiofrequency ablation (Roayaie 2007).

Under MELD allocation, patients must meet the Milan criteria (one tumor  $\leq 5$  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 met 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 a 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 in 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 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 feasibility 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 donor outcome, morbidity rates vary considerably in the 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 ( $\gamma$ GT) 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 faecalis, 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 non-specific, 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.

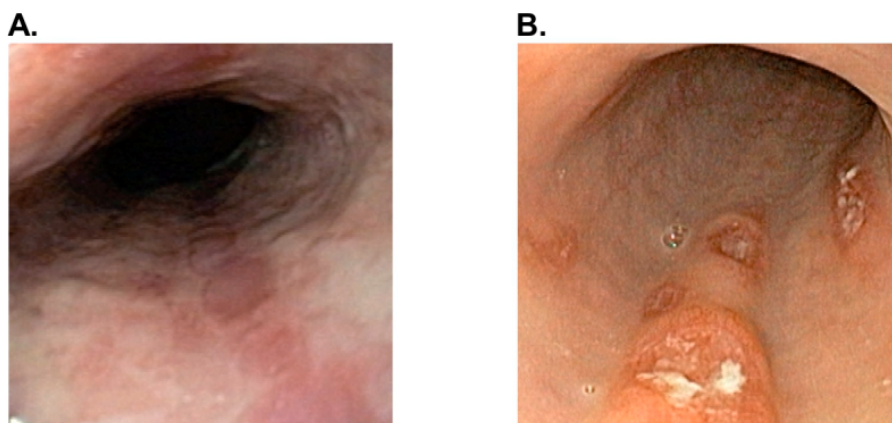
### Opportunistic infections

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 intravenous (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 viral load of Epstein-Barr virus (EBV) infection 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, tonsillitis, 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 transplant 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 abnormalities ( $\gamma$ GT, AP, bilirubin, transaminases). The diagnosis needs to be confirmed by histopathologic examination. Switching the baseline immunosuppression from CSA to TAC and initiating mycophenolate 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).

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) is a macrolide isolated from *Streptomyces hygroscopicus*. 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, leucopaenia, and anaemia. 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 (San-



chez 2005; Neff 2003; Trotter 2003). 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).

A second TOR inhibitor, everolimus (ERL), may exhibit improved bioavailability and a shorter half-life than SRL. 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.

| Immunosuppressant Class         | Immunosuppressive Agent                        |
|---------------------------------|--|
| Corticosteroids                 | Prednisone, prednisolone, methylprednisolone   |
| Calcineurin inhibitors          | Cyclosporin A, tacrolimus                      |
| Antimetabolites                 | Mycophenolate mofetil, azathioprine            |
| TOR Inhibitors                  | Sirolimus, everolimus                          |
| Polyclonal antibodies           | Antithymocyte globulin (ATG)                   |
| Monoclonal anti-CD3 antibodies  | Muromonab-CD3 (OKT3)                           |
| Chimeric monoclonal antibodies  | Anti-IL-2 inhibitors (basiliximab, daclizumab) |
| Monoclonal anti-CD52 antibodies | Alemtuzumab (campath-1H)                       |

**Table 4. Clinically used immunosuppressive agents in liver transplantation.**

## Other side effects of CNI

Beside potential nephrotoxicity, CNI therapy is associated with side effects that 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.

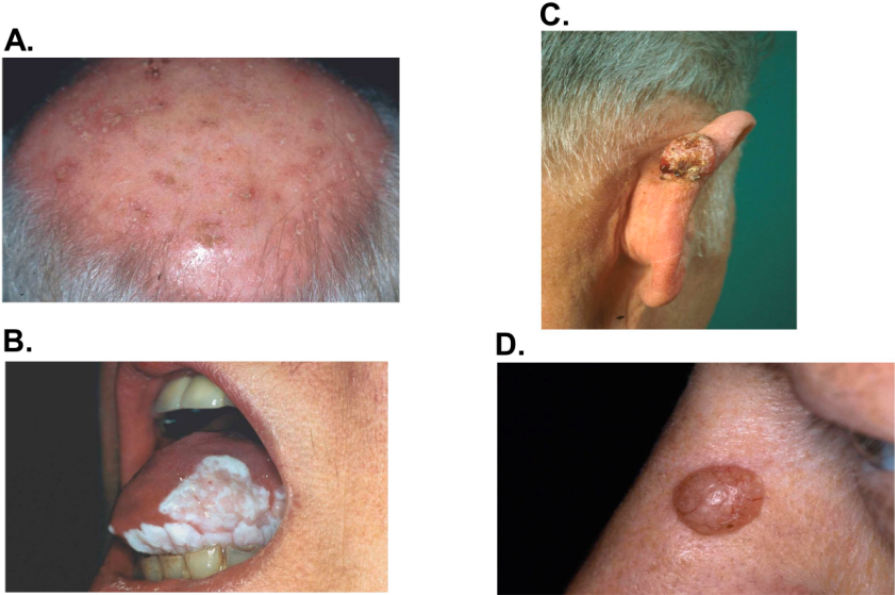
There is ongoing discussion of steroid avoidance due to dyslipidemia, osteoporosis, development of cataracts, weight gain, hypertension, and a deleterious impact on glucose control. The Ochsner Clinic investigated the efficacy of polyclonal rabbit anti-thymocyte globulin (RATG) induction followed by 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 post-transplant dia-

betes, 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 post-transplant 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 the 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 transplantation followed by basal cell carcinoma [D.] (Photographs kindly provided by Dr. Hillen, Transplant Dermatology Outpatient Unit, Department of Dermatology, University Hospital Essen, Germany).

Premalignant 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.

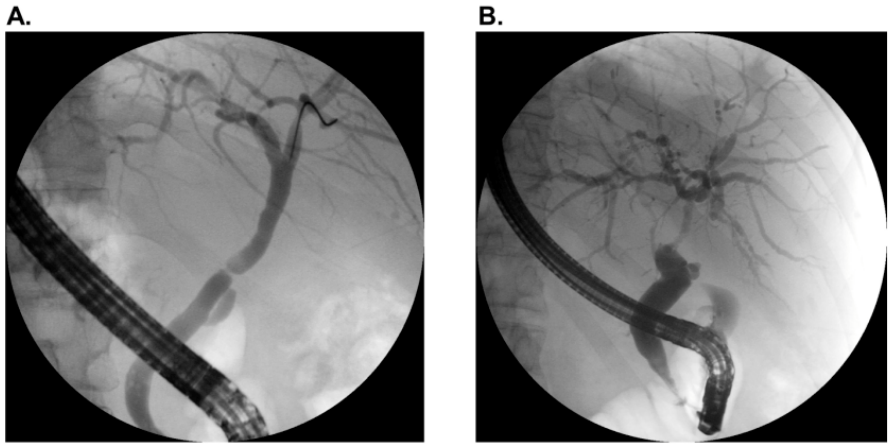
## Biliary complications

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 damage. Novel radiological methods such as magnetic resonance cholangiopancreatography (MRCP) have been introduced as an additional diagnostic tool for biliary complications.

Biliary leaks generally occur as an early post-transplant 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 nonanastomotic 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 earlier. Therapy sessions include dilatation and an increasing number of bile duct endoprotheses at short intervals of every 2-3 months. Prior to endoscopic therapy an endoscopic sphincterotomy 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 anastomotic strictures were retrospectively analyzed (Zoeplf 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 anastomotic 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 are 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; Douzjian 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).

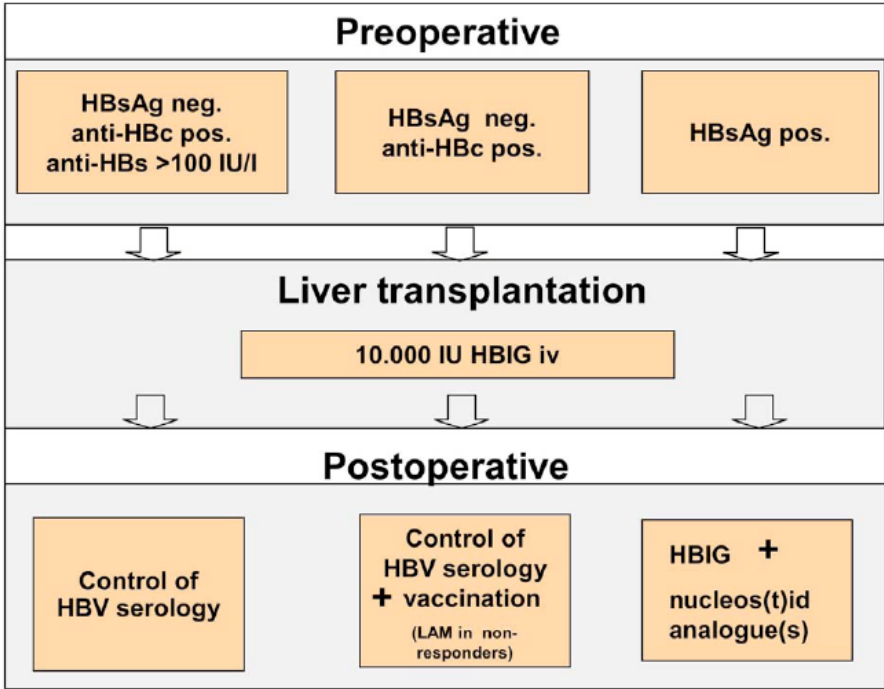
## Recurrent diseases after liver transplantation

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 immunoglobulin (HBIG) and nucleos(t)ide analogues has emerged as 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. 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, see <http://hepatologytextbook.com/link.php?id=9>) (Marzano 2001; Jiao 2007; Zheng 2006) - this monoprophylactic regimen is not sufficient except in patients who are seropositive only for anti-HBc.



**Figure 6. Prophylaxis of HBV recurrence after liver transplantation.**

Combined use of nucleos(t)id 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.

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, no results were given post-therapy switch.

A prospective, open-label, multicenter study was conducted on the safety and efficacy of combined LAM/ADV therapy in HBsAg-positive LT recipients (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.

Similar promising results were obtained by Nath et al. (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.

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  $4.0 \pm 1.6$  and  $4.5 \pm 1.5$   $\log_{10}$  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 was reported 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 (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 plus LAM, TDF plus ETV, or TDF plus emtricitabine are urgently needed.

Experience with ETV in the LT is very limited (Study 109 [<http://www.clinicaltrials.gov>], 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  $\geq 2$   $\log_{10}$  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 (Table 6) has been discussed widely except for listed donor factors, high dose corticosteroids and OKT3 therapy. In particular, there are insufficient and somewhat controversial data regarding the relationship between immunosuppressive agents and clinical expression of HCV recurrence (Table 7). 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. 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).

Induction with MMF is 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). This may be due to the antifibrotic effects of MMF through an antiproliferative effect on myofibroblast-like cells.



|                          |   |
|--------------------------|---|
| <b>Donor factors</b>     | Donor age<br>Liver steatosis  |
| <b>Recipient factors</b> | Surgical factors (cold/warm ischemia time)<br>Recipient age<br>Recipient gender<br>Non-caucasian race<br>High viral load pre-transplant/early posttransplant<br>Genotype 1b<br>Muromonab-CD3 (OKT3)<br>Bolus corticosteroids<br>Rapid tapering of corticosteroids |

**Table 6. Factors that may accelerate histological progression in HCV patients after liver transplantation.**

| Immunosuppressive agent       | Viral load  | Severity of HCV recurrence                          |
|-------------------------------|---|---|
| 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. Impact of immunosuppression on viremia and HCV recurrence.**

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 stage of fibrosis. 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. In addition, despite a few published results there are still a lot of ongoing studies evaluating the role of fibroscan in the LT setting.

There is increasing evidence that IFN- $\alpha$  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- $\alpha$  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 anaemia or leucopaenia 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 posttransplant (Charatcharoenwitthaya 2007). Various other studies reporting recurrent PBC are depicted in Table 8 (see <http://hepatologytextbook.com/link.php?id=10>, 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.

The clinical course of ulcerative colitis was investigated 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  $\geq 5$  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 of 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  $\leq 6.5$  cm, two to three tumors: none  $>4.5$  cm or total diameter  $\leq 8$  cm, no vascular invasion) or even more liberal criteria (no portal inva-

sion, 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  $\leq 5$  cm, HCC number  $\leq 6$ , and no gross vascular invasion) (76%, 75.9% and 76.3%, respectively). Moreover, use of expanded criteria yielded a higher discriminatory power than either the Milan or 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 usually 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 use 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 pre-transplant abstinence as a predictor of

post-transplant abstinence has been widely discussed. Many studies have assessed possible risk factors for alcoholic relapse after LT. The following factors have been identified 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  $\leq 6$  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 which presents as deterioration of cirrhosis not responsive to treatment, as acute or 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 subgroup of **PiZZ** individuals are predisposed to liver damage, due to an insufficient degradation of mutant alpha 1-antitrypsin Z within the endoplasmic reticulum (Perlmutter 1998). A 1-year survival rate of 73% for adults has been reported in the literature (Vennarecci 1996).

In haemochromatosis, the metabolic defect is 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 haemochromatosis is markedly lower in comparison to other indications (Brandhagen 2001). Data on 56 patients with haemochromatosis 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 post-transplant survival in patients with haemochromatosis 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 haemochromatosis 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, coumarins, ecstasy, 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  $\mu\text{mol/L}$  pretransplant, non-white race of the recipient, donor body mass index  $>35 \text{ kg/m}^2$  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  $\geq 6$  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 in the last 20-30 years.

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. Current trends of immunosuppressive strategies include calcineurin-inhibitor-sparing protocols, mTOR inhibitor based-protocols and corticosteroid-avoidance protocols. Finally,



"individually tailored immunosuppressive" protocols may optimize drug efficacy, minimise drug toxicity and improve transplant outcome.

## 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. *Transplant 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.

## 362 Management of patients before and after liver transplantation

- 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, Aguilera 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, Whittington 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.
- 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.
- 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.

## 364 Management of patients before and after liver transplantation

- 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.
- Filippini F, Callea F, Salizzoni M, et al. Double-blind comparison of hepatitis C histological recurrence rate in HCV<sup>+</sup> 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.
- 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, Egtesad 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 (OLT) 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.

## 366 Management of patients before and after liver transplantation

- 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 Transpl* 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, et 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.
- 119.
- 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.

## 368 Management of patients before and after liver transplantation

- 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 HBeAg 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, Snyderman DR, Rubin RH, et al. Cytomegalovirus prophylaxis in solid organ transplant recipients. *Transplantation* 1996;61:1279-89.
- Patel S, Orloff 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.
- Pfützmann 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.

## 370 Management of patients before and after liver transplantation

- 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 leukoencephalopathy 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.
- 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, Bretschneider L, et al. Orthotopic homotransplantation of the human liver. *Ann Surg* 1968; 168 (3):392-415.
- 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.
- 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, Muiresan 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.

## 372 Management of patients before and after liver transplantation

- 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 mono-therapy 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.
- 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.
- Cannon JA. Organs. *Transplant Bull* 1956;3-7.
- 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.

- 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 between 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 monophylaxis 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 endoprosthesis for treatment of anastomotic biliary strictures after liver transplantation. *Liver Transpl* 2006;12:88-94.



## Chapter 23: Liver transplantation in hepatitis B and C and HIV coinfection

José M. Miró, Fernando Agüero, Montserrat Laguno, Montserrat Tuset, Carlos Cervera, Asuncion Moreno, Juan-Carlos García-Valdecasas, Antonio Rimola and the Hospital Clinic OLT in HIV Working Group

### Introduction

Liver disease due to chronic hepatitis B and C is currently a leading cause of morbidity and mortality among HIV-infected patients in the developed world. While patients with chronic hepatitis C without coinfection tend to progress to end stage liver disease (ESLD) over the course of 20-30 years, coinfecting patients have increased 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 subjects coinfecting with HIV and HCV than in HCV-monoinfected subjects (Graham 2001).

ESLD is common in coinfecting patients and this document will review different approaches to treating the condition.

### Epidemiology

Of the estimated 40 million persons infected worldwide with HIV, 4-5 million have chronic HCV and 2-4 million have chronic HBV (Alter 2006).

Prevalence of HCV and/or hepatitis B virus (HBV) coinfection is high in developed countries. Studies performed in European HIV-infected patients show rates of 33% and 9%, respectively (Rockstroh 2003; Konopnicki 2005) while in the USA the figures are very similar, 28% and 9%, respectively (Fung 2004).

There are some studies that have addressed the significance of HCV as a cause of non-AIDS-related death (Palella 2006; Crum 2006; Lewden 2005). One Spanish single-centre study (Martinez 2007) analysed the cause of 235 deaths in 4471 patients (5%) on ART 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-infected patients. In comparison with the general population of a similar age, deaths due to liver disease were 11 times higher in HIV-infected patients. Another prospective multicentre study performed in France (Rosenthal 2007) determined mortality due to ESLD in a nationwide population of HIV-infected patients. It followed a total of 20,940 HIV-infected patients, 4005 (19.9%) of whom were coinfecting, and showed that, in 2003, mortality due to ESLD represented 23.7% of non-AIDS-related deaths. In this population, ESLD deaths were 1.5% in 1995, 6.6% in 1997, 14.3% in 2001, and 12.6% in 2003. Chronic hepatitis C was present in 92.6% of patients who died from ESLD. A prospective, observational study of 11 cohorts was carried out in Europe, the United States, and Australia (The D:A:D Study 2006) and included 23,441 HIV-1-infected patients (22.5% of whom were HCV-positive) followed from December 1999 until February 2004. This study observed that among 1246 deaths, those AIDS-related were the most frequent (31.1%), and 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.

## Clinical features of coinfecting patients with ESLD

One retrospective Spanish study (Pineda 2005) has described the frequency of specific events such as first decompensation and cause of death in HIV-negative and HIV/HCV-coinfecting subjects. Ascites and jaundice were more frequent among HIV-infected patients, while upper gastrointestinal bleeding and hepatocellular carcinoma (HCC) were more frequent in mono-infected patients. Hepatic encephalopathy (HE) as both first decompensation and cause of death was higher in coinfecting patients.

The clinical characteristics and outcome of spontaneous bacterial peritonitis (SBP) were evaluated in an HIV-infected population with cirrhosis. Thirty-five HIV-infected patients with cirrhosis were compared to 70 non-HIV-infected subjects with cirrhosis. This study found that, in HIV-infected patients, an etiologic diagnosis was achieved in almost 80% of cases and bacteraemia was present in more than 50%, with both rates being higher than those observed in HIV-negative patients (Shaw 2006). An important bacteriological finding in this study was the high incidence of *S. pneumoniae* as the etiologic agent of SBP among HIV-infected patients, second only to *E. coli*.

It is well known that hepatocellular carcinoma (HCC) has a faster and worse outcome in HIV/HCV coinfecting people compared with HCV mono-infected patients (Puoti 2004; Bruno 2006).

The findings of HCC in 41 HIV-infected and 2384 noninfected patients were compared in Italy (Puoti 2004). The authors found a more aggressive course of HCC in HIV-infected 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 neoplasms and extrahepatic-extranodal metastases. Furthermore, portal vein thrombosis was more frequent among HIV-infected patients with HCC.

63 HIV-infected HCC patients and 226 HIV-negative HCC patients were compared and it was observed that HIV patients were younger and more frequently symptomatic (Bräu 2007). In this cohort HCC had a bad prognosis (median survival of 6 months) but tumour staging and survival curves were similar.

Survival of HCV/HBV-mono-infected patients with HCC detected by screening has improved in recent years due to increased chance of curative treatment with the advent of liver transplantation and radiofrequency ablation (RFA) (Chan 2008).

## Prognosis after decompensation

Survival of HIV-infected patients with decompensated cirrhosis is much lower than in HIV-negative patients. There is solid data that the survival rate in this specific scenario is about 50% at 1-year of follow up (Pineda 2005; Merchante 2006; Miró 2005). In a recent multicentre case-control study performed in Spain (Pineda 2005), the outcome of cirrhosis after the first decompensation in coinfecting patients is much worse than in the mono-infected population. Survival at one, two, and five years for the coinfecting and mono-infected population was 54%/74%, 40%/61%,



and 25%/44%, respectively. In another study (Merchante 2006), the same group of researchers identified as independent predictors of poor outcome in coinfecting patients the severity of liver disease (Child-Turcotte-Pugh [CTP] classification or HE as the first hepatic decompensation) and the level of cellular immunosuppression ( $< 100$  CD4 cells/mm<sup>3</sup>). On the other hand, ART was associated with a reduced rate of mortality.

Another Spanish study has followed the evolution of 104 HIV-infected patients with cirrhosis after their first hepatic decompensation or hepatocellular carcinoma (Miró 2005). Median survival time of this cohort was 14 months, similar to Merchante's cohort (13 months). This study included HCV and non-HCV infected patients and we did not find significant differences in survival according to the aetiology of cirrhosis, suggesting that HIV-infected patients have an overall poor outcome regardless of the nature of their liver disease. Furthermore, the MELD score was the only factor independently associated with mortality. This is of relevance because over the past few years MELD has been increasingly used to establish the prognosis of patients with cirrhosis and, consequently, to indicate need for liver transplant.

A recent prospective study (Girón-González 2007) that enrolled 92 HIV-infected 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 CTP measured at inclusion, progression of CTP during follow up, more than one decompensation during follow up and absence of ART during follow up.

HIV-infected patients with cirrhosis have a poor prognosis after the development of SBP (Shaw 2006). HIV infection was associated with a more than six-fold increase in the probability of dying within a month of the first SBP episode. Impaired renal function at diagnosis and severity of liver disease were identified as predictors of death. When long-term mortality was considered, HIV-infected 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 hepatic disease rather than to AIDS-related conditions.

High mortality rates among coinfecting patients with ESLD waiting for liver transplantation have also been reported in two studies (Maida 2005; Ragni 2005). In one (Maida 2005), death due to ESLD occurred in 25% of patients during the evaluation period. In the other (Ragni 2005), mortality rates during pre-transplant evaluation in HIV-positive ( $n = 58$ ) and HIV-negative ( $n = 1359$ ) patients were 36% and 15%, respectively ( $p < 0.001$ ).

Nevertheless, these data were not confirmed in a recent US multicentre study. Mortality while on a waiting list was 14% in patients with HIV infection ( $n = 167$ ) and 11% in the control group ( $n = 792$ ) ( $p = 0.30$ ). In a multivariate analysis, a MELD score higher than 25 was the only variable related to death while on the waiting list (Subramanian 2008).

In any case, physicians attending HIV-infected patients with cirrhosis should prospectively follow these patients and they should evaluate them early for Orthotopic Liver Transplantation (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 patient survival until OLT and it should be done as is done in HIV-negative patients (Agüero 2007; Merchante 2007).

## Management of cirrhosis complications

Management of complications (portal hypertension, ascites, gastrointestinal bleeding, encephalopathy, SBP, HCC and hepatorenal syndrome) must be planned, just as with the non-infected population (Cardenas 2005; Han 2006; Arroyo 2008). Medical management also includes the prevention of infection. In view of the short survival associated with development of SBP, primary antibiotic prophylaxis with quinolones or trimethoprim-sulfamethoxazole should be considered (Fernandez 2007).

As far as HCC is concerned, screening is warranted every 3-6 months (Puoti 2004; Bruno 2006). Treatment of HCC could be disappointing 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, hepatorenal syndrome carries a high mortality (Han 2006). The molecular adsorbent recirculating system (MARS) could be a new therapeutic tool in this setting and its association with transplant/retransplantation may be highly effective (Gaspari 2006), although more evidence in coinfecting patients with ESLD and hepatorenal syndrome is needed.

Other issues that may delay the progression of liver disease, such as avoidance of hepatotoxic drugs (i.e., dDI), and vaccination against hepatitis A and B, should be addressed.

## 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 patients who died from ESLD in 2003 (92%), and another study suggests that excess alcohol consumption increases HCV-RNA levels (Cooper 2005).

In addition, recent studies have concluded that daily cannabis smoking is significantly associated with fibrosis progression during chronic hepatitis C and recommend that patients with hepatitis C cirrhosis refrain from regular cannabis use (Hezode 2008).

## HCV/HBV management

Specific treatment for hepatitis B or C virus is possible, although more difficult, at this end stage of cirrhosis, especially for HCV (Soriano 2007; Rockstroh 2008).

One of the objectives in treating HCV-monoinfected patients with advanced liver cirrhosis with pegylated-interferon and ribavirin is to reach undetectable plasma HCV RNA levels at the time of OLT in order to reduce the risk of HCV recurrence after transplantation. One study (Everson 2005) using a low accelerating dosage

regimen (LADR) of anti-HCV therapy in monoinfected patients on an OLT waiting list showed that 30 (24%) of 124 patients achieved sustained virologic 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 coinfecting patients, although some safety data can be extrapolated from an APRICOT substudy (Mauss 2004). Hepatic decompensation was observed only in HIV/HCV-coinfecting patients with markers of advanced cirrhosis and its incidence was 10.4% (14/134). However, six (43%) of the 14 patients died as a result of hepatic decompensation. One of the associated risk factors was antiretroviral treatment with didanosine (ddI). In contrast, no hepatic decompensation was noted in HIV/HCV-coinfecting patients without cirrhosis. Therefore, anti-HCV treatment during the pre-transplant evaluation or on the waiting list should be individualized (e.g., patients with Child A and HCC or genotypes 2/3) and these patients must be monitored closely because of their high risk of hepatic decompensation and death.

In a recent case-control study, 129 HCV-monoinfected patients with decompensated cirrhosis were enrolled. Sixty-six ( $n = 66$ ) of these patients were treated with PEG-IFN plus ribavirin for 24 weeks and compared with the control group ( $n = 63$ ) which was not treated. 13 patients discontinued treatment for intolerance. SVR was observed in 82.6%, 43.5%, 30.2% and 7% for HCV genotypes 2, 3, 1 and 4 respectively. The odds ratio for severe infection or death due to infection was higher in the treated group while ascites, encephalopathy and oesophageal bleeding decreased in the treated group. During follow up there were 15 deaths in the control group and nine in the non-responders group. All the patients who experienced SVR survived and none needed OLT. The authors concluded that HCV clearance by therapy is life-saving and reduces disease progression in HCV-monoinfected patients (Lacobellis 2007).

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 a chronic HBV infection can be treated with lamivudine (or emtricitabine) and tenofovir as part of triple antiretroviral therapy (Soriano 2007; GESIDA/PNS Panel of Experts 2007).

Adefovir and tenofovir have proven useful against HBV and could be used in cases of resistance to lamivudine (Soriano 2007).

## ART

The role of ART in liver disease progression and in overall mortality in HCV/HIV-coinfecting patients is still being debated (Tedaldi 2003; Qurishi 2003). The use of protease inhibitors may offer protection from progression of HCV-related fibrosis (Benhamou 2001; Macias 2006). Antiretroviral drug regimens should be carefully considered in persons with HIV and ESLD. These patients should follow the general recommendations (GESIDA/PNS Panel of Experts 2007; Clumeck 2008) and their liver function must be closely monitored in order to detect hepatotoxicity. Careful consideration of drug prescriptions and possible interactions is essential.

Furthermore, some antiretroviral drugs may be contraindicated in cirrhotic patients (e.g., ddI, nevirapine, full-dose ritonavir) and their dosing should be adjusted according to the degree of hepatic impairment (Wyles 2005; Back 2008; Tuset 2008)

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 CTP and MELD scores, results in patients taking these drugs should be interpreted with caution.

Finally, given the speed with which new antiretrovirals appear and thus generate unknown interactions, physicians should consult updated databases on drug interactions (Back 2008; Tuset 2008).

## **OLT (Orthotopic liver transplantation)**

OLT is the only therapeutic option for patients with ESLD. HIV infection is not a contraindication for liver transplantation (Miró 2007; Stock 2007; Samuel 2008; Norris 2008). There are 3 different classes of criteria for including HIV-infected patients in 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-infected 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 transplant include acute liver failure, decompensated liver disease (with ascites, encephalopathy [it is important to exclude HIV-related dementia] or variceal bleeding that is difficult to manage with standard therapies, and poor liver function [e.g., 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 has been replaced by the MELD score (Chapter 22). The criteria for liver transplant in patients with HCC are as follows: no more than three tumour nodules, none of which is greater than 5 cm in diameter, absence of macroscopic portal vein invasion, and absence of recognizable extrahepatic disease (Milan criteria, Chapter 22).

### **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; Miró 2005; Anonymous 2004).

|  | Spain<br>[Miro 2005] | Italy<br>[Grossi 2005]                  | UK<br>[O'Grady 2005]                                 | USA<br>[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 <sup>3</sup>               | >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                |

BDL: Below detections levels (<200 copies/mL). \* 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 exclusion criteria; \*\*\* Patients with previous OIs should have >200 CD4 cells/mm<sup>3</sup>; \*\*\*\* If PVL was detectable, post-OLT supression with ART should be predicted in all patients.

**Table 1. HIV criteria for OLT in some European countries and the USA.**

## 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, an NIH-sponsored study has recently updated the inclusion criteria for opportunistic complications and only those diseases without therapy are still 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<sup>3</sup> for OLT (Roland 2003; Neff 2004). This figure is lower than for kidney transplantation (CD4 >200 cells/mm<sup>3</sup>) because patients with cirrhosis often have lymphopenia due to hypersplenism, which leads to a lower absolute CD4+ count, despite high CD4 percentages and good virological control of HIV. In Spain and the USA, patients with previous opportunistic infections should have more than 200 cells/mm (Miró 2005; Anonymous 2004).

On the other hand, a CD4 cell count above 200 cells/mm<sup>3</sup> is the cut-off used in Italy (Grossi 2005) and the UK (O'Grady 2005), unless patients have decompensated cirrhosis or portal hypertension, respectively. In these scenarios, they use the same CD4 cell threshold used in Spain and the USA (100 cells/mm<sup>3</sup>).

## Virologic criteria

The essential criterion for OLT is that the patient must be able to have effective, safe and long-lasting ART during the post-transplant period (Neff 2004; Fung 2003). The ideal situation is one in which the patient tolerates ART 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 ART, as they are either long-term non-progressors (LTNP) or do not have immunological criteria ( $CD4^+$  lymphocyte count  $>350$  cells/mm<sup>3</sup>) or clinical criteria to start ART and, therefore, they have viraemia that is detectable in plasma. In this setting, it is unknown whether and when (pre-transplant or post-transplant) it would be beneficial to initiate ART in order to reach an undetectable HIV viral load in plasma.

## Other criteria

To be included on the OLT waiting list, an HIV-infected patient must have a favourable psychiatric evaluation. Patients who actively consume drugs will be excluded. In Spain, a two-year consumption-free period for heroin and cocaine (Miró 2005) and six months without addiction to other drugs (e.g., alcohol) is recommended. Patients who are on stable methadone maintenance programmes are not excluded from transplant and can continue on such programmes after the transplant (Liu 2003). Finally, as is the case with any transplant candidate, HIV-infected patients must show an appropriate degree of social stability to ensure adequate care in the post-transplant period.

## Outcome of OLT in HIV-infected patients

Overall short-term survival rates of HIV-infected patients who have undergone OLT have been reported to be similar to those of HIV-negative patients without HCV coinfection (Fung 2004; Roland 2002; Ragni 2003; Neff 2003; Norris 2004; Duclos-Vallee 2006; De Vera 2006; Schreiber 2007; Coffin 2007; Grossi 2008). HIV-infected patients did not 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). Bacterial infections were common in liver (43%) and kidney (35%) transplant recipients and HCV infection was the only factor associated with increased risk of bacterial infection (in liver transplant recipients only) (Blumberg 2008).

Several hundred patients have undergone OLT in developed countries with good short-term survival (Table 2).

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 with HIV or not. In fact, it is currently the leading cause of death. Some studies have suggested that HCV recurrence in coinfecting patients tends to be more severe and occurs earlier (De Vera 2006; Castells 2006).

The outcomes of 27 coinfecting patients were compared with 54 HCV mono-infected 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 subjects (RR = 2.6, 95%CI, 1.06–6.35;  $p = 0.03$ ). Cumulative 1-, 3- and 5-year survival for coinfecting and mono-infected patients was 67% vs. 76%, 56% vs. 72% and 33% vs. 72%, respectively ( $p = 0.07$ ).

| Author         | Year | Country       | N° cases | Virus                     | Follow-up (months) | Survival rate        |
|----------------|------|---------------|----------|---------------------------|--------------------|----------------------|
| Roland         | 2002 | International | 19       | Most HCV                  | 10                 | 15 (79%)             |
| Ragni          | 2003 | International | 24       | HCV - 62%<br>HBV - 29%    | 17                 | 18 (75%)             |
| Neff           | 2003 | USA           | 16       | HCV or HVB                | 12                 | 14 (87%)             |
| Fung           | 2004 | USA           | 29       | HCV - 90%                 | 18                 | 20 (69%)             |
| Norris         | 2004 | UK            | 14       | HCV - 50%<br>HBV/OH - 50% | 12<br>19           | 2 (29%)<br>7 (100%)  |
| Duclos-Vallée  | 2006 | France        | 41       | HCV - 88%<br>HBV - 12%    | 18                 | 29 (81%)<br>5 (100%) |
| De Vera        | 2006 | USA           | 27       | HCV - 100%                | 27                 | 13 (48%)             |
| Scherei bman   | 2007 | USA           | 15       | HCV - 40%<br>HBV - 33%    | 74                 | 10 (67%)             |
| Coffin         | 2007 | USA           | 16       | HBV - 100%                | 8,5                | 14 (86%)             |
| Spanish study* | 2008 | Spain         | 127      | HCV - 94%                 | 21                 | 89 (74%)             |
| Grossi         | 2008 | Italy         | 60       | HCV - 65%<br>HBV - 12%    | 12                 | 41 (58.3%)           |

\*Unpublished data

**Table 2. Liver transplantation in HIV-infected patients: main series of cases ( $\geq 10$ ) in the later ART era (2002-2008).**

In a recent retrospective study carried out in the USA that enrolled 138 HIV-infected patients with liver transplant in the ART era (1996-2006), the rate of survival at 2 and 3 years was significantly lower in patients with HIV infection (70% and 60%) than in the general population ( $n = 30,520$ ) (81% and 77%) although this difference was observed in the HCV/HIV-HBV/HIV coinfecting group exclusively. None of the 24 transplanted HIV-mono-infected patients died (Mindikoglu 2008). Therefore, liver transplant in HIV-infected 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- (3-5 years) and the long-term (5-7 years).

In France, the data of 35 HIV/HCV coinfecting patients were analysed and compared to 44 HCV mono-infected patients. The rate of survival at 2 and 5 years were 81% and 91% and 51% and 73% in HIV/HCV coinfecting patients and HCV mono-infected patients, respectively ( $p = 0.004$ ) (Duclos-Vallée 2008).

Conversely, in a Spanish multicentre case-control study (Miró 2007), the survival rate of patients and grafts at 3 years was similar in HIV/HCV coinfecting patients ( $n = 51$ ) than in HCV monoinfected patients ( $n = 1177$ ). The survival rate at 1, 2 and 3 years was 88% versus 81%, 75% versus 74% and 64% versus 69%, respectively ( $p = \text{NS}$ ). Although there is no available data at 5 years in this study, the differences observed between the Spanish results and the French and American ones show the real need to implement additional cohort studies to examine donor and recipient characteristics and issues related to the activity of both viruses and the efficacy and safety of antiviral therapies to try to explain the long-term prognosis of this procedure.

After OLT, HCV infection recurrence is universal, regardless of whether the patient is infected by HIV or not. Some studies have suggested that HCV recurrence in coinfecting patients tends to be more severe and occurs earlier (De Vera 2006; Castells 2006).

A rapid progression of HCV-related liver disease in HIV-infected recipients would represent a major drawback and would lead to a shortened life expectancy in these patients. In fact, it is currently the leading cause of death. A French study observed that progression to fibrosis (F2 or higher) was significantly greater in the HIV-infected group ( $p < 0.0001$ ) (Duclos-Vallée 2008) and MELD was the only significant predictor of mortality, though 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 (Miró 2008).

Finally, a recent study has described two cases of spontaneous clearance of HCV RNA after OLT. This phenomenon is very infrequent and its mechanism is not known (Bhagat 2008).

There is insufficient experience on the efficacy and safety of therapy with interferon and ribavirin in coinfecting transplant patients. One study (Miró 2007) summarized the reports evaluating the effectiveness of HCV reinfection treatment in OLT with PEG-IFN plus ribavirin (Fung 2004; Duclos-Vallee 2006; De Vera 2006; Venarecci 2006; Castells 2007; Miró 2007). These patients were treated when they had histological criteria. Only 12 (18.5%) out of 65 HCV/HIV-coinfecting patients achieved an SVR (Table 3).



| Author Year of Publication (Reference) | HIV/HCV coinfecting patients |                            | Non-HIV HCV-monoinfected patients (Control Group) |                            |
|--|------------------------------|----------------------------|---|----------------------------|
|  | No. of cases                 | SVR <sup>a</sup><br>No (%) | No. of cases                                      | SVR <sup>a</sup><br>No (%) |
| Fung 2004                              | 12                           | 2 (17%)                    | -   | -                          |
| Duclos-Vallee 2006                     | 13                           | 2 (15%)                    | -   | -                          |
| de Vera 2006 <sup>b</sup>              | 15                           | 4 (27%)                    | 27  | 7 (28%) <sup>c</sup>       |
| Vennarecci 2006 <sup>d</sup>           | 9                            | 0 (0%)                     | -   | -                          |
| Castells 2007 <sup>e</sup>             | 5                            | 1 (20%)                    | 9   | 1 (11%)                    |
| Spanish study 2007                     | 16                           | 4 (25%)                    | -   | -                          |
| Total                                  | 65                           | 12 (18.5%)                 | -   | -                          |

<sup>a</sup>SVR: sustained virologic response; <sup>b</sup>Most cases were genotype 1. Three patients were treated with classic interferon plus ribavirin; <sup>c</sup>Rate of sustained virologic response was not specified. Data show the rate of virologic response (clearance of HCV RNA from serum); <sup>d</sup> The authors did not specify the type of interferon used; and, <sup>e</sup>These patients were included in the Spanish study and were not taken into account for the overall response rate. Miro et al. *J HIV ther.* 2007; 12(1):24-35.

**Table 3. Summary of studies evaluating the effectiveness of the treatment of HCV re-infection in OLT with pegylated interferon plus ribavirin.**

Krishnan et al. investigated SVR-associated factors in 23 HIV/HCV coinfecting patients who underwent OLT and found that donor age <60 years ( $p = 0.03$ ), genotype other than 1 ( $p = 0.001$ ), and use of Cya ( $p = 0.002$ ) were correlated to SVR. In a logistical regression analysis, viral genotype other than 1 ( $\text{ExpB} = 7.25$ , 95% CI = 2.14-24.49,  $p = 0.001$ ), the use of Cya while on treatment ( $\text{ExpB} = 7.05$ , 95% CI = 2.03-24.51,  $p = 0.002$ ), and donor age <60 years ( $\text{ExpB} = 4.16$ , 95% CI = 1.22-14.22,  $p = 0.02$ ) were independent predictors of SVR (Krishnan 2008).

New strategies are necessary to improve the outcome of HCV recurrence post-OLT. A recent German study showed that SVR was achieved in 6 out of 7 patients treated in the first three months after OLT (Emmelkamp 2007).

Cohorts of HIV/HBV-coinfecting patients are not as large as HIV/HCV-infected cohorts; nevertheless, the outcome of HBV infection after OLT is much better (Terrault 2006; Coffin 2007; Grossi 2008). Probably due to the low incidence of HBV reinfection, the survival rate in the short- and medium-term in HBV/HIV coinfecting patients is high and similar to that observed in HBV monoinfected patients.

Regarding HCC, preliminary Italian experience shows good results in seven HIV-1 infected patients with HCC who underwent OLT. They observed an 86% overall patient and graft survival rate after a mean follow-up period of 8 months. They recommend OLT in HIV-infected patients with early stage HCC (Di Benedetto 2006; Di Benedetto 2008).

## Conclusions

ESLD is becoming a more frequent clinical scenario in the setting of HIV/HCV coinfection and its importance has been on the rise since ART has become 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-infected patients must 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 liver disease.

OLT is a life-saving procedure in this population. It is a safe and effective therapeutic tool in patients with HBV infection. On the other hand, HCV reinfection could play a negative role, affecting graft and mid- and long-term survival in HIV/HCV-coinfected patients. Prospective and larger studies with a longer duration need to 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.
- Arroyo V, Fernandez J, Ginés P. Pathogenesis and treatment of hepatorenal syndrome. *Semin Liver Dis*. 2008 Feb;28(1):8.
- Back D, Gibbons S. The University of Liverpool HIV drug interactions website. Available at: [http://www.hiv-druginteractions.org/frames.asp?pharmacology/pharma\\_main.asp](http://www.hiv-druginteractions.org/frames.asp?pharmacology/pharma_main.asp). June 2008
- 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
- Benhamou Y, Di Martino V, Bochet M et al. Factors affecting liver fibrosis in HIV and hepatitis C coinfecting patients: impact of protease inhibitor therapy. *Hepatology* 2001; 34: 283–287.
- 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.
- 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
- Castells L, Escartin A, Bilbao I, et al. Liver Transplantation in HIV-HCV Coinfected Patients: A Case-Control Study. *Transplantation*. 2007; 83:354-358

- 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
- Clumeck N, Pozniak A, Raffi F; EACS Executive Committee. European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of HIV-infected adults. *HIV Med.* 2008 Feb;9(2):65-71.
- Coffin CS, Berg CL, Dove LM. Survival and risk of hepatitis B virus (HBV) recurrence in HIV-HBV coinfecting 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 transplant patients coinfecting 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 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.
- Di Benedetto F, De Ruvo N, Berretta N, et al. Hepatocellular carcinoma in HIV patients treated by liver transplantation. *EJSO* 34 (2008) 422e427
- Duclos-Vallee JC, Teicher E, Feray C, et al. Liver Transplantation of HIV-HCV and HIV-HBV Coinfecting Patients: A Large Experience in a Single Centre. *Liver Transpl* 2006, 12, No. 5 C-103.
- Duclos-Vallée J, Féray C, Sebah M, et al. Survival and recurrence of hepatitis C after liver transplantation in patients coinfecting with human immunodeficiency virus and hepatitis C virus. *Hepatology.* 2008 Feb;47(2):407-17
- 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 and efficacy. *Transplant Proc* 2006; 38(10):3544-51
- 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 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.

## 388 Liver transplantation in hepatitis B and C and HIV coinfection

- Grossi P, Gabbriellini 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
- 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
- Hezode C, Zafrani ES, Roudot-Thoraval F, et al. Daily cannabis use: a novel risk factor of steatosis severity in patients with chronic hepatitis C. *Gastroenterology*. 2008 Feb;134(2):432-9.
- 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 Feb;46(2):206-12 )
- 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 Euro-SIDA 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 coinfecting candidates: what is the most appropriate time for evaluation? *AIDS Res Hum Retroviruses* 2005; 21: 599-601.
- 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
- 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
- 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-coinfecting patients. *AIDS Rev*. 2007; 9:131-9
- Miro JM, Murillas J, Laguno M et al. Natural history and prognosis of end stage liver disease (ESLD) in Spanish HIV-1 infected patients: A prospective cohort study of 104 pa-

- tients (1999-2004). 10th European AIDS Conference. Dublin (Ireland). November 17-20, 2005. Abstract # PS7/1.
- 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).
- 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.
- Miró 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 coinfecting 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, Aguero F, Laguno M et al. Liver transplantation in HIV/Hepatitis coinfection. *J HIV ther*. 2007; 12(1):24-35.
- Miró JM, Montejo M, Castells L et al. Prognostic Factors of Mortality in HCV-HIV-Coinfected Liver Transplant Recipients From the FIPSE OLT-HIV-05 - GESIDA 45-05 Cohort Study. 15th Conference on Retroviruses and Opportunistic Infections (CROI 2008) February 3-6, 2008, Boston. Abstract P1062.
- Mohsen AH, Easterbook PJ, Taylor 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
- 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.
- Neff GW, Bonham A, Tzakis AG et al. Orthotopic liver transplantation in patients with human immunodeficiency virus and end-stage liver disease. *Liver Transpl*. 003;9(3):239-47.
- 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.
- Norris S, Houlihan D. Liver transplantation in HIV-positive patients. *Expert Rev Gastroenterol Hepatol* 2008, 2, 39-46
- 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 de expertos de GESIDA; Plan Nacional sobre el Sida. Recommendations from the GESIDA/Spanish AIDS Plan regarding antiretroviral treatment in adults with human immunodeficiency virus infection (update January 2007)]. *Enferm Infecc Microbiol Clin*. 2007; 25:32-53.
- 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, 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

## 390 Liver transplantation in hepatitis B and C and HIV coinfection

- 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 Nov;11(11):1425-30
- 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.
- 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* 008 Feb;9(2):82-8.
- Roland ME, Stock PG. Review of solid-organ transplantation in HIV-infected patients. *Transplantation* 2003;75: 425-429.
- Roland M, Stock PG. Liver Transplantation in HIV-Infected Recipients. *Seminar in Liver Disease.* 2006; 26:273-284.
- Roland M, Carlson L, Ragni M et al. Solid organ transplantation in HIV-infected recipients: 47 cases in the HAART era (Abstract MoOrB1060). En: XIV International AIDS Conference. Barcelona, Spain 2002.
- 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
- 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 transplantation 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 coinfecting 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 et al. MELD is the Best Predictor of Pre-Transplant Mortality in HIV-Infected Liver Transplant Candidates. 15th CROI Conference on Retroviruses and Opportunistic Infections Boston, MA. Feb 3-6, 2008. Abstract 64
- 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 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
- Tuset M, Miró JM, Codina C, Ribas J, Ed. Guía de interacciones en HIV. Available at: <http://www.interaccionesHIV.com>. June 2008
- 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 (May), 2006: pp C-115.
- Wyles DL, Gerber J. Antiretroviral drug pharmacokinetics in hepatitis with hepatic dysfunction. *Clin Infect Dis* 2005;40:174-181.

**The members of the Hospital Clinic OLT in HIV Working Group are:**

JM Miró, A. Rimola, A. Moreno, M. Laguno, M. Larrousse, JL Blanco, J Mallolas, C. Cervera, M. Tuset, M. Monras, N. Freixa, M. Brunet, J. Blanch, C. Lanaspa, E. de Lazzari, JC García-Valdecasas, JM Gatell (Hospital Clinic - IDIBAPS. University of Barcelona, Barcelona); C. Tural and D. Fuster (Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain); and, J. Murillas and M. Riera (Hospital Son Dureta, Palma de Mallorca, Spain).





## Part 8

# Autoimmune and Metabolic Liver Disease



## 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.

Spontaneous course of HFE haemochromatosis

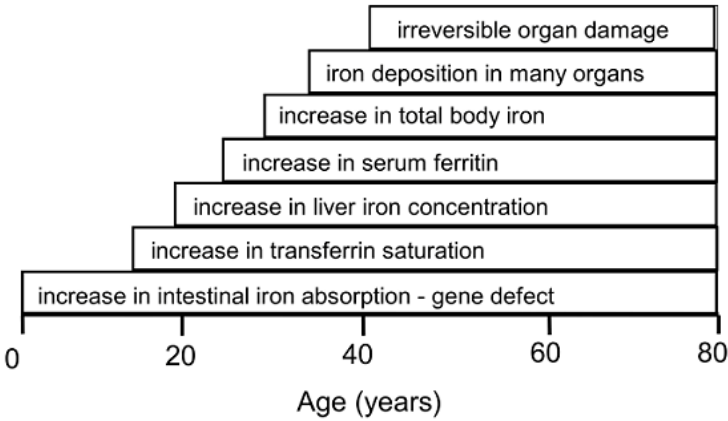


Figure 1. Scheme of natural history of type 1 genetic haemochromatosis.

I) Genetic haemochromatosis

| Types    | Gene defect on | Affected gene          | Inheritance         | High prevalence         |
|----------|----------------|------------------------|---------------------|-------------------------|
| type 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

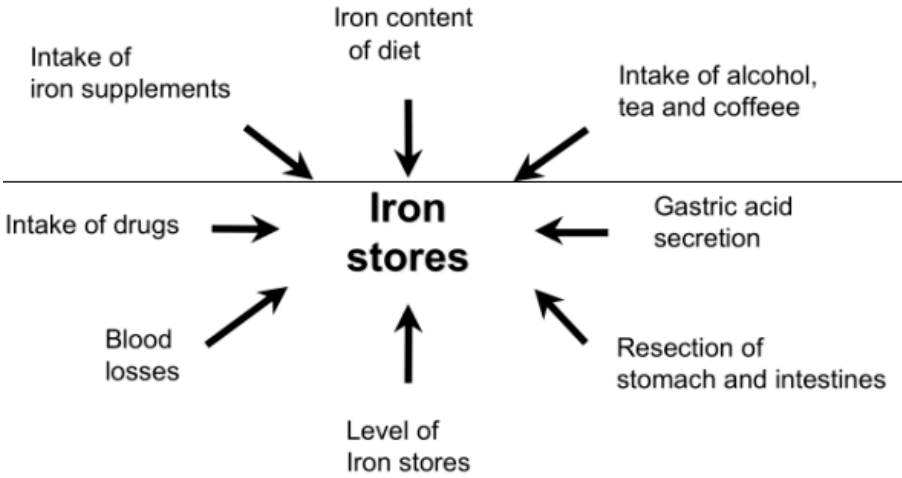
## History

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.

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.



**Figure 2.** Non-genetic factors that may influence iron absorption.

| 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 nor-

mal or only slightly elevated transferrin saturation. Therefore, transferrin saturation should be measured in order to correctly interpret ferritin increases.

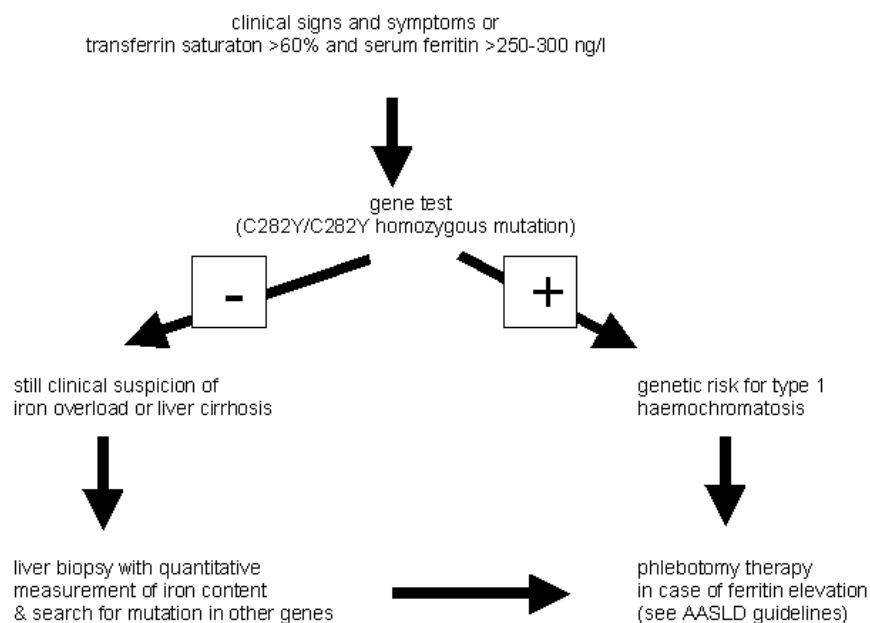
**Liver biopsy and determination of liver iron concentration.** Although simultaneous increases of both serum ferritin and transferrin saturation strongly indicate a risk for haemochromatosis, diagnosis needs to be confirmed by genetic testing or by liver biopsy with a determination of iron content in 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.

**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.





**Table 3. Diagnosis and treatment algorithm for type 1 haemochromatosis.**

**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 dis-



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.

**1. Screening in the general population not recommended**

A screening of HFE gene alterations is not recommended for the general population because it remains unknown how many of the C282Y homozygotes will develop clinical manifestations. Such screening would be meaningful only in Caucasian populations.

**2. Family screening**

Genetic testing can reliably determine who, among the first-degree relatives of a haemochromatotic 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 amenorrhoea of unknown aetiology

**4. Every liver biopsy needs to be checked for iron deposits**

**Table 4. Methods for early diagnosis of haemochromatosis.**

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 (Niederer 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.

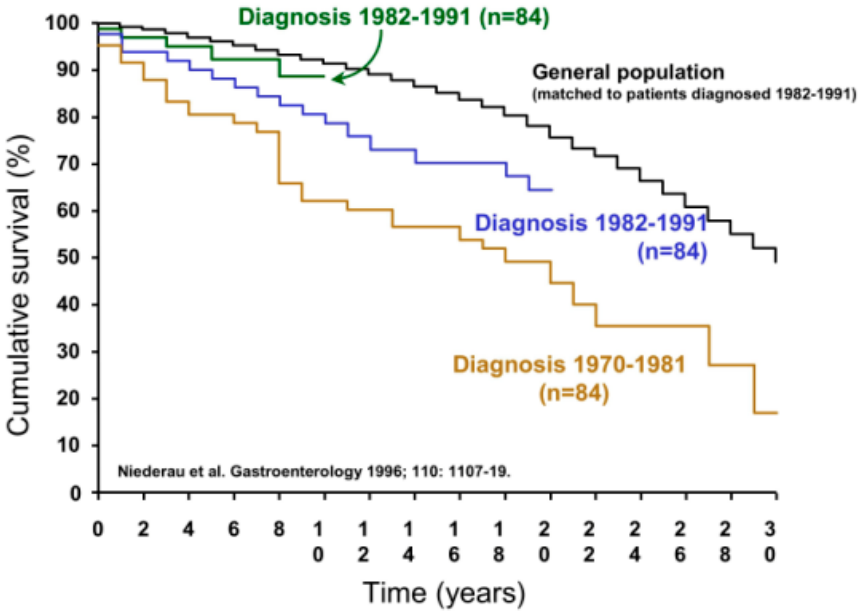


Figure 4. Cumulative survival in 251 patients with genetic haemochromatosis according to the time of diagnosis. Modified from Niederau 1996.

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

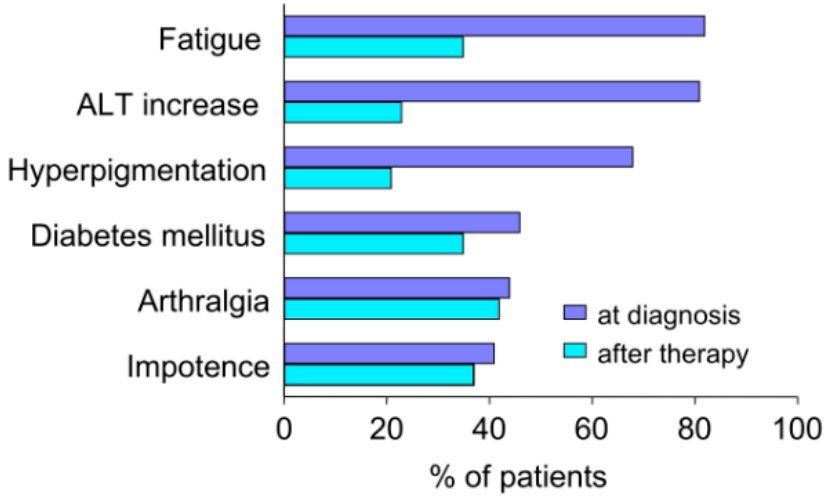
- recommended: avoidance of food with very high iron content (e.g., liver) and iron-supplemented food ;  
a further strict iron-depleted diet is very difficult to adhere to and not recommended
- a single phlebotomy of 500 ml blood is as effective for iron removal as a very rigid iron-restricted diet for a whole year

**Table 5. Therapy of iron overload**

## 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).



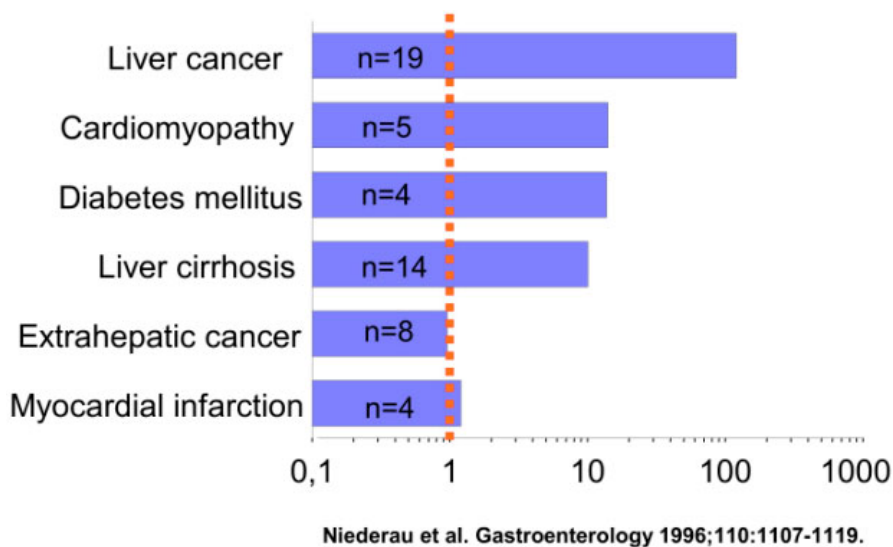
Niederau et al. *Gastroenterology* 1996;110:1107-1119.

**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 Niederrau 1996.

*Diabetes mellitus.* In recent studies the prevalence of diabetes in hereditary haemochromatosis ranges from 20-50% (Niederrau 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 (Niederrau 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 (Niederrau 1985; Niederrau 1996) whereas insulin-dependent diabetes is irreversible (Niederrau 1996). Survival is significantly reduced in patients with diabetes mellitus at diagnosis compared to patients without diabetes (Niederrau 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 amenorrhea 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 metacarpophalangeal joints II and III, in the form of cystic and sclerotic changes, cartilage damage and a narrowing of the intra-articular 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 be 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 amenorrhea 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 porphyria 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 hypogonadism 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 (hemojuvelin mutations) and type 2B (HAMP mutations). Mutations in hemojuvelin are associated with low levels of hepcidin in urine suggesting that hemojuvelin 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

## 414 Metabolic Liver Diseases: Haemochromatosis

- 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. Hcpidin 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, Ekblom 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. *Medicine* 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 syndromes clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2006;4:58-77
- Hanot V, Schachmann M. Sur la cirrhose pigmentaire dans le diabète 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. *Hepato 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 synthetic oral chelator: evaluation in hypertransfused rats with selective radio iron probes of hepatocellular and reticulo-endothelial 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

## 416 Metabolic Liver Diseases: Haemochromatosis

- 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 desferriethiocin to ICL670. *Curr Med Chem* 2003;10:1065-1076
- Niederau C, Berger M, Stremmel W, et al. Hyperinsulinaemia in non-cirrhotic haemochromatosis: 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, Joesse 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. Hemochromatosis. Oxford University Press, 1935, London.
- Short EM, Winkle RA, Billingham ME. Myocardial involvement 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

## 418 Metabolic Liver Diseases: Haemochromatosis

- 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 steatohepatitis (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).

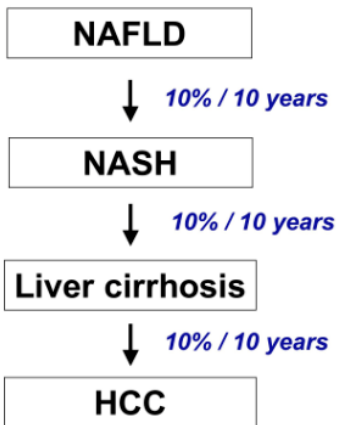


Figure 1. Natural history of NASH.

HAIR index (hypertension; ALT >40 U/L; insulin resistance)

- $\geq 2$  are 80% sensitive, 89% specific for NASH (Dixon 2001)

BAAT index (BMI >28; Age >50 years; ALT >2x UNL; increased triglycerides)

- $\leq 1$  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) (Table 4). 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 than 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).

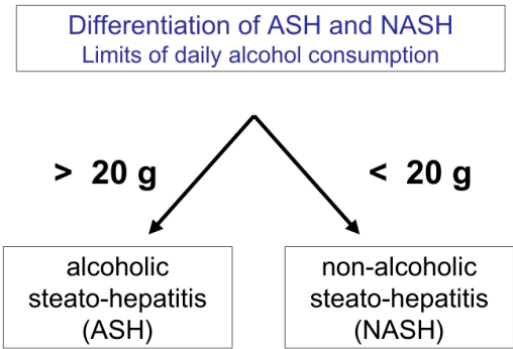


Figure 2. Differentiation of alcoholic liver disease (ASH) and NASH.

**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  $\geq 102$  cm for men and  $\geq 88$  cm for women,
- fasting glucose level  $\geq 6.1$  mmol/L,
- triglyceridemia  $\geq 1.7$  mmol/L,
- increase in high-density lipoprotein cholesterol ( $>1.3$  mmol/L in women;  $>1.03$  mmol/L in men)
- hypertension  $\geq 135/80$  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 (Table 3) (Dixon 2001; Ratzu 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.

| A. Grade | Steatosis | Ballooning of hepatocytes | Degree of inflammation            |
|----------|-----------|---------------------------|-----------------------------------|
| 1        | <33%      | Minimal                   | Mild                              |
| 2        | 34-66%    | Present                   | Moderate                          |
| 3        | >66%      | Marked                    | Portal moderate, lobular moderate |

| B. 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 (Table 5). 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.

## 426 NAFLD and NASH

- 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, non-alcoholic 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, Houghlum K, Chojkier M. Activation of hepatic stellate cells by TGF $\alpha$  and collagen type I is mediated by oxidative stress through c-myc 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 steatohepatitis 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, McCullough 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.

## 428 NAFLD and NASH

- 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.

### 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).



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 symptoms 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).

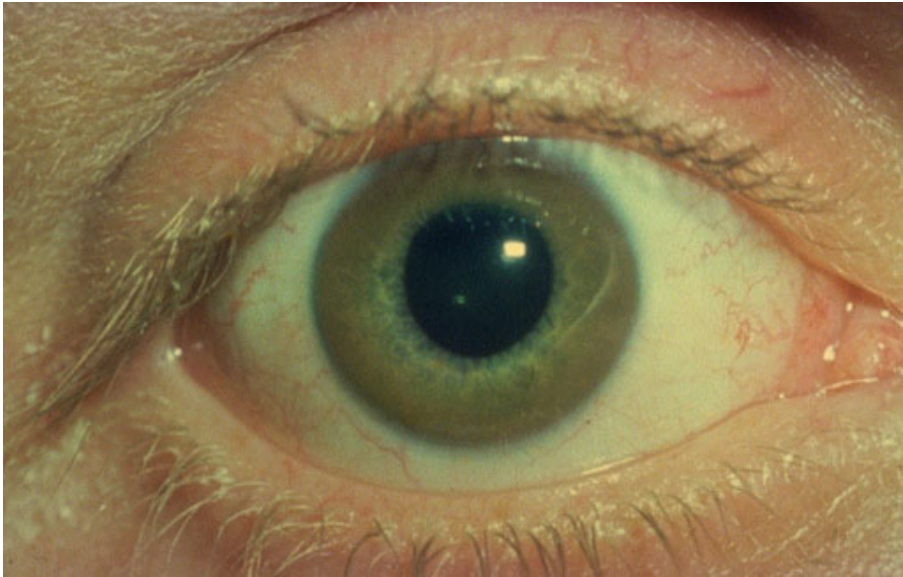
WD has to be excluded in patients with fulminant liver failure of unknown aetiology, especially at ages under 35 years; WD patients with such presentation usually have some sort of liver disease (Rector 1984; Ferlan-Maroult 1999; Roberts 2003) associated with coombs-negative hemolytic anemia and severely 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]).

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).



**Figure 3. Kayser-Fleischer ring in a patient with WD.**



## 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 somewhat 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 µg/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 µg/dL in most untreated patients (normal values are

below 10-15  $\mu\text{g/dL}$ ). The amount of copper associated with ceruloplasmin is 3.15  $\mu\text{g}$  of copper per mg of ceruloplasmin. Thus free copper is the difference between the total serum copper in  $\mu\text{g/dL}$  and 3 times the ceruloplasmin concentration in  $\text{mg/dl}$  (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  $\mu\text{g}/24$  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  $\mu\text{g}/24$  h (Steindl 1997; Giachino 1997; 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  $\mu\text{g}$  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  $\mu\text{g/g}$  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).

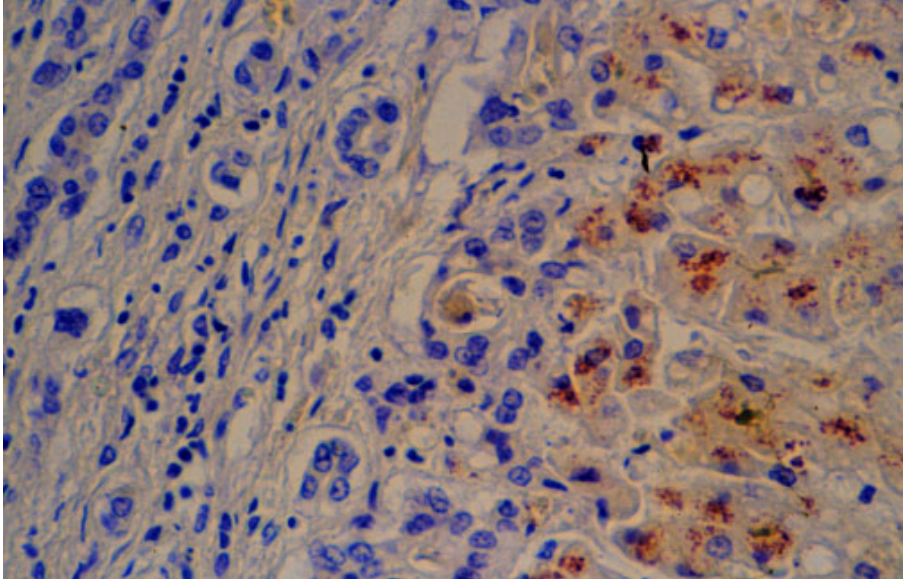
### **Radiolabelled copper**

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 rhodamine using routine histochemistry does not allow a diagnosis of WD (Geller 2000) (Figure 4).



**Figure 4.** Liver histology (rhodamine staining for copper) in a WD patient.

## 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 dysphagia. 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.

## 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](http://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.

penicillamine (600-1800 mg/day)

in case of intolerance to penicillamine:

trientine (900-2400 mg/day)

for combination or maintenance:

zinc acetate/sulfate

for neurologic disease - not yet approved:

tetrathiomolybdate

in acute liver failure/decompensated cirrhosis:

liver transplantation

restriction of food with high copper content

(does not substitute for chelators or zinc!)

**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 (Brewer 1987; 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 **serious** retinitis due to administration of penicillamine.

In order to minimize its side effects penicillamine 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 µg/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 divided 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 µg 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 ug/day under 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.

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 µg; 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.



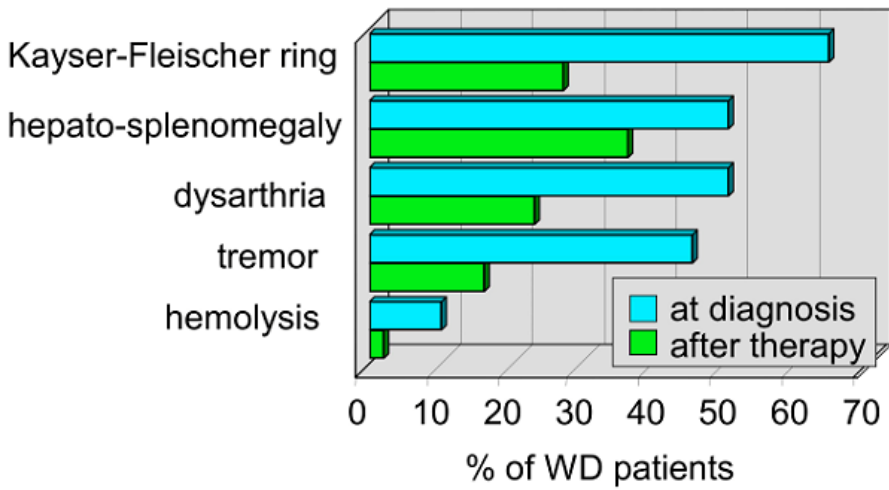


Figure 7. Findings prior to and after beginning chelating therapy in 53 WD patients (modified from [Stremmel 1991]).

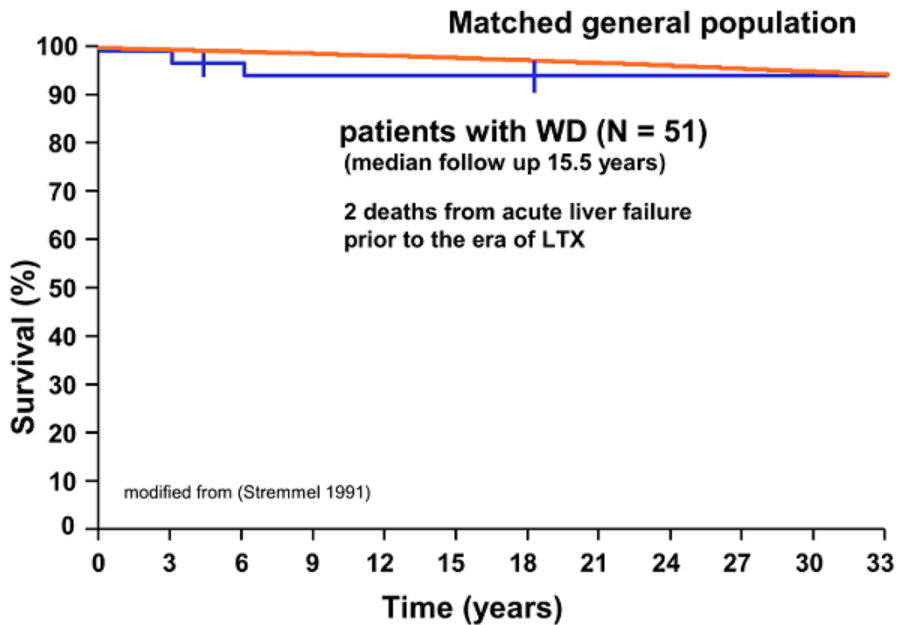


Figure 8. Cumulative survival in 51 WD patients versus a matched general population (modified from [Stremmel 1991]).

## 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, Gavalier 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.
- Cummings 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, Czlonkowska 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. Treatment of 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.

## 444 Wilson's Disease

- 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, Katsiyannakis 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 homology to 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 Wassenaeer-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 vitamin 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 triethylene 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.

## 446 Wilson's Disease

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 (Mackay 1956). 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 sero-immunological 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).

**Table 1. International criteria for the diagnosis of autoimmune hepatitis (Alvarez 1999).**

| 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, $\gamma$ -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)  |       |
| <25 gm/day   | + 2   |
| >60 gm/day   | - 2   |
| Genetic factors: HLA DR3 or DR4                                      | + 1   |
| Other autoimmune diseases  | + 2   |
| Response to therapy  |       |
| complete   | + 2   |
| relapse  | + 3   |
| Liver histology  |       |
| interface hepatitis  | + 3   |
| predominant lymphoplasmacytic infiltrate                             | + 1   |
| rosetting of liver cells   | + 1   |
| none of the above  | - 5   |
| biliary changes  | - 3   |
| other changes  | -3    |
| Seropositivity for other defined autoantibodies                      | + 2   |

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.



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).

**Table 2. Differential diagnosis of autoimmune hepatitis and diagnostic tests.**

|                                      |  |
|--------------------------------------|--|
| Suspected differential diagnosis:    | Test performed to exclude:                           |
| hepatitis C infection (HCV)          | anti HCV ( HCV RNA)                                  |
| hepatitis B and D (HBV, HDV)         | -HBsAg, anti HBc (HBV DNA)                           |
| hepatitis A virus (HAV)              | -anti-HDV, HDV RNA only when HBsAg positive          |
| hepatitis E virus (HEV)              | antibodies, serology: IgG, IgM                       |
| Epstein-Barr virus (EBV)             | only if suspected                                    |
| herpes simplex virus (HSV)           | only if suspected                                    |
| cytomegaly virus (CMV)               | only if suspected                                    |
| varicella zoster virus (VZV)         | only if suspected                                    |
| drug induced hepatitis               | -history, if applicable, withdrawal of drug          |
| primary biliary cirrhosis (PBC)      | -LKM-2, LM autoantibody in selected cases            |
| primary sclerosing cholangitis (PSC) | -anti-mitochondrial antibodies (AMA)                 |
| Wilson's Disease                     | specificity of reactivity: PDH-E2, BCKD-E2           |
| hemochromatosis                      | -liver histology: copper deposition in bile ducts    |
| alpha-1-antitrypsin deficiency       | -unresponsive to steroids                            |
|                                      | cholangiography                                      |
|                                      | Coeruloplasmin, urine copper, eye examination,       |
|                                      | quantitative copper in liver biopsy                  |
|                                      | serum ferritin, serum iron, transferrin saturation,  |
|                                      | liver histology: iron staining, quantitative iron in |
|                                      | biopsy   |
|                                      | genetic testing: C282Y, H63D mutation of HFE         |
|                                      | gene in Caucasoids                                   |
|                                      | Phenotype testing: PiZZ/PiSS/PiMZ/PiSZ               |

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).

**Table 3. Extrahepatic associations of autoimmune hepatitis are present in 10% to 50% of patients.**

**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

Autoantibodies are one of the distinguishing features of AIH. The discovery of autoantibodies directed against different cellular targets (Table 4), including endoplasmic 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).

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.

**Table 4. Heterogeneity of autoimmune hepatitis based on serological findings:** Molecular definitions of the most important autoantigens in serological diagnostics.

| Antibody  | kDa   | Target antigen                            | Disease   |
|---|-------|---|---|
| autoantigens of the endoplasmic reticulum (microsomal autoantigens) |       |   |   |
| LKM-1   | 50    | Cytochrome P450 2D6                       | autoimmune hepatitis Typ 2<br>hepatitis C   |
| LKM-2   | 50    | Cytochrome P450 2C9                       | ticrynafen-induced hepatitis  |
| LKM-3   | 55    | UGT1A                                     | -hepatitis D-associated autoimmunity<br>-autoimmune hepatitis type 2                                      |
| LKM   | 50    | Cytochrome P450 2A6                       | -autoimmune polyendocrine syn-<br>drome type 1 (APS-1)<br>-Hepatitis C                                    |
| LM  | 52    | Cytochrome P450 1A2                       | -Dihydralazine-induced hepatitis<br>-Hepatitis with autoimmune polyendo-<br>crine 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 cy-<br>clocleaminase | autoimmune hepatitis type 2<br>autoimmune hepatitis<br>hepatitis C?                                       |
| SLA/LP  | 50    | UGA repressor tRNA-<br>associated protein | autoimmune hepatitis (type 3)   |
|   |       |   |   |

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 ac-

counts 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 endoplasmic 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 polyglandular 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 likelihood 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.

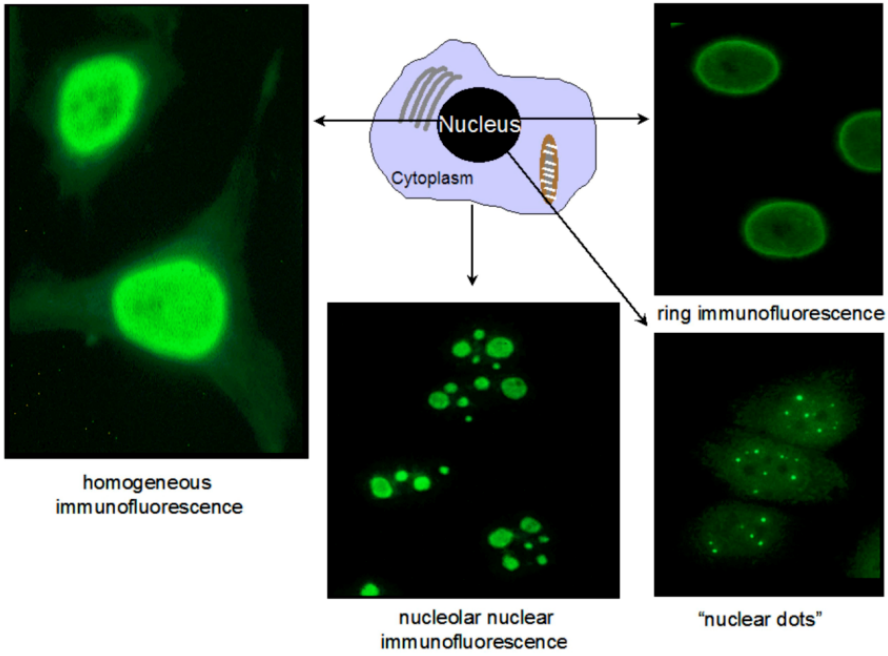
## Autoantibodies in AIH

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).

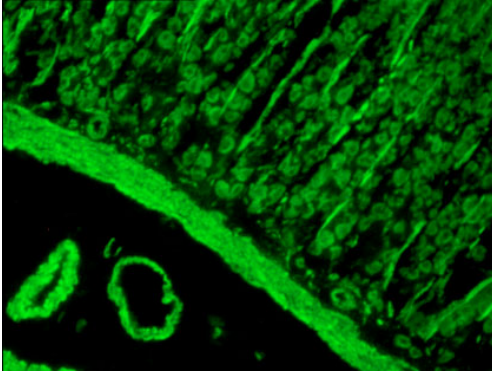
Antinuclear antibodies (ANA) are directed against functional and structural components of the cell nucleus, against nuclear membranes or DNA. The target antigens are a heterogeneous and incompletely defined group of cellular proteins (Tan 1988). To date, subtyping of the various ANA antigens offers no 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 centromeres, 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.

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.

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).

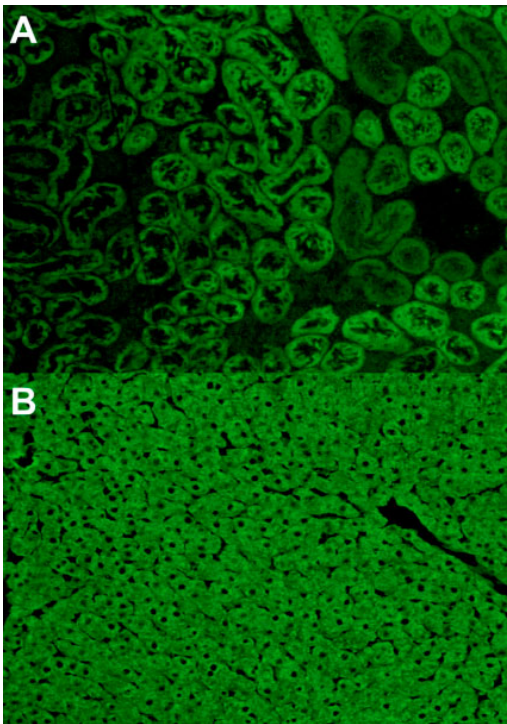


**Figure 1. Indirect immunofluorescence micrographs of a variety of ANA found in autoimmune hepatitis and other autoimmune diseases, detected on immobilized Hep.2 cells.** Aspect of the nuclear membranous (rim) immunofluorescence pattern (top right) found in a patient with autoimmune hepatitis type 1 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).



**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.

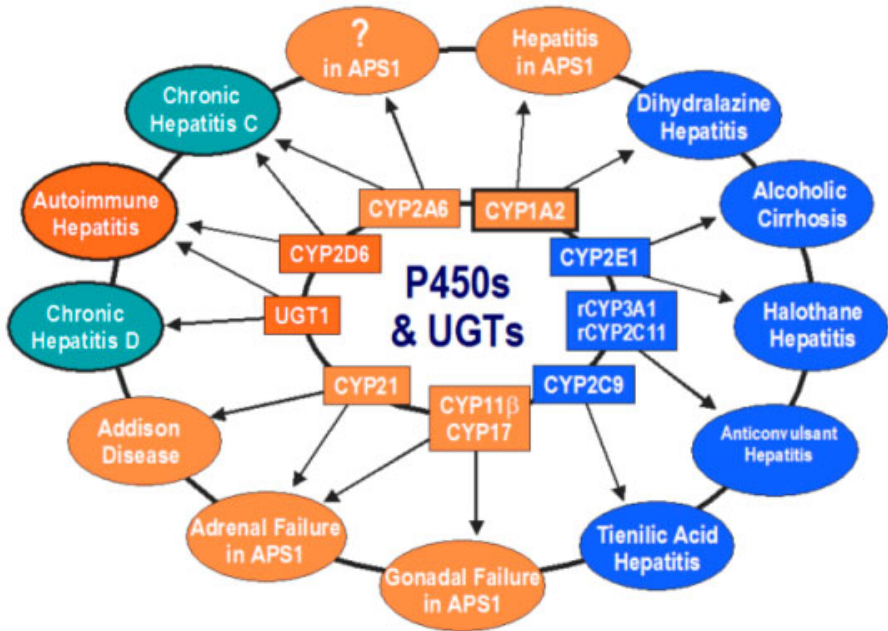


**Figure 3. Indirect immunofluorescence showing LKM-1 autoantibodies on rat kidney and liver cryostat sections.**

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 1991; 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). 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 additionally occur 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.





**Figure 4. Diversity of autoantibodies against endoplasmic 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.**

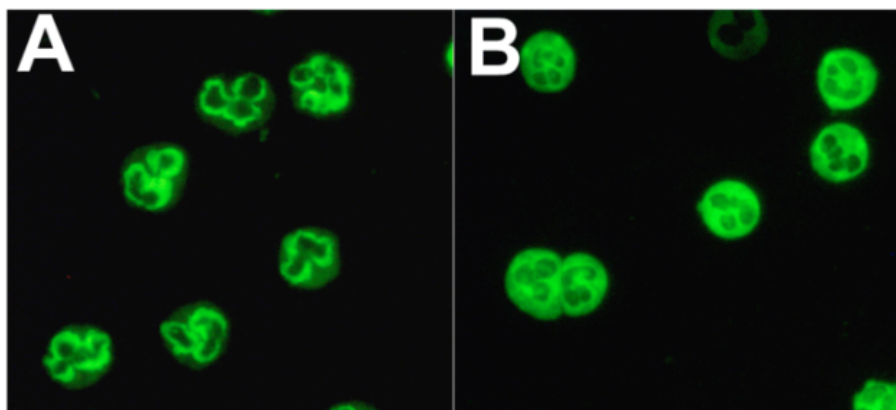
Antibodies against soluble liver antigen (SLA) were detected in a patient with ANA-negative AIH (Manns 1987). It is now clear that the description of liver pancreas (LP) antibodies recognize the same target protein structure leading to the designation SLA/LP autoantibodies (Stechemesser 1993; Wies 2000). 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 selenocysteine incorporation in human cells. Subsequently, SLA/LP antibodies have been identified as being directed against a UGA suppressor serine tRNA-protein complex, and not against cytokeratins 8 and/or 18 or glutathione S transferases, as previously suggested. The exact function and role of this autoantigen in autoimmunity are so far unclear. Regarding the disease specificity, anti-SLA/LP may be linked to the pathogenesis of the autoimmune process.

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 by the more diffuse pattern of LKM-1 antibodies. The antigen recognized by anti-LC1 was identified as formimi-

notransferase 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 hepatitis, drug-induced hepatitis and PBC. Levels of anti-asialoglycoprotein antibodies correlate with inflammatory disease activity and might be used as an additional marker to monitor treatment efficacy.

Antibodies to neutrophil cytoplasmic antigens (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 immunofluorescence, which distinguishes two patterns: cANCA with a diffuse cytoplasmic staining of neutrophils, and pANCA, which exhibit a rim-like staining of the perinuclear cytoplasm. In AIH, atypical pANCA (also termed xANCA) are usually found that display a pANCA immunofluorescence pattern but do not show reactivity with myeloperoxidase, one of the major autoantigens of classical ANCA. The discrimination of ANCA is difficult, because ANA frequently also stain ethanol-fixed neutrophils. The target antigen 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 cytoplasmic 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 TNF $\alpha$  and  $\beta$ , 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- $\alpha$  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). 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.

|   |                         |  |
|---|-------------------------|--|
| HLA   | DR3                     | DR4                                    |
| Genotype                                      | DR B1*0301              | DR B1*0401<br>(DR B1*0405 in Japanese) |
| Age at onset                                  | <30                     | >40                                    |
| Disease activity                              | +++                     | +                                      |
| Treatment response                            | ++                      | ++++                                   |
| Relapse after treatment                       | +++                     | +                                      |
| Liver transplantation                         | +++                     | +                                      |
| DR $\beta$ chain amino acid as<br>Risk factor | lysine at amino acid 71 | ?                                      |

**Table 6. Heterogeneity of autoimmune hepatitis based on genetic markers.**

## 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 weak 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 retrospective 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 oesophageal 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



demographic 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, PSC 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 prednisolone 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.





466 Autoimmune liver diseases: AIH, PBC and PSC

|                |  |  |
|----------------|--|--|
|                | <u>A. monotherapy</u>  | <u>B. combination therapy</u>  |
| Prednis(ol)one | 60 mg<br>Reduction within 4 weeks to<br>maintenance dose 20 mg<br>or lower                               | 30 mg<br>Reduction within 4 weeks to<br>maintenance dose 10 mg<br>or lower |
| Azathioprine   | n.a.   | 50 mg  |
|                | (maintenance with azathioprine:<br>monotherapy: 2 mg/kg body weight)                                     |  |
|                | <u>after remission is reached (treatment length 12-24 Months, histology, avoid premature reduction):</u> |  |
| Prednis(ol)one | Reduction of daily dose by 2.5 mg per week   |  |
| Azathioprine   | n.a.   | Reduction: 25 mg every 3 weeks   |

| 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               | +              | +   | +                                    | +                                    | +                                   |                       |
| Aminotransferases         | +              | +   | +                                    | +                                    | +                                   | +                     |
| Gamma glutamyltransferase | +              | +   | +                                    |                                      |                                     |                       |
| Gammaglobulin             | +              | +   | +                                    | +                                    | +                                   | +                     |
| Bilirubin                 | +              | +   | +                                    | +                                    | +                                   | +                     |
| Coagulation studies       | +              | +   | +                                    | +                                    | +                                   |                       |
| Autoantibodies            | +              | +/-                                       |                                      |                                      |                                     | +                     |
| Thyroid function tests    | +              | +/-                                       |                                      |                                      |                                     | +                     |

n.a. - not applicable. In the elderly patient with low inflammatory activity the indication to treat must be weighed against side effects - 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.

**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 recurrence 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 lipophilic cyclic peptide of 11 residues produced by *Tolypocladium inflatum* that acts on calcium-dependent signaling and inhibits T-cell function via the interleukin 2 gene. Of all 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; Debray 1999). 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; Debray 1999; 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 cir-

rhosis, bleeding esophageal varices 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 transplant 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 (Kerker 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 anti-mitochondrial 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 de-

velop 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. Extrahepatic immune-mediated syndromes in PBC 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). Immunogenetic 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  $\alpha$  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 glutamyltransferase 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 syn-

drome. Extrahepatic syndromes frequently precede hepatic disease manifestation. In later stages pronounced fatigue, pruritus, marked hyperbilirubinemia and the consequences of portal hypertension 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 antibody reactivity (Strassburg 2004).

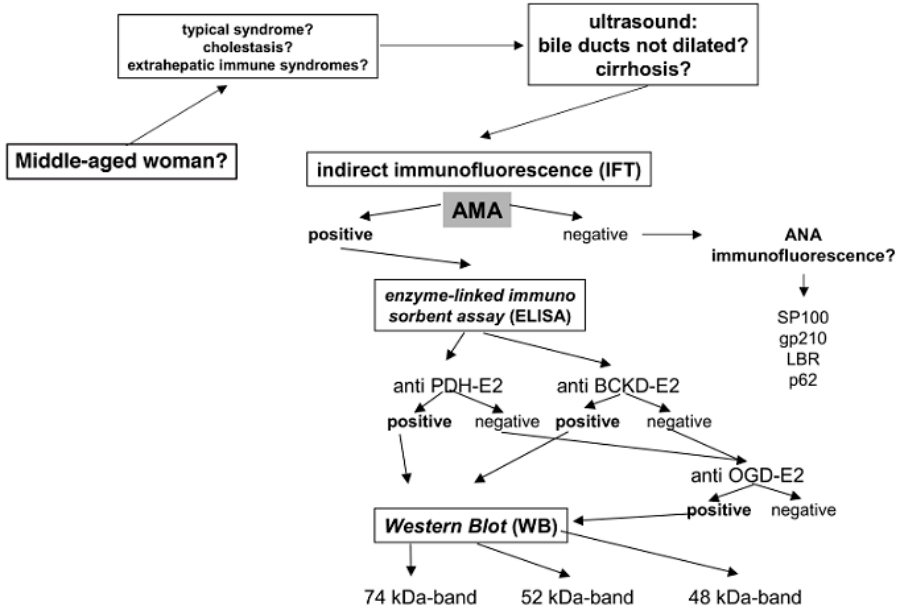
### **Pathophysiology of PBC**

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 antimitochondrial 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).





**Figure 6.** Diagnostic algorithm of PBC including clinical presentation, ultrasound and serology.

## 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 mitochondrial 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; Lindenborn-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).

|   | kDa                           | occurrence | old M-classification |      |
|---|-------------------------------|------------|----------------------|------|
| <u>PYRUVATE DEHYDROGENASE (PDH)</u>                 |                               |            |                      |      |
| PDH-E2  | (Pyruvate decarboxylase)      | 74         | 95%                  | M2 a |
| PDH-E1 $\alpha$                                     | (Pyruvate decarboxylase)      | 41         | 41-66%               | M2 d |
| PDH-E1 $\beta$                                      | (Pyruvate decarboxylase)      | 36         | 2-7%                 | M2 e |
| Protein X   | (Lipoid pomponent of PDH)     | 52         | 95%                  | M2 c |
| <u>BRANCHED-CHAIN KETOACID DEHYDROGENASE (BCKD)</u> |                               |            |                      |      |
| BCKD-E2   | (Acyltransferase)             | 50         | 53-55%               | M2 c |
| BCKD-E1 $\alpha$                                    | (Acyldecarboxylase)           | 46         | ?                    |      |
| BCKD-E1 $\beta$                                     | (Acyldecarboxylase)           | 38         | ?                    |      |
| <u>KETOGLUTARATE DEHYDROGENASE (KGD)</u>            |                               |            |                      |      |
| 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-E1 $\alpha$  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-M1 autoantibodies (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 classification (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 excluding 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 serological diagnosis of PBC (Bloch 1999; Worman 1994). Autoantibodies against a 210 kDa glycoprotein of the nuclear membrane (gp 210) (Lassoued 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 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 calcium 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 bio-

chemistry, delay portal hypertension and varices, and currently has no therapeutic alternative (Poupon 2003). No convincing effect was demonstrable on osteopenia and extrahepatic manifestations 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 re-appearance 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 hypertension 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).

|                           |  |
|---------------------------|--|
| <b>Cholangiocarcinoma</b> | 10-20% of PSC patients<br>Yearly risk 1.5%<br>Frequent within 1 year of diagnosis<br>Bilirubin, male gender, long standing ulcerative colitis, abdominal symptoms, smoking |
| <b>Colorectal cancer</b>  | 10-fold risk (PSC and ulcerative colitis)<br>Yearly colonoscopies in ulcerative colitis<br>In ulcerative colitis and AP-elevation: consider ERC                            |
| <b>Pancreatic cancer</b>  | 14-fold risk in PSC patients<br>Abdominal ultrasound   |

**Table 13. Cancer association of PSC.**

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).

Sulindac 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 Steenberg 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 cytoplasmic 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 contin-

ues 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 occur 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 elevated 10-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).

|                           |   |
|---------------------------|---|
| <b>Uncomplicated PSC</b>  | Ursodeoxycholic acid 15-30 mg/kg per day          |
| <b>Biliary strictures</b> | interventional endoscopy, dilatations (stents)    |
| <b>Cholelithiasis</b>     |   |
| <b>Cholangitis</b>        | 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 prog-

nosis. However, a number of controversies surround the use of UDCA. In two studies an improvement was documented using 20 mg/kg body weight, and 25-30mg/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 25mg/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 individualized 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 interdiscipli-



- 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.

## 488 Autoimmune liver diseases: AIH, PBC and PSC

- 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
36. Debray D, Maggiore G, Giradet Jp et al. (1999) Efficacy of cyclosporin A in children with type 2 autoimmune hepatitis. *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)
- Farrant 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 JI (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, Gaffer 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

## 490 Autoimmune liver diseases: AIH, PBC and PSC

- Joplin R, Wallace LI, 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
- Kerker 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 Ri 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

## 492 Autoimmune liver diseases: AIH, PBC and PSC

- 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 WI, 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 JI, 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 hepatitis 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, Vrouwenraets 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.

## 494 Autoimmune liver diseases: AIH, PBC and PSC

- 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, Sebah 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
- Stretzell 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

## 496 Autoimmune liver diseases: AIH, PBC and PSC

- 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, Vlegaar 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 Steenberg W, Sciort 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.
- Volkman 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, Hohenauer 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 Hj, 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 HI, 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



## Index

- A**
- ACH-806 ..... 224
- Adefovir ..... 131, 147, 281
- Adverse drug reactions .... see Side effects
- Albinterferon ..... 192, 235
- Amantadine ..... 192, 235
- Amplicor ..... 173
- Antiretroviral treatment ..... 287, 379
- Antisense oligonucleotides ..... 230
- Antiviral resistance testing ..... 115
- ART ..... see Antiretroviral treatment
- Autoimmune haemolytic anaemia ..... 264
- Autoimmune hepatitis ..... 447
- B**
- Belerofon ..... 237
- BILB 1941 ..... 212
- BILN 12202 ..... 212
- BMI-790052 ..... 212
- Boceprevir ..... 212, 221
- resistance ..... 232
- C**
- Celgosivir ..... 212, 233
- Cell culture
- HBV ..... 64
- Ciluprevir ..... 212, 216
- resistance ..... 231
- Cirrhosis
- HBV/HCV coinfection ..... 300
- Hepatitis C ..... 42
- Clevudine ..... 161
- Cobas Ampliprep ..... 173
- Coinfection
- HBV/HCV ..... 297
- HBV/HIV ..... 275
- HCV/HIV ..... 285
- Consensus interferon ..... 192
- Copegus ..... 187
- Cryoglobulinaemia ..... 257
- Cutaneous disorders ..... 265
- Cyclophilin B inhibitors ..... 233
- D**
- Dane particle ..... 57
- Debio-025 ..... 212, 233
- Deferasirox ..... 409
- Delta hepatitis ..... see Hepatitis D
- Diabetes mellitus ..... 265
- Diagnostic tests
- Hepatitis B ..... 113
- Hepatitis C ..... 171
- Dialysis
- HCV treatment ..... 197
- Drug resistance
- HBV ..... 143
- HCV ..... 231
- E**
- Emtricitabine ..... 279
- End stage liver disease ..... 375
- Entecavir ..... 133, 147, 281
- Epidemiology
- Hepatitis A ..... 21
- Hepatitis C ..... 37
- Hepatitis D ..... 157
- Hepatitis E ..... 49
- F**
- Famcyclovir ..... 161
- Ferroporin Disease ..... 411
- Flaviviridae ..... 75
- G**
- Glucosidase inhibitors ..... 233
- GS 9190 ..... 212
- GSK625433 ..... 212
- H**
- HAART ..... see Antiretroviral treatment
- Haemochromatosis ..... 395
- juvenile hereditary ..... 410
- secondary ..... 412
- TFR2-related ..... 411
- HBV ..... see Hepatitis B
- HBV DNA assays ..... 114
- HCC ..... see Hepatocellular carcinoma
- HCV-796 ..... 212, 229
- resistance ..... 233
- HCV-SuperQuant ..... 173
- HDV ..... see Hepatitis D
- Hepadnaviridae ..... 55
- Hepatitis A ..... 21
- prophylaxis ..... 99
- vaccination ..... 101
- Hepatitis B ..... 25
- coinfection with HCV ..... 297
- coinfection with HIV ..... 275
- diagnostic tests ..... 113

- genotyping ..... 115  
 occult infection ..... 299  
 post-exposure prophylaxis ..... 104  
 prophylaxis ..... 99  
 superinfection ..... 298  
 treatment ..... 119  
 vaccination ..... 102  
 virology ..... 55  
 Hepatitis C ..... 37, 169  
   coinfection with HBV ..... 297  
   coinfection with HIV ..... 285  
   diagnostic tests ..... 171  
   extrahepatic manifestations ..... 255  
   prophylaxis ..... 100  
   serologic assays ..... 172  
   superinfection ..... 298  
   treatment ..... 183  
   vaccination ..... 105  
   virology ..... 75  
 Hepatitis D  
   diagnostic procedures ..... 155  
   prophylaxis ..... 99  
   treatment ..... 155  
   virology ..... 156  
 Hepatitis E ..... 49  
 Hepatocellular carcinoma ..... 321  
   curative therapy ..... 324  
   HBV/HCV coinfection ..... 300  
   palliative therapy ..... 326  
 HIV  
   coinfection with HBV ..... 275  
   coinfection with HCV ..... 285
- I**
- IFN  $\alpha$ -2bXL ..... 236  
 IMPDH ..... 234  
 Infergen ..... 187  
 Interferon alpha ..... 162, 183  
 Interferons ..... 127  
 Intron ..... 187  
 IRES inhibitors ..... 230  
 ITMN-191 ..... 212, 223  
   resistance ..... 232
- L**
- Lamivudine ..... 130, 145, 279  
 Life cycle  
   HCV ..... 83, 213  
 Liver cancer  
   prophylaxis ..... 328  
 Liver fibrosis ..... 307  
   surrogate markers ..... 310  
 Liver transplantation. see Transplantation  
 Locteron ..... 236
- Lymphoproliferative disorders ..... 262
- M**
- MK-0608 ..... 212  
 MK-7009 ..... 212
- N**
- NAFLD ..... 419  
 NASH ..... 419  
 Natural history  
   Hepatitis B ..... 29  
   Hepatitis C ..... 42  
 Nitazoxanide ..... 212, 234  
 NS3/4A protease inhibitors ..... 215  
 NS4A inhibitors ..... 224  
 NS5B polymerase inhibitors ..... 226  
 Nucleos(t)ide analogues ..... 129
- P**
- Pathogenesis  
   hepadnavirus infections ..... 61  
   Hepatitis D ..... 159  
 Pegasys ..... 187  
 PEG-IFN ..... 128, 183, 185, 289  
   maintenance therapy ..... 201  
 PEG-Intron ..... 187  
 Pegylated interferon ..... see PEG-IFN  
 Penicillamine ..... 436  
 PF-868554 ..... 212  
 Phlebotomy ..... 409  
 Polymerase inhibitors ..... 226, 279  
 Primary biliary cirrhosis ..... 470  
 Primary sclerosing cholangitis ..... 481  
 Prophylaxis  
   viral hepatitis ..... 99  
 Protease inhibitors ..... 215
- R**
- R1626 ..... 212, 228  
 R7128 ..... 212, 229  
 R7227 ..... 223  
 RealTime HCV ..... 173  
 Rebetal ..... 187  
 Resistance ..... see Drug Resistance  
 Ribavirin ..... 161, 185, 289  
 Ribozymes ..... 230  
 Roferon ..... 187
- S**
- Side effects ..... 245  
   HCV treatment ..... 193  
   IFN ..... 193  
   ribavirin ..... 194  
 Standard IFN ..... 127

|                                 |               |
|---------------------------------|---------------|
| <b>T</b>                        |               |
| Taribavirin .....               | 237           |
| Telaprevir .....                | 212, 217      |
| resistance .....                | 231           |
| Telbivudine .....               | 132, 146, 281 |
| Tenofovir .....                 | 133, 148, 279 |
| Tetrathiomolybdate .....        | 438           |
| Thrombocytopaenia .....         | 263           |
| Thyroid disease .....           | 264           |
| TMC435350 .....                 | 212, 223      |
| Transient elastography .....    | 311           |
| Transmission                    |               |
| Hepatitis A .....               | 21            |
| Hepatitis B .....               | 26            |
| Hepatitis C .....               | 37            |
| Hepatitis E .....               | 49            |
| Transplantation                 |               |
| HBV/HCV and HIV coinfection ... | 375           |
| HCV .....                       | 196           |
| HIV/HCV coinfection .....       | 293           |
| Treatment                       |               |
| acute hepatitis B .....         | 123           |
| chronic hepatitis B .....       | 124           |
| Hepatitis B .....               | 119           |
| Hepatitis C .....               | 183           |
| Hepatitis D .....               | 161           |
| Treatment failure               |               |
| HCV .....                       | 198           |
| Trientine .....                 | 437           |
| <b>V</b>                        |               |
| Vaccination .....               | 99            |
| Hepatitis B .....               | 102           |
| Hepatitis C .....               | 105           |
| Hepatitis E .....               | 106           |
| Valopicitabine .....            | 212, 226      |
| resistance .....                | 232           |
| VCH-759 .....                   | 212, 229      |
| Versant .....                   | 173           |
| Virology                        |               |
| HBV .....                       | 55            |
| HCV .....                       | 75            |
| HDV .....                       | 156           |
| VX-497 .....                    | 234           |
| VX-500 .....                    | 212           |
| <b>W</b>                        |               |
| Wilson's Disease .....          | 429           |
| <b>Z</b>                        |               |
| Zinc .....                      | 438           |

**Mauss - Berg - Rockstroh - Sarrazin - Wedemeyer**

**HEPATOLOGY**  
**A clinical textbook**