Management of Patients with Viral Hepatitis

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Editor

Patrick Marcellin
Service d'Hépatologie
Hôpital BEAUJON
University of Paris VII
France

CONTRIBUTING AUTHORS

Alfredo Alberti
Department of Clinical and Experimental Medicine, Clinica Medica 5, University of Padova, Padova, Italy

Luisa Benvegnù
Department of Clinical and Experimental Medicine, Clinica Medica 5, University of Padova, Padova, Italy

Silvia Boccato
Department of Clinical and Experimental Medicine, Clinica Medica 5, University of Padova, Padova, Italy

Ferruccio Bonino
Ospedale Maggiore Policlinico di Milano, Milan, Italy

Jordi Bruix
BCLC Group Liver Unit, Hospital Clinic, University of Barcelona, Barcelona, Spain

Maurizia Rossana Brunetto
Ospedale Maggiore Policlinico di Milano, Milan, Italy

Maria Buti
Liver Unit, Hospital General Universitari Vall d´Hebron, Barcelona, Spain

Pietro Ciccorossi
Ospedale Maggiore Policlinico di Milano, Milan, Italy

Barbara Coco
Ospedale Maggiore Policlinico di Milano, Milan, Italy

Piero Colombatto
Ospedale Maggiore Policlinico di Milano, Milan, Italy

Geoffrey Dusheiko
Centre for Hepatology, Royal Free and University College School of Medicine, London, UK

Rafael Esteban
Liver Unit, Hospital General Universitari Vall d´Hebron, Barcelona, Spain

Giovanna Fattovich
Department of Gastroenterology, University of Verona, Verona, Italy

Alessia Ferrari
Department of Clinical and Experimental Medicine, Clinica Medica 5, University of Padova, Padova, Italy

Stephanos J. Hadziyannis
Department of Medicine and Hepatology, Henry Dunant Hospital, Athens University, School of Medicine, Athens, Greece

E. Jenny Heathcote
Toronto Western Hospital, University Health Network, University of Toronto, Toronto, Canada

Jay H. Hoofnagle
Liver Disease Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA

Elmar Jaeckel
Department of Gastroenterology, Hepatology & Endocrinology, Medizinische Hochschule Hannover, Hannover, Germany

Harry L. A. Janssen
Department of Hepatology and Gastroenterology, Erasmus University Medical Center, Rotterdam, The Netherlands

Michael P. Manns
Department of Gastroenterology, Hepatology & Endocrinology, Medizinische Hochschule Hannover, Hannover, Germany

Patrick Marcellin
Service d'Hépatologie and Inserm U481, Hôpital Beaujon, University of Paris VII, Clichy, France

Filippo Oliveri
Ospedale Maggiore Policlinico di Milano, Milan, Italy

Jean-Michel Pawlotsky
Department of Virology, INSERM U635, Henri Mondor Hospital, University of Paris XII, Créteil, France

Robert P. Perrillo
Section of Gastroenterology and Hepatology, Ochsner Clinic Foundation, New Orleans, USA

Roberta Pistis
Department of Clinical and Experimental Medicine, Clinica Medica 5, University of Padova, Padova, Italy

Stanislas Pol
Unité d' Hépatologie and INSERM U-370, Hôpital Necker, Paris, France

Andrej Potthoff
Department of Gastroenterology, Hepatology & Endocrinology, Medizinische Hochschule Hannover, Hannover, Germany

Bruno Roche
Centre Hépatobiliaire, Hôpital Paul Brousse, Faculté de Médecine Paris Sud, Villejuif, France

Margarita Sala
BCLC Group Liver Unit, Hospital Clinic, University of Barcelona, Barcelona, Spain

Didier Samuel
Centre Hépatobiliaire, Hôpital Paul Brousse, Faculté de Médecine Paris Sud, Villejuif, France

Chiara Scattolini
Department of Gastroenterology, University of Verona, Verona, Italy

Solko W. Schalm
Department of Hepatology and Gastroenterology, Erasmus University Medical Center, Rotterdam, The Netherlands

Eugene R. Schiff
Division of Hepatology, Center for Liver Diseases, University of Miami School of Medicine, Miami, USA

Detlef Schuppan
Department of Medicine I, University of Erlangen-Nuernberg, Erlangen, Germany

Giada Sebastiani
Department of Clinical and Experimental Medicine, Clinica Medica 5, University of Padova, Padova, Italy

Vincent Soriano
Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain

Anaïs Vallet-Pichard
Unité d' Hépatologie and INSERM U-370, Hôpital Necker, Paris, France

Heiner Wedemeyer
Department of Gastroenterology, Hepatology & Endocrinology, Medizinische Hochschule Hannover, Hannover, Germany

Ola Weiland
Department of Medicine, Division of Infectious Diseases, Karolinska University Hospital Huddinge, Stockholm, Sweden

Irene Zagni
Department of Gastroenterology, University of Verona, Verona, Italy

Fabien Zoulim
INSERM Unit 271 and Liver Department, Hotel Dieu, Lyon, France

CONTENTS

Hepatitis B and Hepatitis C in 2004

Patrick Marcellin

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are among the most frequent viral infections of man and represent a major global public health problem [1, 2]. Approximately one third of the world population have serological evidence of past or present infection by HBV and 350 million people are chronically infected. Approximately 3% of the world population, 170 million people, are chronically infected by HCV. HBV and HCV related chronic hepatitis are the main causes of cirrhosis and hepatocellular carcinoma (HCC) which are responsible for a high rate of morbidity and mortality. End-stage HBV and HCV related liver disease and HCC are the main causes of liver transplantation. Indeed, in the last few years, the knowledge of the epidemiology and of the natural history of HBV and HCV infection have markedly improved. Furthermore, considerable progress has been achieved in the efficacy of therapy. New drugs and new therapeutic strategies which are currently under evaluation could further improve the efficacy of therapy in the near future.

HEPATITIS B

Epidemiology

The prevalence of HBV infection is especially high in South-East Asia and Sub-Saharan Africa where more than 8% of the population are hepatitis B surface antigen (HBsAg) chronic carriers [3]. While perinatal transmission or transmission during early childhood are

responsible for the high rate of chronic infection in Asia and Africa, sexual or parenteral exposure account for most cases in industrialised countries [4]. In most developed parts of the world, the prevalence of chronic HBV infection is less than 1%, and the overall infection rate is 5-7%. Within these areas most infections occur among high risk adult populations that include injection drug users, persons with multiple heterosexual partners, men who have sex with men, and health care workers. The risk of perinatal HBV transmission has been well described. This risk is greatest for infants born to women who are hepatitis Be antigen (HBeAg)-positive and ranges from 70-90% at 6 months of age; about 90% of these children remain chronically infected [5]. The risk of perinatal infection among infants born to HBeAg-negative mothers ranges from 10-40%, with 40-70% of these infected infants remaining chronically infected. Children born to HBsAg-positive mothers who do not become infected during the perinatal period remain at high risk of infection during early childhood.

HBV-related end-stage liver disease or HCC are responsible for over 1 million deaths per year and represent currently 5-10% of cases of liver transplantation [1, 3, 4,]. HCC is one of the most common cancers worldwide and HBV is responsible for at least 75% of this cancer [6]. The availability of safe and effective vaccines allowed wide immunization programs which resulted in the reduction of the burden of diseases caused by HBV with clear benefits in terms of prevention of cirrhosis and HCC [7].

Natural history

The natural course of HBV chronic infection is variable, ranging from an inactive HBsAg carrier state to a more or less progressive chronic hepatitis, potentially evolving to cirrhosis and HCC [8-10]. Chronic hepatitis may present as typical HBeAg-positive chronic hepatitis B or HBeAg-negative chronic hepatitis B. Apart from the molecular biology of HBV and host factors, co-infection with other hepatitis viruses, e.g. HCV, hepatitis delta virus, as well as with other not primary hepatotropic viruses, e.g. human immunodeficiency virus (HIV), can affect the natural course of HBV infection as well as the efficacy of antiviral strategies [11]. HBeAg-positive chronic hepatitis is due to wild type HBV; it represents the early phase of chronic HBV infection. HBeAg-negative chronic hepatitis is due to a naturally

occurring HBV variant with mutations in the pre-core or/and basic core promoter regions of the genome; it represents a late phase of chronic HBV infection [12]. The latter form of the disease has been recognized as increasing in many countries within the last decade and it represents the majority of cases in many countries. HBeAg-negative chronic hepatitis B is generally associated with a more severe liver disease with a very low rate of spontaneous disease remission and a low sustained response rate to antiviral therapy [12-14].

Longitudinal studies of patients with chronic hepatitis B indicate that, after diagnosis, the 5-year cumulative incidence of developing cirrhosis ranges from 8-20%. Morbidity and mortality in chronic hepatitis B are linked to evolution to cirrhosis or HCC. The 5-year cumulative incidence of hepatic decompensation is approximately 20% [15]. The 5-year probability of survival being approximately 80-86% in patients with compensated cirrhosis. Patients with decompensated cirrhosis have a poor prognosis (14-35% probability of survival at 5 years). HBV-related end-stage liver disease or HCC are responsible for at least 500,000 deaths per year.

HCC is one of the most common cancers worldwide, about 75% of which are related to chronic HBV infection. The incidence of HCC has increased worldwide and nowadays it constitutes the fifth most frequent cancer representing around 5% of all cancers worldwide. The incidence of HCC appears to vary geographically and correlates with the underlying stage of liver disease [16]. The annual incidence in HBV carriers ranges between 0.2% and 0.6%, but it reaches 2% when hepatic cirrhosis is established [17]. The oncogenic mechanism leading to liver cancer involves different pathways that are not fully elucidated. Prevention through universal vaccination has effectively decreased the incidence of liver cancer and new therapeutic agents may delay or avoid the establishment of cirrhosis. The only chance for long-term survival after HCC diagnosis is to achieve early detection through regular surveillance by ultrasound and alpha-fetoprotein determination [18]. This allows effective therapy such as surgical resection, liver transplantation or percutaneous ablation.

Therapy

Three drugs are currently available for the treatment of chronic hepatitis B: interferon-alpha, lamivudine and adefovir dipivoxil [1, 8, 19].

Conventional interferon-alpha, administered for 4 to 6 months in HBeAg-positive patients and 12-24 months in HBeAg-negative patients, induces a sustained response in only a minority of patients (10-30%) and is associated with a poor tolerability which limits duration of therapy [20, 21]. The nucleoside (lamivudine) and nucleotide (adefovir dipivoxil) analogs have the advantages of oral administration and excellent tolerance. Lamivudine, administered for 12 months, induces a sustained response in approximately 20% of the HBeAg-positive and in 5% of the HBeAg-negative patients [22-26]. Long-term therapy increases the rate of sustained response but is impaired by a high rate of resistance (50% at 3 years) [27-29]. Adefovir dipivoxil, administered for 12 months, induces a sustained response in 12% of HBeAg-positive patients [30]. Adefovir has a similar antiviral efficacy in HBeAg-negative patients [31]. The incidence of resistance to adefovir is low (6% at 3 years) [32]. Adefovir is effective on lamivudine resistant HBV [29]. It has been used successfully in patients with decompensated cirrhosis, in the pre-transplant setting or in transplanted patients developing resistance to lamivudine [33]. In patients with HBV/HIV co-infection with lamivudine resistant HBV, treatment with adefovir has a marked antiviral effect, similar to that observed in HIV negative patients [34].

Perspectives

Indeed, currently available drugs have limited efficacy and new more potent drugs or therapeutic strategies are needed. Recently, pegylated interferon monotherapy and combinations of pegylated interferon, with lamivudine and combination of adefovir and lamivudine have been assessed. The concept of combination therapy has been developed in order to increase efficacy and to decrease resistance. In addition, new nucleoside analogs are at different stages of development.

Pegylated interferon

A recent study of pegylated interferon-alpha-2a has shown a trend to a better efficacy as compared with conventional interferon with HBeAg seroconversion rates of 37% and 25%, respectively [35]. Two recent randomized controlled studies of pegylated interferon (pegylated interferon-alpha-2b in HBeAg-positive and pegylated interferon-alpha-2a in HBeAg-negative chronic hepatitis B) have

confirmed its efficacy with 36% and 43% of 24-week post-treatment response, respectively [36, 37]. Interestingly, relatively high rates of HBsAg loss, which are associated with complete and sustained remission of the disease, were observed in both studies (7% and 4%, respectively).

Combination of adefovir and lamivudine

One randomized study evaluated the efficacy of the combination of adefovir with lamivudine as compared to lamivudine alone or adefovir alone in 59 patients with HBeAg-positive chronic hepatitis B with lamivudine resistant HBV [38]. There was no significant difference in median serum HBV DNA reduction (-3.59 and -4.04 log copies/mL), rates of alanine transaminase (ALT) normalization (53% and 47%) and HBeAg loss (3 patients in each group) between the adefovir-lamivudine combination group and the adefovir monotherapy group.

Another study compared the efficacy of the combination of adefovir with lamivudine versus lamivudine used in monotherapy in 112 treatment-naive patients (107 HBeAg-positive) [39]. There was no significant difference in median serum HBV DNA reduction (-5.41 and -4.80 log copies/mL), rates of undetectable HBV DNA with polymerase chain reaction (39% and 41%) and HBeAg loss (19% and 20%) between the adefovir-lamivudine combination group and the adefovir monotherapy group. Finally, these two studies did not show superior efficacy of the combination versus each drug in monotherapy.

Combination of pegylated interferon and lamivudine

Two randomized controlled trials of the combination of pegylated interferon with lamivudine versus pegylated interferon did not show a superiority of the combination in terms of sustained response [36, 37]. However, noteworthy, higher end-of-treatment response rates were observed with the combination. In addition, in one study, the combination of pegylated interferon-alpha-2a with lamivudine decreased the incidence of lamivudine resistance [37]. Different schedules of combination need to be assessed in order to improve the efficacy.

New nucleoside analogs

A number of nucleoside analogs, with favorable toxicity profiles and a promise of increased effectiveness against HBV, are at various stages

of clinical development. Results of phase II trials of entecavir and emtricitabine (FTC) were encouraging [40, 41]. The results of phase III studies should be available soon. The results of phase II studies of telbivudine and clevudine are promising [42, 43]. Other interesting compounds are at an earlier phase of development (Table 1). These new nucleoside analogs seem to be more potent than lamivudine and adefovir dipivoxil with a good safety profile. However, one may expect that their use in monotherapy could not induce a high rate of sustained response and that long-term therapy or combination should be needed to improve efficacy and/or reduce resistance.

Lamivudine	Approved
Adefovir dipivoxil	Approved
Entecavir	Phase III
Emtricitabine (FTC)	Phase III
Telbivudine (L-dT)	Phase III
Clevudine (L-FMAU)	Phase II
L-dC, L-dA	Phase II

Table 1: Nucleoside and nucleotide analogs for the treatment of chronic hepatitis B.

HEPATITIS C

Epidemiology

The prevalence of chronic hepatitis C ranges from 0.1-5% in different countries [44-46]. It is estimated that there are 4 million in the United States and 5 million of HCV chronic carriers in Western Europe. The prevalence seems to be higher in Eastern Europe than in Western Europe [46]. In industrialized countries, HCV accounts for 20% of

cases of acute hepatitis, 70% of cases of chronic hepatitis, 40% of cases of end-stage cirrhosis, 60% of cases of HCC and 30% of liver transplants [47, 48].

The incidence of new symptomatic infection has been estimated to be 1-3 cases/1,000,000 persons annually. The actual incidence of new infections is obviously much higher (the majority of cases being asymptomatic). The incidence is declining for two reasons: (a) transmission by blood products has been reduced to near zero; (b) universal precautions have markedly reduced transmission in medical settings. Intravenous drug use remains the main mode of transmission; but, even here, the rate of transmission is diminishing due to a heightened awareness of the risk of needle sharing and, in some countries, the availability of needle-exchange programs.

In the United States, in 1999, there were 3,759 deaths attributed to HCV, although this is likely an underestimate [49]. There was a 5-fold increase in the annual number of patients with HCV who underwent liver transplantation between 1990 and 2000. The total direct health care cost associated with HCV is estimated to have exceeded $1 billion in 1998. Future projections predict a 4-fold increase between 1990 and 2015 in persons at risk of chronic liver disease, suggesting a continued rise in the burden of HCV in the United States in the foreseeable future.

In France, the prevalence of anti-HCV positive adults is estimated to be between 1.1% and 1.2%, whose 80% are viremic. Therefore, it is estimated that 400,000 to 500,000 subjects have chronic HCV infection. The prevalence varies widely in different populations: 60% in intravenous drug users, 25% in incarcerated subjects, 25% among HIV-positive patients (25,000 to 30,000 subjects have HCV/HIV co-infection) [48].

Natural history

In the last few years, the natural history of chronic HCV infection has been better understood. The progression of fibrosis determines the ultimate prognosis and thus the need and urgency of therapy. Fibrogenesis is a complex dynamic process, which is mediated by necroinflammation and activation of stellate cells [50]. The liver biopsy remains the gold standard to assess fibrosis. Scoring systems allow a semiquantitative assessment and are useful for cross-sectional and cohort studies and in treatment trials. The rate at which fibrosis

progresses varies markedly between patients. The major factors known to be associated with fibrosis progression are older age at infection, male gender, and excessive alcohol consumption [50-52]. Viral load and genotype do not seem to influence significantly the progression rate. Progression of fibrosis is more rapid in immunocompromised patients [53]. Recently, the importance of hepatic steatosis, obesity, and diabetes have been recognized and studies are in progress to understand the relationship between metabolic disorders, HCV replication and liver steatosis and progression of fibrosis [50]. There are no tests that reliably predict the rate of progression of fibrosis in an individual patient. High serum ALT levels are associated with a higher risk of fibrosis progression. On the contrary, worsening of fibrosis is uncommon in patients with persistently normal serum ALT levels [54]. However, a non-negligible proportion (about 5% each year) of these patients may present an increase of ALT levels and may develop a more progressive liver disease [55]. Serum markers for fibrosis are not fully reliable and need to be improved and validated. Liver biopsy provides the most accurate information on the stage of fibrosis and grade of necroinflammation, both of which have prognostic significance. Repeating the liver biopsy, 3 to 5 years after an initial biopsy, is the most accurate means of assessing the progression of fibrosis [2, 47].

Therapy

Combination of pegylated interferon with ribavirin

The most impressive progress has been achieved in the efficacy of therapy. With the combination of pegylated interferon and ribavirin which is nowadays reference therapy [2, 56]. A sustained virological response (SVR) is observed in roughly 50-60% of patients [57-59]. The absence of detectable serum HCV RNA 6 months after therapy, which defines the sustained virological response, may be considered nowadays as cure of HCV infection since long-term follow-up studies have shown that 97-100% of patients keep undetectable serum HCV RNA [60]. Furthermore, some studies have shown that HCV RNA is no longer detectable in the liver of sustained responders up to several years after therapy [60]. However, further studies with longer follow-up on large populations with very sensitive methods to detect HCV RNA in the serum, peripheral blood mononuclear cells and liver are

needed to confirm the eradication of HCV infection in sustained responders.

The SVR rate is as high as 90% in patients with genotype 2 or 3 and low viral load. The SVR rate is lower, 50%, in the most difficult to treat patients with genotype 1. Even if the presence of bridging fibrosis or cirrhosis is associated with a decreased chance of response, a relatively high rate of response has been observed with the combination of pegylated interferon and ribavirin (50%). The compliance with continuation of therapy with adequate dosing even increases the response rates and studies on adjuvant treatments are needed to improve clinical and haematological tolerability in order to increase compliance and the chance of response.

Treatment of non-responders

In patients who already received therapy, the chance to have a SVR with retreatment depends on the previous therapy and the type of response. In patients who relapsed after interferon or interferon-ribavirin combination therapy, the chance of SVR with retreatment with the pegylated combination is high (about 70% and 50%, respectively). Also, in non-responders to interferon monotherapy, the SVR rate is high (about 30%). In contrast, in those patients who did not respond to standard combination therapy, the rate of SVR is low (around 10%) with retreatment with the combination therapy. Interestingly, the chance of response to retreatment depends mainly on the genotype and the presence of cirrhosis.

Maintenance therapy

In non-responders to current pegylated combination therapy, the concept of maintenance therapy has been developed in the last years [56]. Many studies suggest that long-term treatment of these patients may partially decrease viral load and serum ALT levels associated with improvement in liver necroinflammation which is associated with stabilization or even possible regression of fibrosis. Therefore, maintenance therapy might decrease, at least in some partial responders, the risk of development of cirrhosis and its complications, in particular HCC. However, this hypothesis needs to be proven in prospective randomized trials and the optimal schedule and the subgroup of patients who benefit from this therapeutic strategy need to be determined.

Perspectives

Still, about half of patients do not respond or relapse after therapy and current treatment has significant side-effects and is poorly tolerated. Therefore, new more effective and better tolerated anti-HCV drugs are needed. Many drugs with different mechanisms of action are under investigation. Other types of interferon (beta and gamma) have been disappointing but their use in combination with the current pegylated treatment needs to be assessed. Interleukins (IL2, IL12 and IL10) showed poor antiviral efficacy with limitations related to their toxicity. Inosine monophosphate dehydrogenase (IMPD) inhibitors, which have potentially ribavirin-like mechanisms of action are of interest. Some of them, like mycophenolate mofetyl and levovirin did not show significant efficacy. Other IMPD inhibitors are under evaluation. Preliminary results of therapeutic vaccines are interesting but their efficacy needs to be demonstrated. Newer approaches like antisense nucleotides or ribozymes are limited by the difficulties to reach the target cells (hepatocytes).

Indeed, the enzyme inhibitors appear to be the most promising strategy. In the last years, extensive research has been conducted to elucidate the structure of HCV enzymes in order to produce specific enzyme inhibitors [61]. All of the HCV enzymes (NS2-3 and NS3-4A proteases, NS3 helicase, and NS5B RdRp) are essential for HCV replication, and are therefore potential drug discovery targets. The absence of cell culture model supporting full replication of HCV, and of convenient animal models, has limited the knowledge of HCV life cycle and the testing for antiviral molecules. The recent development of subgenomic HCV RNA replicons capable of replicating in the human hepatoma cell line, Huh 7, has been a significant advance [62]. This model (replicon) is currently the best so far for the study of HCV replication and the testing for antiviral molecules. Target based anti-HCV drugs in development are indicated in Table 1.

Recently, a NS3 protease inhibitor (BILN 2061) demonstrated its ability to inhibit NS3 protease activity in the subgenomic HCV replicon cell model [63]. In phase I studies the administration of BILN 2061 given orally, twice daily, for two days induced a decrease in serum HCV RNA level greater than one $\log_{10}$. These results constitute a major step in the field of HCV drug development since it is the first clinical evidence of an antiviral effect of an enzyme inhibitor in patients with chronic hepatitis C. Many other enzyme inhibitors, in

particular protease inhibitors, have been produced and are currently in preclinical phase or in phase I clinical trials and more are coming. Hopefully, some of these drugs will demonstrate their efficacy and safety and will be good candidates for improving, probably by using them in combinations with interferon, the efficacy of treatment of patients with chronic hepatitis C.

CONCLUSION

HBV and HCV related liver diseases represent a major public health problem. In the last few years, considerable progress has been made in the knowledge of epidemiology, natural history, factors influencing the course of the liver disease and mainly efficacy of therapy. Still important efforts are needed for the early diagnosis in order to improve the management of patients with chronic hepatic B or C.

The understanding of the mechanisms of resistance to therapy and the development of new more potent drugs and new therapeutic strategies are a challenge to decrease in the future the global burden related to chronic viral hepatitis.

REFERENCES

1. EASL International Consensus Conference on Hepatitis B. Consensus Statement. J Hepatol 2003;39:S3-S25.
2. National Institutes of Health Consensus Development Conference Management of Hepatitis C. Hepatology 2002;36:S3-S20.
3. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-1745.
4. Maddrey WC. Hepatitis B: an important public health issue. J Med Virol 2000;61:362-366.
5. McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. J Infect Dis 1985;151:599-603.
6. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61:1942-1956.
7. Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13:S47-S49.
8. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: summary of a workshop. Gastroenterology 2001;120:1828-1853.
9. Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. Hepatology 2004;39:857-861.
10. Liaw Y-F, Leung N, Guan R, Lau GKK, Merican I. Asian-Pacific consensus statement on the management of chronic hepatitis B: an update. J Gastroenterol Hepatol 2003;18:239-245.
11. Di Martino V, Rufat P, Boyer N, Renard P, Degos F, Martinot-Peignoux M, Matheron S, Le Moing V, Vachon F, Degott C, Valla D, Marcellin P. The influence of human immunodeficiency virus coinfection on chronic hepatitis C in injection drug users: a long-term retrospective cohort study. Hepatology 2001;34:1193-1199.
12. Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.
13. Zarski JP, Marcellin P, Cohard M, Lutz JM, Bouche C, Rais A. Comparison of anti-HBe-positive and HBe-antigen-positive chronic hepatitis B in France. J Hepatol 1994;20:636-640.
14. 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;36:263-270.

15. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, Christensen E, Krogsgaard K, Degos F, Carneiro de Moura M. Occurrence of hepatocellular carcinoma and decompensation in Western European patients with cirrhosis type B. Hepatology 1995;21:77-82.

16. Bosch FX, Ribes J, Borras J. Epidemiology of primary liver cancer. Seminars in Liver Disease 1999;19:271-285.

17. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma: conclusions of the Barcelona-2000 EASL Conference. J Hepatol 2001;35:421-430.

18. Okuda K. Hepatocellular carcinoma. J Hepatol 2000;32:225-237.

19. Conjeevaram HS, Lok AS. Management of chronic hepatitis B. J Hepatol 2003;38:S90-S103.

20. Hoofnagle JH, Di Bisceglie AM. The treatment of chronic viral hepatitis. N Engl J Med 1997;226:347-356.

21. Wong DKH, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. Ann Intern Med 1993;119:312-323.

22. Dienstag JL, Schiff E, Wright T, Hann HI, Goodman Z, Crowther L, Condreay LD, Woessner M, Rubinn M, Brown NA for the US Lamivudine Investigator Group. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256-1263.

23. Lai CL, Chien RW, Leung NWY, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray F and the Asia Hepatitis Lamivudine Study Group. A one year trial of lamivudine for chronic hepatitis B. N Engl J Med 1998;339:61-68.

24. Schalm SW, Heathcote J, Cianciara J, Farrell G, Sherman M, Willems B, Dhillon A, Moorat A, Berbar J, Gray DF and the International Lamivudine Study Group. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. Gut 2000;46:562-568.

25. Schiff E, Karayalcin S, Grimm I, Perrillo R, Dienstag J, Husa P, Schalm S, Crowther L, Sullivan M, Wossner M, McPhillips P, Brown N, and the International Lamivudine Investigator Group. A placebo controlled study of lamivudine and interferon alpha 2b in patients with chronic hepatitis B who previously failed interferon therapy. Hepatology 1998;28:S388A.

26. Tassopoulos N, Volpes R, Pastore G, Heathcote J, Buti M, Goldin BD, Hawley S, Barber J, Condreay L, Gray F, and the Lamivudine Precore Mutant Study Group. Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Hepatology 1999;29:889-896.

27. Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KI, Chien RN. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Gastroenterology 2000;119;172-180.

28. Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE. Long-term therapy of chronic hepatitis B with lamivudine. Hepatology 2000;32:828-834.

29. Zoulim F, Trépo C. Drug therapy for chronic hepatitis B: antiviral efficacy and influence of hepatitis B virus polymerase mutations on the outcome of therapy. J Hepatol 1998;29:151-168.

30. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, Jeffers L, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart C for the Adefovir Dipivoxil 437 Study Group. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.

31. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart C for the Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of hepatitis Be antigen- hepatitis B. N Engl J Med 2003;348:800-807.

32. Hadziyannis S, Tassopoulos N, Chang TT, Heathcote J, , Kitis G, Rizzetto M, Marcellin P, Lim SG, Chen SS, Arteburn S, Ma J, Xiong S, Wollman M, James C, Currie G, Brosgart CL. Three year study of adefovir dipivoxil (ADV) demonstrates sustained efficacy in presumed precore mutant chronic hepatitis B (CHB) patients in a long term safety and efficacy study (LTSES). J Hepatol 2004;40:S17.

33. Schiff ER, Lai C-L, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillman HL, Samuel D, Zeuzem S, Lilly L, Rendina M, Villeneuve JP, Lama N, James C, Wulfsohn MS, Namini H, Westland C, Xiong S, Choy GS, Van Doren S, Fry J, Brosgart CL. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post- liver transplantation patients. Hepatology 2003;38:1419-1427.

34. Benhamou Y, Bochet M, Thibault V, Calvez V, Fievet MH, Vig P, Gibbs CS, Brosgart C, Fry J, Namini H, Katlama C, Poynard T. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. Lancet 2001;358:718-723.

35. Cooksley G, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, Chang WY, Zahm F, Pluck N. Peginterferon alpha 2a (40KDa) : an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.

36. Janssen HLA, Sentruk H, Zeuzem S, Schalm SW et al. Peginterferon alfa 2b and lamivudine combination therapy compared with peginterferon alfa 2b for chronic HbeAg-positive hepatitis B: a randomized controlled trial in 307 patients. Hepatology 2003;38:1323.

37. Marcellin M, Lau G, 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. A phase III, partially double-blinded study evaluating the efficacy and

safety of peginterferon alfa 2a (40KD) (Pegasys) alone or in combination with lamivudine vs lamivudine in 546 patients with HBeAg-negative/anti-HBe-positive chronic hepatitis B. Hepatology 2003;38:S1181.

38. Peters MG, Hann HW, Martin P, Heathcote J, Buggisch P, Rubin R, Bourlière M, Kowdley K, Trépo C, Gray DF, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosgart CL. Adefovir dipivoxil alone and in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101.

39. Sung JJY, Lai JY, Zeuzme S, Chow WC, Heathcote E, Perrillo R, Brosgart C, Woessner M, Scott SA, Cambell FM. A randomised double-blind phase II study of lamivudine (LAM) compared to lamivudine plus adefovir dipivoxil (ADV) for treatment naïve patients with chronic hepatitis B (CHB): week 52 analysis. J Hepatol 2003:38:S69.

40. Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, Dehertogh D. Entecavir is superior to lamivudine in reducing HBV DNA inpatients with chronic hepatitis B infection. Gastroenterology 2002;123:1831-1838.

41. Leung N, Gish RG, Wang C, Corey L, Sacks S, Fried M, Wright T, Hut T, Chan F, Rousseau FS. A randomized, double-blind comparison of 3 doses of emtricitabine in patients with chronic hepatitis B given 48 weeks of treatment. Hepatology 2001;34:349A.

42. Han SH, Leung NWY, Teo EK, Tong M, Wong F, Hann HW, Poynard T, Brown NA, Myers M, Chao G, Lloyd D, Lai CL. Results of a one-year international phase IIb trial of LdT, and LdT plus lamivudine, in patients with chronic hepatitis B. J Hepatol 2004;40:16.

43. Marcellin P, Mommeja-Marin H, Sacks SL, Lau GKK, Sereni D, Bronowicki J-P, Conway B, Trepo C, Blum MR, Yoo BC, Mondou E, Sorbel J, Snow A, Rousseau F, Lee HS. A phase II dose-escalating trial of clevudine in patients with chronic hepatitis B. Hepatology 2004;40:140-148.

44. Alter MJ. Epidemiology of hepatitis C in the west. Seminars in liver disease 1995;15:5-14.

45. Trépo C, Pradat P. Hepatitis C virus infection in Western Europe. J Hepatol 1999;31:S80-S83.

46. Naoumov N. Hepatitis C virus infection in Eastern Europe. J Hepatol 1999;31:S84-S87.

47. EASL International Consensus Conference on Hepatitis C. Consensus Statement. J Hepatol 1999;30:956-961.

48. Consensus conference. Treatment of hepatitis C. Guidelines. Gastroentérol Clin Biol 2002;26:B312-B320.

49. Kim WR. The burden of hepatitis C in the United States. Hepatology 2002;36:S30-S34.

50. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology 2002;36:S47-S56.

51. Hoofnagle JH. Course and outcome of hepatitis C. Hepatology 2002;36:S21-S29.

52. Poynard T, Bedossa P, Opolon P for the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825-832.

53. Di martino V, Rufat P, Boyer N, Renard P, Degos F, Martinot-Peignoux M, Matheron S, Le Moing V, Vachon F, Degott C, Valla D, Marcellin P. Influence of human immunodeficiency virus coinfection on chronic hepatitis C in injection drug users: a long term retrospective cohort study. Hepatology 2001;34:1193-1199.

54. Marcellin P, Lévy S, Erlinger S. Therapy of hepatitis C: patients with normal aminotransferase levels. Hepatology 1997;26:133S-137S.

55. Martinot-Peignoux M, Boyer N, Cazals-Hatem D, Pham BN, Gervais A, Le Breton V, Levy S, Degott C, Valla DC, Marcellin P. 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;34:1000-1005.

56. Dhumeaux D, Marcellin P, Lerebours E. Treatment of hepatitis C. The 2002 French consensus. Gut 2003;52:1784-1787.

57. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with IFN-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958-965.

58. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. New Eng J Med 2002;347:975-982.

59. Hadziyannis SJ, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill A. Peginterferon alfa-2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-355.

60. Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Claude Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-a therapy. Annals of Internal Medicine 1997;127:875-881.

61. Kim JL, Morgenstern KA, Lin C, Fox T, Dwyer MD, Landro JA, Chambers SP, Markland W, Lepre CA, O'Malley ET, Harbeson SL, Rice CM, Murcko MA, Caron PR, Thomson JA. Crystal structure of the hepatitis C virus NS3 protease

domain complexed with a synthetic NS4A cofactor peptide. Cell 1996;87:343-355.

62. Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 1999;285:110-113.

63. Lamarre D, Anderson PC, Bailey M, Beaulieu P, Bolger G, Bonneau P, Bos M, Cameron DR, Cartier M, Cordingley MG, Faucher AM, Goudreau N, Kawai SH, Kukolj G, Lagace L, LaPlante SR, Narjes H, Poupart MA, Rancourt J, Sentjens RE, St George R, Simoneau B, Steinmann G, Thibeault D, Tsantrizos YS, Weldon SM, Yong CL, Llinas-Brunet M. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. Nature 2003;426:186-189.

I. Hepatitis C

Clinical Virology of Hepatitis C

Jean-Michel Pawlotsky

INTRODUCTION

There are two types of laboratory tests for the virological diagnosis and monitoring of hepatitis C virus (HCV) infection, namely serologic tests of anti-HCV antibodies (indirect tests), and tests that detect, quantify or characterize viral components such as HCV RNA and core antigen (direct tests). Both direct and indirect tests are useful for diagnosis, treatment choices, and therapeutic monitoring.

HCV MARKERS

The HCV genotype, HCV RNA, HCV core antigen, and anti-HCV antibodies are the four markers of HCV infection.

HCV genotyping

There are six HCV genotypes, which are subdivided into subclades or subtypes identified by lower-case letters (1a, 1b, 1c, etc) [1]. HCV types, subtypes and isolates are distinguished on the basis of average sequence divergences of about 30%, 20% and 10%, respectively [1]. The HCV genotype is an intrinsic characteristic of the infecting HCV strain that does not change over time.

HCV RNA

The detection of HCV RNA in peripheral blood is a reliable marker of HCV replication. HCV RNA becomes detectable about one or two weeks after infection, then usually peaks before disappearing in cases that spontaneously resolve. During chronic infection, HCV RNA levels generally stabilize gradually, although HCV RNA can occasionally disappear for days or weeks before reappearing and reaching a plateau. HCV RNA levels are stable during chronic infection [2]. The HCV RNA level does not correlate to the severity of liver lesions, but it is generally low or undetectable in end-stage liver disease [3, 4].

HCV core antigen

Total HCV core antigen levels correlate with HCV RNA levels [5]. Prior to seroconversion, core antigen is generally detected 1 or 2 days later than HCV RNA [6, 7], and subsequent time course of core antigen levels then matches HCV RNA kinetics [5]. The HCV core antigen titer can therefore be used as an indirect marker of HCV replication.

Anti-HCV antibodies

Specific antibodies can be detected 7 to 8 weeks after infection with current tests [8-10]. From between 50% to 70% of patients have detectable anti-HCV antibodies at clinical onset [11]. Anti-HCV antibodies can persist throughout life in patients with spontaneously resolving infection, although in some cases they may fall slowly, or disappear after several years [12]. In chronically infected patients, antibodies persist for life. They may become undetectable during hemodialysis or profound immunodepression.

VIROLOGICAL TESTS

Antibody tests

Third-generation enzyme immunoassays (EIAs) detect mixed antibodies against HCV core, NS3, NS4 and NS5 antigens. The target antigens are coated on microtiter plates, microbeads or holders designed for “closed” automated devices. The specificity of current EIAs is greater than 99%. There is no gold standard, so sensitivity is

more difficult to determine. In routine use, more than 99% of immunocompetent patients with detectable HCV RNA are positive with current EIAs [13]. EIAs can be negative during hemodialysis and in profoundly immunodeficient patients despite ongoing HCV replication, but this is rare with the most recent tests [14]. Confirmation with immunoblot tests is no longer useful in the clinical setting, because of the excellent sensitivity and specificity of current EIAs [15].

HCV serotyping

The HCV genotype can be determined by competitive EIA testing for type-specific antibodies. One commercial test is available so far (Murex™ HCV Serotyping 1-6 Assay, Murex Diagnostics, Dartford, UK) and is interpretable in about 90% of immunocompetent patients with chronic hepatitis C [16]. Sensitivity is lower in hemodialysis and immunodepression [17, 18]. This test does not identify the subtype. Overall, the results are in agreement with molecular tests in about 95% of cases, although performance is better with genotype 1 than with other genotypes [16, 19, 20]. This test cannot distinguish true mixed infection from cross-reactivity.

Molecular HCV genotyping methods

The gold standard for genotyping is direct sequencing of the NS5B or E1 region. This is followed by sequence alignment with reference sequences and by phylogenetic analysis [1]. In practice, HCV is genotyped by direct sequence analysis, restriction fragment length polymorphism analysis, or reverse hybridization to genotype-specific oligonucleotide probes [21-23]. Two commercial kits are based on PCR amplification of the 5' noncoding region. The Trugene™ HCV 5'NC genotyping kit (Bayer Corporation, Tarrytown, New Jersey) [24, 25] is based on direct sequencing of PCR amplicons and database interpretation. The INNO-LiPA HCV II line-probe test (Innogenetics, Gent, Belgium) [21, 22] is based on reverse hybridization of PCR amplicons, using a nitrocellulose strip coated with genotype-specific oligonucleotide probes, and colorimetric determination. The six HCV types, and also many subtypes, can be identified with the two tests, although subtyping errors occur in 10% to 25% of cases because of variability in the target 5' noncoding region. However, clinical decision-making is solely based on the type, not the subtype.

HCV RNA detection

HCV RNA detection tests are far more sensitive than most quantitative tests, and involve target amplification by PCR or TMA [26, 27]. A commercial PCR-based test is available. It can be fully manual (Amplicor™ HCV v2.0, Roche Molecular Systems, Pleasanton, California) or comprise manual extraction and automated reverse transcription, amplification and reading in the Cobas Amplicor™ device (Cobas Amplicor™ HCV v2.0, Roche Molecular Systems, Pleasanton, California). The detection limit is 50IU of HCV RNA per mL. The manual TMA test (Versant™ HCV RNA Qualitative Assay, Bayer Corporation) has a slightly better detection limit of 10IU/mL. Specificity is 98%-99% with both tests.

HCV RNA quantification

Target amplification (PCR or TMA) and signal amplification ("branched DNA") can be used to determine viral copy numbers. The World Health Organization (WHO) has established an international standard for HCV RNA units [28]. One IU represents the total amount of HCV RNA rather than the number of viral particles. Universal adoption of this reference standard [29] has facilitated recommendations and clinical guidelines.

The detection limits of current tests (30IU/mL to 615IU/mL) are shown in Figure 1. The upper end of the linear range ranges from <500,000IU/mL to 20,000,000IU/mL with the real-time PCR assay (Cobas TaqMan 48 HCV, Roche Molecular Systems, Rotkreuz, Switzerland). Samples exceeding the upper end of the linear range must be diluted 1:10 or 1:100. The specificity of these tests is 98%-99%, regardless of the genotype [30-36]. Variations of less than 0.5 log (less than three-fold) may be due to intrinsic or between-patient variability and should not be taken into account in the clinical setting [37].

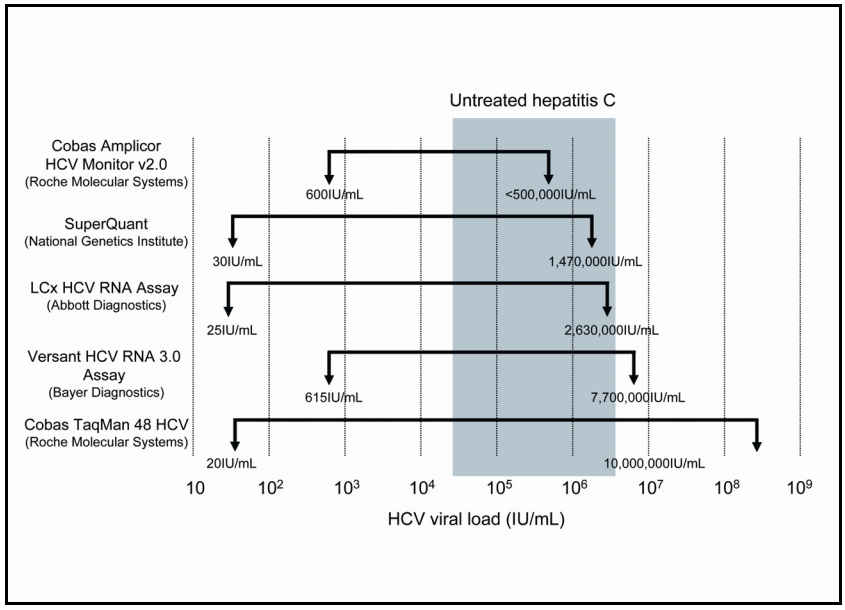

Figure 1: Linear ranges of quantitative HCV RNA tests. HCV RNA levels are shown in IU/mL. About 90% of patients' HCV RNA levels remain in the gray area without antiviral treatment.

HCV core antigen

An EIA test is available that can be used to detect and quantify total HCV core antigen (Ortho-Clinical Diagnostics, Raritan, New Jersey). The HCV core antigen titer (in pg/mL) correlates closely with the HCV RNA level, and can thus be used to monitor viral replication [5]. One pg of total HCV core antigen per mL is equivalent to about 8000IU of HCV RNA in most patients [5]. This test does not currently detect HCV core antigen in samples with HCV RNA levels under 20,000IU/mL, which restricts its clinical use [5].

PRACTICAL USE OF VIROLOGIC TESTS

Diagnosis

Acute hepatitis C

Patients with acute hepatitis of unknown origin must be tested with an anti-HCV EIA and with a sensitive technique (detection limit of 50IU/mL or less) for HCV RNA [38]. HCV RNA positivity in an anti-HCV-negative patient with acute hepatitis strongly indicates acute hepatitis C, and this is subsequently confirmed by seroconversion. Acute hepatitis C is unlikely when both markers are absent, and when anti-HCV is positive and HCV RNA negative; most of these patients have encountered (and cleared) HCV some time previously and therefore have another etiology. Such patients should nonetheless be tested for HCV RNA a few weeks later, as HCV RNA can disappear transiently before chronic replication becomes detectable. The presence of both anti-HCV antibodies and HCV RNA indicates either acute hepatitis C or an acute exacerbation of chronic hepatitis C. It is also difficult to diagnose acute hepatitis due to other causes when the patient also has chronic hepatitis C.

Chronic hepatitis C

A diagnosis of chronic hepatitis C is almost certain when a patient with chronic liver disease has both anti-HCV and HCV RNA (detected with a sensitive technique) [15]. Anti-HCV negativity with HCV RNA positivity is exceptional in immunocompetent patients, but more frequent (albeit still rare with current EIAs) [14] in patients who are on hemodialysis or profoundly immunodepressed.

HCV RNA detection with a sensitive technique confirms chronic HCV infection in patients found to be anti-HCV-positive during blood donation or screening of at-risk populations. Patients who still have antibodies after spontaneously resolving HCV infection in the past are difficult to distinguish from patients with false-positive reactivity when HCV RNA is undetectable on at least two occasions 6 months apart. A high EIA optical density ratio favors a true-positive result, but low optical density ratios are inconclusive, as anti-HCV antibody titers can fall gradually after the virus has been spontaneously cleared. However, all these patients can be reassured that they are not infected.

Diagnosis after occupational exposure

HCV RNA becomes detectable one to two weeks after infection, and diagnosis of acute infection is based on HCV RNA detection with a sensitive technique, starting at least one week after exposure.

Mother-child transmission

Babies born to HCV-infected mothers should be tested for HCV RNA with a sensitive method rather than for anti-HCV, because passively transferred antibodies can persist for up to a year after birth [39-42]. HCV RNA can be detected in the infected infant as little as a few days after delivery, and may then persist or clear spontaneously. There is no consensus on the timing of diagnostic HCV RNA testing, but about 6 to 12 months after birth seems optimal. High titers of anti-HCV antibodies persisting after the first year of life suggest chronic infection, which is confirmed by HCV RNA detection [39-42].

Prognostic value of virologic tests during the course of HCV disease

The HCV RNA level and HCV genotype do not predict the severity of liver damage or fibrosis, or the risk of extra-hepatic disease.

Antiviral treatment of chronic hepatitis C: decision-making and monitoring

The treatment decision

Pegylated interferon-alpha ribavirin combination therapy is only warranted for patients in whom HCV RNA is detected by a sensitive technique. The indication and duration of treatment depends on the genotype. All patients with genotype 2 or 3 infection should be offered this treatment, as they have a good chance of a sustained virologic response (70 to 80%) and treatment only lasts 24 weeks with a low dose of ribavirin [43-45]. The response rate in patients infected by genotype 1 is only 40 to 45%, and treatment lasts 48 weeks with a higher dose of ribavirin [43-45]. The likely risk-benefit ratio must thus be considered case by case. Patients with necroinflammatory activity and/or fibrosis on liver biopsy should be treated, contrary to patients with “mild” hepatitis. Pending further studies, patients infected by genotype 4, 5 or 6 must be treated like patients infected by genotype 1. The baseline HCV RNA level currently has no bearing on the

decision-making process. Baseline HCV RNA assay is not necessary in genotype 2 or 3 infection, but can help when assessing the treatment response at week 12 in patients infected by genotype 1 [45, 46].

Follow-up and treatment monitoring

A sensitive HCV RNA test is used to judge the virologic response at the end of treatment; persistence of HCV RNA is highly predictive of relapse after treatment withdrawal. Non-detection of HCV RNA at the end of treatment defines a virologic response, but such patients should be retested for HCV RNA 24 weeks later, again using a sensitive method.

HCV RNA testing before and after 12 weeks of treatment is used to monitor pegylated interferon-alpha and ribavirin treatment of genotype 1 chronic hepatitis C [45, 46]. Treatment is continued for a total of 48 weeks when, at week 12, a 2-log (100-fold) fall in HCV RNA level occurs or when HCV RNA is undetectable in patients whose baseline HCV RNA level was less than 100 times the detection limit. The likelihood of a sustained virologic response is virtually nil in other patients, and treatment can thus be stopped, or else be continued in an attempt to slow liver disease progression [45, 46]. Total HCV core antigen assay can be used for the same purpose, provided the antigen titer is more than 200pg/mL (detection cut-off 1 to 2pg/mL) [5].

Figure 2 shows a decision algorithm for the use of virologic tests in the treatment of chronic hepatitis C.

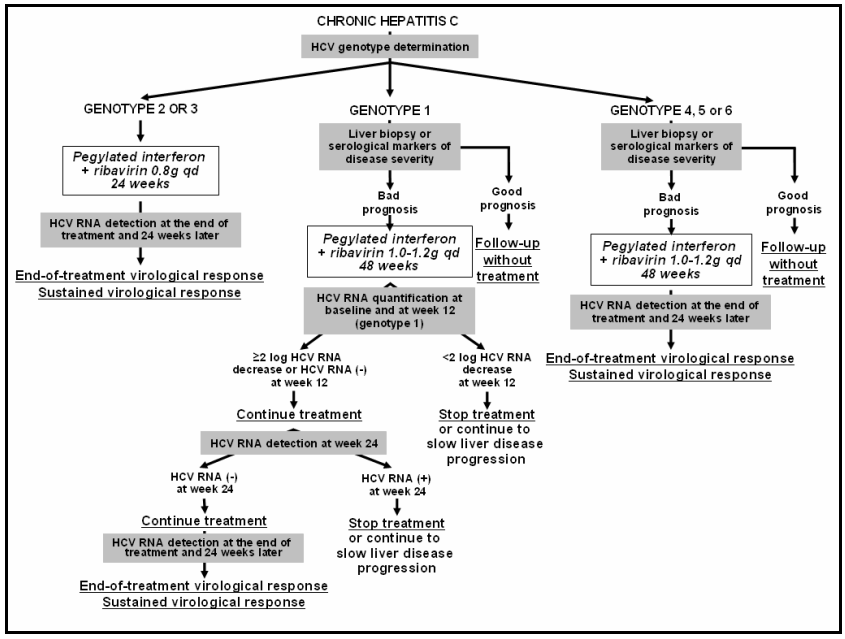

Figure 2: Decision algorithm for the use of virologic tests in the treatment of chronic hepatitis C.

Treatment of acute hepatitis C

Encouraging results were recently obtained with standard interferon alpha monotherapy of acute hepatitis C [47], but the optimal schedule is unknown, and the role of virologic tests in the decision to treat is uncertain [48]. Regardless of the type, dose, and duration of interferon treatment, a sensitive HCV RNA technique must be used to assess the virologic response at the end of treatment. When HCV RNA is negative at the end of treatment, the nature of the response (sustained or transient) should be assessed 24 weeks later; and sustained HCV RNA negativity indicates that treatment has been successful.

Monitoring of untreated patients

Repeat virologic testing has no prognostic value in untreated patients, in whom follow-up is based on regular liver biopsy.

CONCLUSION

The advent of virologic tests has vastly improved the management of HCV infection. These tests can be used to diagnose infection, to guide treatment decisions, and to monitor therapeutic response. They still have to be fully standardized and automated, and more clinically relevant cut-off values are required. The availability of more sensitive and more accurate HCV RNA tests will improve treatment monitoring and help to elucidate the mechanisms of antiviral resistance. Together with the development of new antiviral drugs, these advances should markedly improve the management of HCV infection.

REFERENCES

1. Simmonds P. Viral heterogeneity of the hepatitis C virus. J Hepatol 1999;31:54-60.

2. Nguyen TT, Sedghi-Vaziri A, Wilkes LB, Mondala T, Pockros PJ, Lindsay KL, McHutchison JG. Fluctuations in viral load (HCV RNA) are relatively insignificant in untreated patients with chronic HCV infection. J Viral Hepat 1996;3:75-78.

3. Chazouilleres O, Kim M, Combs C, Ferrell L, Bacchetti P, Roberts J, Ascher NL, Neuwald P, Wilber J, Urdea M, et al. Quantitation of hepatitis C virus RNA in liver transplant recipients. Gastroenterology 1994;106:994-999.

4. Duvoux C, Pawlotsky JM, Bastie A, Cherqui D, Soussy CJ, Dhumeaux D. Low HCV replication levels in end-stage hepatitis C virus-related liver disease. J Hepatol 1999;31:593-597.

5. Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, Hezode C, Picchio G, Dhumeaux D, Neumann AU, McHutchison J, Pawlotsky JM. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatology 2002;36:211-218.

6. Courouce AM, Le Marrec N, Bouchardeau F, Razer A, Maniez M, Laperche S, Simon N. Efficacy of HCV core antigen detection during the preseroconversion period. Transfusion 2000;40:1198-1202.

7. Lee SR, Peterson J, Niven P, Bahl C, Page E, DeLeys R, Giordano-Schmidt D, Baggett D, Green G. Efficacy of a hepatitis C virus core antigen enzyme-linked immunosorbent assay for the identification of 'window-phase' blood donations. Vox Sang 2001;80:19-23.

8. Farci P, Alter HJ, Wong D, Miller RH, Shih JW, Jett B, Purcell RH. A long-term study of hepatitis C virus replication in non-A, non-B hepatitis. N Engl J Med 1991;325:98-104.

9. Puoti M, Zonaro A, Ravaggi A, Marin MG, Castelnuovo F, Cariani E. Hepatitis C virus RNA and antibody response in the clinical course of acute hepatitis C virus infection. Hepatology 1992;16:877-881.

10. Hino K, Sainokami S, Shimoda K, Niwa H, Iino S. Clinical course of acute hepatitis C and changes in HCV markers. Dig Dis Sci 1994;39:19-27.

11. National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. Hepatology 1997;26:2S-10S.

12. Lefrere JJ, Guiramand S, Lefrere F, Mariotti M, Aumont P, Lerable J, Petit JC, Girot R, Morand-Joubert L. Full or partial seroreversion in patients infected by hepatitis C virus. J Infect Dis 1997;175:316-322.

13. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat 2001;8:87-95.

14. Thio CL, Nolt KR, Astemborski J, Vlahov D, Nelson KE, Thomas DL. Screening for hepatitis C virus in human immunodeficiency virus-infected individuals. J Clin Microbiol 2000;38:575-577.

15. Pawlotsky JM, Lonjon I, Hezode C, Raynard B, Darthuy F, Remire J, Soussy CJ, Dhumeaux D. What strategy should be used for diagnosis of hepatitis C virus infection in clinical laboratories? Hepatology 1998;27:1700-1702.

16. Pawlotsky JM, Prescott L, Simmonds P, Pellet C, Laurent-Puig P, Labonne C, Darthuy F, Remire J, Duval J, Buffet C, Etienne JP, Dhumeaux D, Dussaix E. Serological determination of hepatitis C virus genotype: comparison with a standardized genotyping assay. J Clin Microbiol 1997;35:1734-1739.

17. Kobayashi M, Chayama K, Arase Y, Tsubota A, Saitoh S, Suzuki Y, Ikeda K, Matsuda M, Koike H, Hashimoto M, Kumada H. Enzyme-linked immunosorbent assay to detect hepatitis C virus serological groups 1 to 6. J Gastroenterol 1999;34:505-509.

18. Leruez-Ville M, Nguyen QT, Cohen P, Cocco S, Nouyou M, Ferriere F, Deny P. Large-scale analysis of hepatitis C virus serological typing assay: effectiveness and limits. J Med Virol 1998;55:18-23.

19. Sandres K, Dubois M, Pasquier C, Puel J, Izopet J. Determination of HCV genotype using two antibody assays and genome typing. Eur J Clin Microbiol Infect Dis 2001;20:666-669.

20. Prescott LE, Berger A, Pawlotsky JM, Conjeevaram P, Pike I, Simmonds P. Sequence analysis of hepatitis C virus variants producing discrepant results with two different genotyping assays. J Med Virol 1997;53:237-244.

21. Stuyver L, Wyseur A, van Arnhem W, Lunel F, Laurent-Puig P, Pawlotsky JM, Kleter B, Bassit L, Nkengasong J, van Doorn LJ, et al. Hepatitis C virus genotyping by means of 5'-UR/core line probe assays and molecular analysis of untypeable samples. Virus Res 1995;38:137-157.

22. Stuyver L, Wyseur A, van Arnhem W, Hernandez F, Maertens G. Second-generation line probe assay for hepatitis C virus genotyping. J Clin Microbiol 1996;34:2259-2266.

23. Nakao T, Enomoto N, Takada N, Takada A, Date T. Typing of hepatitis C virus genomes by restriction fragment length polymorphism. J Gen Virol 1991;72:2105-2112.

24. Ansaldi F, Torre F, Bruzzone BM, Picciotto A, Crovari P, Icardi G. Evaluation of a new hepatitis C virus sequencing assay as a routine method for genotyping. J Med Virol 2001;63:17-21.

25. Ross RS, Viazov SO, Holtzer CD, Beyou A, Monnet A, Mazure C, Roggendorf M. Genotyping of hepatitis C virus isolates using CLIP sequencing. J Clin Microbiol 2000;38:3581-3584.

26. Pawlotsky JM. Molecular diagnosis of viral hepatitis. Gastroenterology 2002;122:1554-1568.

27. Sarrazin C, Teuber G, Kokka R, Rabenau H, Zeuzem S. 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 2000;32:818-823.

28. Saldanha J, Lelie N, Heath A. Establishment of the first international standard for nucleic acid amplification technology (NAT) assays for HCV RNA. Vox Sang. 1999;76:149-158.

29. Pawlotsky JM, Bouvier-Alias M, Hezode C, Darthuy F, Remire J, Dhumeaux D. Standardization of hepatitis C virus RNA quantification. Hepatology 2000;32:654-659.

30. Pawlotsky JM, Martinot-Peignoux M, Poveda JD, Bastie A, Le Breton V, Darthuy F, Remire J, Erlinger S, Dhumeaux D, Marcellin P. Quantification of hepatitis C virus RNA in serum by branched DNA-based signal amplification assays. J Virol Methods 1999;79:227-235.

31. Yu ML, Chuang WL, Dai CY, Chen SC, Lin ZY, Hsieh MY, Wang LY, Chang WY. Clinical evaluation of the automated Cobas Amplicor HCV Monitor test version 2.0 for quantifying serum hepatitis C virus RNA and comparison to the Quantiplex HCV version 2.0 test. J Clin Microbiol 2000;38:2933-2939.

32. Gerken G, Rothaar T, Rumi MG, Soffredini R, Trippler M, Blunk MJ, Butcher A, Soviero S, Colucci G. Performance of the Cobas Amplicor HCV Monitor test, version 2.0, an automated reverse transcription-PCR quantitative system for hepatitis C virus load determination. J Clin Microbiol 2000;38:2210-2214.

33. Martinot-Peignoux M, Boyer N, Le Breton V, Le Guludec G, Castelnau C, Akremi R, Marcellin P. A new step toward standardization of serum hepatitis C virus-RNA quantification in patients with chronic hepatitis C. Hepatology 2000;31:726-729.

34. Lee SC, Antony A, Lee N, Leibow J, Yang JQ, Soviero S, Gutekunst K, Rosenstraus M. 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 2000;38:4171-4179.

35. Pradat P, Chossegros P, Bailly F, Pontisso P, Saracco G, Sauleda S, Thursz M, Tillmann H, Vlassopoulou H, Alberti A, Braconier JH, Esteban JI, Hadziyannis S, Manns M, Rizzetto M, Thomas HC, Trepo C. Comparison between three quantitative assays in patients with chronic hepatitis C and their relevance in the prediction of response to therapy. J Viral Hepat 2000;7:203-210.

36. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Comparison of 3 quantitative HCV RNA assays--accuracy of baseline viral

load to predict treatment outcome in chronic hepatitis C. Scand J Infect Dis 1998;30:441-446.

37. Pawlotsky JM. Measuring hepatitis C viremia in clinical samples: can we trust the assays? Hepatology 1997;26:1-4.

38. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. J Hepatol 1999;30:956-961.

39. Wejstal R, Widell A, Mansson AS, Hermodsson S, Norkrans G. Mother-to-infant transmission of hepatitis C virus. Ann Intern Med 1992;117:887-890.

40. Roudot-Thoraval F, Pawlotsky JM, Thiers V, Deforges L, Girollet PP, Guillot F, Huraux C, Aumont P, Brechot C, Dhumeaux D. Lack of mother-to-infant transmission of hepatitis C virus in human immunodeficiency virus-seronegative women: a prospective study with hepatitis C virus RNA testing. Hepatology 1993;17:772-777.

41. Ohto H, Terazawa S, Sasaki N, Hino K, Ishiwata C, Kako M, Ujiie N, Endo C, Matsui A, et al. Transmission of hepatitis C virus from mothers to infants. N Engl J Med 1994;330:744-750.

42. Zanetti AR, Tanzi E, Newell ML. Mother-to-infant transmission of hepatitis C virus. J Hepatol 1999;31:96-100.

43. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 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.

44. Hadziyannis SJ, Cheinquer H, Morgan T, Diago M, Jensen DM, Sette H, Ramadori G, Bodenheimer HC, Marcellin P, Lee SD, Roberts PJ, Ackrill AM. Peginterferon alfa-2a (40 KD) (Pegasys) in combination with ribavirin (RBV): efficacy and safety results from a phase III, randomized, double-blind, multicentre study examining effect of duration of treatment and RBV dose. J Hepatol 2002;36:3.

45. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.

46. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645-652.

47. Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, Pastore G, Dietrich M, Trautwein C, Manns M. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med. 2001;345:1452-1457.

48. Hoofnagle JH. Therapy for acute hepatitis C. N Engl J Med 2001;345:1495-1497.

Natural History of Hepatitis C and Prognostic Factors of Disease Progression

Alfredo Alberti, Luisa Benvegnù, Silvia Boccato, Roberta Pistis, Alessia Ferrari, Giada Sebastiani

Infection with the hepatitis C virus (HCV) is one of the main causes of chronic liver disease, cirrhosis and hepatocellular carcinoma worldwide, and is particularly prevalent in Western countries. Acute infection with HCV is often asymptomatic or mild but progresses to chronic infection in more than 50% of the cases. Chronicity rates vary widely and are influenced by many factors, which are mainly related to the host rather than to the virus itself. These include age, immunocompetence and genetic background. There are approximately 170 to 200 million HCV chronic carriers worldwide and most of them are asymptomatic and thus not yet identified. The prevalence of HCV infection in the general population varies greatly in different parts of the world, and is estimated to be between 0.1 and 5%, with a peak prevalence of as high as 20 to 25% in Egypt. According to current estimates, nearly 2 to 4 million individuals in the US and more than 5 million people in Western Europe are chronically infected with HCV. Many have not yet been diagnosed, because of lack of symptoms and major risk factors. Thus, it is expected that many HCV carriers will be diagnosed in the near future and will come to medical attention. This is mainly because of increasing interest in HCV infection from patient advocacy groups, public health advisory boards and institutions that have raised the issue of the "silent epidemic" of HCV and are encouraging individuals at risk to be tested.

The natural history of chronic HCV infection varies greatly and is only partially understood. The rate and speed of progression from initially mild to severe, advanced disease and to end-stage complications vary markedly from individual to individual and are strongly influenced by a number of co-factors. Because of this and because the disease progresses rather slowly and for decades even in the most rapidly evolving cases, it has been difficult to obtain observational data covering the whole course of chronic disease. Thus, the natural history of HCV infection is usually represented as a series of disease stages through a multi-stage model formulation derived from evidence-based transition rates between specific stages [1-5]. This multistage modeling approach predicts that around 20 to 40% of patients with chronic hepatitis C will progress to end-stage liver disease during their lives and that around 10 to 20% will die of liver-related causes. Outcome modeling of the natural cause of hepatitis C is useful for assessing the future burden of the disease in the general population and in specific patient cohorts as well as for economic studies evaluating the cost-effectiveness of different interventional strategies and algorithms. However, to obtain information to assess the prognosis in individual cases in clinical practice, it is more useful to describe the progression rates and outcomes observed in published studies for the main clinical categories of HCV carriers. These include: a) HCV carriers with persistently normal alanine amino-transferase (ALT); b) histologically mild chronic hepatitis C; c) moderate to severe chronic hepatitis C; d) HCV-positive compensated cirrhosis; e) HCV carriers with extrahepatic manifestations.

LIVER DISEASE AND THE OUTCOMES IN ASYMPTOMATIC HCV CARRIERS WITH NORMAL ALT

Recent population-based studies indicate that around 40% of individuals with chronic HCV have persistently normal ALT values when tested serially over a 6-month observation period [6]. A number of studies have been conducted to assess the prevalence, degree and outcome of liver disease in these subjects, after early observations indicating that a subgroup of them may have significant liver damage when evaluated with liver biopsy [7]. The main findings were that significant liver disease, with active inflammation and/or advanced fibrosis, is present in a variable proportion of HCV carriers with normal ALT, with large variations among studies due to different

inclusion criteria and to the baseline follow-up time [8-11]. Indeed, the prevalence of cases with advanced liver disease and/or cirrhosis was significantly higher in studies of HCV carriers with normal ALT who had undergone a liver biopsy after having been tested for ALT for a shorter period or with fewer ALT evaluations, than in those who had been followed for longer periods or with more frequent ALT testing. According to our own recent meta-analysis of published studies [12], 22% of a cumulative 1145 cases with normal ALT had significant liver disease. This figure is consistent with what we have observed recently in our population-based survey showing that significant fibrosis (F2-F3) was detected in 18.7% of HCV carriers with persistently normal ALT [6].

A number of studies have evaluated the short- and long-term outcome in HCV carriers with initially normal ALT [13-15]. All these studies have shown a significant risk of biochemical reactivation, although the frequency varied considerably once again mainly due to different inclusion criteria, number and frequency of ALT evaluation and length of follow-up. In the largest series of HCV carriers published so far, Puoti et al. [10] recently described reactivation of liver damage in 21.5% out of 880 Italian HCV carriers with initially normal ALT levels. Similar findings have been reported by other authors. Interestingly, the probability of having an ALT flare during follow-up was not predictable in most of these studies and did not correlate with initial histologic findings.

Therefore, on the basis of these data it would appear that around 20% of HCV carriers with persistently normal ALT have significant liver disease at liver biopsy and another 20% will develop significant disease reactivation in the medium-term follow-up. However, in other cases the ALT flare is mild and of limited duration and may not affect the overall course of liver disease. Unfortunately, it is difficult to predict at the individual level which patient will show a “clinically significant” ALT flare-up that will affect the progression of liver disease.

“MILD” CHRONIC HEPATITIS C

Chronic hepatitis C is a slowly progressive disease that can be classified as mild, moderate or severe according to liver histology. Most studies indicate that the longer the follow-up, the higher the probability that liver disease will worsen at both the histological and

clinical level in patients who initially presented with histologically mild chronic hepatitis C. This is particularly true in cases with increased ALT levels.

The question of whether liver fibrosis is progressive or not in cases with no or minimal fibrosis (F0/F1) at liver biopsy has recently been addressed in studies where serial liver biopsies were taken during a long-term follow-up in the absence of antiviral treatments. The results of these studies are summarized in Table 1.

Author	**No cases**	**Mean interval between biopsies**	**% with progression of fibrosis**	
			All	Severe fibrosis
Marcellin et al. 2002 [16]	110	3.2 yrs	32%	2%
Ghany et al. 2003 [17]	45	3.8 yrs	42%	4%
Hui et al. 2003 [18]	61	6.3 yrs	33%	10%
Alberti et al. 2004 [19]	105	8.3 yrs	60%	27%

Table 1: Progression of fibrosis in repeated liver biopsies in patients with initial F0/F1

In the report by Marcellin et al. [16], with a shorter follow-up between the initial and final biopsy, (a mean of 3.2 years) only 32% of the patients showed progression of liver fibrosis and only 2% developed severe fibrosis (F3 or F4). Ghany et al. [17] studied 21 patients with no fibrosis and 43 with portal fibrosis on the initial biopsy and reported progression of fibrosis in 13 of the former and in 18 of the latter when followed for a mean period of 3.6 years while untreated. The risk of progression was influenced by the ALT profile, and was significantly higher in patients with initially mild disease and

elevated ALT than in cases with mild disease and persistently normal ALT. Hui et al. [18] described rates of histological progression in 27 patients with F0 (15 with persistently normal ALT and 12 with elevated ALT) and in 34 with F1 (16 with normal ALT and 18 with elevated ALT) in the initial biopsy. These patients underwent a second biopsy after a mean 6.3 (range 2-11.1) years. Progression of fibrosis was seen in 22.5% of cases with normal ALT and in 43% of those with elevated ALT and severe fibrosis developed in 20% of those with elevated ALT. Progression to severe fibrosis or cirrhosis was only seen in patients with F1 in the initial biopsy, while progression in those with F0 was minor. In the group with elevated ALT the cumulative probability of developing severe fibrosis/cirrhosis was estimated to be >50% and >70% 8 years and 10 years respectively after the initial biopsy. In our study [19] in 106 patients with initially mild chronic hepatitis C and with the longest time interval between biopsies in the literature (7-11 years with a mean of 8.3 years) progression of liver fibrosis was seen in 57 cases (60%), including 47 out of 73 (72%) of those with elevated ALT and 10 out of 33 (33%) of those with persistently normal ALT during follow-up. Progression of fibrosis was seen in 6/21 of those with F0 and in 51/85 of those with F1. The corresponding figures for patients with normal and elevated ALT were: normal ALT/F0: 4/18 (22%) with progression of fibrosis; normal ALT/F1: 6/15 (40%); high ALT/F0 2/3 (66%); high ALT/F1: 45/70 (22%). Severe fibrosis only developed in patients with F1 in the initial biopsy and elevated ALT: 12/70 (17%). The mean index of the progression of fibrosis was 0.02/year in those with F0 and normal ALT, 0.11/year in those with F1 and normal ALT, 0.15/year in those with F0 and high ALT and 0.16 in those with F1 and high ALT. By multivariate analysis, the progression of fibrosis correlated with age at diagnosis (P=0.02), mean ALT levels during follow-up (P=0.001), alcohol intake (P=0.05), necroinflammatory activity (P=0.02) and steatosis (P=0.05) in the initial biopsy, but not with the HCV genotype or HCV RNA serum levels (P=NS). Figure 1 describes the rates of progression to severe fibrosis/cirrhosis derived from individual prospective studies with different time intervals between the initial and final biopsy or from published mathematical modeling. Despite the heterogeneity of these studies in terms of included patients and the presence of cofactors, the results of this analysis suggests that there is a significant time-related risk of progression to cirrhosis in patients with F1 in the initial biopsy and abnormal ALT. The corresponding

figures for patients with F0 in the initial biopsy and elevated ALT are much lower, with an estimated incidence of severe fibrosis/cirrhosis <2% at 10 years, <5% at 20 years and <20% at 30 years of follow-up.

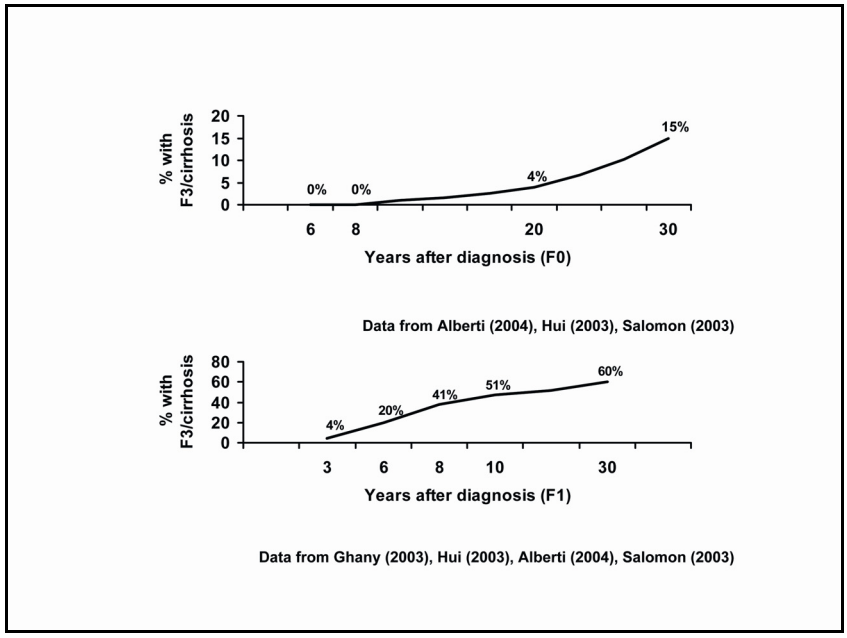

Figure 1: Observed or predicted risk of developing cirrhosis in patients with elevated ALT, according to the initial fibrosis score (F0 or F1).

On the basis of these findings, it is clear that liver disease is progressive in most cases with initially mild chronic hepatitis C in the presence of abnormal ALT levels. According to the available data, more than 50% of patients with F0/F1 in the initial biopsy are expected to progress to more advanced fibrosis (F2/F3) within 5 to 10 years and may develop cirrhosis within 15 to 20 years. Fibrosis may progress even faster in older patients and in the presence of cofactors such as alcohol intake or metabolic abnormalities leading to the accumulation of steatosis in the liver.

The role of cofactors has been clearly demonstrated in recent studies [20-23] and many of the most influential factors such as hepatic steatosis, obesity and moderate alcohol intake, are quite common in the general population and in asymptomatic HCV carriers. Since many studies on the natural course and outcome of initially mild chronic hepatitis C were conducted in selected subgroups of patients, often with exclusion of patients with cofactors that could affect the course of liver disease, these studies could easily have underestimated the risk and speed of the progression of liver disease that may occur in most HCV carriers in the general population, particularly in areas where obesity, metabolic liver steatosis and alcohol consumption are common. Age has been shown to affect the histological progression of chronic hepatitis C, and this is also true for initially mild disease. In our own study [19] conducted in 106 patients with initially mild chronic hepatitis C, the risk of the progression of fibrosis increased by a factor of 1.91 for every 10 years of age, after adjustment for other confounding variables. Thus, age alone appears to directly affect the pathogenicity of HCV. In addition, the progression of liver disease can be further accelerated by increasing age due to the increasing prevalence of metabolic cofactors. Thus, lifetime progression to cirrhosis and to its complications may also occur in middle aged or older patients presenting with histologically mild disease

OUTCOMES IN MODERATE-SEVERE CHRONIC HEPATITIS C

Symptomatic patients with HCV and those with more advanced liver inflammation and fibrosis are at a high risk of progressing to cirrhosis within a relatively short (5-10 years) period of time if left untreated. In mild chronic hepatitis C, a number of cofactors have been shown to influence the rate of progression to cirrhosis. A list of the major cofactors that accelerate the worsening of liver disease are provided in Table 2.

Age at diagnosis
Alcohol intake
Immunogenetics
Iron overload
Liver steatosis
HIV co-infection
HBV co-infection

Table 2: Co-factors shown to influence the rate of progression to cirrhosis in chronic hepatitis C.

NATURAL HISTORY OF HCV-RELATED COMPENSATED CIRRHOSIS

Patients with HCV-related, compensated cirrhosis may remain asymptomatic for many years, and have a normal quality of life. However, recent prospective studies indicate significant morbidity and mortality within the first decade after diagnosis and show that hepatocellular carcinoma is the most frequent and life-threatening complication of initially compensated HCV-positive cirrhosis. We recently described the long-term clinical outcome in a cohort of 269 patients with HCV-related cirrhosis followed prospectively for a mean period of 93 months (range 14-194 months) [24]. During this observation period, 33% developed at least one complication, most frequently and the first to occur being hepatocellular carcinoma. The cumulative incidences of HCC at 5 and 10 years were 7.8% and 28% in patients with HCV alone and 13% and 50% in those with HCV/HBV co-infection. The corresponding cumulative incidences of other complications are described in Table 3.

	Cumulative incidence (%)			
	HCV		HCV/HBV	
Complication	**5 yrs**	**10 yrs**	**5 yrs**	**10 yrs**
HCC	7.8	28	13	50
Ascites	7	20	11	40
GI bleeding	2.5	5	0	5
Encephalopathy	0	2.5	0	5
Liver-related death	5	19	9	8

Table 3: Cumulative incidence of major complications in initially compensated HCV-related cirrhosis.

In this study, as well as in other similar series, the prognosis of HCV-related cirrhosis was worsened by alcohol abuse and HBV co-infection and improved by antiviral therapy.

REFERENCES

1. Davis G, Albright J, Cook S, Rosenberg D. Projecting future complications of chronic hepatitis C in the United States. Liver Transplant 2003;9:331-338.

2. Law M, Dore G, Bath N, Thompson S, Crofts N, Dolan K, Giles W, Gow P, Loveday S, Powell E, Spencer J, Wodak A. Modelling hepatitis C virus incidence, prevalence and long-term sequelae in Australia, 2001. Intern J Epidemiol 2003;32:717-724.

3. Bennett WG, Inoue Y, Beck JR, Wong JB, Pauker SG, Davis GL. Estimates of the cost-effectiveness of a single course of interferon-alpha 2b in patients with histologically mild chronic hepatitis C. Ann Intern Med 1997;127:855-865.

4. Wong JB, Nevens F. Cost-effectiveness of peg-interferon alfa-2b plus ribavirin compared to interferon alfa-2b plus ribavirin as initial treatment of chronic hepatitis C in Belgium. Acta Gastroenterol Belg 2002;65:110-111.

5. Salomon JA, Weinstein MC, Hammitt JK, Goldie SJ. Cost-effectiveness of treatment for chronic hepatitis C infection in an evolving patient population. JAMA 2003;290:228-237.

6. Alberti A. Noventa F, Benvegnù L, Boccato S, Gatta A. Prevalence of liver disease in a population of asymptomatic persons with hepatitis C virus infection. Ann Intern Med 2002;137:961-964.

7. Alberti A, Morsica G, Chemello L, Cavalletto D, Noventa F, Pontisso P, Ruol A. Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. Lancet 1992;340:697-698.

8. Shiffman ML, Stewart CA, Hofmann CM, Contos MJ, Luketic VA, Sterling RK, Sanyal AJ. Chronic infection with hepatitis C virus in patients with elevated or persistently normal serum alanine aminotransferase levels: comparison of hepatic histology and response to interferon therapy. J Infect Dis 2000;182:1595-1601.

9. Pradat P, Alberti A, Poynard T, Esteban J-I, Weiland O, Marcellin P, Badalamenti S, Trepo C. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European Collaborative Study. Hepatology 2002;36:973-977.

10. Puoti C, Castellacci R, Montagnese E, Zaltron S, Stornaiuolo G, Bergami N, Bellis L, Precone DF, Corvisieri P, Puoti M, Minola E, Gaeta GB. 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;37:117-123.

11. Renou C, Halfon P, Pol S, Cacoub P, Jouve E, Bronowicki JP, Arpur Rifflet H, Picon M, Causse X, Canva V, Denis J, Tran A, Bourliere M, Ouzan D, Pariente A, Dantin S, Alric L, Cartier V, Revielle M, Caillat-Zucman S.

Histological features and HLA class II alleles in hepatitis C virus chronically infected patients with persistently normal alanine aminotransferase levels. Gut 2002;51:585-590.

12. Alberti A, Benvegnù L, Boccato S, Ferrari A, Vario A, Sebastiani G. Natural history of initially mild chronic hepatitis C. Dig and Liver Dis 2004 (in press).

13. Martinot-Peignoux M, Boyer N, Cazals-Hatem D, Pham BN, Gervais A, Le Breton V, Levy S, Degott C, Valla DC, Marcellin P. 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;34:1000-1005.

14. Persico M, Persico E, Suozzo R, Conte S, De Seta M, Coppola L, Palmentieri B, Sasso FC, Torella R. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. Gastroenterology 2000;118:760-764.

15. Rumi MG, De Filippi F, Donato MF, Del Ninno E, Colombo M. Progressive hepatic fibrosis in healthy carriers of hepatitis C virus with a transaminase breakthrough. J Viral Hepat 2002;9:71-74.

16. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology 2002;36:S47-S56.

17. Ghany MG, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, Herion D, Park Y, Liang TJ, Hoofnagle JH. Progression of fibrosis in chronic hepatitis C. Gastroenterology 2003;124:97-104.

18. Hui C-K, Belaye T, Montegrande K, Wright TL. A comparison in the progression of liver fibrosis in chronic hepatitis C between persistently normal and elevated transaminase. J Hepatol 2003;38:511-517.

19. Alberti A, Boccato S, Vario A, Pistis R, Ferrari A, Sebastiani G. Histologic progression of liver fibrosis in initially mild chronic hepatitis C. 2004 (in press).

20. Ohata K, Hamasaki K, Toriyama K, Matsumoto K, Saeki A, Yanagi K, Abiru S, Nakagawa Y, Shigeno M, Miyazoe S, Ichikawa T, Ishikawa H, Nakao K, Educhi K. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer 2003;97:3036-3043.

21. Friedenberg F, Pungpapong S, Zaeri N, Braitman LA. The impact of diabetes and obesity on liver histology in patients with hepatitis C. Diabetes Obes Metab 2003;5:150-155.

22. Westin J, Nordlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. J Hepatol 2002;37:837-842.

23. Shiffman ML. Natural history and risk factors for progression of hepatitis C virus disease and development of hepatocellular cancer before liver transplantation. Liver Transpl 2003;9:S14-S20.

24. Benvegnù L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut 2004;53:744-749.

How to Assess the Stage of Fibrosis in Chronic Hepatitis C

Detlef Schuppan

MECHANISMS OF PROGRESSION

Fibrosis and cirrhosis are a result of excess accumulation of extracellular matrix (ECM) molecules (collagens, noncollagenous glycoproteins, glycosaminoglycans, proteoglycans and of elastin [1]. Extensive perisinusoidal fibrosis has marked effects on liver function, due to the blockade of nutrient and metabolite exchange between hepatocytes and the circulation (Figure 1) and the liver is further impaired by the formation of novel intrahepatic vessels via porto-portal and porto-central collaterals that shunt the blood away from hepatocytes. The imbalance of two dynamic processes, fibrogenesis and fibrolysis leads to fibrosis. Activated hepatic stellate cells and myofibroblasts stimulate fibrogenesis by producing most ECM molecules, downregulating the expression of certain matrix metalloproteinases (MMPs), and increasing synthesis of physiological and tissue MMP inhibitors (TIMPs) [1-3] (Figure 2). Even advanced liver fibrosis and cirrhosis are reversible when the causes of fibrogenesis such as viral infection or biliary obstruction, are removed and the liver is given time to recover [4-11]. Furthermore a growing number of gene polymorphisms may either protect against or enhance the development of hepatic fibrosis (Table 1) [12-20]. In addition to the known external factors and the histological and serological markers of fibrosis and its development, these genetic polymorphisms

may provide individual risk profiles for the development of severe fibrosis.

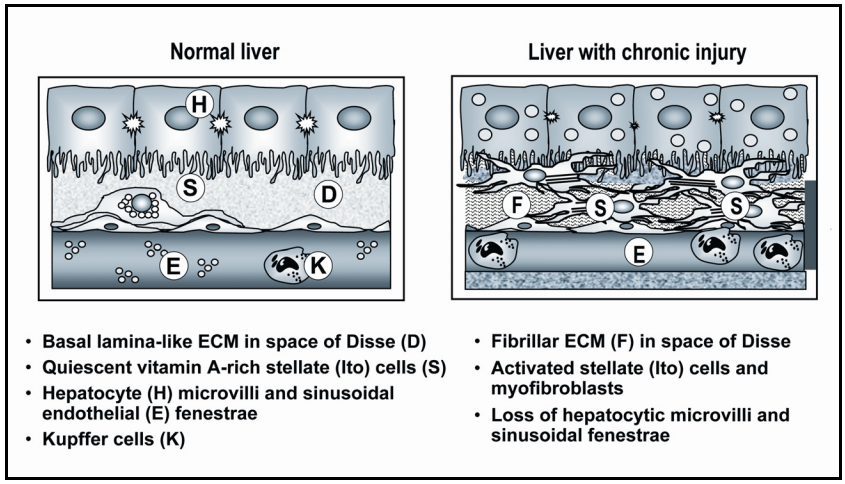

Figure 1: Capillarization of the sinusoids. Illustration of the major cell biological events that determine functionally relevant fibrosis [modified from a sketch kindly provided by Dr. M. Pinzani, Florence, Italy].

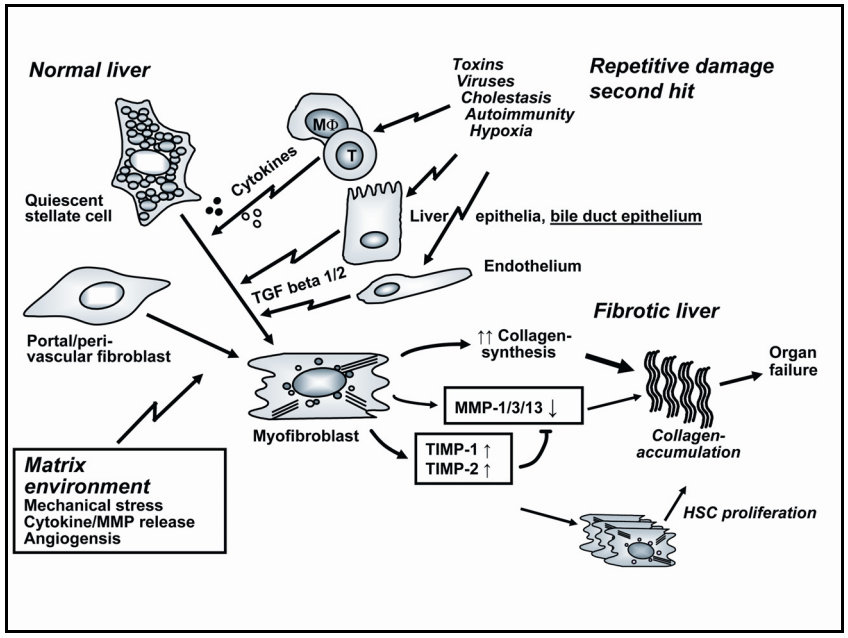

Figure 2: Initiation and maintenance of fibrogenesis. With continuous injury, primarily to hepatocytes or bile duct epithelia, and / or mechanical stress the normally quiescent hepatic stellate cells and portal/perivenular fibroblasts undergo activation and transdifferentiation to myofibroblasts. These myofibroblasts produce excessive amounts of collagens, downregulate certain MMPs and show an enhanced expression of the physiological inhibitors of the MMPs (TIMP-1 and -2). TIMP-1 can also promote myofibroblast proliferation and inhibit their apoptosis.

Gender (protection by high dose estrogens)
Pro/antioxidative enzyme polymorphisms (MnSOD, GSTP1, CYP2D6), e.g., in hemochromatosis
Immune system (profibrogenic Th2 vs. Th1 response)
Single nucleotide-polymorphisms (IL-1beta, IF-gamma, MCP-1, TNF-alpha, Factor V Leiden, MMP-3, TGF beta 1, DQB1*0503)
Genetically determined comorbidities: HFE mutations, metabolic syndrome (NASH)
Regulation of regeneration and apoptosis

Table 1: Genetic predisposition for hepatic fibrosis [12-20].
CYP2D6, cytochrome P450 2D6; GSTP1, glutathione S-transferase P1 [Stickel et al. unpublished data]; MnSOD, manganese superoxide dismutase [Oesterreicher et al. unpublished data]; NASH, non-alcoholic steatohepatitis.

IS THERE A GOLD STANDARD OF LIVER FIBROSIS?

Sequential histological grading of inflammation and particularly staging of fibrosis are still considered the gold standard to assess progression. However, certain studies have demonstrated sampling errors not only in patients with liver diseases with a high degree of intrahepatic heterogeneity such as biliary fibrosis, but also in patients with alcoholic or hepatitis C virus (HCV)-induced fibrosis and inflammation. Thus, when the well accepted, easy to use, 4 stage METAVIR score is used to stage fibrosis [21], roughly one third of the scores differed by at least one stage in the same patient when biopsies from the left and right liver lobes were compared [22]. Similar results were obtained when laparoscopic assessment of cirrhosis vs. non-cirrhosis (which is questionable as a gold standard) was matched to histological findings [23] (Table 2 and 3). Similar results were obtained for the grading of inflammation. This discrepancy was confirmed and systematically investigated in a recent study using the overall scoring of large surgical liver specimens from

patients with chronic hepatitis C as a gold standard. Results of this study showed that small, virtual biopsies derived from these large sections were correctly categorized in only 65% vs. 75% of cases when the biopsies were 15mm and 25mm long [24]. Moreover, a further increase in length from 25-45mm did not significantly increase accuracy. Therefore, although it is indispensable for many reasons, liver biopsy cannot be considered the ultimate gold standard for the assessment of stage and grade and thus the progression of fibrosis. This uncertainty complicates the search for non-invasive (serological) markers of the progression of fibrosis.

Homogeneity of staging & grading in chronic hepatitis C. HCV, laparoscopic biopsy of right and left liver n=124, METAVIR score

Difference	n	%
≥1 stage	41/124	33.1
≥2 stages	3/124	2.4
≥1 grade	30/124	24.2
≥2 grade	2/124	1.6
cirrhosis vs. stage 3	18/124	14.5

Table 2: Sampling error in chronic hepatitis C [22].

Laparoscopy vs. Histology Retrospective, 1992-1994, 434 consecutive patients. HCV 52%, HBV 8%, FL 8%, PBC 4%, AIH 3%, others 25%			
	Laparoscopy	**Histology**	**Error**
Cirrhosis	169	115	32%
No cirrhosis	265	263	0.8%
Detection of cirrhosis (gold standard laparoscopy)			
Sensitivity of biopsy		68%	
Specificity of biopsy		0.8	

Table 3: Sampling error in chronic liver diseases [23].

IMAGING TECHNIQUES

At present imaging techniques lack the sensitivity and specificity necessary for the assessment of the stage of fibrosis in patients with chronic liver diseases. Structural, non-homogenous findings at ultrasound are not associated with the stage of fibrosis, and liver echogenicity can only be used for the detection or exclusion of moderate to extensive fatty infiltration [25]. Although the hepatic artery resistance index as measured by Doppler ultrasound was slightly higher in severe than in mild fibrosis, and no correlation was found with histological inflammation, necrosis or portal flow velocity, the method lacks sensitivity [26]. A slightly better differentiation between slight and severe fibrosis is found with magnetic resonance (MR)-techniques, such as superparamagnetic iron oxide-enhanced MR, which shows hypersignal intensities with a reticular pattern in most patients with advanced fibrosis (METAVIR F2-4), while the signal from non-fibrotic areas where more Kupffer cells are present is decreased [27]. The fibroscan, an interesting new technique using both ultrasound and low-frequency (50Hz) elastic waves whose propagation velocity are directly related to elasticity, was evaluated to

quantify liver fibrosis in 106 patients with chronic hepatitis C. The areas under the (ROC) curves were 0.88 and 0.99 for the diagnosis of significant fibrosis or cirrhosis (METAVIR F2-4 and F4) [28]. Further prospective studies are needed to determine whether this technique can be used to detect changes in the stage of fibrosis in individual patients, e.g. during antifibrotic therapy.

SEROLOGICAL MARKERS OF PROGRESSION

Several studies have been performed with combinations of known serum markers of synthetic, metabolic or excretory liver functions, to derive an algorithm that predicts the histological severity (stage and grade) of chronic liver diseases. These algorithms were retrospectively determined and prospectively validated. Examples are the fibroscore, using alpha 2-macroglobulin, haptoglobin, gamma glutamyl transferase (GGT), gamma-globulin and bilirubin [29-31], and another score using platelet count, GGT, age and cholesterol [32] in patients with chronic hepatitis C (Table 4 and 5). Although these scores can be used instead of liver biopsy in a certain number of patients when a decision to treat or not must be made, they do not appear to be suitable for scientific studies requiring greater accuracy and an assessment of the dynamics of fibrogenesis and fibrolysis. Thus, when making a treatment decision, simple indicators may suffice. For example a single increase in alanine aminotransferase (ALT) during a 6 month observation period in patients with chronic hepatitis C indicated ≥stage 1 fibrosis allowing treatment to begin. These results occurred in 90% of patients (Table 6) [33]. Other indices are the PGA (prothrombin time, GGT, apolipoprotein A with or without alpha 2-macroglobulin) which has been validated in patients with alcoholic liver disease (Table 7) [34, 35].

Non-connective tissue markers as predictors of relevant liver fibrosis in hepatitis C (Fibroscore)
205 retrospective, 134 prospective patients with hepatitis C
METAVIR F0-1 vs. F2-4
5/11 serum markers predictive: alpha-2 macroglobulin haptoglobin gamma-globulin GGT bilirubin
Index 0-0.1: 100% negative predictive of F2-4 (12%)
Index 0.6-1.0: 90% positive predictive of F2-4 (34%)
Index 0.1-0.6: no assignment possible (54%)

Table 4: Diagnostic value of the fibroscore to predict fibrosis stage in patients with chronic hepatitis C [29].

Score to predict absent/little fibrosis (F0-1) in hepatitis C		
351 retrospective, 125 prospective patients with hepatitis C		
METAVIR F0-1 vs. F2-4		
Score: 7.811-3.131 ln (platelet count) + 0.781 ln (GGT) + 3.647 ln (age) – 0.014 (cholesterol)		
Score <4.2	**Stage 0-1**	**Stage 2-4**
Estimation	120/266	5/125
Validation	47/92	2/49
Score >6.9	**Stage 0-1**	**Stage 2-4**
Estimation	10/47	37/85
Validation	5/15	10/33
Score <4.2: sensitivity 51%, NPV 96%		
Score >6.9: sensitivity 30%, PPV 66%		

Table 5: Alternative index for prediction of fibrosis in patients with chronic hepatitis C [32].

Prediction of absent/little fibrosis (F0-1) by ALT					
864 retrospective patients with hepatitis C					
METAVIR F0-1 vs. F2-4					
ALT normal vs. ALT elevated during 6 months					
Stage	**0**	**1**	**2**	**3**	**4**
ALT normal	34.8	51.5	12.1	0	1.5
ALT elevated	0.8	23.7	50.5	17	8
ALT persistently normal (n=66): 65% ≥F1, 26% >A1F1					
ALT elevated: 99% ≥F1, 88% >A1F1					
Cut-off ALT >2.25 ULN: clear indication for treatment					
All patients with elevated ALT can be treated					
Biopsy only for patients with normal ALT					

Table 6: ALT as a predictor of relevant fibrosis or inflammation in patients with chronic hepatitis C [33].

PGA- or PGAA-index and alcoholic liver disease
Patients with alcoholic liver disease: n=333 retrospective, n=291 prospective
METAVIR F0-1 vs. F4
Serum markers: prothrombin time gamma GT apolipoprotein A
Index 0-2: 100% neg. pred. for F3/F4, 83% pos. pred. for F0/F1
Index 9-12:0% neg. pred. for F0/F1, 86% pos. pred. for F3/F4
Correct classification of 65% of patients (Poynard et al. 1991 [34])
PGAA-Index (incl. alpha-2 macroglobulin n=316 prospective):
Correct classification of 70% of patients (Naveau et al. 1994 [35])

Table 7: PGAA and PGA indices to predict the severity of alcoholic liver fibrosis [34-35].

Measuring circulating metabolites of the ECM appears be a more straightforward approach to assess fibrogenesis and fibrolysis, especially in studies on the inhibition or reversal of liver fibrosis (Figure 3) [36-38]. However, serum levels of these markers are influenced by their excretion via the kidney or in bile, and by their uptake by endothelial cells, especially by liver sinusoidal endothelial cells. In addition, other organs with a high ECM turnover can contribute to these serum levels. Cross-sectional studies suggest a significant, but insufficient predictive value of single ECM markers for the stage of fibrosis [39-41]. Meanwhile the cross-sectional evaluation of the European liver fibrosis consortium (ELF) study using 10 automatized ECM parameters in more than 1000 patients with various chronic liver diseases provided algorithms of 3-4 ECM markers with a better predictive value than an assessment by an independent expert pathologist who was not trained as well as two reference pathologists [42]. As in other studies correlating histology

with noninvasive markers, the problem of validation for bioptical sampling errors remains (see chapter above) which introduces an error of one stage (METAVIR scale) in 25% of biopsies; this is expected to increase when liver diseases other than chronic hepatitis C are included (as in the ELF study). The results of the two-year follow-up arm of the ELF study have not yet been published.

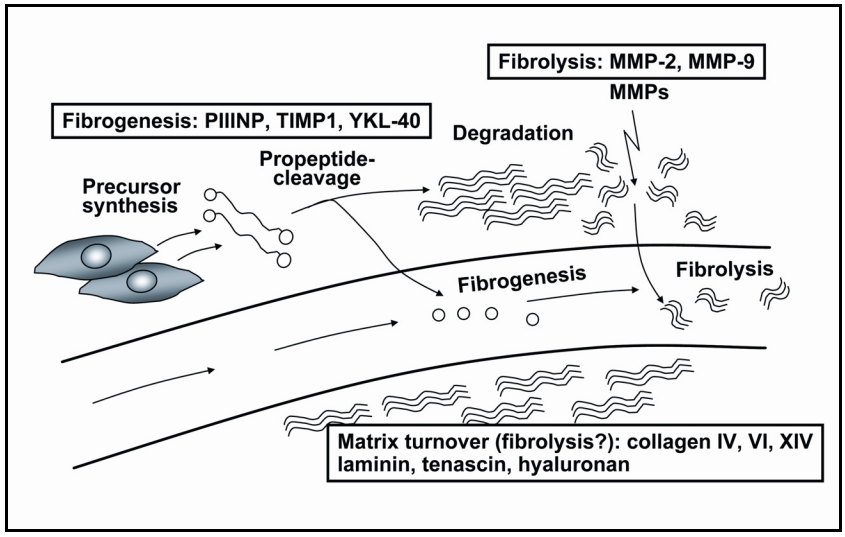

Figure 3: Circulating matrix proteins related to fibrogenesis and fibrolysis. Procollagen precursors released by fibrogenic cells are processed by procollagen peptidases. Only removal of the bulky propeptides allows the formation of collagen fibrils in the extracellular space. Thus circulating propeptide levels should reflect de novo synthesis and deposition of collagen, i.e. fibrogenesis. On the other hand, action of MMPs is expected to generate fragments of already deposited matrix proteins the levels of which should reflect matrix dissolution, i.e. fibrolysis. Most other molecules appear to rather represent an accelerated matrix turnover.
The two large multicenter studies that evaluate the predictive value of circulating matrix markers as predictors of fibrosis stage are mentioned (ELF: patients with all chronic liver diseases; Prometheus: patients with chronic hepatitis C). The ELF study also assesses the predictive value as to fibrosis progression.

A more direct approach to validate the true serum markers of fibrogenesis and fibrolysis, which is nevertheless equally prone to sampling errors, is the use of real time quantification of fibrosis-relevant mRNA expression from liver biopsies compared to serum fibrosis markers. In a study of 50 patients with various types of liver disease, we found a fairly good correlation between liver procollagen I or TIMP-1 expression and serum levels of the aminoterminal procollagen type III peptide or TIMP-1 (data not shown). These results need to be confirmed in larger studies. The availability of serum markers of hepatic fibrogenesis (or fibrolysis) will provide a quick and frequent assessment of the antifibrotic potential of drugs in patients with progressive liver disease. If these reliable serological tests can be combined with drugs that inhibit or revert fibrosis [43] the desire to revert fibrosis or even cirrhosis may be fulfilled.

REFERENCES

1. Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. Sem Liver Dis 2001;21:351-372.

2. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000;275:2247-2250.

3. Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liver Dis 2001;21:373-384.

4. Soyer MT, Ceballos R, Aldrete JS. Reversibility of severe hepatic damage caused by jejunoileal bypass after re-establishment of normal intestinal continuity. Surgery 1976;79:601-604.

5. Dufour JF, DeLellis R, Kaplan MM. Reversibility of hepatic fibrosis in autoimmune hepatitis. Ann Intern Med 1997;127:981-985.

6. Hammel P, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Flejou JF, Degott C, Belghiti J, Bernades P, Valla D, Ruszniewski P, Levy P. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. N Engl J Med 2001;344:418-423.

7. Muretto P, Angelucci E, Lucarelli G. Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med 2002;136:667-672.

8. Crone J, Moslinger D, Bodamer OA, Schima W, Huber WD, Holme E, Stockler Ipsiroglu S. Reversibility of cirrhotic regenerative liver nodules upon NTBC treatment in a child with tyrosinaemia type I. Acta Paediatr 2003;92:625-628.

9. Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002;122:1303-1313.

10. Arif A, Levine RA, Sanderson SO, Bank L, Velu RP, Shah A, Mahl TC, Gregory DH. Regression of fibrosis in chronic hepatitis C after therapy with interferon and ribavirin. Dig Dis Sci 2003;48:1425-1430.

11. Arthur MJ. Reversibility of liver fibrosis and cirrhosis following treatment for hepatitis C. Gastroenterology 2002;122:1525-1528.

12. Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, Purdie DM, Jonsson JR. Host genetic factors influence disease progression in chronic hepatitis C. Hepatology 2000;31:828-833.

13. Bahr MJ, el Menuawy M, Boeker KH, Musholt PB, Manns MP, Lichtinghagen R. Cytokine gene polymorphisms and the susceptibility to liver cirrhosis in patients with chronic hepatitis C. Liver Int 2003;23:420-425.

14. Muhlbauer M, Bosserhoff AK, Hartmann A, Thasler WE, Weiss TS, Herfarth H, Lock G, Scholmerich J, Hellerbrand C. A novel MCP-1 gene polymorphism is associated with hepatic MCP-1 expression and severity of HCV-related liver disease. Gastroenterology 2003;125:1085-1093.

15. Hellier S, Frodsham AJ, Hennig BJ, Klenerman P, Knapp S, Ramaley P, Satsangi J, Wright M, Zhang L, Thomas HC, Thursz M, Hill AV. Association of genetic variants of the chemokine receptor CCR5 and its ligands, RANTES and MCP-2, with outcome of HCV infection. Hepatology 2003;38:1468-1476.

16. Satsangi J, Chapman RW, Haldar N, Donaldson P, Mitchell S, Simmons J, Norris S, Marshall SE, Bell JI, Jewell DP, Welsh KI. A functional polymorphism of the stromelysin gene (MMP-3) influences susceptibility to primary sclerosing cholangitis. Gastroenterology 2001;121:124-130.

17. Wright M, Goldin R, Hellier S, Knapp S, Frodsham A, Hennig B, Hill A, Apple R, Cheng S, Thomas H, Thursz M. Factor V Leiden polymorphism and the rate of fibrosis development in chronic hepatitis C virus infection. Gut 2003;52:1206-1210.

18. Yoshizawa K, Ota M, Saito S, Maruyama A, Yamaura T, Rokuhara A, Orii K, Ichijo T, Matsumoto A, Tanaka E, Kiyosawa K. Long-term follow-up of hepatitis C virus infection: HLA class II loci influences the natural history of the disease. Tissue Antigens 2003;61:159-165.

19. Erhardt A, Maschner-Olberg A, Mellenthin C, Kappert G, Adams O, Donner A, Willers R, Niederau C, Haussinger D. 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.

20. Silvestri L, Sonzogni L, De Silvestri A, Gritti C, Foti L, Zavaglia C, Leveri M, Cividini A, Mondelli MU, Civardi E, Silini EM. CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer. Int J Cancer 2003;104:310-317.

21. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289-293.

22. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614-2618.

23. Poniachik J, Bernstein DE, Reddy KR, Jeffers LJ, Coelho-Little ME, Civantos F, Schiff ER. The role of laparoscopy in the diagnosis of cirrhosis. Gastrointest Endosc 1996;43:568-571.

24. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449-1457.

25. Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U, Fryden A, Bodemar G. Increased liver echogenicity at ultrasound examination

reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis 2002;34:516-522.

26. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705-1713.

27. Lucidarme O, Baleston F, Cadi M, Bellin MF, Charlotte F, Ratziu V, Grenier PA. Non-invasive detection of liver fibrosis: Is superparamagnetic iron oxide particle-enhanced MR imaging a contributive technique? Eur Radiol 2003;13:467-474.

28. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705-1713.

29. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T; MULTIVIRC Group. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001;357:1069-1075.

30. Poynard T, McHutchison J, Manns M, Myers RP, Albrecht J. Biochemical surrogate markers of liver fibrosis and activity in a randomized trial of peginterferon alfa-2b and ribavirin. Hepatology 2003;38:481-492.

31. Myers RP, Benhamou Y, Imbert-Bismut F, Thibault V, Bochet M, Charlotte F, Ratziu V, Bricaire F, Katlama C, Poynard T. Serum biochemical markers accurately predict liver fibrosis in HIV and hepatitis C virus co-infected patients. AIDS 2003;17:721-725.

32. Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, Bruguera M, Sanchez-Tapias JM, Rodes J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986-992.

33. Pradat P, Alberti A, Poynard T, Esteban JI, Weiland O, Marcellin P, Badalamenti S, Trepo C. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. Hepatology 2002;36:973-977.

34. Poynard T, Aubert A, Bedossa P, Abella A, Naveau S, Paraf F, Chaput JC. A simple biological index for detection of alcoholic liver disease in drinkers. Gastroenterology 1991;100:1397-1402.

35. Naveau S, Poynard T, Benattar C, Bedossa P, Chaput JC. Alpha-2-macroglobulin and hepatic fibrosis. Diagnostic interest. Dig Dis Sci 1994;39:2426-2432.

36. Schuppan D, Stölzel U, Oesterling C, Somasundaram R. Serum markers for liver fibrosis. J Hepatol 1995;22:82-88.

37. Oh S, Afdhal NH. Hepatic fibrosis: are any of the serum markers useful? Curr Gastroenterol Rep 2001;3:12-18.

38. Trinchet JC. Clinical use of serum markers of fibrosis in chronic hepatitis. J Hepatol 1995;22:89-95.

39. Nojgaard C, Johansen JS, Krarup HB, Holten-Andersen M, Moller A, Bendtsen F; Danish Viral Hepatitis Study Group. Effect of antiviral therapy on markers of fibrogenesis in patients with chronic hepatitis C. Scand J Gastroenterol 2003;38:659-665.

40. Stickel F, Poeschl G, Schuppan D, Conradt C, Strenge-Hesse A, Fuchs FS, Hofmann WJ, Seitz HK. Serum hyaluronate correlates with histological progression in alcoholic liver disease. Eur J Gastroenterol Hepatol 2003;15:945-950.

41. Patel K, Lajoie A, Heaton S, Pianko S, Behling CA, Bylund D, Pockros PJ, Blatt LM, Conrad A, McHutchinson JG. Clinical use of hyaluronic acid as a predictor of fibrosis change in hepatitis C. J Gastroenterol Hepatol 2003;18:253-7.

42. Rosenberg WMC, Voelker M, Thiel R, Becka M, Burt, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJP, and the European Liver Fibrosis Group A cohort study of serum markers as predictors of human hepatic fibrosis assessed with histology and image analysis, submitted.

43. Schuppan D, Strobel D, Hahn EG. Hepatic fibrosis - therapeutic strategies. Digestion 1998;59:385-390.

Treatment of Acute Hepatitis C

Michael P. Manns, Andrej Potthoff, Elmar Jaeckel,
Heiner Wedemeyer

Hepatitis C Virus (HCV) infection is a common cause of chronic liver disease but is rarely diagnosed at the stage of acute hepatitis C. HCV becomes chronic in about 85% of individuals and leads to cirrhosis in 5 to 30 percent of cases [1]. The chronic course of acute infection should be avoided to prevent the potential risk of cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis C. Overall, approximately 3% of the world's population is thought to be infected with HCV. In the US, the National Health and Nutrition Examination Study III (NHANES III) showed an anti-HCV prevalence of 1.8% corresponding to 3.9 million anti-HCV-positive patients [2]. In Germany, approximately 350,000 individuals (0.4% of the population) are HCV carriers [3].

EPIDEMIOLOGY OF ACUTE HEPATITIS C

HCV is a blood-borne virus. Until 1990, hepatitis C was the most important post-transfusion hepatitis, but the epidemiology of HCV infection has changed dramatically. Nowadays, most patients with acute HCV infection are intravenous drug users. The risk of acquiring HCV by blood products has been reduced to less than 1/500,000. In Germany all blood products are screened for HCV RNA by PCR and thus, the risk of acquiring HCV through contaminated blood is close to zero.

NATURAL HISTORY

Regarding the acute phase of hepatitis C infection, it is currently estimated that 25-30% of people infected with HCV will clear the virus spontaneously 2-6 months after acquiring infection. Although there is no way of predicting viral clearance on an individual basis, it has been suggested that people with clinical symptoms of acute hepatitis (e.g. jaundice), are more likely to clear the virus [4]. These data support the findings of Gerlach et al. [5], and Nomura et al. [6], showing that symptomatic icteric patients more often clear the virus spontaneously than asymptomatic individuals. In the Nomura trial the incidence of spontaneous HCV clearance during the first two months was rather low (12% of cases), compared to other recent trials. The rate of spontaneous clearance of HCV in acute hepatitis C was also analysed by Hofer et al. [7] in a recent study. 75% (n=12) of patients with acute icteric hepatitis showed clearance of HCV in a repeated testing of HCV viral load. The authors concluded that early HCV viral kinetics are a useful tool for distinguishing between patients who require treatment and those who do not. Moreover, Lehmann et al. [8] suggest that spontaneous clearance of HCV may be higher in patients with HCV genotype 3 than in HCV genotype 1. There are also results suggesting that women, babies and young adults are also more likely to clear the virus than men [9].

DIAGNOSIS OF ACUTE HEPATITIS C

The symptoms of acute hepatitis C are usually mild and variable, and can include malaise, nausea, loss of appetite, weakness, abdominal discomfort, pale stools and dark urine. However, most patients do not have clinical symptoms or jaundice in the phase of acute viral infection and clinical symptoms are not different from other types of hepatitis. The asymptomatic patient is often detected via surveillance, such as following needle-stick exposure to a known carrier. Moreover, there is still no prophylactic vaccine for HCV.

Unfortunately, there is no specific diagnostic test to identify acute HCV infection, to distinguish it from reactivation phases that may occur in chronic infection and to predict spontaneous clearance in patients with acute HCV. Without an accepted serologic definition of acute hepatitis C, and because many individuals do not have a previously documented negative anti-HCV test, the diagnosis of acute

HCV is usually based on surrogate markers (e.g. the existence of a potential infection event, previously normal ALT values, exclusion of other liver diseases etc.). Thus acute hepatitis C has been difficult to study and there is still limited information about its natural history and optimal management strategies.

TREATMENT OF ACUTE HEPATITIS C

Unlike the treatment of chronic hepatitis, controlled trials and practice guidelines for the treatment of acute HCV are lacking. The NIH Consensus Conference stated that treatment of acute hepatitis C "is warranted" [10], and several meta-analyses of published studies have concluded that antiviral therapy during acute HCV infection significantly reduces evolution to chronic hepatitis [11]. This was supported by the 2003 German Hep-Net/DGVS consensus conference, which advises antiviral treatment of acute hepatitis C with interferon for 24 weeks, to prevent chronicity.

Several studies have assessed treatment with interferon in patients with acute hepatitis C, but there are problems with the accurate timely diagnosis of acute HCV and thus the comparison of research results. Different variables of the studies are represented in Figure 1. Furthermore, randomized controlled trials are difficult to perform, because of problems with patient enrolment, such as in patients with intravenous drug addiction, ethical issues, and the small number of people diagnosed with acute HCV.

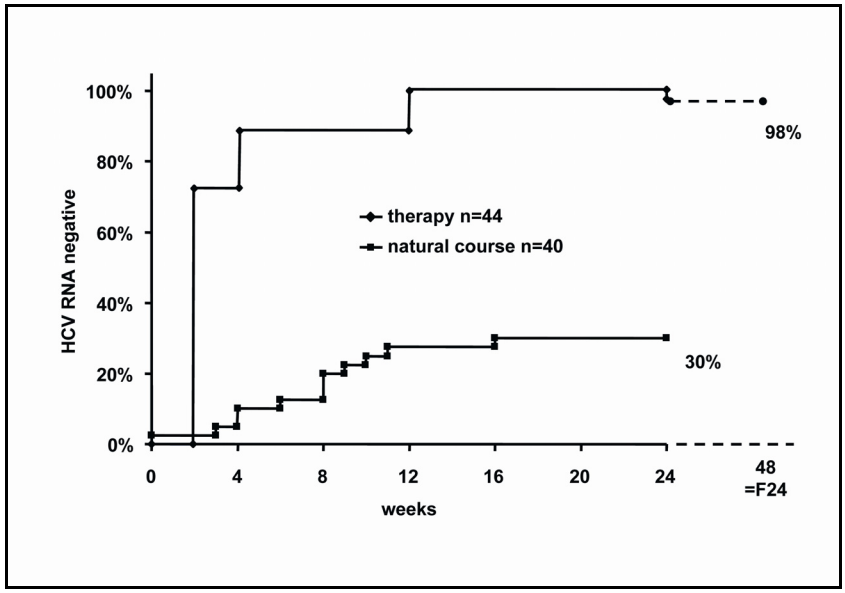

Figure 1: Early treatment of acute HCV-infection.
Santantonio et al. (Bari, Italy).

In order to standardize and optimize treatment of acute HCV, we performed a prospective controlled trial in 1998 in Germany [12, 13] and showed that monotherapy with interferon-alpha-2b for 24 weeks prevented chronicity of acute hepatitis C in 98% of cases (n=44 patients).

One issue is when to begin treatment? Should treatment of acute HCV be delayed 2-3 months after diagnosis or started immediately? Starting immediately seems reasonable, before the infection is established. In the Jaeckel study the favorable outcome of patients with acute HCV could be due to the fact that treatment was started a mean 89 days post-infection. In other studies [6, 7], a good starting point for therapy in HCV-infected, HCV RNA-positive patients was between day 70 and 100 after exposure, corresponding to day 20-50 after the onset of symptoms. Nevertheless, in the Jaeckel trial [12] one

third of patients who were treated might have cleared the virus spontaneously before starting treatment.

To study the efficacy of pegylated interferon in acute hepatitis C, a second German trial was started in February 2001 with pegylated interferon-alpha-2b alone for 24 weeks. Results were similar in this study to those in the Jaeckel study with virological response rates of >90% at least in the protocol analysis [14].

Thus far, there are no available data on the efficacy of antiviral therapy for asymptomatic acute hepatitis C. While many uncertainties remain, there are clear guidelines for management after injuries with HCV-contaminated needles [15]. All expert panels agree that there is no rationale for immediate post-HCV-exposure prophylactic treatment as is currently performed after HIV exposure. Monthly monitoring of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and a single measurement of the HCV RNA with PCR after 4-8 weeks are sufficient (Figure 2). Overall, it has been estimated that only 1-3% of needle stick injuries lead to infection.

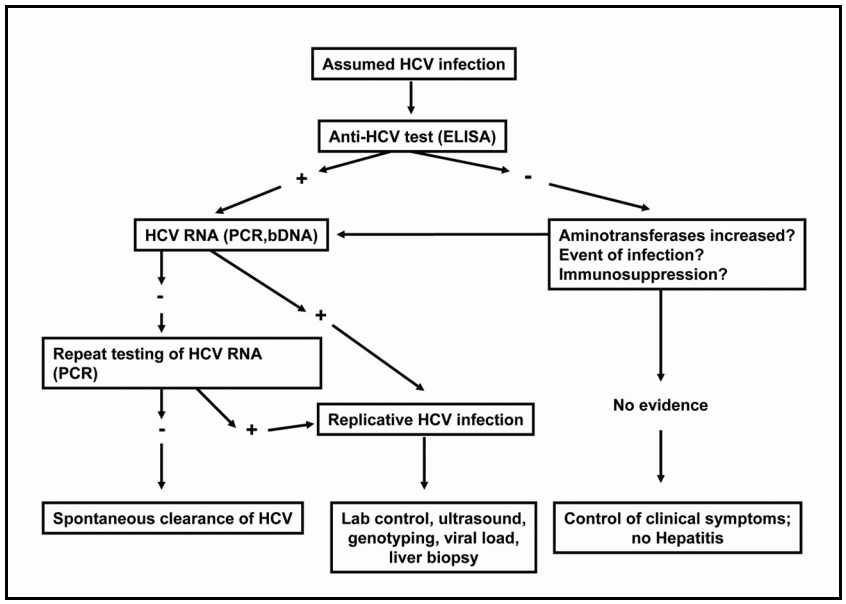

Figure 2: Diagnostic algorithm.

PERSPECTIVES

Numerous uncertainties remain about treatment of acute hepatitis C infection. When is the best time to start therapy; when the patient is first HCV RNA-positive before the onset of symptoms, at the peak of ALT elevation, or when HCV RNA is already declining? Or even later, after waiting for spontaneous HCV clearance, so that only persistently viremic patients undergo treatment? Because the only available data are for symptomatic acute HCV infection, the best treatment regimen for asymptomatic individuals is unknown. Should patients infected with HCV genotype 2 or 3 be treated since chronic HCV with genotype 2 or 3 can be cured in more than 50% of patients? What role should interferon therapy play in intravenous drug abuse patients, methadone substitution programs or concurrent infections with HIV or HBV?

In order to obtain new data about the advantages of the "wait and see" strategy on one hand, and immediate treatment, on the other, a nationwide upcoming randomised trial by the German network of excellence for viral hepatitis (Hep-Net: www.kompentenznetz-hepatitis.de) will compare these two treatment strategies (Figure 3). It is our hope that this Hep-Net acute hepatitis C III trial will provide answers to most of these open questions.

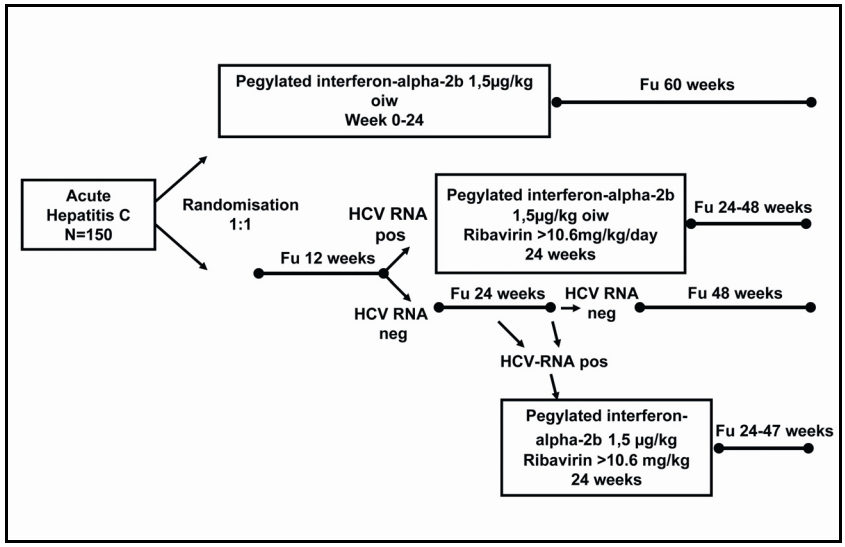

Figure 3: Immediate monotherapy vs. delayed combination therapy.
Acute hepatitis C No. 3 Trial by the German Network of Viral Hepatitis "HEP-NET" www.kompetenznetz-hepatitis.de.

REFERENCES

1. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. J Hepatol. 1999;30:956-961.
2. Alter MJ. Prevention of spread of hepatitis C. Hepatology 2002;36:S93-S98.
3. Schreier E, Höhne M. Hepatitis C-Epidemiologie und Prävention. Bundesgesundheitsbl Gesundheitsforsch-Gesundheitsschutz 2001;554-561.
4. Poynard T, Regimbeau C, Myers RP, Thevenot T, Leroy V, Mathurin P, Opolon P, Zarski JP. Interferon for acute hepatitis C. Cochrane Database Syst Rev. 2002;(1):CD000369.
5. Gerlach TJ, Zachoval R, Gruener N, Jung MC. Acute hepatitis: Natural course and response to antiviral treatment. Hepatology 2001;34:341A.
6. Nomura H, Sou S, Tanimoto H, Nagahama T, Kimura Y, Hayashi J, Ishibashi H, Kashiwagi S. Short-term interferon-alfa therapy for acute hepatitis C: a randomized controlled trial. Hepatology 2004;39:1213-1219.
7. Hofer H, Watkins-Riedel T, Janata O, Penner E, Holzmann H, Steindl-Munda P, Gangl A, Ferenci P. Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. Hepatology 2003;37:60-64.
8. Lehmann M, Meyer MF, Monazahian M, Manns MP, Wedemeyer H. High rate of spontaneous clearance of acute hepatitis C virus genotype 3 infection. J Med Virol 2004 (in press).
9. Farrell G. 'Hepatitis C liver disorders and liver health", MacLennan and Petty, Sydney, Philadelphia, London. 2002
10. NIH Consensus Development Conference Statement: Management of Hepatitis C: 2002. Jun 10-12. Hepatology 2002;36:S3-S20.
11. Alberti A, Boccato S, Vario A, Benvegnu L. Therapy of acute hepatitis C. Hepatology 2002;36:S195-S200.
12. Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M Pastore G, Dietrich M, Trautwein C, Manns MP; German Acute Hepatitis C Therapy Group. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001;345:1452-1457.
13. Jaeckel E, Manns MP. The course and therapy of acute hepatitis C viral infection. Is a prevention of its becoming chronic possible?. Z Gastroenterol 2000;38:387-395.
14. Wiegand J et al. 24 weeks of monotherapy with pegylated interferon-alfa-2b in patients with acute hepatitis C. Hepatology 2003;38:277A.

15. Wedemeyer H, Jackel E, Wiegand J, Cornberg M, Manns MP. Whom? When? How? Another piece of evidence for early treatment of acute hepatitis C. Hepatology 2004;39:1201-1203.

Treatment of Chronic Hepatitis C in Naive Patients

Ola Weiland

Treatment of naive patients with chronic hepatitis C has already been reviewed in international consensus meetings [1, 2]. The first consensus was held in Paris and standard interferon in combination with ribavirin was judged to be the best treatment for naive patients [1]. Later in 2002 the National Institutes of Health (NIH) consensus meeting stated that pegylated interferon in combination with ribavirin was the optimal treatment for chronic hepatitis C [2, 3]. There is no doubt that pegylated interferon is better than standard interferon and results in higher response rates, is easier to administer, and generally allows a better quality of life for patients during treatment, both as monotherapy and in combination with ribavirin [4-9]. Thus, at present the gold standard for treatment of naive patients with chronic hepatitis C is pegylated interferon in combination with ribavirin [2].

TREATMENT WITH THE COMBINATION OF PEGYLATED INTERFERON WITH RIBAVIRIN

The pivotal trials evaluating pegylated interferons have used either pegylated interferon-alpha-2b or -2a, different doses of pegylated interferon and ribavirin [6-8]. In the study by Manns et al. pegylated interferon-alpha-2b with a low 0.5µg/kg body weight once weekly (BW QW) (after a 4 weeks induction period with high dose) in combination with ribavirin 1000/1200mg q.d. or a high dose pegylated interferon-alpha-2b (1.5µg/kg BW QW) in combination with a fixed

800mg dose of ribavirin was compared with standard combination therapy (interferon-alpha-2b 3MU t.i.w. plus ribavirin 1000/1200mg depending on weight) [6]. The 1.5µg pegylated interferon-alpha-2b dose arm resulted in the highest overall sustained virologic response (SVR) rate (Figure 1). In the study by Fried et al., pegylated interferon-alpha-2a with a fixed dose (180µg QW) in combination with ribavirin 1000 or 1200mg depending on weight was compared with pegylated interferon-alpha-2a monotherapy, and standard combination therapy [7]. The overall SVR rate was highest in the pegylated interferon plus ribavirin arm (Figure 2). Treatment lasted 48 weeks for all patients in these studies, and a 24 week treatment schedule, which had been recommended for genotypes 2 and 3 at the Paris consensus meeting with standard combination therapy, was not evaluated. The length of treatment (24 versus 48 weeks) and ribavirin dose (800mg vs. 1000-1200mg) was further evaluated in a 4-armed study with pegylated interferon-alpha-2a with a fixed dose (180µg QW). In this study randomized patients were stratified according to genotype and viral load, with a pre-planned unequal distribution of difficult-to-treat patients (genotype 1 and or a high viral load) to the longer treatment making the overall results not applicable for comparison with other studies, but allowing assessment of response by genotype and viral load [8]. This study confirmed that a 24 week treatment was sufficient for genotypes 2 and 3 whereas 48 weeks was needed for genotype 1. A 24-week treatment schedule for genotype 2 or 3 has also recently been evaluated for pegylated interferon-alpha-2b (dosed 1.5µg/kg BW QW) in combination with ribavirin (800–1400mg q.d. depending on BW), and has been found to provide the same results as a 48-week schedule in the registration study [10]. No head to head comparison of the two approved pegylated interferons has been conducted but the above mentioned studies indicate that both pegylated interferons when combined with ribavirin are better than standard combination therapy.

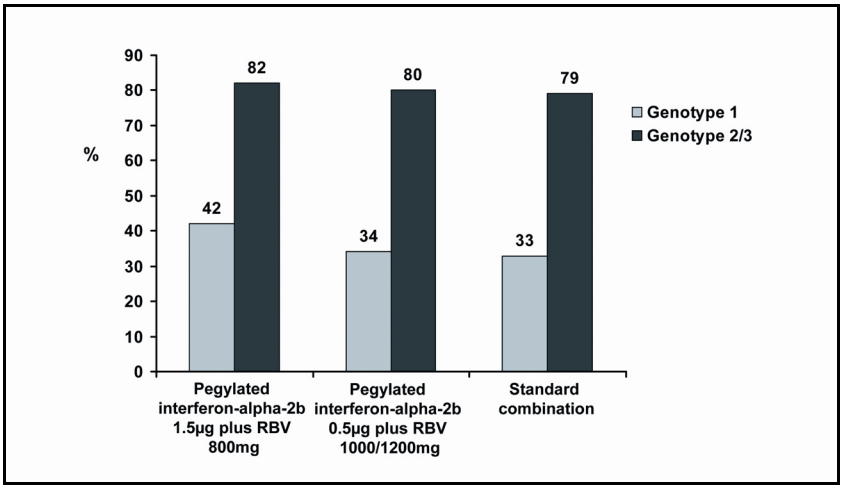

Figure 1: Sustained virologic response (SVR) (%) in registration study on pegylated interferon-alpha-2b by Manns et al. [6] according to treatment arm.

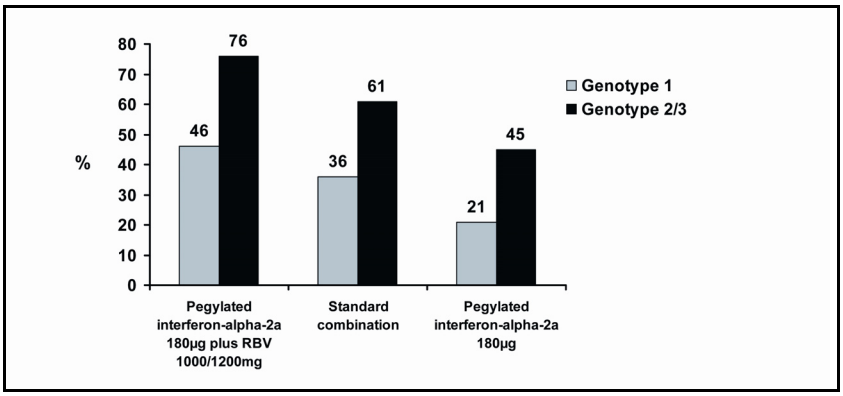

Figure 2: Sustained virologic response (SVR) (%) in registration study on pegylated interferon-alpha-2a by Fried et al. [7] according to treatment arm.

In these previous pivotal studies genotype was the strongest baseline factor to predict a sustained virologic response followed by viral load, extent of fibrosis, race, weight, alanine aminotransferase (ALT) quotient and gender. Furthermore, sustained virologic response has been the primary end-point in these studies and is the best indicator of a favorable response as well as an indication of eradication and cure for most patients during long-term follow-up [11]. Hence, this paper will review SVR separately according to genotype based primarily on these pivotal trials.

TREATMENT OF CHRONIC HEPATITIS C CAUSED BY GENOTYPE 2 OR 3

In the 2 pivotal registration studies [6, 7] only 48-week schedules were used, and not a 24-week schedule which had been judged to be sufficient for genotypes 2 and 3 with standard combination therapy [1]. However, a 24 week combination treatment with pegylated interferon-alpha-2a 180µg QW in combination with ribavirin 800mg or weight based 1000-1200mg q.d. was shown to result in the same SVR as the 48 week treatment schedule, 81-84% versus 79-80%, respectively, in patients with genotype 2 or 3 [8]. The same findings were found for pegylated interferon-alpha-2b when dosed 1.5µg/kg BW and given in combination with ribavirin dosed 800-1400mg q.d. according to weight [10]. This latter study only included a 24 week treatment arm but the overall 81% SVR rate was comparable to the 82% reached in the 48-week treatment arm in the registration study [6]. Thus, the optimal treatment length for genotypes 2 and 3 is 24 weeks and not 48 weeks since the shorter treatment reaches the same SVR rate as the longer, and is more cost-effective. Although treatment periods of less than 24 weeks seem to be sufficient in a subset of patients with genotype 2 or 3, for the moment, this has only been evaluated in small studies reported in abstract form.

On the other hand, certain monotherapy studies have indicated that lower pegylated interferon doses might be sufficient for genotypes 2 and 3 since 1.0µg/kg BW of pegylated interferon-alpha-2b and 135µg of interferon-alpha–2a have resulted in SVR rates that are similar or better than higher doses [4, 12]. However, lower doses of pegylated interferon in combination with ribavirin have only been investigated in small uncontrolled studies which have showed similar

results to those in randomized controlled studies with higher doses [13].

The SVR for genotype 2 and 3 with pegylated interferon-alpha-2a 180µg plus ribavirin are given according to ribavirin dose and viral load (low ≤800,000IU/mL versus high >800,000IU/mL) in Table 1a [8], and with pegylated interferon-alpha-2b 1.5µg/kg BW plus ribavirin 800-1400mg depending on BW according to viral load (low ≤600,000IU/mL versus high >600,000IU/mL) in Table 1b [10]. Results in patients with genotype 2 or 3 are also given separately in Table 1b.

Table 1: SVR at follow-up 24 weeks after treatment has been discontinued in patients with chronic hepatitis C caused by genotype 2 and 3 with pegylated interferon-alpha-2a (Table 1a) and pegylated interferon-alpha-2b (Table 1b) according to viral load.

		Sustained virologic response (SVR)		
Ribavirin dose	**Treatment length**	**All**	**LVL**	**HVL**
800mg	24 weeks	84%	85%	84%
1000/1200mg	24 weeks	81%	83%	80%
800mg	48 weeks	79%	88%	74%
1000/1200mg	48 weeks	80%	77%	82%

Table 1a: SVR (%) in patients with genotype 2 and 3 treated with pegylated interferon-alpha-2a 180µg according to ribavirin dose, treatment length, and viral load (low ≤800,000IU/mL (LVL) versus high >800,000IU/mL (HVL), Cobas Amplicor HCV Monitor Test, version 2.0, Roche Diagnostics, Brannchburg, New Jersey) [8].

		Sustained virologic response (SVR)		
Genotype	**Treatment length**	**All**	**LVL**	**HVL**
2 and 3	24 weeks	81%	87%	74%
2	24 weeks	93%	95%	91%
3	24 weeks	79%	86%	70%

Table 1b: SVR (%) in patients with genotype 2 or 3 treated with pegylated interferon-alpha-2b 1.5µg/kg BW plus ribavirin 800-1400mg depending on body weight according to viral load (low ≤600,000IU/mL (LVL) versus high >600,000IU/mL (HVL), real time polymerase chain reaction technology, lower limit of detection 29IU/mL) [10].

The overall SVR results in these studies are very similar, 84 and 81%, respectively. Results from the Hadziyannis study clearly indicate that 24 weeks of treatment with a fixed pegylated interferon-alpha-2a dose of 180µg QW is sufficient when combined with a 800mg ribavirin dose (low compared to the 1000/1200mg standard dose). This indicates that the dose of ribavirin can be lower in genotype 2 and 3 infections than in genotype 1. The SVR rate was the same in patients with high and low viral load in this study when genotypes 2 and 3 were analysed together. In the study by Zeuzem et al. both pegylated interferon-alpha-2b and ribavirin were dosed according to body weight. The overall SVR was in the same range as with pegylated interferon-alpha-2a plus ribavirin in the Hadziyannis study when genotype 2 and 3 were combined, but patients with high viral loads (>600,000IU/mL) responded less well and had an SVR of 74%. When the SVR was analysed separately for genotypes 2 and 3, a lower SVR was found but only in patients with genotype 3 who had high viral loads (Table 1b). The SVR rates in genotype 3 patients with high viral baseline loads was not published separately in the pegylated interferon-alpha-2a study by Hadziyannis et al. so no data are available. In the pegylated interferon-alpha-2b study by Zeuzem et al. the lower SVR rate in genotype 3 patients with high viral loads mainly seemed to be caused by a higher relapse rate in this category (23%) compared to patients with genotype 2 with low or high viral loads and genotype 3 with low viral loads (5-9%) [10]. These two studies indicate that different doses of ribavirin, a lower 800mg fixed dose versus a higher weight-based dose (800-1400mg) respectively are optimal when combined with pegylated interferon-alpha-2a and -2b respectively. The optimal dosing of ribavirin, however, has not yet been fully clarified, and results from population pharmacokinetic analysis suggest that it may be better to dose ribavirin according to renal function and not to body weight alone [14].

TREATMENT OF CHRONIC HEPATITIS C CAUSED BY GENOTYPE 1

The likelihood of achieving an SVR is predicted by pre-treatment patient characteristics, as well as the early virologic response. The strongest predictor for response is genotype. For the more difficult to treat genotype 1 infections, a 48 week treatment schedule is necessary, and improves results obtained with shorter treatment schedules [8].

The results obtained in three pivotal pegylated interferon plus ribavirin studies [6-8] are shown in Table 2.

In the registration pegylated interferon-alpha-2a study, a fixed 180µg interferon dose was used in combination with a 1000-1200mg weight based ribavirin dose, a treatment schedule which also provided the highest SVR rate in the 4-armed study evaluating ribavirin dose (800mg versus 1000/1200mg) and treatment length (24 versus 48 weeks) [7, 8]. The overall SVR with pegylated interferon-alpha-2a 180µg plus the weight based ribavirin dose in these studies was 46-52% (Table 2a). The corresponding SVR rates in patients with low or high baseline viral loads were 56-65% versus 41-47%, respectively.

Table 2: SVR at follow-up 24 weeks after treatment has been discontinued in patients with chronic hepatitis C caused by genotype 1 with pegylated interferon-alpha-2a (Table 2a) and pegylated interferon-alpha-2b (Table 2b) according to viral load and ribavirin dose.

		Sustained virologic response (SVR)		
Ribavirin dose	**Treatment length**	**All**	**LVL**	**HVL**
1000/1200mg	48 weeks	46%	56%	41%
800mg	48 weeks	41%	55%	36%
1000/1200mg	48 weeks	52%	65%	47%

Table 2a: SVR (%) in patients with genotype 1 treated with pegylated interferon-alpha-2a 180µg according to ribavirin dose, and viral load (low ≤800,000IU/mL (LVL) versus high >800,000IU/mL (HVL), Cobas Amplicor HCV Monitor Test, version 2.0, Roche Diagnostics, Brannchburg, New Jersey) [7, 8].

		Sustained virologic response (SVR)		
Ribavirin dose	**Treatment length**	**All**	**LVL**	**HVL**
800mg	48 weeks	42%	73%	30%
≤10.6mg/kg BW	48 weeks	38%	74%	27%
>10.6mg/kg BW	48 weeks	48%	71%	37%

Table 2b: SVR (%) in patients with genotype 1 treated with pegylated interferon-alpha-2b 1.5µg/kg BW and ribavirin 800mg q.d. (with a post hoc calculation of ribavirin dose ≤10.6mg versus >10.6mg per BW) according to viral load (low ≤2,000,000 copies/mL corresponding to ≤800,000IU/mL (LVL) versus high >2,000,000 copies/mL corresponding to >800,000IU/mL (HVL), quantitative PCR assay National Genetics Institute, Los Angeles, CA, USA, lower limit of detection 100 copies/mL) [6].

In the registration pegylated interferon-alpha-2b study, the best results were obtained in the arm using a fixed 800mg dose of ribavirin in combination with a 1.5µg pegylated interferon-alpha-2b dose/kg BW [6]. An overall SVR of 42% was reached, and the highest SVR (73%) was found in patients with low baseline viral loads and the lowest SVR in patients with high baseline viral loads (30%) (Table 2b). The low ribavirin dose was selected because of concern that a higher dose of pegylated interferon-alpha-2b might be associated with anemia that would exacerbate that caused by ribavirin. A post hoc evaluation showed that patients who received a ribavirin dose of >10.6mg/kg BW had a better response than those receiving less than 10.6mg/kg. Nevertheless, only 37% of patients with genotype 1 and high viral loads reached SVR in the high ribavirin dose group (http://www.emea.eu.int/index/indexh1.htm), and only 27% of those in the low ribavirin dose group [6].

In these randomized, controlled studies the SVR was lower in patients with high baseline viral loads, who were older, heavier, and had bridging fibrosis or cirrhosis.

TREATMENT OF CHRONIC HEPATITIS C CAUSED BY GENOTYPE 4

Genotype 4 seems to be more difficult to treat than genotypes 2 or 3. A combined analysis of data from patients infected with HCV genotype 4 who were enrolled in the Hadziyannis and Fried pivotal studies revealed that SVR was obtained in 17/24 (79%) of the patients treated with pegylated interferon-alpha-2a 180µg QW plus ribavirin 1000/1200mg QD for 48 weeks [7, 8]. In contrast, none of the 5 patients treated with a lower dose of ribavirin for 24 weeks obtained an SVR [15]. This suggests that if genotype 4 is treated in the same way as genotype 1 with high dose ribavirin for 48 weeks results can be obtained that are similar to those with HCV genotype 2 or 3 infections.

EARLY VIROLOGIC RESPONSE (EVR)

The predictability of an SVR based on EVR has been assessed in studies with both pegylated interferon-alpha-2a and-2b [7, 16]. An EVR, defined as a decline of HCV RNA levels by at least 2 logs at week 12 of treatment, is a good indication that an SVR will occur. Conversely, and perhaps more important, in those without an EVR, 97 to 100% will not have an SVR. Thus lack of an EVR at week 12 is used as a criteria to stop treatment of pegylated interferon plus ribavirin.

COMPLIANCE TO TREATMENT

Treatment compliance is of major importance for the outcome [17]. There is a higher rate of SVR in patients who take 80% of the pegylated interferon dose and 80% of the ribavirin dose for 80% of the scheduled treatment period than in patients who do not. Compliance to treatment period is particularly important. This is especially true in patients with HCV genotype 1 and in patient populations with low virologic response. It is therefore extremely important to treat side-effects promptly and effectively to avoid unnecessary discontinuation of therapy [18]. Hence, 80/80/80 compliance increased SVR in genotype 1 infections from an overall 42% with pegylated interferon-alpha-2b/ribavirin to 63% [6, 17].

ADVERSE EVENTS

In general, the incidence and types of side-effects with pegylated interferons combined with ribavirin are similar to those for standard interferon plus ribavirin.

Adverse events associated with pegylated interferon-alpha are bone marrow suppression with neutropenia and thrombocytopenia which seem to be somewhat more pronounced with pegylated interferon than standard interferon. The initial flu-like symptoms and decline in health related quality of life during treatment seem to be less pronounced with pegylated interferons than with standard interferons [9, 18].

Side-effects typically associated with ribavirin are hemolytic anemia, fatigue, itching and rash. Ribavarin-induced reactions depend primarily on serum concentrations of ribavirin, and not on the dose per kg BW [19].

CONCLUSIONS

Treatment for both HCV genotype 1 and probably genotype 4 with pegylated interferon-alpha plus ribavirin should be for 48 weeks, with a standard ribavirin dose (1000mg for patients weighing ≤75 kg and >1200mg for those >75kg). Either pegylated interferon-alpha-2a 180µg or pegylated interferon-alpha-2b 1.5µg/kg body weight once a week can be used. Quantification of serum HCV RNA should be performed at baseline and at week 12 during therapy to evaluate if a reduction in HCV RNA levels of at least 2 logs has occurred corresponding to an early viral response. Treatment should be discontinued in patients with no EVR. For genotypes 2 and 3, pegylated interferon and ribavirin for 24 weeks is sufficient and the ribavirin dose can be reduced to 800mg if needed. Further studies should be performed to see if lower pegylated interferon doses and shorter treatment times can be used for genotype 2 and 3 infections.

REFERENCES

1. Anonymous. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. J Hepatol 1999;30:956-961.

2. Anonymous. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002-June 10-12, 2002. Hepatology 2002;36:S3-S20.

3. Di Bisceglie A, Hoofnagle J. Optimal therapy of hepatitis C. Hepatology 2002;36:S121-127.

4. Lindsay K, Trepo C, Heintges T, Shiffman M, Gordon S, Hoefs J, Shiffman ML, Gordon SC, Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK; Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34:395-403.

5. Zeuzem S, Feinman S, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J, Brunda MJ. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343:1666-1672.

6. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 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.

7. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.

8. 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-alpha-2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-355.

9. Hassanein T, Cooksley G, Sulkowski M, Smith C, Marinos G, Lai MY, Pastore G, Trejo-Estrada R, Horta E, Vale A, Wintfeld N, Green J. The impact of peginterferon alfa-2a plus ribavirin combination therapy on health-related quality of life in chronic hepatitis C. J Hepatol 2004;40:675-681.

10. Zeuzem S, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, Sarrazin C, Harvey J, Brass C, Albrecht J. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. J Hepatol 2004;40:993-999.

11. Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. Ann Intern Med 1997;127:875-881.

12. Pockros P, Carithers R, Desmond P, Dhumeaux D, Fried M, Marcellin P, Shiffman ML, Minuk G, Reddy KR, Reindollar RW, Lin A, Brunda MJ; For the PEGASYS International Study Group. Efficacy and safety of 2-dose regimens of peginterferon alfa-2a compared with interferon alfa-2a in chronic hepatitis C: a multicenter randomized controlled trial. Am J Gastroenterol 2004:(in press).

13. Weiland O, Hollander A, Frisell K, Sandell S. Cost-effective therapy for genotype 2 and 3 chronic hepatitis C. Hepatology 2003;38:739A.

14. Bruchfeld A, Lindahl K, Schvarcz R, Stahle L. Dosage of ribavirin in patients with hepatitis C should be based on renal function: a population pharmacokinetic analysis. Ther Drug Monit 2002;24:701-708.

15. Diego M, Hassanein T, Rodes J, Ackrill A, Sedarati F. Optimized virologic response in hepatitis C virus genotype 4 with peginterferon-alpha 2a and ribavirin. Ann Intern Med 2004;140:72-73.

16. Davis G, Wong J, McHutchison J, Manns M, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645-652.

17. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK; International Hepatitis Interventional Therapy Group. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061-1069.

18. Fried MW. Side-effects of therapy of hepatitis C and their management. Hepatology 2002;36:S237-S244.

19. Lindahl K, Schvarcz R, Bruchfeld A, Stahle L. Evidence that plasma concentration rather than dose per kilogram body weight predicts ribavirin-induced anaemia. J Viral Hepat 2004;11:84-87.

Treatment of Chronic Hepatitis C in Non-Responders

Jay H. Hoofnagle

INTRODUCTION

The treatment of chronic hepatitis C has evolved markedly over the past 10 to 15 years [1]. With the initially approved regimens of standard interferon-alpha given alone for 24 weeks, sustained virological response (SVR) rates were only 6 to 12% [1, 2]. These rates were increased by prolonging therapy to 48 weeks, but the response rates were only 12 to 18% [2, 3]. A major improvement in response rates to interferon therapy came with the addition of ribavirin. Combination therapy for 48 weeks yielded sustained response rates of 38 to 47%, more than twice that of interferon alone [3, 4]. The introduction of pegylated interferons [5, 6] provided further increases in response rates, and combination therapy with ribavirin yielded SVR rates in the range of 54 to 56% [7-9]. Retrospective analyses and subsequent prospective controlled trials demonstrated that response rates and optimal dose-regimens varied with different genotypes of HCV. In patients with genotype 1 infection, the optimal regimen was full doses of pegylated interferon (180ug/week of alpha-2a or 1.5ug/kg/week of alpha-2b) combined with ribavirin (1000 to 1200mg/day) for 48 weeks to achieve response rates of 41 to 52% [1, 9]. In patients with non-1 genotypes (particularly genotypes 2 & 3), the optimal response rates (75 to 81%) could be achieved with a 24-

week course of full doses of pegylated interferon and reduced doses of ribavirin (800mg/day).

With each improvement in treatment regimen, the issue arises of whether patients who fail to respond to a previous course of therapy should be retreated with the more effective regimen [10]. Although early studies demonstrated that retreatment with the same regimen was associated with poor response rates, a proportion of non-responder patients responded to the more effective regimen [11, 12]. Unfortunately, response rates to retreatment are often low, and retreatment exposes patients to the added side-effects and expense of another course of therapy. Furthermore, if retreatment is attempted after each advance in therapy, many patients would undergo repeated courses of treatment without a sustained benefit. Clearly, the potential for efficacy and relative risks of retreatment regimens require careful assessment.

In discussing treatment of non-responders, two major issues must be analyzed separately: first, retreatment of patients who have failed to respond to a previous, non-optimal course of therapy; second, retreatment of patients who have failed to respond to the current optimal regimen. Furthermore, it is also important to consider the type of previous non-response for each category, whether it is a virological response and relapse or a documented virological non-response [10].

RETREATMENT OF NON-RESPONDERS TO STANDARD INTERFERON WITH OR WITHOUT RIBAVIRIN

Response rates with pegylated interferon have been consistently higher than those with standard interferon with or without ribavirin [1, 5-9]. Results of the major registration trials of standard interferon with and without ribavirin and of pegylated interferon with and without ribavirin are given in Table 1 for patients with genotype 1 and in Table 2 for patients with genotypes 2 and 3 (or in some instances "non-1"). When analyzed by genotype, SVR rates to pegylated interferon and ribavirin were 17 to 25% higher than those to standard interferon and ribavirin and 35 to 48% higher than those to standard interferon alone. From these results one can calculate a hypothetical response rate to retreatment:

$$\text{Expected rate} = [(\text{SVR of current regimen}) - (\text{SVR to previous regimen})] / (1 - \text{SVR to previous regimen}).$$

Author (yr)	**IFNα type**	**IFNα**	**IFNα**	**IFNα & RBV**	**IFNα & RBV**	**Peg IFN**	**Peg IFN & RBV**	**Peg IFN & RBV**
		24 wks	**48 wks**	**24 wks**	**48 wks**	**48 wks**	**24 wks**	**48 wks**
McHutchison (1998)	alpha-2b	1.8%	6.8%	15.9%	27.7%	-	-	-
Poynard (1998)	alpha-2b	-	11.2%	18.1%	31.1%	-	-	-
Lindsay (2001)	alpha-2b	-	6.5%	-	-	14.0%	-	-
Heathcote (2000)	alpha-2a	-	2.1%	-	-	12.5%	-	-
Manns (2001)	alpha-2b	-	-	-	33.2%	-	-	41.7%
Fried (2002)	alpha-2a	-	-	-	36.1%	20.7%	-	46.3%
Hadziyannis (2004)	alpha-2a	-	-	-	-	-	42.4%	52.0%
Average		**1.8%**	**7.6%**	**17%**	**32.8%**	**15.4%**	**42.4%**	**49.2%**

Table 1: Sustained virological response rates in large trials in chronic hepatitis C, genotype 1.
IFNα = interferon-alpha, RBV = ribavirin.

Author (yr)	IFNα type	IFNα	IFNα	IFNα & RBV	IFNα & RBV	Peg IFN	Peg. IFN & RBV	Peg. IFN & RBV
		24 wks	48 wks	24 wks	48 wks	48 wks	24 wks	48 wks
McHutchison [a] (1998)	alpha -2b	15.6%	28.6%	68.8%	67.2%	-	-	-
Poynard [b] (1998)	alpha -2b	-	33.3%	64.0%	63.9%	-	-	-
Lindsay [b] (2001)	alpha -2b	-	28.4%	-	-	48.1%	-	-
Heathcote [a] (2000)	alpha -2a	-	14.6%	-	-	51.3%	-	-
Manns [b] (2001)	alpha -2b	-	-	-	78.8%	-	-	81.0%
Fried [b] (2002)	alpha -2a	-	-	-	60.7%	44.9%	-	75.7%
Hadziyannis [b] (2004)	alpha -2a	-	-	-	-	-	82.5%	79.4%
Average		15.6%	28.2%	65.9%	68.2%	47.7%	82.5%	79.3%

Table 2: Sustained virological response rates in large trials in chronic hepatitis C, genotype 2 & 3 or non-1.
[a] Non-1 genotype (thus genotypes 2, 3, 4, 5 and 6), [b] Genotypes 2 and 3
IFNα = interferon-alpha, RBV = ribavirin.

Thus, for a patient with genotype 1 who previously received a 48-week course of interferon alone (SVR=7.6%) and is retreated with pegylated interferon and ribavirin for 48 weeks (SVR=49.2%), the expected response rate would be [(0.492-0.076)/(0.924)=45%]. Similarly for patients with genotype 1 who previously received a 48-week course of combination therapy (SVR=33%), the expected response rate to retreatment with 48 weeks of pegylated interferon and ribavirin would be [(0.492-0.328)/(0.672)=24%]. For genotype 2 and 3 infected patients, the expected response rates to retreatment with pegylated interferon and ribavirin would be 71% in patients who had received monotherapy and 35% in those who had received combination therapy using standard interferon. This analysis is clearly

oversimplified and requires prospective assessment. It is based on several assumptions: a lack of bias in patients selected for retreatment, an expected clinical and virologic profile and response rate that is similar to patients in the published registration studies, and a lack of change in the likelihood of response with time or with previous treatment. Thus, patients willing to undergo retreatment are likely to have tolerated therapy well during the first course and had at least a partial response to treatment or even a virological response and relapse. Patients who have had poor tolerance to interferon therapy are unlikely to accept retreatment. Perhaps even more important, there are specific clinical factors associated with response and an overall average response rate may not apply to the individual patient being retreated.

Although there have been many studies of retreatment, few have used the current recommended regimen of therapy for hepatitis C. The largest study to date was recently published based upon the lead-in phase of the HALT-C trial (Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis) in which patients with advanced fibrosis or cirrhosis who were non-responders (remaining HCV RNA-positive on therapy) to a previous course of standard interferon with or without ribavirin were retreated with pegylated interferon and ribavirin for at least 24 weeks [13]. The overall SVR was 18% and further analyses showed that SVR rates were 12% in patients who had previously received combination therapy and 28% in those who had received interferon alone (P<0.0001). Furthermore, SVR rates varied by genotype, and were 14% with genotype 1, 65% with genotype 2, and 54% with genotype 3 (P<0.0001). Thus, response rates were generally lower than the estimated rates based on the calculations given above. It is important to note that patients retreated in the HALT-C trial all had advanced fibrosis or cirrhosis, which has been shown to be associated with lower response rates. Furthermore, all were non-responders to a previous course of therapy, and relapse patients were not enrolled. Finally, the HALT-C trial was conducted in the United States and had a high proportion of older, overweight or obese, African-American patients, all factors that have been consistently associated with lower rates of response to interferon- based therapy of hepatitis C [1, 13].

Thus, the recommendation of retreatment for non-responders to a previous less-than-optimal course of therapy, resulted in SVR rates that are generally lower than predicted. Favorable clinical,

biochemical, histological and virological factors should be considered in the decision to retreat patients. Thus, retreatment might be recommended with some optimism for a young patient with genotype 2 or 3 who previously received a short course of interferon monotherapy. In contrast, retreatment may not be appropriate for the older, overweight patient with genotype 1 who previously had a virological nonresponse to the combination of standard interferon and ribavirin. Patients who relapse after an initial course of standard interferon with or without ribavirin are probably good candidates for retreatment with pegylated interferon and ribavirin. In this situation, longer courses of therapy might be considered as the duration of treatment is correlated strongly with a relapse [3, 9, 11]. Clearly, more studies of retreatment using pegylated interferon and ribavirin for different periods of time are needed to provide more reliable recommendations on retreatment.

RETREATMENT OF NON-RESPONDERS TO AN OPTIMAL REGIMEN OF THERAPY

Patients who have already received pegylated interferon and ribavirin and who have not had a virological response to therapy should not be retreated with the same regimen. Previous studies with standard interferon have demonstrated that retreatment with the same regimen usually results in the same non-response, unless there was a major lack of compliance or another unrelated adverse event during the initial therapy [10-12].

An alternative approach, however, is to retreat, not for virus eradication, but to ameliorate disease activity and prevent disease progression. This approach is based on long-term, maintenance therapy with either interferon (or pegylated interferon) or ribavirin or the combination. An important factor to mention when discussing these studies is that prevention of disease progression is a less well defined endpoint for antiviral therapy of hepatitis C than sustained virus eradication. The criteria for documentation of prevention of progression have not been clearly established, and results of studies of maintenance therapy must be viewed with caution. Disease progression in chronic hepatitis C is slow, variable and difficult to document. The proof that maintenance therapy can delay progression of hepatitis C requires large, randomized, controlled trials with well characterized cohorts of patients followed for several years with

careful documentation of disease activity and stage. Endpoints in these studies need to be carefully selected and focus on the prevention of fibrosis and cirrhosis and ultimately clinical decompensation. These requirements have not yet been met by any of the published studies on long-term maintenance therapy of chronic hepatitis C with interferon, ribavirin or both.

Maintenance therapy with interferon

Maintenance therapy with standard interferon alpha was evaluated by Shiffman and coworkers in a preliminary study of patients who had histological improvement without a complete virological response (remaining HCV RNA-positive) during 24 weeks of interferon monotherapy [14]. Patients underwent liver biopsy at the end of interferon treatment and 65 of 167 non-responder patients were found to have had a 50% decline in hepatic inflammatory scores compared to baseline. These patients were considered to be eligible for the study, and 53 agreed to be enrolled: 26 were assigned to stop interferon therapy and be followed on no treatment and 27 were continued on maintenance interferon at doses of 3 million units thrice weekly for an additional 2 years, with follow-up liver biopsies at 12 and 24 months. Thus, only virological non-responders who appeared to have had histological improvement during therapy were enrolled in this study. Eligible patients constituted approximately 39% of all non-responders who were evaluated for inclusion and only 32% agreed to enroll in the study and be treated for an additional 2 years.

The results of this trial showed that continuing interferon therapy maintained improvements in serum aminotransferase levels, HCV RNA titers and histological necroinflammatory scores in most patients. In contrast, discontinuation of treatment was followed by a shift of the serum biochemical, virological and histological improvements towards baseline. Thus, the biochemical and histological improvements that occur in approximately one-third of virological non-responders can be maintained by continuous interferon therapy. Of course, the major issue is whether these maintained responses result in reversal or retardation of disease progression. In this study fibrosis scores were improved by maintenance therapy but the differences between treated and untreated patients were not statistically significant. Thus, mean fibrosis scores increased in the control patients from 2.2 $\pm$ 0.3 to 2.4 $\pm$ 0.4 (P=0.11) and declined in

the patients on maintenance interferon from 2.5 $\pm$ 0.3 to 1.7 $\pm$ 0.4 (P=0.07). This study provided valuable preliminary results which supported the need for larger and more ambitious studies on maintenance interferon therapy.

Maintenance therapy with pegylated interferon

The possibility that maintenance therapy with standard or pegylated interferon will result in long-term improvement of hepatitis C despite lack of virus eradication is the focus of several ongoing trials, including the National Institutes of Health-supported trial entitled HALT-C. In that study, over 1500 patients with chronic hepatitis C and advanced fibrosis or cirrhosis (Ishak fibrosis scores 3-4 and 5-6) who previously failed to respond to a course of standard interferon with or without ribavirin were retreated with pegylated interferon and ribavirin for 24 weeks [13]. Patients who remained HCV RNA-positive despite therapy with this optimal regimen were then randomized to be treated with low doses of pegylated interferon alpha-2a (90mg/week) or to be followed on no specific therapy. Treatment was scheduled to last for 4 years with repeat liver biopsies at 2 and 4 years. The endpoints in this trial are progression to liver-biopsy determined cirrhosis (in patients with bridging fibrosis initially) and/or the clinical endpoints of death, liver transplantation, clinical decompensation or hepatocellular carcinoma. This trial is in its fifth year and most patients have completed the first two years of treatment and follow-up evaluation.

Maintenance therapy with ribavirin

The use of ribavirin alone is another approach to maintenance therapy to improve disease and prevent progression. Ribavirin monotherapy has been shown to improve serum aminotransferases and liver histology in approximately one-third of patients [15-18]. Patients treated with ribavirin for one to two years have shown improvements in necroinflammatory activity on liver biopsy [15, 18]. The question is whether these biochemical and histological improvements are also associated with prevention of disease progression whose major surrogate marker is hepatic fibrosis.

Recently, a randomized controlled trial evaluated the benefits of continuing ribavirin monotherapy when there was no virological response to a 24-week course of standard interferon and ribavirin [18].

In this study 108 patients with chronic hepatitis C were treated with a standard regimen of interferon alpha-2b and ribavirin for 24 weeks. Patients who did not become HCV RNA-negative on therapy were randomized to continue placebo or ribavirin alone (1000 to 1200mg/da**y**) without interferon. Fifty patients were non-responders at 24 weeks, and 34 agreed to be enrolled into the double-blind part of the study. The results showed that serum aminotransferase levels returned to baseline in most of the 17 patients randomized to receive placebo, but remained normal or near normal in most of the 17 patients randomized to continue ribavirin. After a year of placebo or ribavirin therapy, repeat liver biopsies showed improvements in histological activity among ribavirin- in comparison to placebo-recipients. The degree of histological improvement on ribavirin monotherapy was not as great as that in sustained virological responders. Furthermore, fibrosis scores improved significantly among the virological responders but did not change in the ribavirin recipients. Thus, ribavirin was able to maintain biochemical and histological responses in patients who continued to be viremic (at least in a proportion of patients) but did not appear to improve fibrosis. Patients with marked improvement in histological scores had normal or nearly normal serum aminotransferases levels after therapy.

Thus, maintenance therapy with ribavirin or interferon or both is an attractive approach to treat virological nonresponders but has yet to be shown to have a significant effect on the course of chronic hepatitis C. While both approaches appear to induce or maintain biochemical and histological responses, neither has been shown to delay the progression of disease or reverse fibrosis.

Maintenance therapy with ribavirin and pegylated interferon must be considered experimental and of unproven benefit at present. If this therapy is to be used outside of controlled trials, it should be limited to patients who tolerate maintenance therapy well and who exhibit and maintain a biochemical response while on treatment. Better definition of patients who may benefit from maintenance therapy and optimal means of monitoring and directing therapy is likely to arise from the ongoing trials of this approach in the near future.

Future therapies of hepatitis C

The importance of hepatitis C as a liver disease and the limited efficacy of current therapeutic regimens have led to a search for more effective and better tolerated therapies. These approaches have included non-specific therapies and recommendations; immune modulatory agents and cytokines, and specific antiviral drugs. These approaches are particularly appropriate for patients who have failed to respond to the optimal current regimen of pegylated interferon and ribavirin or who have specific contraindications to this therapy. The recent description of marked inhibitory effects of a HCV-specific serine protease inhibitor provides great promise that safe and effective small molecule therapies for hepatitis C will eventually be developed [19]. Furthermore, multiple alternative and innovative approaches to the treatment of hepatitis C are under active investigation and are likely to bear fruit [20]. At present these therapies remain experimental [21]. A patient who has failed to respond to optimal current therapy of hepatitis C is a prime target for new therapies of this important disease.

REFERENCES

1. Di Bisceglie AM, Hoofnagle JH. Optimal therapy of hepatitis C. Hepatology 2002;36:S121-S127.

2. Carithers RL Jr, Emerson SS. Therapy of hepatitis C: meta-analysis of interferon alpha-2b trials. Hepatology 1997;26:S83-S88.

3. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alpha-2b alone or in combinatin with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med 1998;339:1485-1492.

4. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha-2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha-2b plus placebo for 48 weeks for the treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426-1432.

5. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, Reindollar R, Reddy RK, Wright TL, Lin A, Hoffman J, De Pamphilis J. Pegylated interferon alpha-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673-1680.

6. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK; Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alpha-2b to interferon alpha-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34:395-403.

7. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD. Pegylated interferon alpha-2b plus ribavirin compared with interferon-alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001;358:958-965.

8. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alpha 2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.

9. 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 alpha-2a and ribavirin combination therapy in chronic hepatitis C. A randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-355.

10. Shiffman ML. Retreatment of patients with chronic hepatitis C. Hepatology 2002;36:S128-S134.

11. Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, Shiffman ML, Zeuzem S, Craxi A, Ling MH, Albrecht J. Recombinant interferon alfa-2b alone or in combination with ribavirin for retreatment of interferon relapse in chronic hepatitis C. N Engl J Med 1998;339:1493-1499.

12. Cummings KJ, Lee SM, West ES, Cid-Ruzafa J, Fein SG, Aoki Y, Sulkowski MS, Goodman SN. Interferon and ribavirin vs. interferon alone in the re-treatment of chronic hepatitis C previously nonresponsive to interferon: a meta-analysis of randomized trials. JAMA 2001;285:193-199.

13. Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, Dienstag JL, Ghany MG, Goodman ZD, Everhart JE; Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial Group. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. Gastroenterology 2004;126:1015-1023.

14. Shiffman ML, Hofmann CM, Sterling RK, Luketic VA, Contos MJ, Sanyal AJ. A randomized, controlled trial of maintenance interferon therapy for patients with chronic heptitis C virus and persistent viremia. Gastroenterology 1999;117:1164-1172.

15. Di Bisceglie AM, Conjeevarum HS, Fried MW, Sallie R, Park Y, Yurdaydin C, Swain M, Kleiner DE, Mahaney K, Hoofnagle JH. Ribavirin as therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 1995;123:897-93.

16. Dusheiko G, Main J, Thomas H, Reichard O, Lee C, Dhillon A, Rassam S, Fryden A, Reesink H, Bassendine M, Norkrans G, Cuypers T, Lelie N, Telfer P, Watson J. Ribavirin treatment for patients with chronic hepatitis C: results of a placebo- controlled study. J Hepatol 1996;25:591-598.

17. Bodenheimer HC Jr, Lindsay KL, Davis GL, Lewis JH, Thung SN, Seeff LB. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. Hepatology 1997;26:473-477.

18. Hoofnagle JH, Lau D, Conjeevaram H, Kleiner D, Di Bisceglie AM. Prolonged therapy of chronic hepatitis C with ribavirin. J Viral Hepatitis 1996;3:247-252.

19. Hoofnagle JH, Ghany MG, Kleiner DE, Doo E, Heller T, Promrat K, Ong J, Khokhar F, Soza A, Herion D, Park Y, Everhart JE, Liang TJ. Maintenance therapy with ribavirin in patients with chronic hepatitis C who fail to respond to combination therapy with alpha interferon and ribavirin. Hepatology 2003;38:66-74.

20. Lamarre D, Anderson PC, Bailey M, Beaulieu P, Bolger G, Bonneau P, Bos M, Cameron DR, Cartier M, Cordingley MG, Faucher AM, Goudreau N, Kawai SH, Kukolj G, Lagace L, LaPlante SR, Narjes H, Poupart MA, Rancourt J, Sentjens RE, St George R, Simoneau B, Steinmann G, Thibeault D, Tsantrizos YS, Weldon SM, Yong CL, Llinas-Brunet M. An NS3 protease

inhibitor with antiviral effects in humans infected with hepatitis C virus. Nature 2003;426:186-189.

21. Pawlotsky J-M, McHutchison JG. Hepatitis C. Development of new drugs and clinical trials: promises and pitfalls. Hepatology 2004;39:554-567.

Case Study in the Management of Chronic Hepatitis C in Non-responders to Antiviral Therapy

Jenny Heathcote

CASE

A 39 year old white business man was first found to have abnormal liver biochemical tests in 1990 when he went to see his family physician for a routine checkup. At the age of 17 he had begun injecting drugs and at age 18 he had an episode of acute hepatitis. Despite this he continued to be an injecting drug user for another 3 years. He had never been a heavy drinker, consuming no more than 3 beers per month. He has been overweight since he was a teenager (Body Mass Index 32). He has asthma, and takes local medication to relieve the symptoms. His mother is said to have died of liver cancer.

The patient was first referred to a hepatology clinic in 1997 after being tested and found positive for hepatitis C. At that time, his liver function tests were normal; serum bilirubin 11µmol/L, serum albumin 48g/L, and international normalized ratio (INR) of 1.2. His liver biochemical tests revealed an aspartate aminotransferase (AST) of 81IU/L and an alanine aminotransferase (ALT) of 160IU/L, serum alkaline phosphatase levels were normal. His hemoglobin was 146g/L, white cell count was 4.2×10^6/L, and platelet count was 148×10^6/L. Ultrasound suggested that the liver texture was heterogeneous without focal lesions and the size of the liver and spleen were normal. Bile

ducts were normal. A percutaneous liver biopsy performed in 1998 showed grade 2 activity and stage 4 fibrosis (METAVIR). Genotyping and viral load were not available at that time.

The patient was treated with interferon 3mU 3 times/week for 12 weeks. However his liver biochemical tests never returned to normal, he had headaches, fever and nausea and his white blood count decreased, so after 12 weeks he stopped treatment. The following year he was identified as genotype 1a with a viral load of $9x10^4$IU/L. In 2001 he was retreated with pegylated interferon alpha-2a (180µg/wk) and ribavirin 1200mg daily for a full year with no improvement in either liver biochemistry or viral load. He described the treatment as "brutal".

In 2003 he was reassessed because he was anxious about undergoing long-term therapy. He was recruited for the European prospective investigation into cancer and nutrition (EPIC) program and received a further 12 weeks of treatment with pegylated interferon alpha-2b 1.5µg/kg/wk + ribavirin 1200mg/day. At the end-point of these 12 weeks, his white blood cell count was only $1.5x10^6$ (absolute neutrophil count [ANC] 0.9) and his platelet count $70x10^9$/L. Liver function tests (Alb 4.1g/L, bilirubin 17mmol/L) were normal and his serum aminotransferases remained elevated (AST 149, ALT 252). There was a <2 log decrease in his viral load after 12 weeks of therapy. Treatment was stopped and he was randomized to long-term observation rather than long-term low dose pegylated interferon alpha-2b.

This 46-year old male has a 30 year history of hepatitis C and currently has compensated cirrhosis. He has failed to respond to interferon monotherapy, or pegylated interferon alpha + ribavirin. This case history raises a number of questions.

What are the risk factors for progressive disease?

Factors recognized to influence the rate of progression in chronic hepatitis C include age at acquisition, male gender, co-infection with hepatitis B and/or HIV, regular alcohol consumption and hepatic steatosis. In addition, there may be genetic factors which are unknown as yet [1].

What factors influence response to antiviral therapy?

Lack of response to treatment is influenced by viral genotype (genotype 1a responds particularly poorly), the pattern of quasi species development during treatment, and the viral titer. Genetic factors such as ethnicity (African-Americans respond poorly) and genetic factors controlling interferon responsiveness play a role. There may also be viral factors which influence interferon response genes, e.g. IRF3 inhibited by genotype 1. Host factors influencing antiviral therapy include the degree of hepatic fibrosis, hepatic steatosis, continued alcohol consumption and central obesity [2, 3].

Is antiviral therapy beneficial in patients with persistent viremia?

Short-term follow-up of individuals treated with interferon and ribavirin indicate that in some individuals, there may be a reduction in the degree of hepatic fibrosis, despite persistent viremia. However, long-term follow-up studies of therapy do not show that this improvement is maintained.

In patients with cirrhosis the risk of hepatocellular carcinoma (HCC) is only slightly reduced even in those with a sustained virological response to treatment, i.e. antiviral therapy needs to be given prior to the development of cirrhosis to effectively reduce the risk of HCC [4-7].

What treatment strategies are there for hepatitis C patients with cirrhosis who are non-responders to antiviral therapy?

Preventive strategies such as regular screening for hepatocellular carcinoma on a semiannual or annual basis (and early treatment if HCC is detected) as well as screening for esophageal varices and prophylactic therapy with non-selective beta blockers when varices develop may improve overall survival.

General strategies for patients with cirrhosis include advice on avoiding certain drugs, e.g. benzodiazepines, nonsteroidal anti-inflammatory drugs (NSAIDs), and aminoglycosides. Patients should also always be reminded to have infections treated rapidly. In patients who are not immune to hepatitis A and B, vaccination is recommended to avoid the risk of developing a superimposed acute hepatitis.

Because the risk factors for HCC in cirrhosis due to hepatitis C include iron overload, diabetes/obesity and smoking, attempts should be made to reduce these additional risks.

There are some data which suggest that the use of triple therapy (interferon, ribavirin and amantadine) may be effective in non-responders to antiviral therapies [8, 9].

REFERENCES

1. 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;349:825-832.

2. Layden-Almer JE, Ribeiro RM, Wiley T, Perelson AS, Layden TJ. Viral dynamics and response differences in HCV-infected African-American and white patients treated with IFN and ribavirin. Hepatology 2003;37:1343-1350.

3. Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. Hepatology 2003;38:639-644.

4. Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002;122:1303-1313.

5. Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000;132:517-524.

6. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Ann Intern Med 1999;131:174-181.

7. Farci P, Strazzera R, Alter HJ, Farci S, Diogioannis D, Coiana A, Peddis G, Usai F, Serra G, Chessa L, Diaz G, Balestrieri A, Purcell RH. Early changes in hepatitis C viral quasispecies during interferon therapy predict therapeutic outcome. Proc Natl Acad Sci USA 2002;99:3081-3086.

8. Teuber G, Pascu M, Berg T, Lafrenz M, Pausch J, Kullmann F, Ramadori G, Arnold R, Weidenbach H, Musch E, Junge U, Wiedmann KH, Herrmann E, Zankel M, Zeuzem S. Randomized controlled trial with IFN-alpha combined with ribavirin with and without amantadine sulphate in non-responders with chronic hepatitis C. J Hepatol 2003;39:606-613.

9. Foy E, Li K, Wang C, Sumpter R, Masanori I, Lemon S, Gale M. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science 2003;300:1145-1148.

Management of Patients with HCV/HIV Co-infection

Vincent Soriano

INTRODUCTION

Both HIV and hepatitis C virus (HCV) infections are global public health problems. Currently, more than 42 million people are estimated to have HIV worldwide, while HCV infection is found in 2-3% of the world's population, which represents around 175 million people [1]. Overall, nearly 10 million people are co-infected with both HIV and HCV.

Transmission of either HCV or HIV is frequent throughout parenteral exposure to contaminated blood and blood products, with HCV being 10 times more infectious than HIV. Co-infection with both viruses is therefore frequent in this population. For instance, HCV can be found in 70-90% of HIV-positive hemophiliacs and in 60-80% of HIV-positive intravenous drug users (IDUs) [2]. In contrast, sexual transmission of hepatitis C is rare, which explains the low 4-8% rate of HCV co-infection among HIV-positive homosexual men [3]. However, small epidemics of acute hepatitis C have been reported among homosexuals in London and Berlin, which seem to be associated with many sexual partners and blood shedding sexual practices [4, 5] Taking these differences in transmission among different risk groups into account, around one third of HIV-positive persons are estimated to be co-infected with HCV in Europe and the United States [6, 7].

Around 85% of HCV antibody-positive HIV co-infected individuals show HCV viremia [8, 9], a rate that is slightly above the 75% reported among HIV-negative individuals with HCV-positive serology. Thus, HIV seems to favor hepatitis C chronicity after initial exposure. Moreover, higher plasma HCV RNA levels (1 log on average) are found in HIV-positive individuals compared to hepatitis C patients without HIV [10]. In one study conducted among hemophiliacs, plasma HCV RNA levels increased 10-fold within the first 2 years after HIV seroconversion [11].

The distribution of HCV genotypes in the HIV population reflects the main route of HCV transmission. Genotype 1b accounts for more than two thirds of post-transfusion HCV infections and accordingly is the predominant genotype among hemophiliacs [12]. In contrast, genotypes 1a and 3a are much more frequent among IDUs [13]. However, recent evidence suggests that HCV genotypes 1 and 4 are becoming more frequent than genotype 3 in Europe, while the frequency of HCV-3 is steadily decreasing [14]. Given the prognostic value of HCV genotypes and HCV load on treatment response, co-infected patients should be generally considered as a difficult-to-treat population.

NATURAL HISTORY OF CHRONIC HEPATITIS C IN HIV-POSITIVE PATIENTS

There is no doubt that HIV accelerates the progression of HCV liver disease, especially when HIV-associated immunodeficiency progresses [15, 16]. In the American Multicenter Hemophilia Cohort study, liver failure occurred in 9% of multitransfused HCV/HIV co-infected hemophiliacs with no AIDS-defining condition [15]. In contrast, during the same period, no cases of liver failure were observed among HCV-positive hemophiliacs without HIV infection. Subsequently, several studies have confirmed the unfavorable course of hepatitis C in HIV co-infected patients, particularly in the setting of advanced CD4 depletion [16, 17]. The time interval between HCV acquisition and the development of cirrhosis is significantly shortened in co-infected subjects. Overall, within 10-15 years after the initial HCV infection, 15-25% of HCV/HIV co-infected patients develop cirrhosis [18-21], compared to 4-6% of HIV-negative patients with hepatitis C. It is important to note that co-infected hemophiliacs who died from advanced liver disease were 10 years younger than HIV-

negative hemophiliacs with hepatitis C [22]. Moreover, several reports have also emphasized the occurrence of hepatocellular carcinoma at a younger age and after a shorter duration of hepatitis C infection in HCV/HIV co-infected individuals [23]. Finally, in a recent European study including 914 patients co-infected with hepatitis C and HIV, who underwent liver biopsy, the distribution of METAVIR liver fibrosis stages was F0 in 10% of patients, F1 in 33%, F2 in 22%, F3 in 22% and F4 in 13%, clearly showing the increased severity of liver fibrosis in this population [24]. In that study, the best predictor of severe liver fibrosis was age: nearly 50% of HCV/HIV co-infected patients over 40 years had bridging fibrosis or cirrhosis (Figure 1). It should also be stated that the mean age of HIV-positive individuals being treated in European and North American clinics is 40-45 years old.

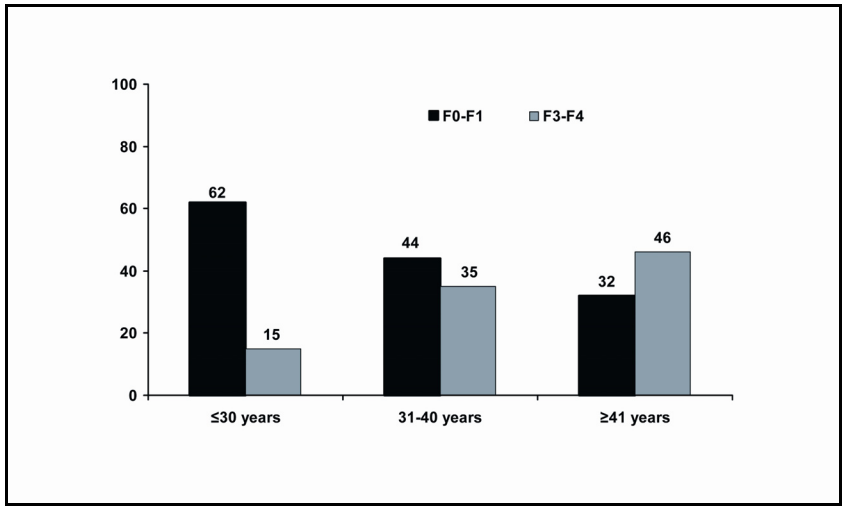

Figure 1: Liver fibrosis stage according to age in HCV/HIV co-infected patients [24].

Recent evidence suggests that the immune restoration that follows the use of antiretroviral therapy might reverse the unfavorable course of hepatitis C in co-infected patients. A study in 162 HCV/HIV

co-infected individuals who underwent liver biopsy, demonstrated that use of protease inhibitors as part of the highly active antiretroviral therapy (HAART) regimens resulted in significantly reduced rates of fibrosis progression [25]. These findings have been further confirmed by a recent long-term cohort analysis showing that HCV/HIV co-infected individuals on HAART had significantly lower liver-related mortality than patients receiving either suboptimal (1 or 2 HIV drugs) or no antiretroviral therapy [26]. Overall, the data available suggest that HAART has a favorable impact on the further course of hepatitis C in co-infected patients. These benefits of HAART seem to largely outweigh the risks of increased liver toxicity in this population [7]. Thus, HAART should be offered to all HCV/HIV co-infected patients using the general guidelines of antiretroviral treatment [27].

However the short and long-term success of HAART in HCV/HIV co-infected patients, is limited by an increased risk of hepatotoxicity [28, 29]. Multiple studies have demonstrated that underlying hepatitis C is an independent predictor of liver enzyme elevations after initiating HAART [30-33]. Further studies have shown a significantly increased risk of liver toxicity in co-infected patients especially with the protease inhibitor ritonavir (at doses of 600mg bid) and with nevirapine [34, 35]. Moreover, the use of "d-nucleosides" (didanosine [ddI], Zerit [d4T], Hivid [ddC]), especially the combination of d4T plus ddI has been found to result in an increased rate of hepatic steatosis in co-infected patients [36-39]. Therefore, d-nucleosides (especially the combination of d4T plus ddI) should be avoided, when possible, in HCV/HIV co-infected patients.

Liver disease due to chronic hepatitis C is now a leading cause of morbidity and mortality among HIV infected patients in the developed world, where the classic opportunistic complications of severe immunodeficiency have declined dramatically thanks to the widespread use of potent antiretroviral therapies [40-43]. Initial trials with interferon and much later with interferon plus ribavirin provided disappointing response rates and high drop-out rates due to adverse events in HCV/HIV co-infected patients [44-48]. However, the recent availability of the new pegylated forms of interferon (pegylated interferon) provides better maintenance of effective interferon levels for over a week after a single injection, allowing weekly subcutaneous administration of the drug. The first results with the pegylated interferon plus ribavirin combination are now available and the

improved performance of this combination in several trials is encouraging for HIV-positive patients with hepatitis C. As a result, new guidelines about how to manage HCV/HIV co-infected patients have recently been released [49] and will be the main body of discussion of this review.

THE BEST HIV-POSITIVE CANDIDATES FOR HCV THERAPY

All HIV infected individuals should be screened for HCV antibodies in serum or plasma. HCV-antibody negative but HCV RNA-positive cases may exist, mainly in patients with severe cellular immune suppression due to HIV [10, 50, 51]. Those with repeatedly elevated aminotransferase levels should be tested for HCV load and HCV genotype, to assess anti-HCV therapy.

All HIV-positive patients with chronic HCV infection should be considered as potential candidates for anti-HCV therapy, due to the higher risk of progression to end-stage liver disease and increased risk of liver toxicity after beginning antiretroviral therapy, compared to HIV-negative patients [49]. Since response to HCV therapy is dependent on the CD4 count [44, 52], ideally it should only be prescribed when the CD4 count is above 350 cells/μL, a threshold which is relatively easy to obtain in most instances when antiretroviral therapy is properly used. Besides, this is currently the immunological cut-off for beginning antiretroviral therapy in drug-naive patients [27]. In subjects with CD4 counts between 200 and 350 cells/μL, who are already receiving long-term antiretroviral therapy, the decision to treat HCV should take into account other factors, such as the estimated length of HCV infection, the severity of liver disease, the extent of suppression of HIV replication, and classical predictors of response to anti-HCV therapy, such as HCV genotype and HCV load [53, 54].

Finally, anti-HCV therapy should be postponed in individuals with less than 200 CD4+ T cells/μL, since the response rate is very low in this subgroup of patients [44, 52]. Moreover, the risk of opportunistic infections in the short-term may be high and may worsen with HCV therapy [55, 56]. Therefore, these patients should be treated with antiretroviral therapy and receive prophylaxis for opportunistic infections first. Later on, when their CD4 counts have risen and their plasma HIV RNA is under control, the possibility of HCV therapy should again be assessed.

Patients with prior liver decompensation (ascites, gastrointestinal bleeding, hepatic encephalopathy, etc) should not be treated, due to the higher risk of serious side-effects using the current approved drugs, pegylated interferon and ribavirin. These patients should be assessed for liver transplantation. However, patients with compensated cirrhosis (Child-Pugh class A) must be treated, since their chance of response is currently relatively high and are ultimately those who will benefit most from HCV clearance.

Individuals with a prior history of severe neuropsychiatric disorders should not be treated, since interferon can exacerbate these conditions. Individuals who have heavy alcohol intake and/or are addicted to illegal drugs should delay treatment, and all efforts should be made to put them onto detoxification programs. Patients on methadone are acceptable candidates for anti-HCV therapy. Up to one third of patients may require adjustment in methadone dosage [57]; however, this is for psychological reasons rather than pharmacological interactions between HCV drugs and methadone. Ideally, a multidisciplinary team, including experts in addiction, psychologists/ psychiatrics and infectologists should treat these patients [58, 59].

Based on the 2002 NIH Consensus Conference recommendations [60], subjects with repeated normal liver enzymes may benefit from current HCV therapy, particularly those infected with HCV genotypes 2 or 3. However, more data on liver damage in this subgroup of HCV/HIV co-infected patients are needed to balance the cost-benefit of anti-HCV therapy [49]. Preliminary data from the APRICOT trial suggest that liver fibrosis may be recognized in a substantial proportion of co-infected patients with normal ALT levels, although treatment response rates seem to be lower in this population.

In drug-naive individuals with HCV/HIV co-infection, chronic hepatitis C should be treated first if the CD4 count does not require antiretroviral therapy. However, in patients with CD4 counts above 350 cells/μL but high plasma HIV RNA (i.e., above 50,000 copies/mL), it is not clear whether suppression of HIV replication should be done first, postponing anti-HCV therapy until after HIV viremia becomes undetectable. In these patients, a possible greater efficacy of HCV therapy should be weighed against a greater risk of interactions between antiretroviral and HCV drugs [49].

THE ROLE OF LIVER BIOPSY IN TREATMENT DECISIONS

The value of liver biopsy before prescribing HCV therapy is under debate [61-64]. Liver histology allows staging of HCV liver damage and predicts the development of cirrhosis in the short-mid term. At the same time, it may rule out other causes of liver damage, such as hemochromatosis, alcoholic steatosis, Wilson's disease, autoimmune hepatitis, etc., although these conditions may be identified by other non-invasive means [61-64].

This controversy is less a problem in HCV/HIV co-infected patients, because the rate of advanced liver fibrosis is much higher than in HCV monoinfected persons [20-24]. Anti-HCV therapy will almost always be justified because of the extent of histological damage in HCV/HIV co-infected patients [65]. Moreover, nearly half of co-infected patients may show unexpected cirrhosis or pre-cirrhosis [24]. The main predictor of advanced fibrosis stages seems to be age, reflecting the estimated duration of HCV infection. On average, nearly half of patients will have cirrhosis 25 years after first exposure to HCV. The mean age of co-infected patients is currently 40 years old, and most are former intravenous drug users who began to exchange needles when they were about 20 years old, thus many of them should now have significant liver fibrosis. Therefore, if they are not treated, a rapid increase in liver complications among HIV infected persons should occur over the next decade.

Those in favor of a liver biopsy before treating chronic hepatitis C in HIV co-infected patients argue that side-effects, the risk of interactions with antiretroviral treatment and the relatively low efficacy of current anti-HCV therapy in this population are major limitations so that medication should only be prescribed for those who histologically really need it. However, liver damage is a dynamic process and progression of fibrosis is accelerated in HCV/HIV co-infected patients [66, 67], so those who support this point of view should be reminded that if treatment is not offered to patients without or with minimal fibrosis, liver biopsy should be repeated at 2-3 year intervals. However, this option would be refused by many patients and may significantly increase costs. Accordingly, a recent analysis has demonstrated the cost-effectiveness of therapy in co-infected individuals [68].

TREATMENT RESULTS IN HCV/HIV CO-INFECTED PATIENTS

In the last few months, the final results of large trials assessing the efficacy and safety of pegylated interferon plus ribavirin in co-infected patients have been released. Most of these studies have been performed by European investigators [69-72]. However, the trials about this treatment modality in co-infected patients that had the greatest public impact appeared in February 2004, when three large comparative trials were presented orally at the 11th Retrovirus Conference, in San Francisco, CA. In contrast to many prior studies, these three pivotal trials all provided treatment for 12 months to all patients, irrespective of their HCV genotype. Besides, due to concerns on drug interactions and further toxicities, lower than recommended doses of ribavirin were prescribed. Moreover, only patients with a relatively good immunologic status were recruited into these trials, acknowledging that severely immunosuppressed patients should not be treated.

Table 1 summarizes the main treatment schedules and results of the main trials conducted in co-infected patients assessing the efficacy and safety of pegylated interferon plus ribavirin.

Study	No.	Treatment schedule	Discont. due to adverse events	End-of-treatment response*	Sustained virological response*
Pérez-Olmeda et al. [69]	68	pegylated interferon alpha-2b 1.5µg/kg/week + ribavirin 800mg/d 48 weeks (genos 1 & 4) and 24 weeks (genos 2 & 3)	15%	40%	28%
Voigt et al. [70]	72	pegylated interferon alpha-2b 1.5µg/kg/week + ribavirin 800mg/d 48 weeks (genos 1 & 4) and 24 weeks (genos 2 & 3)	17%	46%	26%
Ballesteros et al. [71]	28	pegylated interferon alpha-2b 1.5µg/kg/week + ribavirin 800mg/d 48 weeks (genos 1 & 4) and 24 weeks (genos 2 & 3)	29%	25%	29%
Moreno et al. [72]	35	pegylated interferon alpha-2b 0.5µg/kg/week + ribavirin 800mg/d 48 weeks (all genos)	17%	40%	31%
Chung et al. [73]	66	pegylated interferon alpha-2a 180µg/week + ribavirin 600mg/d (increased to 1000mg/d at week 12) 48 weeks (all genos)	12%	41% (geno 1:29%) (geno 3:80%)	27% (geno 1:14%) (geno 3:73%)
Perronne et al. [74]	205	pegylated interferon alpha-2b 1.5µg/kg/week + ribavirin 800mg/d 48 weeks (all genos)	38%	36%	27% (genos 1-4:16%) (geno 2-3:43%)
Torriani et al. [75]	289	pegylated interferon alpha-2a 180µg/week + ribavirin 800mg/d 48 weeks (all genos)	12%	49% (geno 1:38%) (genos 2-3:64%)	40% (geno 1:29%) (genos 2-3:62%)

*results based on intent-to-treat analyses.

Table 1: Response to pegylated interferon plus ribavirin in HCV/HIV co-infected patients; results of pivotal studies.

The AIDS Clinical Trials Group (ACTG) 5071 included 66 co-infected patients from several centers located in the United States. Subjects were treated with a fixed dose of 180µg/week of pegylated interferon-alpha-2a (Pegasys) plus ribavirin [73]. All subjects began ribavirin at doses of 600mg/day and increased up to 1000mg over the last weeks if the tolerance was acceptable. In this trial, 77% of patients carried HCV genotype 1, which tends to respond less well to HCV therapy. End-of-treatment-response (EOTR) was reached in 41% of patients, but sustained virological response (SVR) only was maintained by 27% (14% in subjects with HCV genotype 1 and 73% in those with other genotypes).

The RIBAVIC trial was a multicenter French study performed by the Agence Nationale de la Recherche Scientifique, in which 205 co-infected patients were treated with a weight adjusted dose (1.5µg/kg/week) of pegylated interferon-alpha-2b (pegylated Intron) plus a fixed dose of 800mg of ribavirin [74].

AIDS Pegasys Ribavirin International Co-infection Trial (APRICOT) is the largest trial so far in co-infected patients assessing the response to current HCV therapy. A total of 289 co-infected patients from several countries and continents received at least one dose of pegylated interferon-alpha-2a (Pegasys) 180µg/week plus a fixed dose of 800mg of ribavirin per day [75]. The overall rate of SVR was 40%, but it dropped to 29% in patients with HCV genotype 1. Close monitoring of patients and strict inclusion criteria provided a relatively low discontinuation rate in this trial (25%), whereas in the French RIBAVIC trial up to 38% of patients did not complete therapy [74].

Much better response rates were obtained for HCV genotypes 2 or 3 compared to genotype 1 in all these trials. For instance, in the APRICOT trial the rate of EOTR was 64%, with a rate of SVR of 62%. This low relapse rate for these genotypes should be mentioned, and suggests that extending treatment beyond 24 weeks for those particular genotypes appears necessary to avoid relapses in the setting of HIV infection [76]. In prior studies in which co-infected patients with HCV genotypes 2 or 3 were treated for only 24 weeks, relapse rates were recognized in more than one third of patients [76].

In summary, the use of pegylated interferon plus ribavirin improves the rate of SVR in HIV-positive patients with chronic hepatitis C and therefore should be considered the best treatment choice in this population, as it is in HCV monoinfected individuals

[60]. The best response rates were seen in the APRICOT trial, although some differences existed between the studies. For example, the lower ribavirin dosages of 600mg per day given initially in ACTG A5071 could explain the lower response rates. Similarly, discontinuation rates of nearly 40% significantly penalized the response in the RIBAVIC trial. Other features, such as the proportion of patients with HCV genotype 1, with cirrhosis, or who were intravenous drug users could also further explain the lower response rates in those trials (see Table 2).

	ACTG 5071	**APRICOT**	**RIBAVIC**
No. on pegylated + ribavirin	67	289	205
IDUs	80%	62%	81%
Cirrhotics	11%	15%	18%
Genotypes 1-4	77%	67%	69%
Mean CD4 count	492	520	525
With HAART	85%	84%	82%
Discontinuations	–	25%	42%
EOTR (ITT)	41%	49%	36%
SVR (ITT)	27%	40%	27%

Table 2: Mean characteristics of the study populations and results of the three pivotal studies reported at the 11th Retrovirus Conference (February 2004).

The reason why anti-HCV therapy obtains such a poor response in the setting of HIV infection are multiple (Table 3) [49]. Since both pegylated interferon and ribavirin act, at least partially, as immunomodulatory agents, subtle immune defects due to HIV infection might have a negative influence on the performance of these drugs, even in patients with high CD4 counts and undetectable plasma HIV RNA under antiretroviral therapy.

Use of lower than optimal doses of ribavirin in most trials.
Less activity of anti-HCV therapy in the setting of HIV-related immune dysfunction.
More advanced liver fibrosis stage.
Higher rate of steatosis (alcohol, nucleoside analogs).
Unfavorable HCV virological features (high HCV RNA titers).
Lower initial HCV RNA clearance on treatment.
More frequent relapses after treatment discontinuation.
Higher rate of treatment withdrawals due to side-effects.
Lower drug compliance.

Table 3: Factors explaining the lower response rates to HCV therapy in HCV/HIV co-infected patients.

In addition, we also mentioned that there was a high rate of HCV treatment discontinuation in some of the trials performed in HIV co-infected patients, sometimes more than one third of recruited patients. Although this may reflect a higher rate of serious adverse events in this population compared to HIV-negative patients, which is usually less than 15% [77, 78], it might also suggest that some HIV physicians are not familiar with the management of side-effects of anti-HCV therapy. Thus, efforts to minimize side-effects with preventive symptomatic treatments and appropriate management of complications are critical to ensure completion of HCV therapy in most patients.

MONITORING THE RESPONSE TO HCV THERAPY IN HIV-POSITIVE PATIENTS

Individuals with HCV alone who will clear HCV RNA with HCV treatment show a virological response soon after beginning therapy [60, 78, 79]. Therefore, early assessment of serum or plasma HCV RNA titers after starting treatment may help identify who will benefit

from prolonging therapy and who will not. Those HIV-negative patients who show a decline in HCV RNA greater than 2 logs and/or to undetectable levels at 12 weeks of therapy, may eventually reach a sustained response. In contrast, almost none of those who have HCV RNA reductions of less than 2 logs at 12 weeks achieve this goal. Therefore, HCV therapy may be discontinued at week 12 based on this virological criteria in early non-responders [60]. This guide to HCV therapy can spare side-effects and cost in individuals with no chance of cure. In HCV/HIV co-infected patients these considerations are even more crucial, since interactions between antiretrovirals and HCV drugs are frequent and issues related to poor compliance in subjects under polymedications are highly relevant [49].

Kinetic studies suggest that HCV clearance after beginning therapy with interferon may be delayed in the setting of HIV infection [80] (Figure 2a). Therefore, concern exists about the reliability of the 2 log HCV RNA reduction rule at 12 weeks: it might not work in HCV/HIV co-infected patients. However, data from several recent trials, included those from ACTG A5071, RIBAVIC, APRICOT and others suggest that despite a slower decay in HCV/RNA in HIV co-infected patients after beginning HCV therapy, all subjects who will reach SVR show a greater decline than 2 logs at week 12 of therapy [71-75]. Furthermore, a more recent report has demonstrated the predictive value of the 2 log rule at week 12 in co-infected patients in a better designed study [81]. In this study, the only difference between HIV-positive and HIV-negative subjects with hepatitis C was that the proportion of patients reaching virological response at any given time point was much lower in the co-infected population (see Figure 3), but it did not deny the predictive value of SVR using early virological assessments. Therefore, the principles guiding anti-HCV therapy in HIV-negatives may also apply to HIV co-infected patients (see Figure 4).

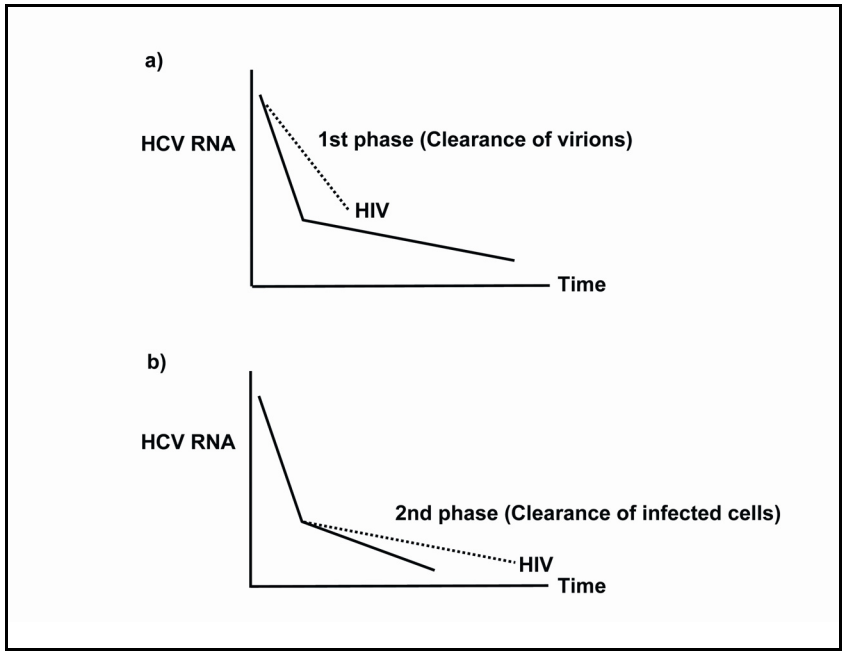

Figure 2: Hepatitis C virus kinetics under interferon therapy. Influence of HIV infection [49]: a) early phase; b) second phase.

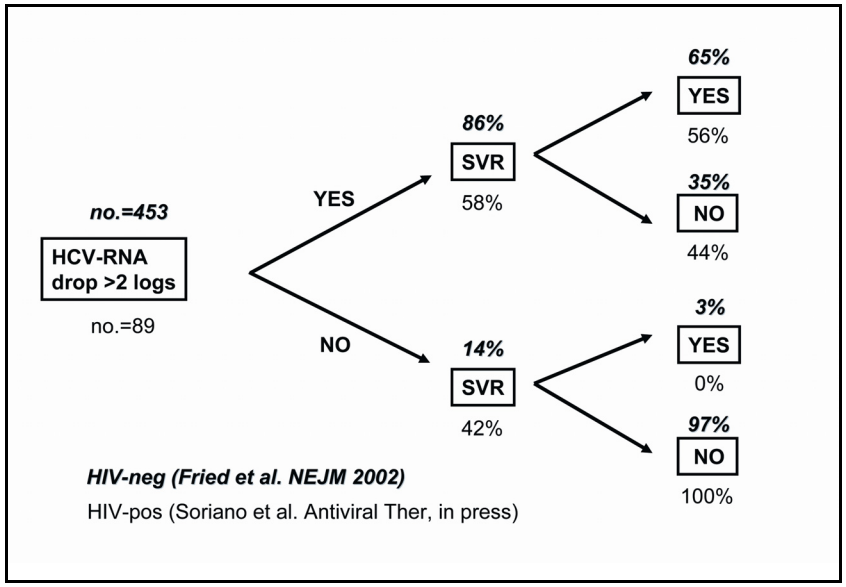

Figure 3: Virological response at different time points in HIV-positive [81] versus HIV-negative patients [78].

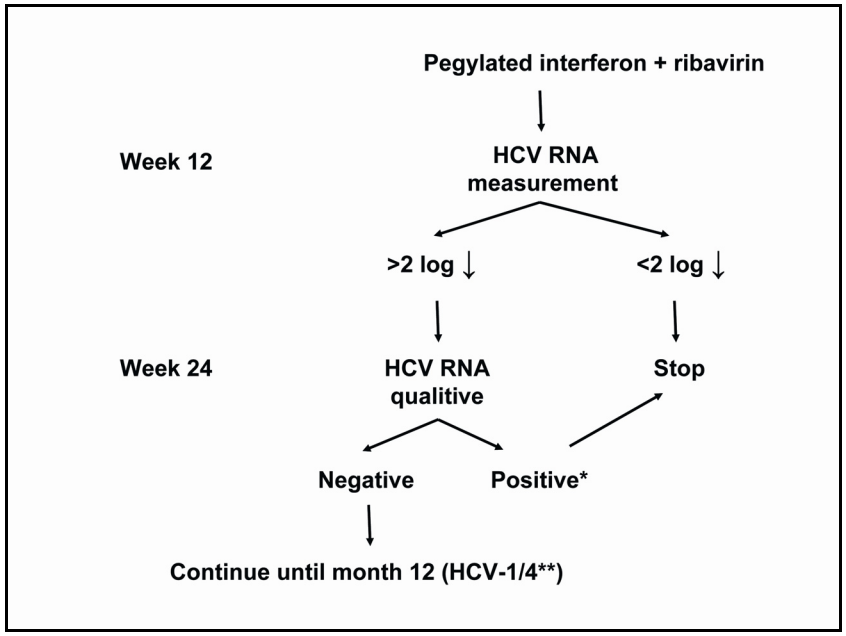

Figure 4: Algorithm for the treatment of chronic hepatitis C [60].
* In patients with high baseline HCV loads, treatment might be prolonged beyond 24 weeks despite the recognition of detectable viremia at that time if a reduction greater than 2 logs was observed at week 12 of therapy.
** In the light of higher relapse rates in the setting of HCV-HIV co-infection, patients with HCV genotypes 2-3 showing good virological responses at earlier time-points should be advised to prolong therapy up to 12 months.

Patients with high HCV loads may have a good early virological response but may not reach undetectable viremia at week 24, even though they will clear HCV much later [82]. This subset of patients represents less than 3% of HCV monoinfected individuals, but may be larger in HIV co-infected patients, who frequently have higher baseline HCV RNA titers and who may have slower HCV RNA decays on treatment [10, 80]. In this situation, extending treatment for 12 months may be advisable since it may allow the patient to reach SVR.

There is a second phase of clearance of HCV RNA in subjects on long-term HCV therapy, which explains the steady destruction of infected cells (hepatocytes) [83, 84]. A slower decay in HCV RNA in the presence of HIV infection (see Figure 2b) could explain why the early discontinuation of therapy might result in higher relapse rates in virological responders. We have already mentioned that the most recent data support this notion, and make it necessary to reconsider how long to continue HCV therapy in HCV/HIV co-infected patients with an early virological response. This particularly applies to HCV genotype 3, since relapses are uncommon in HIV-negative subjects infected with this genotype while it may occur in one third of HCV/HIV co-infected patients treated for only 6 months [76], based on what is recommended in HIV-negative patients [60]. Recent studies such as RIBAVIC and APRICOT, which provided treatment for 12 months to patients with HCV genotypes 2 and 3, have proven that relapses are markedly reduced using the extended period of therapy. Therefore, co-infected individuals with HCV genotypes 2 or 3 should be treated for 12 months instead of for shorter periods. Relapses in HCV genotypes 1 or 4 in co-infected patients treated for 12 months occur in 20-35% of patients. In this population, the benefit of long periods of therapy, at least among early virological responders, should be investigated, since results from patients with HCV alone have recently shown a reduction in relapse rate to less than 15% when HCV treatment is extended to 18 months [85].

Patients with HCV alone who do not clear HCV RNA during HCV treatment might benefit from long-term therapy with interferon alone [86-88]. Maintenance therapy with interferon may provide histological improvement and even reduce the risk of hepatocellular carcinoma. It is currently being investigated as an alternative approach in large trials (i.e., hepatitis long-term treatment against cirrhosis (HALT-C) and EPIC). Whether this strategy could be considered in some HCV/HIV co-infected individuals with advanced fibrosis who did not respond virologically to HCV therapy, should be further investigated. The use of lower doses of pegylated interferon (half those recommended at first line) may improve tolerance and facilitate long-term administration of the drug. However, this potential benefit should be weighed against an expected reduction in the quality of life due to the long-term prescription of a drug administered subcutaneously, which often causes side-effects, including a reduction

in the CD4 count which is considered undesirable in most HIV-positive patients.

MANAGEMENT OF SIDE-EFFECTS OF HCV THERAPY IN HIV-POSITIVE PATIENTS

Side-effects of HCV treatments are common, and include five main categories: influenza-like symptoms (headache, fever, asthenia, myalgias, decreased appetite), hematologic abnormalities, neuropsychiatric disorders (depression, irritability, insomnia), gastrointestinal symptoms (nausea, diarrhea), and inflammation at injection sites. In addition, other adverse events are rare but include alopecia and thyroid dysfunction [60, 89]. Overall, they result in treatment discontinuation in around 15% of patients with HCV infection alone, and to dose reductions of either pegylated interferon and/or ribavirin in another 20-25% [77, 78]. Higher treatment discontinuation rates have been found in some studies in HIV co-infected persons [71, 74]. The lack of expertise in the management of HCV treatment-related side-effects by doctors as well as insufficient information to patients both help explain these high drop-out rates. These aspects should therefore be properly addressed in the future. When possible, hepatitis C in HIV co-infected patients should be treated by medical teams with expertise in the field.

The hematologic abnormalities may be due to either pegylated interferon or ribavirin. Anemia due to ribavirin typically is mild and due to extravascular hemolysis, and is accompanied by an increase in reticulocytes. Although ribavirin dose reductions may reduce anemia, the usefulness of recombinant erythropoietin (r-EPO) has been shown in these patients [90]. Supplements of folinic acid are advisable. Otherwise, the dose of ribavirin should be reduced to half when hemoglobin (Hb) drops below 10g/dL, and it needs to be discontinued if it goes below 8.5g/dL. However, ribavirin exposure appears crucial to obtain higher sustained response rates, especially in patients with HCV genotype 1 [77, 91]; therefore any efforts to keep patients on adequate doses of the drug (i.e., using r-EPO) should be favored.

Leukopenia, especially neutropenia and less frequently lymphocytopenia, may develop with pegylated interferon. Patients should be informed about the risk of reduced CD4+ counts [44, 55, 56] which mostly affect absolute CD4 number but not the percentage of cells. Moreover, it reverses after discontinuing interferon therapy

[92]. For neutropenia, the use of therapeutic growth factors, such as granulocyte colony stimulating factor (GCS-F), may be considered and may be better than reducing pegylated interferon doses, especially in patients with HCV genotype 1, who seem to be particularly sensitive to pegylated interferon doses.

INTERACTIONS BETWEEN ANTIRETROVIRAL DRUGS AND HCV MEDICATIONS

Since anemia is a frequent side-effect during ribavirin treatment, attention should be paid to patients who are taking azidothymidine (AZT), which is also known to cause anemia. Thus, in patients with AZT-related anemia this drug should be discontinued before prescribing ribavirin. Alternatively, Hb values should be closely monitored during the first 6 weeks of therapy [49].

Mitochondrial damage is a result of the inhibition of mitochondrial polymerase gamma by nucleoside analogs [93, 94]. Ribavirin can enhance intracellular concentrations of phosphorylated ddI metabolites, and result in a higher risk of toxicity [95-97]. Several cases of pancreatitis and/or lactic acidosis have been reported, and the FDA now warns against the risk of giving ribavirin and ddI concomitantly. Therefore, subjects who begin treatment with ribavirin should not use ddI concomitantly [49]. The role of d4T in the development of lactic acidosis in these patients has also been shown in the RIBAVIC and APRICOT trials, mainly when used concomitantly with ddI [74, 98].

More recently, cases of liver decompensation, some fatal, have been reported in subjects receiving ribavirin with ddI [74, 98]. All these cases occurred in patients with cirrhosis, and hypothetically ddI and ribavirin acted synergistically leading to liver failure. Therefore, the concomitant administration of ddI and ribavirin should be contraindicated in subjects with advanced liver fibrosis.

Finally, several observations have shown that ribavirin could potentiate subcutaneous fat loss when used concomitantly with some nucleoside analogs, mainly d4T [99]. In this form, severe weight loss mimicking progression of lipoatrophy could be another characteristic side-effect due to the interaction of ribavirin and antiretroviral drugs. Patients should be informed in advance about the risk of this complication and, when possible, drugs with a lower lipodystrophic profile should be prescribed.

REFERENCES

1. Alter M. Epidemiology of hepatitis C. Hepatology 1997;26:62–5.
2. Backmund M, Meyer K, von Zielonka M, Eichenlaub D. Treatment of chronic hepatitis C infection in injection drug users. Hepatology 2001;34:188-193.
3. Diamond C, Thiede H, Perdue T, Secura GM, Valleroy L, Mackellar D, Corey L; Seattle Young Men's Survey Team. Viral hepatitis among young men who have sex with men: prevalence of infection, risk behaviors and vaccination. Sex Transm Dis 2003;30:425-432.
4. Nelson M, Browne R, Asboe D, Gilleece Y, Atkins M, Gazzard B. Increasing incidence of acute hepatitis C in HIV-positive men secondary to sexual transmission, epidemiology and treatment. 9th European AIDS Conference, October 25-29, Warsaw, Poland 2003 Abstract F12/3..
5. Vogel M, Bieniek B, Jessen H, et al. The treatment of acute hepatitis C infection in HIV-coinfected patients. A retrospective analysis of 8 cases in a multicenter setting. 9th European AIDS Conference, October 25-29, Warsaw, Poland 2003 Abstract 13.2/6
6. Rockstroh J, Konopnicki D, Soriano V, et al. Hepatitis B and hepatitis C in the EuroSIDA cohort: prevalence and effect on mortality, AIDS, progression and response to HAART. 11th Conference on Retroviruses and Opportunistic Infections, San Francisco 2004 Abstract 799.
7. Soriano V, Sulkowski M, Bergen C, Hatzakis A, Cacoub P, Katlama C, Cargnel A, Mauss S, Dieterich D, Moreno S, Ferrari C, Poynard T, Rockstroh J. Care of patients with chronic hepatitis C and HIV co-infection: Recommendations from the HIV/HCV International Panel. AIDS 2002;16:813-828.
8. Daar E, Lynn H, Donfield S, Gomperts E, Hilgartner MW, Hoots WK, Chernoff D, Arkin S, Wong WY, Winkler CA. Hemophilia Growth and Development Study. Relation between HIV-1 and HCV load in patients with hemophilia. J Acquir Immun Def Syndr 2001;26:466-472.
9. Strasfeld L, Lo Y, Netski D, Thomas D, Klein R. The association of hepatitis C prevalence, activity and genotype with HIV infection in a cohort of New York city drug users. J Acquir Immun Def Syndr 2003;33:356-364.
10. Cribier B, Rey D, Schmitt C, Lang JM, Kirn A, Stoll-Keller F. High hepatitis C viremia and impaired antibody response in patients coinfected with HIV. AIDS 1995;9:1131-1136.
11. Eyster M, Fried M, Di Bisceglie A, Goedert J. Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to HIV infection and liver disease. Blood 1994;84:1020-1023.

12. Pol S, Thiers V, Losbaum J, et al. Changing distribution of HCV genotypes in Europe in the last decades. J Hepatol 1994;2:13-17.

13. Pawlotsky JM. HCV genetic variability: pathogenic and clinical implications. Clin Liver Dis 2003;7:45-66.

14. Perez-Olmeda M, Rios P, Nuñez M, Garcia-Samaniego J, Romero M, Soriano V. Virological characteristics of hepatitis C virus infection in HIV-infected patients with chronic hepatitis C: implications for treatment. AIDS 2002;16:493-495.

15. Eyster M, Diamondstone L, Lien J, Ehmann WC, Quan S, Goedert JJ. Natural history of hepatitis C virus infection in multi-transfused hemophiliacs: effect of co-infection with HIV. A multicenter hemophilia cohort study. J Acquir Immun Defic Syndr 1993;6:602-610.

16. Rockstroh J, Spengler U, Sudhop T, Ewig S, Theisen A, Hammerstein U, Bierhoff E, Fischer HP, Oldenburg J, Brackmann HH, Sauerbruch T. Immunosuppression may lead to progression of hepatitis C virus associated liver disease in hemophiliacs coinfected with HIV. Am J Gastroenterol 1996;91:2563-2568.

17. Puoti M, Bonacini M, Spinetti A, Putzolu V, Govindarajan S, Zaltron S, Favret M, Callea F, Gargiulo F, Donato F, Carosi G; HIV-HCV Co-infection Study Group. Liver fibrosis progression is related to CD4 cell depletion in patients coinfected with HCV and HIV. J Infect Dis 2001;183:134-137.

18. Sabin C, Telfer P, Phillips A, Bhagani S, Lee C. Association between hepatitis C virus genotype and HIV disease progression in a cohort of hemophiliac men. J Infect Dis 1997;175:164–168.

19. Garcia-Samaniego J, Soriano V, Castilla J, Bravo R, Moreno A, Carbo J, Iniguez A, Gonzalez J, Munoz F. Influence of hepatitis C virus genotypes and HIV-infection on histological severity of chronic hepatitis C – Hepatitis/HIV Spanish Study Group. Am J Gastroenterol 1997;92:1130-1134.

20. Soto B, Sanchez-Quijano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, Rey C, Abad MA, Rodriguez M, Sales Gilabert M, Gonzalez F, Miron P, Caruz A, Relimpio F, Torronteras R, Leal M, Lissen E. HIV infection modifies the natural history of chronic parenteral acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol 1997;26:1-5.

21. Sanchez-Quijano A, Andreu J, Gavilan S, Luque F, Abad MA, Soto B, Munoz J, Aznar JM, Leal M, Lissen E. Influence of HIV type 1 infection on the natural history of chronic parenteral acquired hepatitis C. Eur J Clin Microbiol Infect Dis 1995;14:949-953.

22. Darby S, Ewart D, Giangrande P, Spooner RJ, Rizza CR, Dusheiko GM, Lee CA, Ludlam CA, Preston FE. Mortality from liver cancer and liver disease in hemophiliac men and boys in the UK given blood product contaminated with hepatitis C. Lancet 1997;350:1425-1431.

23. Garcia-Samaniego J, Rodriguez M, Berenguer J, Rodriguez-Rosado R, Carbo J, Asensi V, Soriano V. Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis C. Am J Gastroenterol 2001;96:179-183.

24. Martin-Carbonero L, Benhamou Y, Puoti M, Berenguer J, Mallolas J, Quereda C, Arizcorreta A, Gonzalez A, Rockstroh J, Asensi V, Miralles P, Laguno M, Moreno L, Giron JA, Vogel M, Garcia-Samaniego J, Nunez M, Romero M, Moreno S, de la Cruz JJ, Soriano V. Incidence and predictors of severe liver fibrosis in HIV-infected patients with chronic hepatitis C - a European collaborative study. Clin Infect Dis 2004;38:128-133.

25. Benhamou Y, De Martino V, Bochet M, Colombet G, Thibault V, Liou A, Katlama C, Poynard T; MultivirC Group: Factors affecting liver fibrosis in HIV and hepatitis C virus coinfected patients: impact of protease inhibitor therapy. Hepatology 2001;34:283-287.

26. Qurishi N, Kreuzberg C, Lüchters G, Effenberger W, Kupfer B, Sauerbruch T, Rockstroh JK, Spengler U. Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C co-infection. Lancet 2003;362:1708-1713.

27. Yeni P, Hammer S, Carpenter C, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hirsch MS, Jacobsen DM, Katzenstein DA, Montaner JS, Richman DD, Saag MS, Schechter M, Schooley RT, Thompson MA, Vella S, Volberding PA. Antiretroviral treatment for adult HIV-infection 2002: update recommendations of the IAS-USA Panel. JAMA 2002;288:222-235.

28. Rodriguez-Rosado R, García-Samaniego J, Soriano V. Hepatotoxicity after introduction of highly active antiretroviral therapy. AIDS 1998;12:1256.

29. Spengler U, Lichterfeld M, Rockstroh J. Antiretroviral drug toxicity - a challenge for hepatologists? J Hepatol 2002;36:283-294.

30. Saves M, van den Torren S, Daucourt V, Marimoutou C, Dupon M, Couzigou P, Bernard N, Mercie P, Dabis F. Severe hepatic cytolysis: incidence and risk factors of patients treated by antiretroviral combinations. Aquitaine Cohort, France, 1996-1998. AIDS 1999;13:F115-F121.

31. den Brinker M, Wit F, Wertheim-van Dillen P, Jurriaans S, Weel J, van Leeuwen R, Pakker NG, Reiss P, Danner SA, Weverling GJ, Lange JM. Hepatitis B and C virus co-infection and risk for hepatotoxicity of highly active antiretroviral therapy in HIV-1 infection. AIDS 2000;14:2895-2902.

32. Martinez E, Blanco J, Arnaiz J, Perez-Cuevas JB, Mocroft A, Cruceta A, Marcos MA, Milinkovic A, Garcia-Viejo MA, Mallolas J, Carne X, Phillips A, Gatell JM. Hepatotoxicity in HIV-1 infected patients receiving nevirapine-containing antiretroviral therapy. AIDS 2001;15:1261-1268.

33. Núñez M, Lana R, Mendoza JL, Martín-Carbonero L, Soriano V. Risk factors for severe liver toxicity following the introduction of HAART. J Acquir Immune Def Syndr 2001;27:426-431.

34. Sulkowski M, Thomas D, Chaisson R, Moore D. Hepatotoxicity associated with antiretroviral therapy in adults infected with HIV and the role of hepatitis C or B virus infection. JAMA 2000;283:74-80.

35. González de Requena D, Núñez M, Jiménez-Nácher I, Soriano V. Liver toxicity caused by nevirapine. AIDS 2002;16:290-291.

36. Montessori V, Harris M, Montaner J. Hepatotoxicity of nucleoside reverse transcriptase inhibitors. Sem Liver Dis 2003;23:167-172.

37. Lonergan J, Behling C, Pfander H, Hassanein T, Mathews W. Hyperlactatemia and hepatic abnormalities in 10 HIV-infected patients receiving nucleoside analogue combination regimens. Clin Infect Dis 2000;31:162-166.

38. Batisse D, van Huyen J, Piketty C, Canali GG, Karmochkine M, Weiss L, Bruneval P, Kazatchkine MD. Severe liver mitocondriopathy with normal liver histology and normal lactate levels in patients receiving nucleoside analogs. AIDS 2002;16:2370-2371.

39. Sutinen J, Hakkinen AM, Westerbacka J, Seppala-Lindroos A, Vehkavaara S, Halavaara J, Jarvinen A, Ristola M, Yki-Jarvinen H. Increased fat accumulation in the liver in HIV-infected patients with antiretroviral therapy-associated lipodystrophy. AIDS 2002;16:2183-2193.

40. Martin-Carbonero L, Soriano V, Valencia E, Garcia-Samaniego J, Lopez M, Gonzalez-Lahoz J. Increasing impact of chronic viral hepatitis on hospital admission and mortality among HIV-infected patients. AIDS Res Hum Retrovir 2001;17:1467-1471.

41. Bica I, McGovern B, Dahr R, Stone D, McGowan K, Scheib R, Snydman DR. Increasing mortality due to end-stage liver disease in patients with HIV infection. Clin Infect Dis 2001;32:490-497.

42. Puoti M, Spinetti A, Ghezzi A Donato F, Zaltron S, Putzolu V, Quiros-Roldan E, Zanini B, Casari S, Carosi G. Mortality for liver disease in patients with HIV-infection: a cohort study. J Acquir Immune Defic Syndr 2000;24:211-217.

43. Rosenthal E, Poiree M, Pradier C, Perronne C, Salmon-Ceron D, Geffray L, Myers RP, Morlat P, Pialoux G, Pol S, Cacoub P; GERMIVIC Joint Study Group. Mortality due to hepatitis C-related liver disease in HIV-infected patients in France (Mortavic 2001 study). AIDS 2003;17:1803-1809.

44. Soriano V, García-Samaniego J, Bravo R, Gonzalez J, Castro A, Castilla J, Martinez-Odriozola P, Colmenero M, Carballo E, Suarez D, Rodriguez-Pinero FJ, Moreno A, del Romero J, Pedreira J, Gonzalez-Lahoz J. Interferon alpha for the treatment of chronic hepatitis C in patients infected with HIV. Hepatitis-HIV Spanish Study Group. Clin Infect Dis 1996;23:585-591.

45. Landau A, Batisse D, Piketty C, Duong Van Huyen JP, Bloch F, Belec L, Bruneval P, Weiss L, Jian R, Kazatchkine MD. Long-term efficacy of

combination therapy with interferon–α 2b and ribavirin for severe chronic hepatitis C in HIV-infected patients. AIDS 2001;15:2149-2155.

46. Sauleda S, Juarez A, Esteban JI, Altisent C, Ruiz I, Puig L, Esteban R, Guardia J. Interferon and ribavirin combination therapy for chronic hepatitis C in HIV-infected patients with congenital coagulation disorders. Hepatology 2001;34:1035-1040.

47. Nasti G, Di Gennaro G, Tavio M, Cadorin L, Tedeschi RM, Talamini R, Carbone A, Tirelli U. Chronic hepatitis C and HIV-infection: feasibility and sustained efficacy of therapy with interferon-α 2b and ribavirin. AIDS 2001;15:1783-1787.

48. Perez-Olmeda M, Soriano V, Asensi V, Morales D, Romero M, Ochoa A, Sanchez-Montero F, Santin M, Guardiola J, Blanch J, Nunez M, Jimenez-Nacher I, Garcia-Samaniego J; The HCV/HIV Spanish Study Group. Treatment of chronic hepatitis C in HIV-infected patients with interferon plus ribavirin. AIDS Res 2003;19:1083-1089.

49. Soriano V, Puoti M, Sulkowski M, Mauss S, Cacoub P, Cargnel A, Dieterich D, Hatzakis A, Rockstroh J. Care of patients with hepatitis C and HIV-co-infection. Updated recommendations from the HCV-HIV International Panel. AIDS 2004;18:1-12.

50. Beld M, Penning M, van Putten M, van den Hoek A, Damen M, Klein MR, Goudsmit J. Low levels of HCV RNA in serum, plasma, and PBMCs of IDUs during long antibody-undetectable periods before seroconversion. Blood 1999;94:1183-1191.

51. George S, Gebhardt J, Klinzman D, Foster MB, Patrick KD, Schmidt WN, Alden B, Pfaller MA, Stapleton JT. Hepatitis C viremia in HIV-infected individuals with negative HCV antibody tests. J Acquir Immun Def Syndr 2002;31:154-162.

52. Mauss S, Klinker H, Ulmer A, Willers R, Weissbrich B, Albrecht H, Haussinger D, Jablonowski H. Response to treatment of chronic hepatitis C with interferon alpha in patients infected with HIV-1 is associated with higher CD4+ cell count. Infection 1998;26:16-19.

53. Gao B, Hong F, Radaeva S. Host factors and failure of interferon-alpha treatment in hepatitis C virus. Hepatology 2004;39:880-890.

54. Zeuzem S. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who responds less well? Ann Intern Med 2004;140:370-381.

55. Vento S, Di Perri G, Cruciani M, Garofano T, Concia E, Bassetti D. Rapid decline of CD4+ cells after interferon treatment in HIV-1 infection. Lancet 1993;341:958-959.

56. Pesce A, Taillan B, Rosenthal E. Opportunistic infections and CD4 lymphocytopenia with interferon treatment in HIV-1 infected patients. Lancet 1993;341:1597.

57. Sylvestre D. Treating hepatitis C in methadone maintenance patients: an interim analysis. Drug Alcohol Depend 2002;67:117-123.

58. Backmund M, Meyer K, von Zielonka V, Eichenlaub D. Treatment of hepatitis C infection in injection drug users. Hepatology 2001;34:188-193.

59. Edlin B. Prevention and treatment of hepatitis C in injecting drug users. Hepatology 2002;36:210-219.

60. NIH Consensus Development Conference Statement: Management of Hepatitis C. Gastroenterology 2002;123:2082-2099.

61. Wong J, Bennett W, Koff R, Pauker S. Pre-treatment evaluation of chronic hepatitis C. Risks, benefits, and costs. JAMA 1998;280:2088-2093.

62. Spinzi G, Minoli G. To biopsy or not to biopsy. Hepatology 2001;34:438-439.

63. Andriulli A, Mangia A, Niro G, Caturelli E. To biopsy or not to biopsy. Hepatology 2001;34:438.

64. Dienstag J. The role of liver biopsy in chronic hepatitis C. Hepatology 2002;36:152-160.

65. Soriano V, Martín-Carbonero L, García-Samaniego J. Treatment of chronic hepatitis C virus infection: we must target the virus or liver fibrosis? AIDS 2003;17:751-753.

66. Benhamou Y, Bochet M, Di Martino V, Calvez V, Fievet MH, Vig P, Gibbs CS, Brosgart C, Fry J, Namini H, Katlama C, Poynard T. Liver fibrosis progression in HIV and hepatitis C virus coinfected patients. Hepatology 1999;30:1054-1058.

67. Martínez-Sierra C, Arizcorreta A, Díaz F, Roldan R, Martin-Herrera L, Perez-Guzman E, Giron-Gonzalez JA. Progression of chronic hepatitis C to liver fibrosis and cirrhosis in patients coinfected with HCV and HIV. Clin Infect Dis 2003;36:491-498.

68. Kuehne F, Bethe U, Freedberg K, Goldie S. Treatment for hepatitis C virus in HIV-infected patients: clinical benefits and cost-effectiveness. Arch Intern Med 2002;162:2545-2556.

69. Perez-Olmeda M, Nuñez M, Romero M, Gonzalez J, Castro A, Arribas JR, Pedreira J, Barreiro P, Garcia-Samaniego J, Martin-Carbonero L, Jimenez-Nacher I, Soriano V. Pegylated interferon-α 2b + ribavirin as therapy for chronic hepatitis C in HIV-infected patients. AIDS 2003;17:1023-1028.

70. Voigt E, Schulz C, Mauss S. Factors related to outcome of treatment with pegylated interferon-α 2a (PEG-INF) + ribavirin (RBV) in HCV/HIV-coinfected patients. 2nd IAS Conference on HIV Pathogenesis and Treatment, Paris 2003;976.

71. Ballesteros A, Franco S, Fuster D, Planas R, Martinez MA, Acosta L, Sirera G, Salas A, Tor J, Rey-Joly C, Clotet B, Tural C. Early HCV dynamics on

peg-interferon and ribavirin in HIV/HCV co-infection: indications for the investigation of new treatment approaches. AIDS 2004;18:59-66.

72. Moreno L, Quereda C, Morena A, Perez-Elias MJ, Antela A, Casado JL, Dronda F, Mateos ML, Barcena R, Moreno S. Pegylated interferon-α 2b + ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. AIDS 2004;18:67-73.

73. Chung R, Andersen J, Volberding P. A randomized controlled trial of peg-interferon-α 2a + ribavirin versus interferon-α 2a + ribavirin for chronic hepatitis C virus infection in HIV-coinfected persons: follow-up results of ACTG A5071. 11th Conference on Retroviruses and Opportunistic Infections, San Francisco 2004 Abstract 110.

74. Perronne C, Carrat F, Bani-Sadr F. Final results of ANRS HCO2-Ribavic: a randomized controlled trial of pegylated interferon-α 2b + ribavirin versus interferon-α 2b + ribavirin for the initial treatment of chronic hepatitis C in HIV-coinfected patients. 11th Conference on Retroviruses and Opportunistic Infections, San Francisco 2004 Abstract 117LB.

75. Torriani F, Rockstroh J, Rodriguez-Torres M, Gonzalez J, Dietrich D. Final results of APRICOT: a randomized partially blinded international trial evaluating peg-interferon-α 2a + ribavirin versus interferon-α 2a + ribavirin in the treatment of HCV in HIV/HCV-co-infection. 11th Conference on Retroviruses and Opportunistic Infections, San Francisco 2004 Abstract 112.

76. Soriano V, Perez-Olmeda M, Rios P, Nuñez M, Garcia-Samaniego J, Gonzalez-Lahoz J. Hepatitis C virus (HCV) relapses after anti-HCV therapy are more frequent in HIV-infected patients. AIDS Res Human Retroviruses 2004;20:471-475.

77. Manns M, McHutchison J, Gordon S. 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.

78. Fried M, Shiffman M, Reddy R. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347:975-982.

79. Davis G, Albright J, Cook S, Rosenberg D. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645-652.

80. Torriani F, Ribeiro R, Gilbert T, Ribeiro R. Schrenk UM, Clauson M, Pacheco DM, Perelson AS. HCV and HIV dynamics during HC treatment in HIV/HCV co-infection. J Infect Dis 2003;188:1498-1507.

81. Soriano V, Pérez-Olmeda M, Martín-Carbonero L, Rios P, Núñez M, González-Lahoz J. Predictive value of early virological response (12 weeks) to pegylated interferon plus ribavirin in HIV-HCV coinfected patients. Antiviral Ther 2004 (in press).

82. Buti M, Valdes A, Sanchez-Avila F, Esteban R, Lurie Y. Extending combination therapy with peginterferon alfa-2b plus ribavirin for genotype 1

chronic hepatitis C late responders: a report of 9 cases. Hepatology 2003;37:1226-1227.

83. Neumann A, Lam N, Dahari H, Davidian M, Wiley TE, Mika BP, Perelson AS, Layden TJ. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon alpha therapy. Science 1998;282:103-107.

84. Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36:65-73.

85. Brouwer J, Nevens F, Bekkering F, Bourgeois N, Van Vlierberghe H, Weegink CJ, Lefebvre V, Van Hattum J, Henrion J, Delwaide J, Hansen BE, Schalm SW. for The Benelux Study Group On Treatment Of Chronic Hepatitis C. Reduction of relapse rates by 18-month treatment in chronic hepatitis C: a Benelux randomised trial in 300 patients. J Hepatol 2004;40:689-695.

86. Alri L, Duffaut M, Selves J. Maintenance therapy with gradual reduction of the interferon dose over one year improves histological response in patients with chronic hepatitis C with biochemical response: results of a randomized trial. J Hepatol 2001;35:272-278.

87. 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.

88. Yoshida H, Arakawa Y, Sata M, Nishiguchi S, Yano M, Fujiyama S, Yamada G, Yokosuka O, Shiratori Y, Omata M. Interferon therapy prolongs life expectancy among chronic hepatitis C patients. Gastroenterology 2002;123:483-491.

89. Fried M. Side-effects of therapy of hepatitis C and their management. Hepatology 2002;36:237-244.

90. Dieterich D. Treatment of hepatitis C and anemia in HIV-infected patients. J Infect Dis 2002;185:128-137.

91. Hadziyannis S, Sette H, Morgan T, 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 alfa-2a and ribavirin combination therapy in chronic hepatitis C. Ann Intern Med 2004;140:346-355.

92. Soriano V, Bravo R, García-Samaniego J, Gonzalez J, Odriozola PM, Arroya E, Vicario JL, Castro A, Colmenero M, Carballo E et al. CD4+ T-lymphocytopenia in HIV-infected patients receiving interferon therapy for chronic hepatitis C. AIDS 1994;8:1621-1622.

93. Brinkman K, Smeitink J, Romijn J, Reiss P. Mitochondrial toxicity induced by nucleoside analogue RT inhibitors is a key factor in the pathogenesis of antiretroviral therapy-related lipodystrophy. Lancet 1999;354:1112-1115.

94. Moyle G. Hyperlactatemia and lactic acidosis during antiretroviral therapy: causes, management and possible etiologies. AIDS Rev 2001;3:150-156.

95. Lafeuillade A, Hittinger G, Chapadaud S. Increased mitochondrial toxicity with ribavirin in HIV/HCV co-infection. Lancet 2001;357:280-281.

96. Kakuda T, Brinkman K. Mitochondrial toxic effects of ribavirin. Lancet 2001; 357:1803.

97. Salmon-Ceron D, Chauvelot-Moachon L, Abad S, Silbermann B, Sogni P. Mitochondrial toxic effects of ribavirin. Lancet 2001;357:1803.

98. Mauss S, Larrey D, Valenti W, et al. Risk factors for hepatic decompensation in cirrhotic patients with HIV-HCV co-infection treated with PEG interferon or interferon and ribavirin or placebo. Sixth International Congress on Drug Therapy in HIV Infection. Glasgow, Nov 2002 Abstract PL12.4.

99. García-Benayas T, Blanco F, Soriano V. Weight loss in HIV-infected patients. N Engl J Med 2002;347:1287-1288.

Treatment of Chronic Hepatitis C in Special Populations

Anaïs Vallet-Pichard, Stanislas Pol

INTRODUCTION

We will focus on the antiviral treatment of HCV infection in special populations, namely patients with hematological diseases, psychiatric disorders or kidney diseases (hemodialyzed patients and kidney recipients). In these patients, the prevalence of HCV infection is high (10 to 90%), mainly related to blood transfusions (before 1990) or clotting factor transfusions before 1986 and to the solvent-detergent procedure of viral inactivation (genotype 1b), as well as nosocomial transmission with frequent mixed infections. In hemophiliacs [1, 2] or hemodialysis patients [3], the natural history of HCV infection is similar to that of the general population, while it is accelerated in alcoholic patients [4] and kidney recipients [3,5] with increased viral replication and a higher frequency of cirrhosis. Early treatment is logical and cost effective by decreasing the prevalence of cirrhosis and hepatocellular carcinoma before irreparable severe liver lesions have occurred and before highly complex populations of HCV genomes have been generated.

TREATMENT OF CHRONIC HEPATITIS C IN PATIENTS WITH HEMATOLOGICAL DISEASES

Hemophiliac patients

Hemophilia does not modify the recommended therapeutic strategies because there are risks of liver-related mortality, whatever the HIV status [1]. The tolerance and efficacy of interferon alone and combination therapy with ribavirin are similar to that in the general population [6, 7], even with HIV co-infection [8] and a risk of lactic acidosis associated with the use of nucleoside analogue reverse transcriptase inhibitor (NRTI). To date there is no experience of combination therapy with pegylated interferon and ribavirin, but this should be the first line treatment. Even if there are limited data about the risk of developing anti-FVIII antibodies in hemophiliac patients treated with interferon [9], monitoring of these patients should be recommended.

Thalassemic patients

In thalassemic patients, a sustained virologial response is obtained in 40 to 57% of cases with interferon monotherapy with no more side-effects than in other populations [10-16]. There is limited data about interferon and ribavirin but efficacy seems to be comparable to that observed in the general population [16]. Ribavirin therapy may be difficult to manage because of pre-existing anemia but treatment is relatively well tolerated with enhancement of blood transfusions. Finally, iron overload may limit the efficacy of treatment [17].

As in the general population, pegylated interferon-alpha in association with low and increasing doses of ribavirin should be recommended in thalassemic patients. The place of associated erythropoietin therapy in these patients needs be discussed.

TREATMENT OF CHRONIC HEPATITIS C IN PATIENTS WITH PSYCHIATRIC DISEASES

Psychiatric diseases, such as depression, are more frequently found in HCV-infected patients than in the general population [18]; this seems to be related both to chronic disease and to fear of the future [19] as well as to past medical history because some HCV-infected patients are alcoholic or ex-intravenous drug abusers, which may be associated

with psychiatric disorders [20, 21] such as schizophrenia [22]. Treatment with pegylated interferon and ribavirin is associated with mental side-effects in 20 to 40% of cases [23]. Results of therapy in this population are limited and contradictory [24-25] and a psychiatric evaluation, clear explanations to the patient and his family and close follow-up are recommended before treating patients with severe liver disease. The use of preventive or therapeutic antidepressant medication also helps decrease or control side-effects [25-31].

In a recent study including patients with psychiatric disorders, ex-intravenous drug users using methadone or who had stopped drug abuse for at least 3 months and a control group, the mental side-effects were neither more frequent nor more severe in the psychiatric population than in the control group and compliance to treatment was comparable [32]. No increase in underlying psychiatric disease was noted.

In summary, antiviral treatment in psychiatric patients, including psychotic patients may be proposed but should include multidisciplinary management, with a psychiatric evaluation before treatment and close follow-up as well as possible preventive antidepressant therapy.

TREATMENT OF CHRONIC HEPATITIS C IN PATIENTS WITH EXCESSIVE ALCOHOL CONSUMPTION

Chronic alcohol consumption increases HCV viremia [4, 33, 34]. No data are available about compliance to antiviral therapy in heavy drinkers, but it may be decreased as was found in the HIV-infected patients studied in highly active anti-retroviral therapy (HAART) [35]. Little is known about the effects of alcohol on the safety of anti-HCV treatment, but one study has suggested that interferon may trigger alcoholic hepatitis [36]. The efficacy of interferon therapy is decreased in heavy drinkers [37-39]. Patients should therefore be asked to reduce or stop alcohol during the 3 to 6 months preceding anti-HCV combination treatment of pegylated interferon and ribavirin to improve treatment efficacy and safety.

TREATMENT OF CHRONIC HEPATITIS C IN PATIENTS WITH RENAL DISEASES

HCV infection is frequent in patients with end-stage renal failure who receive chronic hemodialysis with a prevalence varying from 10 to 65% according to the geographical area [40]. The prevalence is significantly associated with the duration of dialysis and the number of transfused blood products [41]. It has dramatically declined with hemovigilance [42, 43] even if, despite the high efficiency of blood screening and erythropoietin therapy, there is a continued yearly incidence of HCV contamination of 1.4% [43] suggesting nosocomial transmission. HCV contamination may result in cirrhosis in 10% of dialysis patients. Immunosuppressive regimens for the prevention of allograft rejection results in: (1.) increased HCV viral replication [44]; (2.) frequent histopathological deterioration with a 25% prevalence of biopsy-proven extensive fibrosis or cirrhosis within a mean 5 years after transplantation) [45]; (3.) rare fibrosing cholestatic hepatitis [46]. Liver disease results in a significant decrease in survival [47].

In dialysis patients, liver biopsy (or biochemical markers) should be performed to assess the histopathological impact: most patients will have mild liver disease which does not require antiviral treatment compared to those with significant liver disease (fibrosis score ≥2). In dialysis patients, ribavirin is contraindicated for pharmacokinetic reasons (accumulation of ribavirin metabolites in erythrocytes); a ribavirin/interferon-alpha combination should not be used due to the risk of deep and long-lasting hemolytic anemia in dialysis patients with a poor secretion of erythropoietin [48].

Thus, standard interferon-alpha therapy appears to be the only alternative in dialysis patients: it is feasible with a standard schedule, 3MU subcutaneously three times a week after hemodialysis. In dialysis patients, the biochemical and virological efficacy (summarized in Table 1) is, at least as good as in the general population with a 20 to 90% rate of viral eradication depending on the dose and duration of treatment [49-55] and on virological factors. Moreover, histological improvement is common, even without virological efficacy [50]. Tolerance is poorer than in non-hemodialyzed patients since treatment discontinuation is necessary in 20 to 40% of cases with a high incidence of cardiovascular side-effects, anemia, erythropoietin resistance and general symptoms (weight loss) [55]. It should be noted that persistent detectable viremia

2 months after the beginning of treatment suggests that there will be no lasting viral eradication [55]. Nevertheless, treatment could be continued if the therapeutic aim is improvement of the disease in hemodialysed patients with severe liver disease (palliative treatment to reduce fibrosis in the absence of virological efficacy (Figure 1).

Authors	**N**	**Mos**	**ALT N (%)**	**PCR (%)**	**Relapse (%)**	**SVR** (%)**
Koenig et al.	23	5	50	65	33	43
[49]	14*					
Pol et al.	19	6	85	53	62	20
[50]	1*					
Casanovas et al.	10	12	90	10	0	20
[54]						
Izopet et al.	23	6	85	92	54	42
[51]	3*	12		90	0	90
Degos et al.	37	12	70	66	NT	19***
[55]	21*					

Table 1: Treatment of chronic hepatitis C by interferon-alpha in hemodialysis patients.
* number of treatment discontinuation or reduction.
**SVR=sustained virological response (negative PCR at least 6 months after discontinuation).
*** 38% of patients who received a 12-month course (n=12).

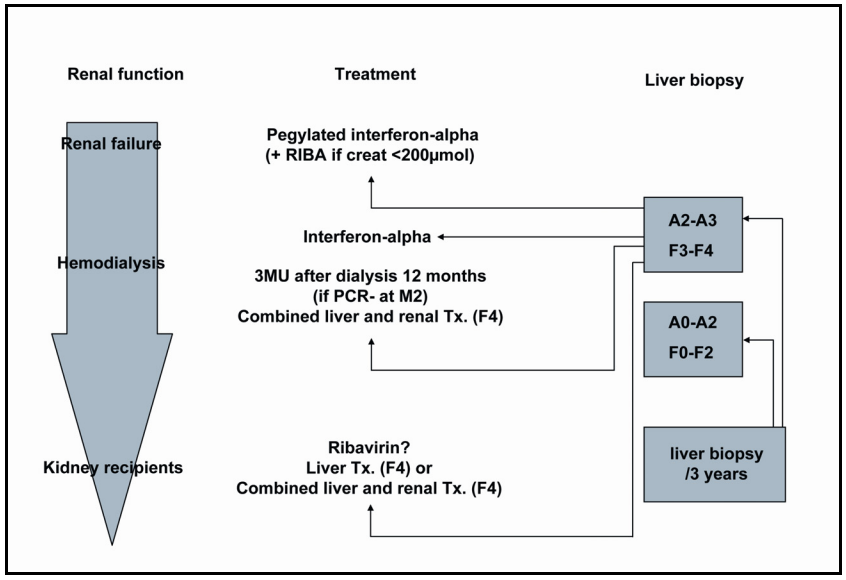

Figure 1: Therapeutic options in HCV-infected patients with renal disorders. Antiviral therapies should be proposed to patients with active and/or fibrotic liver lesions while patients with low fibrotic activity will not be treated and will receive regular liver biopsies (every 3 years) for early detection of histopathological deterioration. Patients with cirrhosis may benefit from liver transplantation or combined renal and liver transplantation depending on renal function.

The greater efficacy and poorer tolerance could be due to the significantly greater pharmacokinetic area under the curve of interferon-alpha showing an increased half life (10 hours vs. 6 hours) and to the upper concentration because of the decrease in renal clearance in dialysis patients [56]. Because of the specific pharmacokinetics of interferon in dialysis patients the use of pegylated interferon is unclear and is under evaluation [57].

In acute hepatitis C, which may occur in dialysis patients at a yearly incidence of 2.6% [58], interferon may be less effective than in the general population [59]: viral clearance is obtained in 26% and 51% of hemodialyzed patients treated by 3MU and 6-10MU for 3 months (compared to spontaneous clearance in 5.6%) [60].

In essential mixed cryoglobulinemia associated with HCV infection, interferon-alpha may improve urinary protein excretion, renal failure and hematuria but recurrence of the nephrotic syndrome is common after treatment discontinuation [61, 62]. The real benefit of higher doses or durations of interferon-alpha or of a combination with ribavirin (in the absence of renal failure) is anecdotal considering the high rate of relapse after treatment discontinuation and should be confirmed in large series.

Finally, interferon-alpha is not recommended in kidney allograft recipients since it is not effective and associated with an unacceptably high rate of allograft rejection (15 and 29%) [63, 64]. Interferon-alpha-related nephrotoxicity has also been reported in the absence of graft rejection with glomerular nephropathy or acute interstitial nephritis. Among the 42 reported kidney recipients who were treated with interferon-alpha therapy for HCV infection, 47.6% had acute renal failure after a mean 3.6 months of therapy (range 11 days to 9 months) and 65.0% resumed dialysis; in contrast, only 5.9% had a long-term virological response. This is also why the treatment of HCV in kidney recipients must be discussed before the renal transplantation even if anecdotal encouraging results of combination therapy have been reported in kidney recipients.

REFERENCES

1. Darby SC, Ewart DW, Giangrande PLF, Spooner RJD, Rizza CR, Dusheiko GM Lee CA, Ludlam CA, Preston FE. For the UK Haemophilia Center Directors' Organisation. Mortality from liver cancer and liver disease in haemophiliac men and boys in UK given blood products contaminated with hepatitis C. Lancet 1997;350:1425-1431.

2. Lethagen S, Widell A, Berntorp E, Verbaan H, Lindgren S. Clinical spectrum of hepatitis C-related liver disease and response to treatment with interferon and ribavirin in haemophilia or von Willebrand disease. Br J Haematol 2001;113:87-93.

3. Zylberberg H**,** Pol S. Reciprocal interactions between human immunodeficiency virus and hepatitis C virus infections. Clin Infect Dis 1996;23:1117-1125.

4. Pessione F, Degos F, Marcellin P, Duchatelle V, Njapoum C, Martinot-Peignoux M, Degott C, Valla D, Erlinger S, Rueff B. Effect of alcohol consumption on serum hepatitis C virus RNA and histological lesions in chronic hepatitis C. Hepatology 1998;27:1717-1722.

5. Pereira BJ, Wright TL, Schmid CH, Levey AS. The impact of pretransplantation hepatitis C infection on the outcome of renal transplantation. Transplantation 1995;60:799-805.

6. Sauleda S, Esteban JI, Altisent C, Puig L, Esteban R, Guardia J. Treatment with interferon plus ribavirin in anti-HIV-negative patients with congenital coagulation disorders and chronic hepatitis C. Thromb Haemot 2000;83:807-810.

7. Santagostino E, Rumi MG, Rivi M, Colombo M, Mannucci PM; Hepatitis Study Group of the Association of Italian Hemophilia Centers. Sustained suppression of hepatitis C virus by interferon and ribavirin in hemophilic patients not responding to interferon monotherapy. Blood 2002;99:1089-1091.

8. Sauleda S, Juarez A, Esteban JI, Altisent C, Ruiz I, Puig L, Esteban R, Guardia J. Interferon and ribavirin combination therapy for chronic hepatitis C in human immunodeficiency virus-infected patients with congenital coagulation disorders. Hepatology 2001;34:1035-1040.

9. Vianello F, Tison T, Tagariello G, Zerbinati P, Zanon E, Scarano L, Girolami A. Serological markers of autoimunity in patients with hemophilia A: the role of hepatitis C virus infection, alpha-interferon and factor VIII treatment in skewing the immune system toward autoreactivity. Blood Coagul Fibrinolysis 1999;10:393-397.

10. Spiliopoulou I, Repanti M, Katinakis S, Karana-Ginopoulou A, Papanastasiou DA. Response to interferon alfa-2b therapy in multitransfused children with

beta-thalassemia and chronic hepatitis C. Eur J Clin Microbiol Infect Dis 1999;10:709-715.

11. Marcellini M, Kondili LA, Comparcola D, Spada E, Sartorelli MR, Palumbo M, Rapicetta M. High dosage alpha-interferon for treatment of children and young adults with chronic hepatitis C disease. Pediatr Infect Dis J 1997;16:1049-1053.

12. Wonke B, Donohue SM, Hoffbrand AV, Scheuer PJ, Brown D, Dusheiko G. Recombinant alpha2b interferon in the treatment of chronic hepatitis C disease in thalassaemia major. Bone Marrow Transplant 1993;12:24-25.

13. Di Marco V, Lo Iacono O, Capra M, Grutta S, Ciaccio C, Gerardi C, Maggio A, Renda D, Almasio P, Pisa R. Alpha-interferon treatment of chronic hepatitis C in young patients with homozygous beta-thalassemia. Haematologica 1992;77:502-506.

14. Di Marco V, Lo Iacono O, Almasio P, Ciaccio C, Capra M, Rizzo M, Malizia R, Maggio A, Fabiano C, Barbaria F, Craxi A. Long-term efficacy of alpha-interferon in beta-thalassemics with chronic hepatitis C. Blood 1997;90:2207-2212.

15. Donohue SM, Wonke B, Hoffbrand AV, Reittie J, Ganeshaguru K, Scheuer PJ, Brown D, Dusheiko G. Alpha interferon in the treatment of chronic hepatitis C infection in thalassaemia major. B J Haematol 1993;83:491-497.

16. Telfer PT, Garson JA, Whitby K, Grant PR, Yardumian A, Hoffbrand AV, Wonke B. Combination therapy with interferon alpha and ribavirin for chronic hepatitis C virus infection in thalassaemic patients. Br J Haemotol 1997;98:850-855.

17. Ikura Y, Morimoto H, Johmura H, Kukui M, Sakurai M. Relationship between hepatic iron deposits and response to interferon in chronic hepatitis C. J Hepatol 1994;91:1367-1373.

18. Lee CH, Jamal H, Regenstein FG, Perrillo RP. Morbidity of chronic hepatitis C as seen in a tertiary care medical center. Dig Dis Sc 1997;42:186-191.

19. Rodger AJ, Jolley D, Thomson SC, Lanigan A, Crofts N. The impact of diagnosis of hepatitis C on quality of life. Hepatology 1999;30:1299-1301.

20. Johnson ME, Gisher DG, Fenaughty A, Theno SA. Hepatitis C virus and depression in drug users. Am J Gastroenterol 1998;93:785-789.

21. Kendall JC, Sherman MI, Bigelow GE. Psychiatric symptoms in polysubstance abusers: relationship to race, sex and age. Addict Behav 1995;20:685-690.

22. Cheung R, Ahmed A. Treating chronic hepatitis C patients with psychiatric disorders: an uphill battle. Am J Gastroenterol 2001;96:3-4.

23. Fontana RJ. Neuropsychiatric toxicity of antiviral treatment in chronic hepatitis C. Dig Dis 2000;18:107-116.

24. Van Thiel D, Friedlander L, Molloy PJ, Fagiuoli S, Kania RJ, Caraceni P. Interferon alpha can be used successfully in patients with hepatitis C virus-positive chronic hepatitis who have a psychiatric illness. Eur J Gastroenterol Hepatol 1995;7:165-168.

25. Ho SB, Nguyen H, Tetrick LL, Opitz GA, Basara ML, Dieperink E. Influence of psychatric diagnoses on interferon alpha treatment for chronic hepatitis C in a veteran population. Am J Gastroenterol 2001;96:157-164.

26. Schafer M, Schramm TM, Lawford BR, Macdonald GA, Cooksley WG. Sertraline treatment of interferon-alfa-induced depressive disorder. Med J Aust 2000;173:359-361.

27. Goldman LS. Sucessful treatment of interferon alpha induced mood disorder with nortriptyline. Psychosomatics 1994;35:412-413.

28. Levenson J, Fallon H. Fluoxetine treatment of depression caused by IFN-α. Am J Gastroenterol 1993;88:760-761.

29. Gleason OC, Yates WR. Five cases of interferon alpha-induced depression treated with antidepressant therapy. Psychosomatics 1999;40:510-512.

30. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, Greiner K, Nemeroff CB, Miller AH. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001;344:961-966.

31. Kraus MR, Schafer A, Scheurlen M. Paraxetine for the prevention of depression induced by interferon alfa. N Engl J Med 2001;345:375-376.

32. Schaefer M, Schmidt F, Folwaczny C, Lorenz R, Martin G, Schindlbeck N, Heldwein W, Soyka M, Grunze H, Koenig A, Loeschke K. Adherence and mental side-effects during hepatitis C treatment with interferon alfa and ribavirin in psychiatric risk groups. Hepatology 2003;37:443-451.

33. Sawada M, Takada A, Takase S, Takada N. Effects of alcohol on the replication of hepatitis C virus. Alcohol 1993;28:85-90.

34. Oshita M, Hayashi N, Kasahara A, Hagiwara H, Mita E, Naito M, Katayama K, Fusamoto H, Kamada T. Increased serum hepatitis C virus RNA levels among alcoholic patients with chronic hepatitis C. Hepatology 1994;20:1115-1120.

35. Miguez MJ, Burbano X, Morales G, Shor-Posner G. Alcohol use and HIV infection in the HAART era. Am Clin Lab 2001;20:20-23.

36. Zylberberg H, Fontaine H, Thepot V, Nalpas B, Brechot C, Pol S. Triggering of acute alcoholic hepatitis by interferon therapy. J Hepatol 1999;30:722-725.

37. Mochida S, Ohnishi K, Matsuo S, Kakihara K, Fujiwara K. Effect of alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. Alcohol Clin Exp Res 1996;20:371A-377A.

38. Ono K, Sata M, Murashima K, Fukuizumi K, Suzuki H, Tanikawa K. Biological responses to administered interferon in alcoholics. Alcohol Clin Exp Res 1996;20:1560-1563.

39. Ohnishi K, Matsuo S, Matsutani K, Itahashi M, Kakihara K, Suzuki K, Ito S, Fujiwara K. Interferon therapy for chronic hepatitis C in habitual drinkers: comparison with chronic hepatitis C in infrequent drinkers. Am J Gastroenterol 1996;91:1374-1379.

40. Zeldis JB, Depner TA, Kuramoto IK, Gish RG, Holland PV. The prevalence of hepatitis C virus antibodies among hemodialysis patients. Ann Intern Med 1990;112:958-960.

41. Chan TM, Lok ASF, Cheng IKP, Chan RT. Prevalence of hepatitis C virus infection in hemodialysis patients: a longitudinal study comparing the results of RNA and antibody assays. Hepatology 1993;17:5-8.

42. Donahue JG, Munoz A, Ness PM, Brown DE Jr, Yawn DH, McAllister HA Jr, Reitz BA, Nelson KE. The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med 1992;327:369-373.

43. Jadoul M, Cornu C, Van Ypersele De Strihou C, and the UCL Collaborative Group. Incidence and risk factors for hepatitis C virus seroconversion in hemodialysis. A prospective study. Kidney Int 1993;44:1322-1326.

44. Magrin S, Craxi A, Fabiano C, Simonetti RG, Fiorentino G, Marino L, Diquattro O, Di Marco V, Loiacono O, Volpes R. Hepatitis C viremia in chronic liver disease: relationship to interferon alpha or corticosteroid treatment. Hepatology 1994;19:273-279.

45. Zylberberg H, Nalpas B, Carnot F, Skhiri H, Fontaine H, Legendre C, Kreis H, Brechot C, Pol S. Severe evolution of chronic hepatitis C in renal transplantation: a case control study. Nephrol Dial Transplant 2002;17:129-133.

46. Zylberberg H, Carnot F, Mamzer MF, Blancho G, Legendre C, Pol S. Hepatits C virus-related fibrosing cholestatic hepatitis after renal transplantation. Transplantation 1997;63:158-160.

47. Mathurin P, Mouquet C, Poynard T, Sylla C, Benalia H, Fretz C, Thibault V, Cadranel JF, Bernard B, Opolon P, Coriat P, Bitker MO. Impact of hepatitis B and C virus on kidney transplantation outcome. Hepatology 1999;29:257-263.

48. Tan AC, Brouwer JT, Van Leusen R, Kauffmann RH, Schalm SW, de Vries RA, Vroom B. Safety of interferon and ribavirin therapy in dialysis patient with chronic hepatitis C: results of a pilot study. Hepatology 1999;30:364A.

49. Koenig P, Vogel W, Umlauft F, Weyrer K, Prommegger R, Lhotta K, Neyer U, Stummvoll HK, Gruenewald K. Interferon treatment for chronic hepatitis C virus infection in uremic patients. Kidney Int 1994;45:1507-1509.

50. Pol S, Thiers V, Carnot F, Zins B, Romeo R, Berthelot P, Brechot C. Efficacy and tolerance of 2b interferon therapy on HCV infection of hemodialyzed patients. Kidney Int 1995;47:1412-1418.

51. Izopet J, Rostaing L, Moussion F, Alric L, Dubois M, That HT, Payen JL, Duffaut M, Durand D, Suc JM, Puel J. High rate of hepatitis C virus clearance in hemodialysis patients after interferon alpha therapy. J Infect Dis 1997;176:1614-1617.

52. Chan TM, Wu PC, Lau JY, Lok AS, Lai CL, Cheng IK. Interferon treatment for hepatitis C virus infection in patients on haemodialysis. Nephrol Dial Transplantation 1997;7:1414-1419.

53. Uchihara M, Izumi N, Sakai Y, Yauchi T, Miyake S, Sakai T, Akiba T, Marumo F, Sato C. Interferon therapy for chronic hepatitis in hemodialysis patients: increased serum levels of interferon. Nephron 1998;80:51-56.

54. Casanovas-Taltavull T, Baliellas C, Benasco C, Serrano TT, Casanova A, Perez JL, Guerrero L, Gonzalez MT, Andres E, Gil-Vernet S, Casais LA. Efficacy of interferon for chronic hepatitis C virus-related hepatitis in kidney transplant candidates on hemodialysis: results after transplantation. Am J Gastroenterol 2001;96:1170-1177.

55. Degos F, Pol S, Chaix ML, Laffitte V, Buffet C, Bernard PH, Degott C, Carnot F, Riffaud PC, Chevret S. The tolerance and efficacy of interferon alpha in haemodialysis patients with HCV infection: a multicenter, prospective study. Nephrol Dial Transplant 2001;16:1017-1023.

56. Buisson C, Degos F, Daniel F, Dupuy A, Sari R, Simon N. Pharmacokinetics of interferon alpha-2b in haemodialysis. Nephrol Dial Transplant 1994;9:977-978.

57. Lamb MW, Marks IM, Wynohradnyk L, Modi MW, Preston RA, Pappas C. 40KDA peginterferon alfa-2A (Pegasys) can be administered safely in patients with end-stage renal disease. Hepatology 2001;34:326A.

58. Furuyso N, Hayashi J, Kakuda K, Ariyama I, Kanamoto-Tanaka Y, Shimizu C, Etoh Y, Shigematsu M, Kashiwagi S. Acute hepatitis C among Japanese hemodialysis patients: a prospective 9-year study. Am J Gastroenterol 2001;96:1592-1600.

59. Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, Pastore G, Dietrich M, Trautwein C, Manns MP; German Acute Hepatitis C Therapy Group. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001;345:1452-1457.

60. Gursoy M, Gur G, Arslan H, Ozdemir N, Boyacioglu S. Interferon therapy in haemodialysis patients with acute hepatitis C virus infection and factors that predict response to treatment. J Viral Hepat 2001;8:70-77.

61. Misiani R, Bellavita P, Fenili D, Vicari O, Marchesi D, Sironi PL, Zilio P, Vernocchi A, Massazza M, Vendramin G. Interferon alpha-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 1994;330:751-756.

62. Sarac E, Bastacky S, Johnson JP. Response to high-dose interferon-α after failure of standard therapy in MPGN associated with hepatitis C virus infection. Am J Kidney Dis 1997;30:113-115.

63. Thervet E, Pol S, Legendre C, Gagnadoux MF, Cavalcanti R, Kreis H. Low-dose recombinant leukocyte interferon alpha treatment of hepatitis C viral infection in renal transplant recipients: a pilot study. Transplantation 1994;58:625-628.

64. Rostaing L, Izopet J, Baron E, Duffaut M, Puel J, Durand D. Treatment of chronic hepatitis C with recombinant interferon alpha in kidney transplant recipients. Transplantation 1995;59:1426-1431.

Treatment of Hepatitis C in Liver Transplant Patients

Didier Samuel, Bruno Roche

INTRODUCTION

Liver disease caused by the hepatitis C virus (HCV) is the main indication for liver transplantation (OLT) in Europe and the United States. Recurrence of hepatitis C on the graft is a major issue and may lead to graft loss. In the absence of effective prophylaxis, recurrent HCV infection is almost constant. Recurrence of HCV leads to chronic active hepatitis in most patients and may lead to cirrhosis or cholestatic hepatitis in some with a risk of graft failure at medium or long-term. Thus effective treatment for recurrent HCV is mandatory. In this review, current knowledge on the treatment of HCV graft infection after liver transplantation is discussed.

LIVER TRANSPLANTATION FOR HCV-RELATED CIRRHOSIS

The effect of HCV infection on patients and graft survival after liver transplantation is controversial. However, recent data have confirmed that HCV infection impairs patient and allograft survival [1]. HCV recurrence is almost universal and 60-80% of patients will develop lesions of chronic hepatitis on the graft [1-4]. Cholestatic hepatitis can occasionally (2-8%) result in progressive liver dysfunction. Overall, the course of HCV graft disease is accelerated in liver transplant

recipients compared to that observed in immune competent patients, with a 5-year rate of cirrhosis of around 10-20% [1, 3-5] reaching 28-40% [6]. When cirrhosis occurs on the graft, there is a high risk of decompensation in the following years and a 60% risk of death within the year after the first episode of decompensation [7]. At least 10% of patients transplanted for HCV cirrhosis will require retransplantation for hepatitis C graft failure. The factors which influence disease severity and the consequent progression of graft injury or survival remain unclear. Factors clearly associated with the severity of recurrent hepatitis C are: high pre-transplant and early post-transplant serum HCV RNA levels [8, 9], severe early histological recurrence [10], rejection episodes and treatment with more potent immunosuppression (methylprednisone boluses, anti-CD3 monoclonal antibody (OKT3), use of mycofenolate mofetil (MMF) [6, 11-13] and the increasing age of donors [14, 15]. Some of these factors are negative predictors for a virological response to interferon. In the long-term, HCV RNA levels are related to the level of immunosuppression and correlate with the severity of liver injury [16]. Strategies to reduce the impact of immunosuppression on recurrent HCV infection include an overall reduction in immunosuppression, discontinuation of individual agents and the use of immunosuppressive agents with possible antiviral effects. Current data have failed to show differences in the incidence or severity of HCV recurrence using tacrolimus or cyclosporine [3, 8]. Many studies have shown a strong correlation between multiple rejection episodes, exposure to pulse solumedrol, greater daily exposure to steroids or OKT3 and the incidence and severity of HCV recurrence [6, 12, 13, 17]. Despite general acceptance of early steroid withdrawal in patients with chronic hepatitis C, data are limited on the effectiveness of this approach and more recent data suggest that this strategy may have a harmful effect. The post transplantation use of MMF has not been associated with consistent beneficial or deleterious effects [18]. The effects of induction immunosuppression with anti-IL2 receptor antibodies in HCV-infected transplant recipients have not been clearly determined [18].

It therefore appears to be legitimate to offer antiviral therapy to patients with recurrent chronic hepatitis C to stop hepatitis disease progression on the graft. However, certain points should be kept in mind before starting antiviral treatment: (1) 20-30% of patients have a

benign or mild long-term course of HCV hepatitis on the graft and may not require treatment. (2) Optimal treatment is a combination of interferon and ribavirin, which is not well tolerated in transplant patients and which may cause serious side-effects (i.e., hemolytic anemia, risk of rejection). Antiviral therapy could be used: (1) before transplantation to suppress viral replication and reduce the risk of recurrence, (2) early post-transplantation to prevent hepatitis disease progression (3) at time of HCV recurrence.

PRE- AND POST-TRANSPLANTATION TREATMENT OF HCV INFECTION

Pre-transplantation antiviral therapy

Interferon alone or in combination with ribavirin has been shown to reduce viral levels in patients with cirrhosis but its use is very difficult in this setting due to the risk of severe decompensation of cirrhosis and the development of cytopenia or uncontrolled sepsis [19]. Forns et al. evaluated the efficacy and safety of antiviral therapy in 30 patients with HCV cirrhosis awaiting OLT (Child A n=15, Child B/C n=15, genotype 1b n=25) [20]. Treatment with interferon-alpha-2b 3MU/day and ribavirin 800mg/day was initiated when the expected time for OLT was less than 4 months (median duration of treatment 12 weeks). Virological response was observed in 9 patients (30%). After OLT 6 of them (20%) remain free of reinfection after a median follow-up of 46 weeks and HCV infection recurred in 3. A viral load decrease >2 logs at week 4 of treatment was the strongest predictor of virologic response. Side-effects were frequent and dose reduction was necessary in 63% of patients. Everson et al. reported on 102 HCV-cirrhotic patients treated with interferon and ribavirin for one year with a low accelerating dose regimen [21]. The end-of-treatment virological response was 40% and the sustained virological response 20%. None of the 10 sustained responders who underwent OLT had recurrent HCV infection. There are no data on the safety and efficacy of pegylated interferon with or without ribavirin in patients with decompensated HCV cirrhosis. In conclusion, antiviral therapy in patients awaiting OLT should be considered as a strategy to prevent HCV recurrence in patients without severe hepatocellular insufficiency.

Preventive therapy in the early post-transplantation period

HCV RNA is present in the serum of more than 95% of those who are HCV RNA-positive before transplantation, which is the vast majority of patients. HCV RNA is detected in serum as early as the first post-transplant hours [22]. However, HCV RNA is at the lowest level in serum during the first post-transplant week, which is the rationale for starting treatment early [23]. Treatment is generally considered prophylactic if it is started during the 3 first post-transplant weeks. Indeed acute hepatitis on the graft may occur around 3 weeks, with a median at 4 months [4]. Few studies have been performed on prophylactic antiviral treatment. In one study, 86 patients were randomized within 2 weeks after transplantation to receive either interferon alone (n=38) or placebo (n=48) for one year [24]. Patient and graft survival at 2 years and HCV viremia were not affected by treatment, but histological disease recurrence was less frequent in interferon treated patients than in those who were not treated (26% vs. 53%, P=0.01). Interferon and 1-month HCV RNA levels were independent predictors of recurrence. Interferon was stopped in 30% of patients because of adverse effects (acute rejection n=1, thrombopenia n=4, other n=3). In a second trial, 24 patients were randomized 2 weeks after transplantation to receive interferon (n=12) or placebo (n=12) for 6 months [25]. No difference in graft or patient survival, incidence or severity of histological recurrence or 6 months HCV RNA levels were observed. However, interferon significantly delayed the occurrence of HCV hepatitis in treated patients (408 vs. 193 days, P=0.05). Although the use of interferon was not associated with rejection, adverse effects that were probably due to interferon were observed in 50% of the patients (leucopoenia 17%, headache and/or fatigue 33%). In a non-randomized pilot study, 36 patients were treated with interferon-alpha-2b and ribavirin started during the 3 post-transplant weeks [26] and were followed up for a median of 4.5 years. HCV RNA clearance was obtained in 12 patients (33%) at the end of treatment. All these patients remained HCV RNA-negative 6 months after the completion of therapy. Six of the 12 patients who became HCV RNA-negative were infected with genotype 1b (20% response rate), whereas 6 had genotype 2 (100% response rate). Of the remaining 24 patients, only 7 developed recurrent hepatitis with significant fibrosis in 4. Dose reduction because of drug toxicity was needed in 25% of patients but no patients were withdrawn from the

treatment regimen. A subsequent pilot study of combination interferon and ribavirin therapy failed to obtain these excellent results because of high dropout rates (48% related to severe ribavirin-induced hemolysis and interferon-induced neutropenia). Sustained virologic response was achieved in only 16% of patients [27]. Multicenter studies are currently underway and should provide further data on the safety and efficacy of pegylated interferon with or without ribavirin as prophylaxis against recurrent hepatitis C after liver transplantation. In conclusion from the published studies combination therapy is probably more effective on viral load than monotherapy with interferon. The occurrence of hepatitis may be delayed using antiviral therapy. The main drawbacks are the high risk of poor hematological tolerance, the risk of rejection and sepsis. With existing drugs, results in intent to treat are disappointing. Indeed most patients have contraindications to treatment during the first post-transplant weeks.

Treatment of established infection

The treatment of patients with HCV graft reinfection is necessary when the disease is severe to avoid progression of the hepatitis. As in the non-transplant setting, the decision to treat should take into account all parameters: Age, general status, genotype, severity of hepatitis, risk of graft loss, and expected tolerance to treatment. There are some patients that absolutely must be treated: those with fibrosing cholestatic hepatitis due to the poor short-term prognosis and those with rapidly evolving fibrosis on successive biopsies. For the latter reason, we suggest routine yearly biopsies to determine the rate of HCV-related progression of fibrosis.

Interferon or ribavirin monotherapy (Table 1)

Interferon is an immunostimulating agent enhancing the expression of HLA class I and II molecules on hepatocytes and has been reported to facilitate the occurrence of rejection in transplant recipients [28-30]. In our experience, a histological disappearance of interlobular bile duct suggestive of chronic rejection was observed in 5 patients. Three of them were retransplanted [28]. Interferon at doses of 3MU thrice weekly for 6 months had a sustained virologic effect in 0 to 7% of patients and had a minor effect on liver histology [28, 31-33]. Using ribavirin, a biochemical improvement was observed in 44 to 93% of patients but virological clearance in none [32, 34, 35]. The main side-

effect was hemolysis and dosage had to be adapted to renal function since the incidence of hemolysis was significantly associated with higher serum creatinine and decreased creatinine clearance [36].

Author [ref.]	Patients (N)	Treatment (duration)	Interval from transplan -tation (months)	Biochemical response [a] (%)	EOTR (%)	SVR (%)	Histological [a] improvement (%)	Rejection (%)	Cessation of therapy/ side effects (%)
Wright [31]	18	IFN-alpha-2b 3MU x3/week (4 months)	15	28	0	0	0	4	11
Feray [28]	14	IFN-alpha-2b 3MU x3/week (6 months)	44	23	7	7	14	35	28
Cotler [33]	8	IFN-alpha-2a 3MU/day (12 months)	34	14	12.5	12.5	0	12.5	25
Gane [32]	14	IFN-alpha-2b 3MU x3/week (6 months)	6	43	46	NA	21	0	0
Gane [32]	16	RBV (6 months)	7	93	17	NA	64	0	12.5
Gane [34]	7	RBV (6 months)	10	57	0	0	57	0	0
Cattral [35]	9	RBV (6 months)	6	44	0	0	22	0	0

Table 1: Treatment of HCV recurrence: interferon or ribavirin monotherapy.
EOTR = end-of-treatment-response, SVR = sustained virological response, NA = not available, [a] = end of therapy, IFN = interferon, RBV = ribavirin, 3x/week = thrice weekly.

Combination therapy (Table 2)

Combination therapy is more effective than monotherapy with interferon or ribavirin. In a non-randomized pilot study, 21 patients with early recurrent hepatitis (median time from transplantation: 9 months, 3-24) received a combination of interferon and ribavirin for 6 months and then ribavirin alone for an additional 6 months [37]. After 6 months of combination therapy, all patients had normal ALTs and histological improvement. Ten patients (48%) cleared HCV RNA from serum. During maintenance ribavirin monotherapy, ALTs remained normal in all but one patient and HCV RNA reappeared in 5. The main side-effect was anemia, which required cessation of ribavirin therapy in 3 patients. No patient experienced graft rejection. Off-treatment response rates were not reported in this study. In a randomized controlled trial we compared 12 months of combination therapy vs. no treatment in 52 patients with HCV reinfection [38]. Intent to treat analysis for loss of serum HCV RNA showed a sustained virologic response of 21% in the treated group, vs. no patient in the control group (P=0.019). Twelve treated patients (43%) were withdrawn from the study for anemia in 7, chronic rejection in 1, insomnia in 1, depression in 1 and irritability in 2 patients. Lavezzo et al. reported 57 patients treated with interferon and ribavirin for 6 or 12 months [39]. Six additional months of ribavirin monotherapy was given to virologic responders who had tolerated the drug well during combination therapy (n=7). End of treatment and 12 months post-therapy, the sustained virological response was 33 and 22% respectively for 6 months of therapy and 23 and 17% for 12 months of therapy (P=0.4). Genotype non-1 compared to genotype 1 was a significant predictor of sustained virologic response (43% vs. 12% P=0.02) and HCV RNA level below 2meq/mL correlated with a higher rate of end of treatment virologic response. The principal side-effects were anemia and leucopoenia, which required a dose reduction in 51% of patients. Several recent studies of combination therapy have shown that the sustained virological response rate was between 8 to 33% (Table 2) [40-47]. Bizollon et al. described the virological and histological course of 14 liver transplant patients with a sustained virological response to antiviral therapy (combination therapy for 6 months and maintenance ribavirin monotherapy for 12 months) [48].

A complete response was obtained in 93% for 3 years after cessation of therapy and was associated with an absence of detectable intrahepatic HCV RNA and marked histological improvement (marked reduction of necroinflammatory activity, stabilization of the stage of liver fibrosis).

The optimal duration of therapy is uncertain. In contrast to an immunocompetent population, the increase in efficacy seems limited in patients treated for 12 months vs. 6 months [37-39]. The efficacy and duration of additional ribavirin monotherapy in patients with a sustained response to the combination of interferon and ribavirin needs to be determined [49]. As in the non-transplant setting, patients with HCV genotype non-1 responded better than patients with genotype 1 [39]. Other factors such as interval between transplantation to the start of therapy and the type and amount of immunosuppression could influence treatment efficacy.

All these studies showed a high incidence of side-effects compared to that observed in non-transplant patients. Between 20% and 50% of patients could not complete treatment because of side-effects. The most important side-effect of ribavirin is hemolysis, which required dose reductions or cessation of therapy. The use of erythropoietin may be effective in the treatment of anemia during combination therapy. Common side-effects of interferon such as neutropenia, thrombocytopenia or depression are also present. In addition the risk of rejection in patients receiving interferon plus ribavirin seems lower than in patients receiving interferon alone. This may be because ribavirin has an immunosuppressive effect.

There is little information about the potential benefit of pegylated interferon versus interferon. Pegylated interferon is more effective in immunocompetent patients, however its long half-life and its main renal clearance may be a risk in transplant patients. In a randomized-trial 32 liver transplant recipients were treated with pegylated interferon-alpha-2a monotherapy 180µg/week for 48 weeks vs. no treatment [50]. At the end of treatment, 35% of patients had undetectable HCV RNA. Post-treatment data are awaited. Preliminary results of treatment with pegylated interferon and ribavirin showed virological response during treatment in 33% of naive patients and in 18% of non-responders to interferon-alpha-2b and ribavirin patients [51, 52]. We report a sustained virologic response rate of 26% using pegylated interferon-alpha-2b and ribavirin [53]. Recently the group in

Lyon treated 20 patients with increasing doses of pegylated interferon (from 0.5m/kg/week to 1mg/kg by week) plus increasing doses of ribavirin (from 400mg/day to 1000mg/day). 4 patients (20%) were withdrawn from the study and 13 patients required dose reduction of ribavirin because of anemia. The sustained virological response rate was 9/20 (80% of patients were infected with genotype 1).

Authors [ref.]	Patients (N)	Treatment (duration)	Interval from transplan -tation (months)	Biochemical response [a] (%)	EOTR (%)	SVR (%)	Histolo-gical [a] improv-ement (%)	Rejection (%)	Cessation of therapy/ side effects (%)
Bizollon [37]	21	IFN 3MU 3x/week + RBV (6 months) then RBV (6 months)	9	100	48 (6 months) 24 (12 months)	NA	94	0	14
Fischer [40]	8	IFN 3MU 3x/week + RBV (6 months)	5.5	87	12.5	0	NA	0	37.5
Samuel [38]	28	IFN 3MU 3x/week + RBV (12 months)	56	NA	25	21.4	NA	3.5	43
Gopal [41]	12	IFN 3MU 3x/week + RBV (1-17 months)	9	75	50	8.3	NA	8	8
De Vera [44]	32	IFN 1.5-3MU 3x/week + RBV (3-18 months)	NA	77	9	9	0	0	46.8
Alberti [43]	18	IFN 3MU 3x/week + RBV (12 months) then RBV (18-73 months)	9	83	44 (12 months)	33 (18 months)	73	5.5	22.2

Table 2: Treatment of HCV recurrence: interferon plus ribavirin combination therapy.

Ahmad [42]	40	IFN 3-5MU 3x/week (6 months)	24	20	15	2.5	0	0	25
	20	IFN 3-5MU 3x/week + RBV (12 months)	38	25	40	20	0	0	25
Lavezzo [39]	27	IFN 3MU 3x/week + RBV (6 months)[b]	9	66	33	22	52	1.7	2
	30	IFN 3MU 3x/week + RBV (12months)[b]	(3-60)	53	23	17	NA	NA	NA
Menon [45]	26	IFN 3MU 3x/week + RBV (12 months)	14.6	42	35	30.7	75	0	50
Shakil [46]	38	IFN 3MU 3x/week + RBV (12 months) then RBV (6 months)	23	18	13	5	0	0	42
Firpi [47]	54	IFN 3MU 3x/week + RBV (12 months)	31.2	39	38	30	30	5.5	12.9
Dumortier [57]	20	Peglyated IFN 0.5-µg/kg/week + RBV (12 months)	28	75	55	45	NA	25	20

Table 2: (Cont.) Treatment of HCV recurrence: interferon plus ribavirin combination therapy.
EOTR = end-of-treatment-response, SVR = sustained virological response, NA = not available,
[a] = end of therapy, [b] = 6 additional months of ribavirin

Retransplantation

Recurrence of HCV infection may lead to graft failure and an indication for retransplantation in a minority of cases (5-10% of patients). Early reports suggested that the outcome was worse following retransplantation for HCV reinfection than in patients undergoing retransplantation for other indications [54]. However, the natural history of recurrent HCV disease in the second graft seems to be unrelated to that observed in the first graft. Recent studies reported an improved outcome when retransplantation was performed before the development of infectious and renal complications [55, 56]. Due to increased organ shortage and uncertainty regarding the natural history of HCV recurrence, retransplantation is still the subject of debate and requires further studies.

CONCLUSION

Most patients with HCV infection will develop recurrence after transplantation. Although recurrence of HCV on the liver graft does not significantly reduce the medium-term survival of the patient and the graft, HCV infection impairs long-term patient and graft survival. Treatment of recurrent HCV disease with interferon or ribavirin as single agents has been disappointing, but results with combination therapy are encouraging with sustained virologic response in about 25% of patients. Preventive therapy in the early post-transplant period is limited by the high rate of side-effects. Treatment of established infection on the graft is a matter of controversy and several questions should be raised. What is the best treatment? Combination therapy with interferon and ribavirin, or combination pegylated interferon + ribavirin? The duration of therapy and doses are not yet known. The need for ribavirin monotherapy following interferon discontinuation is unclear. Which patients should be treated and what is the optimal timing for initiation of treatment? Further studies are required to resolve these questions. Future research should also focus on improving the tolerance of treatment; this can be achieved with the administration of erythropoietin during ribavirin treatment.

REFERENCES

1. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002;122:889-896.

2. Wright TL, Donegan E, Hsu H Ferrell L, Lake JR, Kim M, Combs C, Fennessy S, Roberts JP, Ascher NL. Recurrent and acquired hepatitis C viral infection in liver transplant recipients. Gastroenterology 1992;103:317-322.

3. Gane EJ, Portmann BC, Naoumov NV, Smith HM, Underhill JA, Donaldson PT, Maertens G, Williams R. Long-term outcome of hepatitis C infection after liver transplantation. N Engl J Med 1996;334:815-820.

4. Feray C, Gigou M, Samuel D, Paradis V, Wilber J, David MF, Urdea M, Reynes M, Brechot C, Bismuth H. The course of hepatitis C infection after liver transplantation. Hepatology 1994:20:1137-1143.

5. Feray C, Caccamo L, Alexander GJM, Ducot B, Gugenheim J, Casanovas T, Loinaz C, Gigou M, Burra P, Barkholt L, Esteban R, Bizollon T, Lerut J, Minello-Franza A, Bernard PH, Nachbaur K, Botta-Fridlund D, Bismuth H, Schalm SW, Samuel D. European collaborative study on factors influencing outcome after liver transplantation for hepatitis C. Gastroenterology 1999;117:619-625.

6. Prieto M, Berenguer M, Rayon JM, Cordoba J, Arguello L, Carrasco D, Garcia-Herola A, Olaso V, De Juan M, Gobernado M, Mir J, Berenguer J. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. Hepatology 1999;29:250-256.

7. Berenguer M, Prieto M, Rayon JM, Mora J, Pastor M, Ortiz V, Carrasco D, San Juan F, Burgueno MD, Mir J, Berenguer J. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. Hepatology 2000;32:852-858.

8. Charlton M, Seaberg E, Wiesner R, Everhart J, Zetterman R, Lake J, Detre K, Hoofnagle J. Predictors of patient and graft survival following liver transplantation for hepatitis C. Hepatology 1998;28:823-830.

9. Sreekumar R, Gonzalez-Koch A, Maor-Kendler Y Batts K, Moreno-Luna L, Poterucha J, Burgart L, Wiesner R, Kremers W, Rosen C, Charlton MR. Early identification of recipients with progressive histologic recurrence of hepatitis C after liver transplantation. Hepatology 2000;32:1125-1130.

10. Berenguer M, Ferrell L, Watson J, Prieto M, Kim M, Rayon M, Cordoba J, Herola A, Ascher N, Mir J, Berenguer J, Wright TL. HCV-related fibrosis progression following liver transplantation: increase in recent years. J Hepatol 2000;32:673-684.

11. Rosen HR, Shackleton CR, Higa L, Gralnek IM, Farmer DA, McDiarmid SV, Holt C, Lewin KJ, Busuttil RW, Martin P. Use of OKT3 is associated with early and severe recurrence of hepatitis C after liver transplantation. Am J Gastroenterol 1997;92:1453-1457.

12. Sheiner PA, Schwartz ME, Mor E, Schluger LK, Theise N, Kishikawa K, Kolesnikov V, Bodenheimer H, Emre S, Miller CM. Severe or multiple rejection episodes are associated with early recurrence of hepatitis C after orthotopic liver transplantation. Hepatology 1995;21:30-34.

13. Berenguer M, Prieto M, Cordoba J, Rayon JM, Carrasco D, Olaso V, San-Juan F, Gobernado M, Mir J, Berenguer J. Early development of chronic active hepatitis in recurrent hepatitis C virus infection after liver transplantation: association with treatment of rejection. J Hepatol 1998;28:756-763.

14. Berenguer M, Prieto M, San Juan F, Rayon JM, Martinez F, Carrasco D, Moya A, Orbis F, Mir J, Berenguer J. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. Hepatology 2002;36:202-210.

15. Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248-252.

16. Gane E, Naoumov N, Qian K, Mondelli MU, Maertens G, Portmann BC, Lau JY, Williams R. A longitudinal analysis of hepatitis C virus replication following liver transplantation. Gastroenterology 1996;110:167-177.

17. Papatheodoridis G, Davies S, Dhillon A, Teixeira R, Goulis J, Davidson B, Rolles K, Dusheiko G, Burroughs AK. The role of different immunosuppression in the long-term histological outcome of HCV reinfection after liver transplantation for HCV cirrhosis. Transplantation 2001;72:412-418.

18. Everson T. Impact of immunosuppressive therapy on recurrence of hepatitis C. Liver Transpl 2002;8:S19-S27.

19. 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;8:350-355.

20. Forns X, Garcia-Retortillo M, Serrano, T Feliu A, Suarez F, de la Mata M, Garcia-Valdecasas JC, Navasa M, Rimola A, Rodes J. Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. J Hepatol 2003;39:389-396.

21. Everson G, Trotter J, Kugelmas M. Long-term outcome of patients with chronic hepatitis C and decompensated liver disease treated with the LADR protocol (low accelerating-dose regimen). Hepatology 2002;36:297A.

22. Garcia–Retortillo M, Forns X, Feliu A, Moitinho E, Costa J, Navasa M, Rimola A, Rodes J. Hepatitis C virus kinetics during and immediately after liver transplantation. Hepatology 2002;35:680-687.

23. Samuel D, Feray C. Recurrence of hepatitis C virus after liver transplantation. J Hepatol 1999;31:217-221.

24. Sheiner PA, Boros P, Klion FM, Thung SN, Schluger LK, Lau JY, Mor E, Bodian C, Guy SR, Schwartz ME, Emre S, Bodenheimer HC Jr, Miller CM. The efficacy of prophylactic interferon alfa-2b in preventing recurrent hepatitis C after liver transplantation. Hepatology 1998;28:831-838.

25 Singh N, Gayowski T, Wannstedt CF, Shakil AO, Wagener MM, Fung JJ, Marino IR. Interferon alfa for prophylaxis of recurrent viral hepatitis C in liver transplant recipients: a prospective, randomized, controlled trial. Transplantation 1998;65:82-86.

26. Mazzaferro V, Tagger A, Schiavo M, Regalia E, Pulvirenti A, Ribero ML, Coppa J, Romito R, Burgoa L, Zucchini N, Urbanek T, Bonino F. Prevention of recurrent hepatitis C after liver transplantation with early interferon and ribavirin treatment. Transplant Proc 2001;33:1355-1357.

27. Reddy R, Fried M, Dickson R, Martin P, Schiff E, Torres M, Weppler D, Ruiz P, Tzakis A. Interferon α-2b and ribavirin vs. placebo as early treatment in patients transplanted for hepatitis C end-stage liver disease: results of a multicenter randomized trial. Gastroenterology 2002;122:199A.

28. Feray C, Samuel D, Gigou M, Paradis V, David MF, Lemonnier C, Reynes M, Bismuth H. An open trial of interferon alfa recombinant for hepatitis C after liver transplantation: antiviral effects and risk of rejection. Hepatology 1995;22:1084-1089.

29. Dousset B, Conti F, Houssin D, Calmus Y. Acute vanishing bile duct syndrome after interferon therapy for recurrent HCV infection in liver-transplant recipients. N Engl J Med 1994;330:1160-1161.

30. Gaidano AC, Mosnier JF, Durand F, Martinot-Peignoux M, Marcellin P, Degott C, Belghiti J, Erlinger S, Benhamou JP, Bernuau JR. Alpha-interferon-induced rejection of a hepatitis C virus-infected liver allograft tolerated with a low dosage immunosuppressive regimen. Transplantation 1995;59:1627-1629.

31. Wright TL, Combs C, Kim M, Ferrell L, Bacchetti P, Ascher N, Roberts J, Wilber J, Sheridan P, Urdea M. Interferon-alpha therapy for hepatitis C virus infection after liver transplantation. Hepatology 1994;20:773-779.

32. Gane EJ, Lo SK, Riordan SM, Portmann BC, Lau JY, Naoumov NV, Williams R. A randomized study comparing ribavirin and interferon alfa monotherapy for hepatitis C recurrence after liver transplantation. Hepatology 1998;27:1403-1407.

33. Cotler SJ, Ganger D, Kaur S, Rosenblate H, Jakate S, Sullivan DG, Ng KW, Gretch DR, Jensen DM. Daily interferon therapy for hepatitis C virus infection in liver transplant recipients. Transplantation 2001;71:261-266.

34. Gane EJ, Tibbs CJ, Ramage JK, Portmann BC, Williams R. Ribavirin therapy for hepatitis C infection following liver transplantation. Transpl Int 1995;8:61-64.

35. Cattral MS, Krajden M, Wanless IR, Rezig M, Cameron R, Greig PD, Chung SW, Levy GA. A pilot study of ribavirin therapy for recurrent hepatitis C virus infection after liver transplantation. Transplantation 1996;61:1483-1488.

36. Jain A, Eghtesad B, Venkataramanan R, Fontes PA, Kashyap R, Dvorchik I, Shakil AO, Kingery L, Fung JJ. Ribavirin dose modification based on renal function is necessary to reduce hemolysis in liver transplant patients with hepatitis C virus infection. Liver Transpl 2002;8:1007-1013.

37. Bizollon T, Palazzo U, Ducerf C, Chevallier M, Elliott M, Baulieux J, Pouyet M, Trepo C. Pilot study of the combination of interferon alfa and ribavirin as therapy of recurrent hepatitis C after liver transplantation. Hepatology 1997;26:500-504.

38. Samuel D, Bizollon T, Feray C, Roche B, Ahmed SN, Lemonnier C, Cohard M, Reynes M, Chevallier M, Ducerf C, Baulieux J, Geffner M, Albrecht JK, Bismuth H, Trepo C. Interferon-alfa 2b plus ribavirin in patients with chronic hepatitis C after liver transplantation: a randomized study. Gastroenterology 2003;124:642-650.

39. Lavezzo B, Franchello A, Smedile A, David E, Barbui A, Torrani M, Ottobrelli A, Zamboni F, Fadda M, Bobbio A, Salizzoni M, Rizzetto M. Treatment of recurrent hepatitis C in liver transplants: efficacy of a six versus a twelve month course of interferon alpha 2b with ribavirin. J Hepatol 2002;37:247-252.

40. Fischer L, Sterneck M, Valentin-Gamazo C, Feucht HH, Malago M, Broelsch CE. Treatment of severe recurrent hepatitis C after liver transplantation with ribavirin plus interferon alpha. Transplant Proc 1999;31:494-495.

41. Gopal DV, Rabkin JM, Berk BS, Corless CL, Chou S, Olyaei A, Orloff SL, Rosen HR. Treatment of progressive hepatitis C recurrence after liver transplantation with combination interferon plus ribavirin. Liver Transpl 2001;7:181-190.

42. Ahmad J, Dodson SF, Demetris AJ, Fung JJ, Shakil A. Recurrent hepatitis C after liver transplantation: a non-randomized trial of interferon alpha alone versus interferon alpha and ribavirin. Liver Transpl 2001;10:863-869.

43. Alberti A, Belli L, Airoldi A, de Carlis L, Rondinara G, Minola E, Vangeli M, Cernuschi A, D'Amico M, Forti D, Pinzello G. Combined therapy with interferon and low-dose ribavirin in post transplantation recurrent hepatitis C: A pragmatic study. Liver Transpl 2001;10:870-876.

44. De Vera M, Smallwood G, Rosado K, Davis L, Martinez E, Sharma S, Stieber AC, Heffron TG. Interferon alpha and ribavirin for the treatment of recurrent hepatitis C after liver transplantation. Transplantation 2001;71:678-686.

45. Menon K, Poterucha J, El-Amin O, El-Amin OM, Burgart LJ, Kremers WK, Rosen CB, Wiesner RH, Charlton M. Treatment of post transplantation recurrence of hepatitis C with interferon and ribavirin: lessons on tolerability and efficacy. Liver Transpl 2002;8:623-629.

46. Shakil A, McGuire B, Crippin J, Teperman L, Demetris AJ, Conjeevaram H, Gish R, Kwo P, Balan V, Wright TL, Brass C, Rakela J. A pilot study of interferon alfa and ribavirin combination in liver transplant recipients with recurrent hepatitis C. Hepatology 2002;36:1253-1258.

47. Firpi R, Abdelmalek M, Soldevila-Pico C, Reed A, Hemming A, Howard R, Van Der Werf W, Lauwers G, Liu C, Crawford JM, Davis GL, Nelson DR. Combination of interferon alfa-2b and ribavirin in liver transplant recipients with histological recurrent hepatitis C. Liver Transpl 2002;8:1000-1006.

48. Bizollon T, Ahmed SN, Radenne S, Chevallier M, Chevallier P, Parvaz P, Guichard S, Ducerf C, Baulieux J, Zoulim F, Trepo C. Long-term histological improvement and clearance of intrahepatic hepatitis C virus RNA following sustained response to interferon-ribavirin combination therapy in liver transplanted patients with hepatitis C virus recurrence. Gut 2003;52:283-287.

49. Bizollon T, Trepo C. Ribavirin and interferon combination for recurrent post-transplant hepatitis C: which benefit beyond 6 months? J Hepatol 2002;37:274-276.

50. Vogel W, Ferenci P, Fontana R, Arbor A, Saab S, LaBrecque D, Pappas SC, Sidhu RK, Harb GE, Roche Laboratoire. Peginterferon alfa-2a (Pegasys) in liver transplant recipients with established recurrent hepatitis C: interim results of an ongoing randomized multicenter trial. Hepatology 2002;36:312A.

51. Neff G, Montalbano M, Lee Y, Slapak G, Safdar K, Morrero R, Nery J, Weppler D, De Modina M, Schiff E, Tzakis A. Naïve treatment results in liver transplant recipients with hepatitis C virus recurrence using pegylated interferon alpha-2b combined with ribavirin. Preliminary results. Hepatology 2002;36:183A.

52. Neff G, Montalbano M, Lee Y, Slapak G, Safdar K, Morrero R, Nery J, Weppler D, De Modina M, Schiff E, Tzakis A. Preliminary treatment results of liver transplant recipients with recurrent hepatitis C virus nonresponsive to interferon alpha-2b and ribavirin using combination pegylated interferon alpha-2b with ribavirin. Hepatology 2002;36:183A.

53. Samuel D. Treatment of patients with recurrent hepatitis C after liver transplantation with pegylated interferon and ribavirin. Hepatology 2003;34:531A.

54. Sheiner PA, Schluger LK, Emre S, Thung SN, Lau JY, Guy SR, Schwartz ME, Miller CM. Retransplantation for recurrent hepatitis C. Liver Transpl Surg 1997;3:130-136.

55. Rosen HR, Martin P. Hepatitis C infection in patients undergoing liver retransplantation. Transplantation 1998;66:1612-1616.

56. Rosen HR, Madden JP, Martin P. A model to predict survival following liver retransplantation. Hepatology 1999;29:365-370.

57. Dumortier J, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. J Hepatol 2004;40:669-674.

Management of Hepatocellular Carcinoma

Margarita Sala, Jordi Bruix

The incidence of hepatocellular carcinoma (HCC) has increased worldwide and is now the 5th most frequent cancer representing approximately 5% of all cancers worldwide. More than 500,000 new cases are diagnosed per year and it is the third cause of cancer-related death and the first cause of death in patients with cirrhosis [1]. The incidence of HCC has major geographical differences, but most patients diagnosed with HCC have underlying cirrhosis. The highest risk is observed in cirrhosis from chronic infection by the hepatitis B virus (HBV) or hepatitis C virus (HCV) [2]. In patients with HCV infection the risk increases with the confirmation of cirrhosis, when the yearly incidence varies between 3-5% and the 5-year cumulative incidence ranges from 15-20% [2]. Since vaccination against HCV is not available, prevention of HCV infection is based on preventing transmission by blood products. Progression from chronic HCV infection to advanced fibrosis or cirrhosis may be prevented in 40% of patients who are sustained responders to new antiviral strategies, such as pegylated interferon and ribavirin [3]. Thus, the prevention of cirrhosis can prevent the development of HCC. On the other hand, in patients with confirmed cirrhosis, the preventive effect of these agents has not been proven [4].

DIAGNOSIS

When cirrhosis has been confirmed, surveillance is the only available strategy to limit tumor-related mortality. If early stage HCC are detected, treatment and cure are possible [2]. Nevertheless, despite surveillance programs, only 30% of HCC are diagnosed at an early stage [1].

A panel of experts organized by the European Association for the Study of the Liver (EASL) proposed surveillance based on abdominal ultrasound (US) and serum alpha-fetoprotein (AFP) every 6 months. Only patients with cirrhosis that could be treated and potentially cured for HCC should undergo surveillance [2]. This includes Child-Pugh A and B patients. Child-Pugh C patients should be evaluated for liver transplantation. If this is not possible, surveillance is a cost-effective choice for early detection and treatment will not improve survival. The following strategy was recommended to diagnose nodules detected by US during surveillance (Figure 1) [2]. If the nodule is <1cm, close follow-up is recommended since less than 50% of the cases are malignant and a reliable diagnosis is not possible with current diagnostic techniques. In 1-2cm nodules, diagnosis of HCC is based on positive cytology or histology. However, false negative biopsies may occur in 30-40% of cases. Thus, a negative biopsy does not clearly exclude malignancy. If the nodule is >2cm and the underlying liver is cirrhotic, diagnosis of HCC can be determined by non-invasive criteria proposed by the EASL experts: two coincident imaging techniques showing a focal lesion >2cm with characteristic arterial hypervascularization, or one imaging technique with a specific pattern associated with AFP >400ng/mL [2]. After detection and diagnosis of HCC the extent of the tumor must be properly staged based on state of the art computed tomography (CT) or magnetic resonance imaging (MRI) [5]. Angiography is not helpful for diagnosis and staging and lipiodol CT is not reliable [2]. Prognosis of HCC depends on the stage of the neoplasm at diagnosis, liver function impairment and the treatment received.

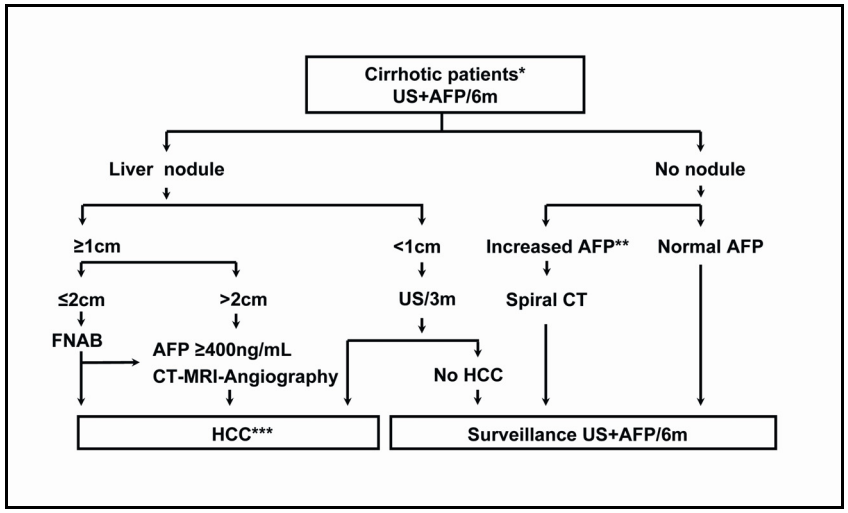

Figure 1: Surveillance and recall strategy for HCC
(reproduced from Bruix et al, J Hepatol 2001 [2], with permission).
FNAB: Fine needle aspiration biopsy.
* Available for curative treatments if diagnosed with HCC.
** AFP levels to be defined.
*** Pathological confirmation or non-invasive criteria.

PROGNOSIS

HCC is generally considered to be a neoplasm with a poor prognosis. However, at present diagnosis occurs earlier; thus certain patients are now successfully treated resulting in an encouraging disease-free survival at 5 years. Nevertheless, prediction of prognosis is still debatable. Several scoring systems exist that divide patients according to expected survival [5]. Almost all of them take into account tumor stage and liver function parameters, but unfortunately predictive accuracy is limited and there is no link between estimated prognosis and treatment indication. Thus, we have developed the Barcelona-Clinic Liver Cancer group (BCLC) staging system which links staging, treatment indication and predicted outcome [5]. With this

system, patients are stratified into four categories (early, intermediate, advanced and end-stage) and the best possible treatment and outcome within each category are established according to specific parameters.

EARLY HCC

The definition of early HCC has varied over time as size limit has steadily decreased; Patients with single tumors ≤5cm or with up to 3 nodules ≤3cm each are usually included. However, pathological and clinical data show that some of these tumors are not early at all, while some are very early HCC or carcinoma in situ (CIS). CIS is a small, very well differentiated HCC with an ill-defined nodular appearance with no invasion of malignant cells in any structure. Cancer invasion and spread (microvascular invasion and satellites) may occur even in tumors <2cm but others are CIS [6]. Both entities may be detected by US, but CIS may be identified if there is no arterial supply as it is a minute, non-arterial enhanced nodule.

The natural history of untreated early HCC is not known because these patients are usually treated. The few available studies report a 65% 3-year survival in Child-Pugh A patients with single tumors [7]. Since survival may exceed 50% at 5 years with proper treatment, effective treatment of early stage HCC is thought to improve patient survival [5]. Effective long-term treatments include surgical resection, liver transplantation and percutaneous ablation.

Surgical Resection

This is the first treatment option in non-cirrhotic patients. However, few cirrhotic patients may receive this treatment [8] because it is limited to those patients with a single HCC ≤5cm and well preserved liver function to prevent morbidity and mortality after resection [5]. Japanese researchers use the indocyanine-green retention rate to identify the best candidates [9], whereas portal pressure and bilirubin are used in Europe. Clinically relevant portal hypertension is defined as the presence of a hepatic vein pressure gradient >10mmHg, esophageal varices and/or splenomegaly with a platelet count $<100x10^9$/L. Patients without portal hypertension and with normal bilirubin have a 70% 5-year survival rate, while those with an adverse profile have 50% or less, even if they are Child-Pugh stage A [5].

The main drawback of surgical resection is tumor recurrence, which may exceed 70% at 5 years [10]. This is the main argument in support of resection instead of transplantation as the first treatment option. Tumor recurrence includes true recurrence secondary to tumor dissemination and *de novo* tumors. Microvascular invasion and the presence of additional nodules or satellites are the best predictors of recurrence due to tumor dissemination [11]. Because of this high recurrence rate these patients are the best patients for evaluation of preventive agents. These include agents that prevent true recurrence such as intraarterial lipiodol-I^{131} or adoptive immunotherapy or those that prevent metachronic tumors such as retinoids or interferons. Nevertheless, despite promising results in randomized studies, all of these substances require further validation before being accepted as standard preventive agents after resection [10].

Liver transplantation

Liver transplantation (LT) is supposed to simultaneously cure the tumor and the underlying cirrhosis if it is limited to carefully selected patients and restrictive criteria. Most groups limit transplantation to patients with single HCC ≤5cm or with up to 3 tumors ≤3cm each. This policy results in a 70% 5-year survival rate with <15% of recurrence during follow-up [5]. Nevertheless, the main concern is the shortage of donors leading to a long waiting time, tumor progression and drop-out from LT. This problem concerns 15% to 50% of enlisted patients depending on the waiting time and has a severe impact on patient survival if outcome is analyzed according to intention to treat [5].

Thus, most programs have established priority policies to decrease the drop-out rate. The United Network of Organ Sharing (UNOS) bases organ allocation on the model for end-stage liver disease (MELD). This model does not give any points to HCC patients who are thus granted a fixed score: patients in stage I (single <2cm) received 24 points and patients in stage II (single 2-5cm or 3 ≤3cm) 29 points. However, this policy unfairly increased the proportion of HCC patients that were transplanted and points were thereafter reduced to 20 and 24 respectively [12].

Living donor liver transplantation (LDLT) using the right hepatic lobe is the most feasible alternative to cadaveric LT and may help overcome the shortage of donors [13]. Analysis of cost-effectiveness

based on the exclusion rate (4% monthly), the morbidity/mortality of donors (0.3-0.5% mortality) and costs has shown that live donation for early HCC in patients enlisted for cadaveric LT is adequate for waiting times of more than 7 months [14].

The availability of LDLT has allowed patients with more advanced HCC to undergo transplantation. The definition of acceptance criteria is a major controversy with critical ethical considerations. In the Barcelona Liver Unit, we have proposed a moderate expansion of criteria to achieve a 50% survival at 5 years: 1) Single HCC ≤7cm; 2) Multinodular HCC with 3 nodules ≤5cm or 5 nodules ≤3cm each; 3) Downstaging to cadaveric criteria by locoregional treatment lasting >6 months [5]. Long-term follow-up will determine whether this strategy is adequate.

Adjuvant therapies (resection, percutaneous ablation, chemoembolization) have also been proposed to reduce tumor progression while waiting for a donor. Since there are no randomized controlled trials (RCTs) in the field, there is no proof of the benefit of these therapies. Cohort studies and cost-effectiveness analysis suggest that there is improved survival if the waiting time exceeds 6 months both for resection and percutaneous treatments [5].

Reinfection of the graft with HCV is a major and unsolved problem in HCV carriers treated by transplantation. It affects almost all patients and leads to cirrhosis in half of them. Antiviral treatments while waiting, during, or after transplantation is only effective in a few patients.

Percutaneous ablation

Percutaneous ablation can be considered for patients with early stage HCC who are not suitable for surgical therapies. HCC foci can be necrosed by the injection of chemical substances (alcohol, acetic acid or hot saline) or by modifying the temperature [radiofrequency (RF), microwave, laser and cryoablation]. Percutaneous Ethanol Injection (PEI) is the gold standard treatment. It is inexpensive, easy to perform and has few adverse events. Complete tumor necrosis (complete response (CR) in oncologic terms) is achieved in 90-100% of HCC <2cm, while the efficacy is reduced as tumor size increases [5]. The best outcome is achieved in Child-Pugh A patients, with a 5-year survival rate of approximately 50% [15]. RF ablation is the most extensively used alternative to PEI. It can be applied percutaneously,

laparoscopically or during laparotomy, and is claimed to result in the same objective responses as PEI, but in significantly fewer sessions [16]. In addition, RF may ablate a 1cm safety margin in surrounding parenchyma and also eliminate satellites. However, there is no evidence that this results in better survival [16]. The side-effects of RF are more severe than those of PEI. For example while tumor seeding is infrequent after PEI, treatment of subcapsular HCC by RF may induce peritoneal dissemination [17] and thus, RF should be avoided in these tumors.

INTERMEDIATE-ADVANCED HCC

Most HCC patients are diagnosed with advanced stage HCC, thus preventing radical treatments. The natural outcome of these patients if left untreated is better known now than two decades ago when patients did not survive any more than 1 year after diagnosis. Nevertheless, modern reported figures of untreated patients in 25 RCTs are extremely heterogeneous with 1- and 2-year survival rates ranging from 10-72% and 8-50%, respectively [18]. This heterogeneity suggests that these patients need to be stratified into separate categories. This was done by our group by joining two control groups of two RCTs in a cohort of 102 patients. Their 1, 2, and 3 year survival was 54%, 40% and 28% and the independent prognostic factors were the presence of cancer-related symptoms (Performance status 1-2) and of an invasive pattern defined as vascular invasion or extrahepatic spread. When patients were divided according to the absence (intermediate stage) or presence (advanced stage) of these prognostic factors the survival at 1, 2 and 3 years was 80%, 65% and 50% vs. 29%, 16% and 8% respectively [19]. This finding is highly relevant when assessing new therapeutic options. Patients are frequently recruited because they cannot receive surgical treatment, but clearly, non-surgical patients represent a very broad spectrum of the disease.

Palliative treatment

These treatments are for patients who cannot undergo radical therapies. Although there is a large list of options that have been tested in patients with HCC, unfortunately the scientific evidence about their use in conventional clinical practice is limited. Since no treatment is

accepted as the standard of care in patients with advanced HCC, the only way to demonstrate an advantage in survival is to perform an RCT comparing active intervention vs. best supportive care. The review of RCTs published in the last 25 years showed 63 trials assessing primary treatments for HCC but only 26 including a control group with conservative treatment [18]. The most extensively evaluated interventions were arterial embolization, with or without chemotherapy, and estrogen blockade. A meta-analytical assessment was possible for both of these techniques, since there are enough trials and patients to obtain robust conclusions. This analysis showed improved survival with transarterial chemoembolization (TACE) in well selected candidates. Accordingly, TACE is now the standard treatment in patients with intermediate stage HCC [18]. In contrast, no improvement in survival was found for tamoxifen [18].

The lack of improved survival with available therapies in patients who are not candidates for TACE, suggests that any new agent proposed for HCC patients should be compared to the best conservative support or placebo. Comparisons with a control arm of a proven inactive treatment such as systemic chemotherapy should not be accepted for scientific and ethical reasons [5].

1. Transarterial embolization

This is the most extensively used treatment for unresectable HCC. Acute obstruction of hepatic artery blood flow nourishing the HCC induces different degrees of tumor necrosis. Gelatin, coils, alcohol, spheres and blood clots have been used to block blood flow and the most common is to inject chemotherapy (doxorubicin, mitomycin and cisplatin are the most usual agents) mixed with lipiodol before arterial obstruction. This treatment induces partial response in 15-55% of patients and delays tumor progression and vascular invasion [18]. Seven RCTs have compared arterial embolization with no treatment [18]. TACE with doxorubicin or cisplatin was assessed in five of them. Only two of them showed significant improvement in survival and in one of them, treatment response was shown to be an independent predictor of survival. Cumulative meta-analysis showed that TACE improved survival compared to no treatment. The data for embolization without chemotherapy were not conclusive due to the few studies and recruited patients. It is important to note that selection of candidates for TACE is critical to avoid side-effects leading to liver failure and death. The optimal candidates have preserved liver

function and asymptomatic multinodular tumors without vascular invasion. Ongoing investigations should define the best chemotherapeutic agents or combinations, as well as the optimal treatment schedule. It is well known, for example, that after extensive necrosis, the tumor is revascularized thus indicating need for new treatment sessions. It has not been clearly established whether treatment should be administered at regular intervals or on a case by case basis and the timing for evaluation of response to treatment and follow-up monitoring requires further studies.

2. Estrogen blockade

Because some HCC present wild or mutant estrogen receptors, antiestrogenic therapy has been tested in patients with advanced disease. Initial studies were encouraging, but large double-blind trials and cumulative meta-analysis of the seven RCTs comparing tamoxifen vs. no treatment failed to show that tamoxifen affected patient outcome [18]. A recent RCT with higher dosage of tamoxifen has also failed to identify any benefit [20], thus confirming that tamoxifen is not active in HCC patients.

Several other treatments such as systemic chemotherapy, internal radiation with lipiodol-I^{131}, proton beam radiotherapy, immunotherapy or octreotide have either been shown to be ineffective or if they have a marginal activity, were only assessed in small sample size studies. Thus, they should not be proposed or should be properly tested to reach enough statistical power to provide solid conclusions [5].

BCLC TREATMENT STRATEGY

Very early stage (Stage 0) or early stage (stage A) HCC patients are candidates for radical treatment. Resection is the first option in patients with single tumors, without clinically relevant portal hypertension and with normal bilirubin. LT is considered in patients with 3 lesions <3cm each or with single tumors <5cm with liver function impairment. If the waiting time is more than 6 months, adjuvant treatments are recommended and LDLT can be considered. Percutaneous ablation is proposed in small non-surgical HCC. Asymptomatic patients with large/multinodular tumors without vascular invasion or extrahepatic spread (Stage B) are candidates for TACE if they have underlying compensated cirrhosis. Patients with advanced tumors (symptomatic and/or invasive pattern) or with

decompensated liver disease (Stage C) can be considered for entry into trials assessing new antitumoral agents. Finally, patients with terminal stage cancer (Stage D) with impaired physical status (Performance status >2) or tumor burden (Okuda stage III) should only receive symptomatic treatment (Figure 2) [5].

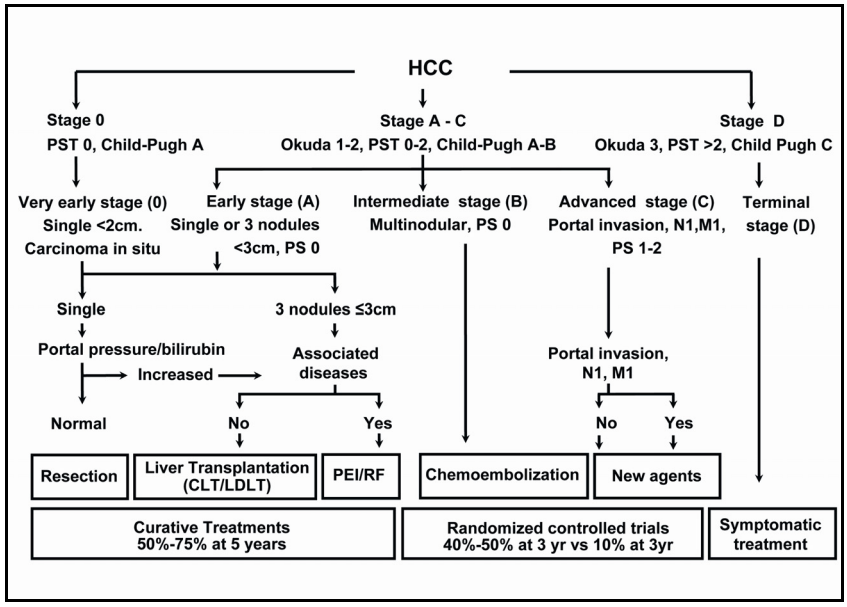

Figure 2: Barcelona-Clinic Liver Cancer (BCLC) staging classification and treatment schedule. Stage 0: Patients with very early HCC are optimal candidates for resection. Stage A: Patients with early HCC are candidates for radical therapies (resection, liver transplantation or percutaneous treatments); Stage B: Patients with intermediate HCC may benefit from chemoembolization; Stage C: Patients with advanced HCC may receive new agents in the setting of RCT; Stage D: Patients with end-stage disease will receive symptomatic treatment. (Llovet JM et al. Lancet 2003 [5]). Reprinted with permission from Elsevier (Lancet 2003, 362, pp 1907-1917).

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REFERENCES

1. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, de Franchis R, Colombo M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. Gastroenterology 2004;126:1005-1014.

2. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma: conclusions of the Barcelona-2000 EASL Conference. J Hepatol 2001;35:421-430.

3. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 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.

4. 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.

5. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907-1917.

6. Nakashima Y, Nakashima O, Tanaka M, Okuda K, Nakashima M, Kojiro M. Portal vein invasion and intrahepatic micrometastasis in small hepatocellular carcinoma by gross type. Hepatol Res 2003;26:142-147.

7. Barbara L, Benzi G, Gaiani S, Fusconi F, Zironi G, Siringo S, Rigamonti A, Barbara C, Grigioni W, Mazziotti A. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology 1992;16:132-137.

8. Bismuth H, Majno PE. Hepatobiliary surgery. J Hepatol 2000;32:208-224.

9. Torzilli G, Makuuchi M, Inoue K, Takayama T, Sakamoto Y, Sugawara Y, Kubota K, Zucchi A. No-mortality liver resection for hepatocellular carcinoma in cirrhotic and noncirrhotic patients: is there a way? A prospective analysis of our approach. Arch Surg 1999;134:984-992.

10. Schwartz JD, Schwartz M, Mandeli J, Sung M. Neoadjuvant and adjuvant therapy for resectable hepatocellular carcinoma: review of the randomised clinical trials. Lancet Oncol 2002;3:593-603.

11. Okada S, Shimada K, Yamamoto J, Takayama T, Kosuge T, Yamasaki S, Sakamoto M, Hirohashi S. Predictive factors for postoperative recurrence of hepatocellular carcinoma. Gastroenterology 1994;106:1618-1624.

12. Freeman RB. Liver allocation for HCC: a moving target. Liver Transpl 2004;10:49-51.

13. Trotter JF, Wachs M, Everson GT, Kam I. Adult-to-adult transplantation of the right hepatic lobe from a living donor. N Engl J Med 2002;346:1074-1082.

14. Sarasin FP, Majno PE, Llovet JM, Bruix J, Mentha G, Hadengue A. Living donor liver transplantation for early hepatocellular carcinoma: A life-expectancy and cost-effectiveness perspective. Hepatology 2001;33:1073-1079.

15. Livraghi T, Giorgio A, Marin G, Salmi A, de Sio I, Bolondi L, Pompili M, Brunello F, Lazzaroni S, Torzilli G. Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology 1995;197:101-108.

16. Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235-240.

17. Llovet JM, Vilana R, Bru C, Bianchi L, Salmeron JM, Boix L, Ganau S, Sala M, Pages M, Ayuso C, Sole M, Rodes J, Bruix J. Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. Hepatology 2001;33:1124-1129.

18. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology 2003;37:429-442.

19. 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;29:62-67.

20. Chow PK, Tai BC, Tan CK, Machin D, Win KM, Johnson PJ, Soo KC. High-dose tamoxifen in the treatment of inoperable hepatocellular carcinoma: A multicenter randomized controlled trial. Hepatology 2002;36:1221-1226.

Case Study in the Management of Patients with Hepatocellular Carcinoma

Eugene R. Schiff

This 50-year-old married man with three children has a history of chronic hepatitis C that progressed to cirrhosis. Risk factors for hepatitis C were intravenous drug use in the 1960's and multiple transfusions given for trauma in 1975. Cirrhosis had been complicated by bleeding esophageal varices in 2001 but treatment with endoscopic banding was effective. Furthermore he developed ascites and hepatic encephalopathy. A computed tomography (CT) scan in November 2001 demonstrated a 6x6.5x7cm mass lesion in segment 7 of the liver. The mass increased in size to 11.2x9.2x5.8cm on a repeat CT scan two months later (Figure 1). Serum alpha-fetoprotein (AFP) was above 16,000ng/mL.

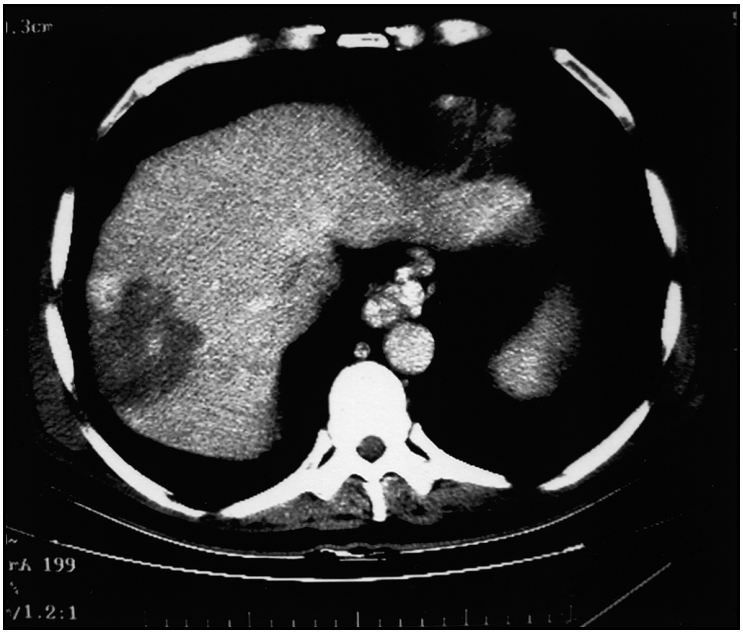

Figure 1: CT scan demonstrating mass in right hepatic lobe.

What therapy would you recommend?

Chemoembolization was undertaken with mitomycin, adriamycin and carboplatinum in February of 2002. By late July 2002 the CT scan showed an area of decreased enhancement 3-4cm in diameter in segment 7 associated with a dramatic drop in serial AFP values.

Would you recommend liver transplantation at this point?

An orthotopic liver transplant was performed in August of 2002. The explant showed advanced cirrhosis without any histologic evidence of residual hepatocellular carcinoma (Figure 2, Figure 3).

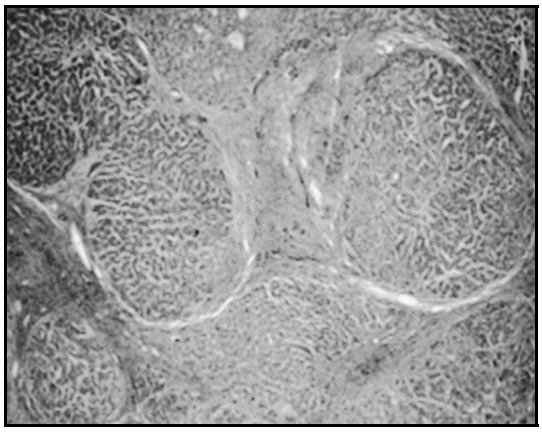

Figure 2: Liver biopsy showing presence of cirrhosis.

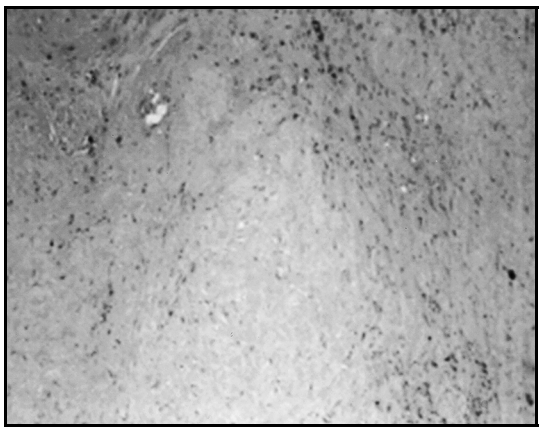

Figure 3: Liver tissue from site chemoembolization.

Would you treat the patient with chemotherapy post-liver transplant?

The decision was made not to treat the patient with chemotherapy. Five months post transplant he was maintained on tacrolimus 7mg b.i.d. and medrol 16mg q.d. after several episodes of acute rejection. He was generally feeling fine but had developed diabetes and was started on insulin. Laboratory values included total bilirubin 1.2mg/dL, alanine aminotransferase (ALT) 32U/L, aspartate aminotransferase (AST) 42U/L, alkaline phosphate 102U/L. hepatitis C virus (HCV) RNA >500,000IU/mL. Liver biopsy was consistent with chronic hepatitis C (Grade 3) and mild rejection.

Would you treat this patient with pegylated interferon and ribavirin?

Treatment was not started until 6 months later when he became increasingly icteric and developed diarrhea. A repeat liver biopsy suggested recurrent hepatitis C and mild rejection. At that time the total bilirubin was 21.5mg/dl, AST 417U/L, ALT 218UL, alkaline phosphate 267U/L, albumin 2.7g/dL, creatinine 0.8mg/dL, AFP 14.3. He was treated with pegylated interferon-alpha-2a, 90µg weekly and ribavirin 200mg b.i.d. He continued to deteriorate and a repeat liver biopsy one month later was consistent with fibrosing cholestatic hepatitis and without significant rejection. He succumbed 1.5 years post liver transplantation. There was never any evidence of recurrent hepatocellular carcinoma.

CASE DISCUSSION

This patient presented with a hepatocellular carcinoma more than 5cm in diameter that grew to 11cm in diameter. Liver transplantation was contraindicated at that stage. However he underwent chemoembolization, which literally eradicated the hepatocellular carcinoma which was absent in the explant. Hepatic artery chemoembolization for hepatocellular carcinoma in patients listed for transplantation is beneficial but must be considered in relation to the risk of chemoembolization induced deterioration. The latter was a transient problem in this patient prior to transplantation. The impact of the Model for End Stage Liver Disease (MELD) for patients with hepatocellular carcinoma has significantly improved the probability of

a timely orthotopic liver transplant. Recurrence of hepatocellular carcinoma after a liver transplant develops in approximately 20% of patients with a median of one year. Recurrence of hepatocellular carcinoma significantly shortens post transplant survival but as many as 20% of these patients survive for at least 5 years compared to 65% in patients without recurrent hepatocellular carcinoma. Recurrence of hepatitis C is inevitable in almost 100% of patients with endstage liver disease secondary to chronic hepatitis C who undergo liver transplantation. Unfortunately current antiviral therapy for hepatitis C is contraindicated in patients with decompensated cirrhosis. Furthermore there is no antiviral regimen comparable to that used in decompensated cirrhosis secondary to hepatitis B prior to liver transplantation, e.g. nucleoside analogs and hepatitis B immunoglobulin. The development of protease and polymerase inhibitors for HCV is in a relatively early stage and thus far no high titer anti-HCV preparation has been shown to successfully prevent hepatitis C. Although the use of pegylated interferon and ribavirin post liver transplant is being assessed, efficacy is limited and the side-effect profile is high in this immunosuppressed group. As many as 40% of post transplant patients with recurrent hepatitis C develop cirrhosis within 5 years after transplantation. Retransplantation of patients with fibrosing cholestatic hepatitis as in the present case has been shown to be ineffective as has antiviral therapy. Retransplantation of recurrent hepatitis C patients who have progressed to cirrhosis has been associated with relatively poor survival rates but these patients would do better if they were retransplanted with MELD scores less than 16 and with livers from donors who are under 60 years old.

REFERENCES

1. Berenguer M. Natural history of recurrent hepatitis C. Liver Transpl 2002;8:S14-18.

2. Dumortier J, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. J Hepatol 2004;40:669-674.

3. Gores GJ. Hepatocellular carcinoma: gardening strategies and bridges to transplantation. Liver Transpl 2003;9:199-200.

4. Hayashi PH, Ludkowski M, Forman LM, Osgood M, Johnson S, Kugelmas M, Trotter JF, Bak T, Wachs M, Kam I, Durham J, Everson GT. Hepatic artery chemoembolization for hepatocellular carcinoma in patients listed for liver transplantation. Am J Transplant 2004;4:782-787.

5. 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:S115-120.

6. Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GI, Kreiger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. Liver Transpl 2004;10:534-540.

7. Ross AS, Bhan AK, Pascual M, Thiim M, Benedict Cosimi A, Chung RT. Pegylated interferon alpha-2b plus ribavirin in the treatment of post-liver transplant recurrent hepatitis C. Clin Transplant 2004;18:166-173.

8. Van Thiel DH, Yong S, Li SD, Kennedy M, Brems J. The development of de novo hepatocellular carcinoma in patients on a liver transplant list: Frequency, size and assessment of current screening methods. Liver Transpl 2004;10:631-637.

9. Yao FY, Bass NM, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: Lessons from the first year under the Model of End-Stage Liver Disease (MELD) organ allocation policy. Liver Transpl 2004;10:621-630.

10. Yao FY, Saab S, Bass NM, Hirose R, Ly D, Terrault N, Lazar AA, Bacchetti P, Ascher NL, Roberts JP. Prediction of survival after liver retransplantation for late graft failure based on preoperative prognostic scores. Hepatology 2004;39:230-238.

II. Hepatitis B

Clinical Virology of Hepatitis B

Fabien Zoulim

NATURAL HISTORY OF HEPATITIS B VIRUS INFECTION [1, 2, 3]

The natural history of hepatitis B infection varies according to the age that the infection is contracted. In adults with normal immunological status less than five percent of individuals with acute hepatitis B infection develop chronic infection, characterized by persistent circulating viral antigens and viral DNA and most acute infections resolve spontaneously because the immune system eliminates infected cells while developing antibodies to viral surface antigens. However, acute infection is associated with clinical manifestations in approximately one third of patients. On the other hand, the rate of progression to chronic hepatitis is much higher when the infection is acquired in childhood, even though the initial infection is usually clinically asymptomatic. Moreover, progression to chronic hepatitis is more frequent in subjects with immunodepression.

The clinical manifestations of the chronic phase of the disease depend on the immune attack on infected cells, resulting in elevated serum transaminase levels and in some cases clinical symptoms. The natural course of infection is characterized by distinctive phases that differ in the replicative activity of the virus and the intensity of the immune response (Table 1). Generally, in the early stages of infection, the infected cells do not stimulate an immune response and continue to shed viral particles. This immunotolerant phase is characterized by

high serum levels of viral antigens and DNA and normal transaminase levels. Then the immune system mounts an attack on infected hepatocytes. During this immunoactive stage, serum levels of viral DNA fall, transaminase levels rise, and clinical symptoms may appear. If this stage persists because the immune system fails to control viral infection, liver damage will lead to chronic hepatitis. However chronic infection may remain clinically silent for significant periods. In many patients, hepatitis Be antigen (HBeAg) seroconversion occurs and HBe antigens disappear while anti-HBe antibodies appear, although low levels of DNA may persist. Biological markers of hepatic function normalize. This is predictive of a good clinical outcome, and indicates good immunological control of liver infection (Figure 1). In this phase, patients are inactive carriers of hepatitis B virus. As viral supercoiled DNA persists in the liver, episodes of viral reactivation may occur either spontaneously or because of immune suppression. It should be noted that certain individuals carry hepatitis B strains that bear mutations and prevent HBe antigen expression. These individuals may thus present with active chronic hepatitis without serum HBeAg. The prevalence of chronic HBeAg-negative hepatitis varies from region to region, and is more prevalent in the Mediterranean basin, for example, than elsewhere in Europe. There is also some evidence that this form of hepatitis is associated with more severe liver disease than HBeAg-positive hepatitis. In patients with chronic infection, the incidence of cirrhosis and of hepatocellular carcinoma is less than ten cases per hundred patient years. The five-year mortality rate for uncomplicated chronic hepatitis is 0-2%, for chronic hepatitis with compensated cirrhosis is 14-20% and for chronic hepatitis with decompensation is 70-86%.

Infection phase	Serum markers	Clinical risk
Immunotolerant phase	HBV DNA high HBsAg high HBeAg high Transaminases normal	High risk of transmission to the household
Immunoactive phase Chronic hepatitis	HBV DNA decrease HBsAg high HBeAg decrease Transaminases high	Development of cirrhosis and HCC
Low-replicative phase Inactive carrier state	HBV DNA very low HBsAg decrease HBeAg absent, anti-Hbe-positive Transaminases normal	Development of HCC Viral reactivation: wild type or pre-core mutant
HBsAg clearance phase	HBsAg-negative Anti-HBc-positive Anti-HBs-positive or -negative Normal ALT HBV DNA may be positive by PCR	Occult HBV infection Viral reactivation (immune suppression) Transmission of occult HBV (nosocomial infection)

Table 1: Evolution of serum markers in the different phases of chronic hepatitis B virus infection.

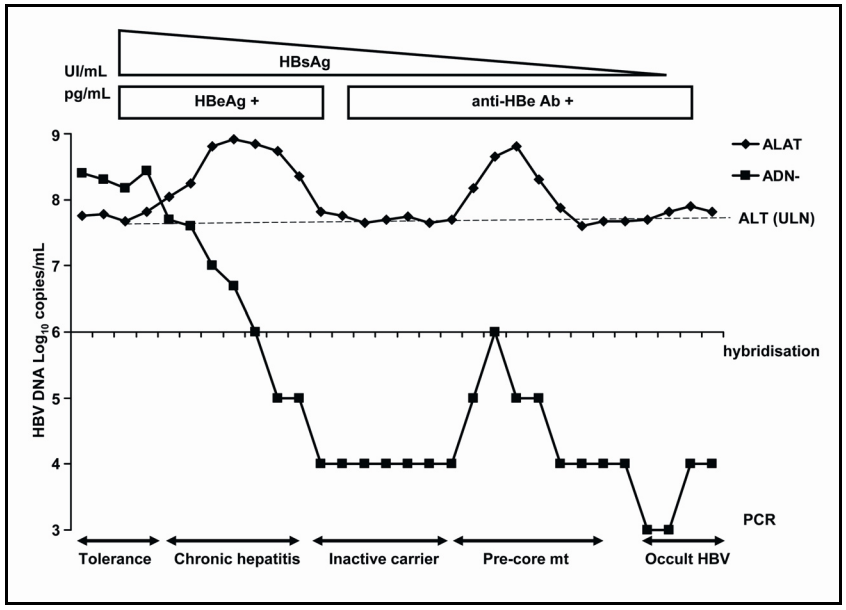

Figure 1: Evolution of virological markers during the natural history of chronic hepatitis B.

MEASURING VIRAL LOAD

Since hepatitis B virus infection can remain clinically silent, it is important to have accurate methods to determine the presence of viral replication to monitor treatment outcomes and to identify changes in viral activity before they provoke clinical symptoms. The most rigorous way of determining viral replication is to measure circulating viral DNA. The threshold for risk of liver damage is around 10^4 virions/mL.

A surrogate measure of viral load is the presence of circulating hepatitis B virus e antigen; this method has been extensively used because it is inexpensive and relative simple compared to DNA measurement. Loss of this antigen and the appearance of anti-HBe

antibody (HBeAg seroconversion) is associated with a decrease in viral DNA titers to below 10^4 copies/mL and clinical remission. However, certain variants of the hepatitis B virus carry mutations in the pre-core region of the HBV genome encoding the HBeAg that prevent determination of serological status.

MONITORING TREATMENT EFFICACY AND RESISTANCE

Patients with hepatitis B treated with antiviral drugs must be closely monitored to assess virological efficacy and to detect any treatment resistance as early as possible. This requires the measurement of viral load, genotyping of viral DNA and assessment of hepatic function (Figure 2).

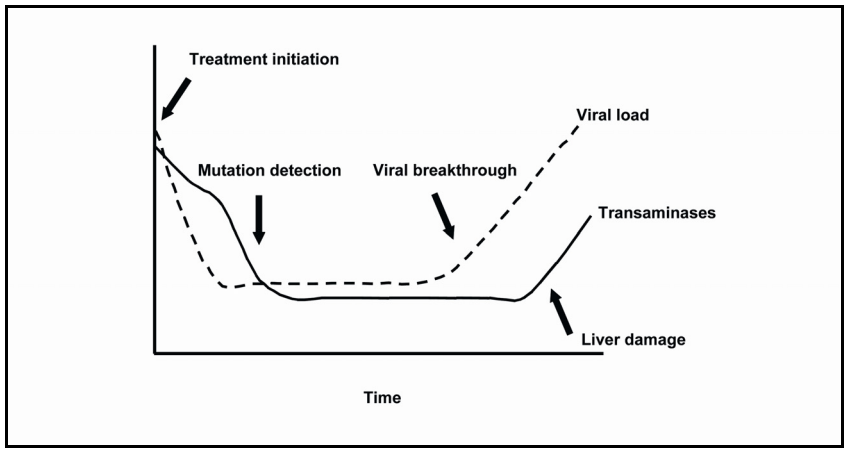

Figure 2: Time-course of emergence of antiviral resistance as measured by evolution of viral load (- -) and serum transaminases (—).

SEROLOGICAL ASSAYS

Regular monitoring of viral markers is necessary to evaluate the virological response to therapy and treatment end-points. In wild type

virus infected patients, HBeAg seroconversion is one of the major aims of antiviral therapy. Usually, it follows a rapid decline in serum viral load that may lead to a restored hepatitis B virus (HBV) specific T cell immune response [4, 5]. In patients infected with a pre-core mutant, hepatitis B surface antigen (HBsAg) clearance is a major endpoint.

VIRAL LOAD

Measuring viral load in serum is important to characterize the early virological response and to detect viral breakthrough. Viral breakthrough is generally defined as an increase in viral load of one log unit compared to the nadir value (lowest viral load obtained during therapy). Breakthrough indicates that the resistant strain has become dominant and that the proportion of hepatocytes infected with this strain and actively shedding the virus has become significant. This is a sign of treatment failure and indicates that the patient is again at risk of developing clinically symptomatic hepatitis (Figure 2).

The most recent assays to quantify intrahepatic viral covalently closed circle DNA (cccDNA) show that the kinetics of clearance during adefovir dipivoxil therapy are much slower than that of serum viral load and total intrahepatic viral DNA. These studies suggest that viral cccDNA decline is mainly a result of the inhibition of intracellular recycling and not of a non-cytolytic process. Mathematical modeling suggests that 14 years of therapy would be required to clear viral cccDNA from the liver [6].

GENOTYPING

Genotyping is the only way to confirm that clinical resistance is due to the emergence of a drug-resistant variant strain of hepatitis B. Genotyping has several functions. First, the use of very sensitive assays is the best way to detect resistant strains before viral breakthrough has taken place. This is because resistant strains mainly infect uninfected cells to become the dominant strain while hepatocytes containing the previously dominant drug-sensitive strain are eliminated from the liver. Thus there is a lag between the appearance of a drug-resistant variant that actively sheds virions, the colonization of the liver by this variant and viral breakthrough. The length of time of this lag period depends on the rate that the immune

system removes the wild type virus infected hepatocytes so that they are replaced with healthy cells. Thus, resistant strains may be detected in plasma before any noticeable change occurs in viral load. The interval between detection of drug-resistant strains in patients treated with lamivudine has been shown to precede viral breakthrough by 4 to 6 months [7]. This is important because suitable treatment may be started to control replication of the resistant strain and to prevent worsening of liver disease.

The second reason to use genotyping to identify resistant strains rather than waiting for viral breakthrough is to determine the genetic variant, and to choose the most appropriate alternative treatment in relation to the sensitivity of the variant to antiviral drugs. This will become increasingly relevant as new antiviral drugs with different resistance profiles become available. Furthermore, genotyping confirms the diagnosis of drug resistance by identifiying specific viral mutants thus ruling out any questions about patient compliance.

Different methods are available for genotyping viral strains (Table 2). Direct sequencing identifies all possible mutations including previously unknown mutations. This is particularly pertinent in patients receiving new nucleoside analogs that do not yet have any resistance profiles. However, genotyping can only be used if the proportion of the strain in the total viral population is significant, around 20%. In addition, if multiple mutations are identified direct sequencing cannot determine if they originate from one or multiple strains. This problem can be solved by cloning individual viral strains before sequencing, although this technique is too labor intensive to be used in routine screening. Hybridization with oligonucleotide probes or restriction enzyme polymorphism can be easily automated, but these techniques only identify previously characterized mutations. Hybridization with specific oligonucleotide probes is more sensitive than direct sequencing of Polymerase Chain Reaction (PCR) products, since a minor mutant can be detected even if it is as little as 5% of the total viral population [8]. Although it is only under development for the moment, DNA chip technologies would allow large scale screening of multiple mutations in viral sequences (Table 3).

Direct sequencing using RT-PCR
Sequencing of cloned viral DNA
Hybridization using specific oligonucleotide probes (Lipa)
Size polymorphism of fragments after restriction enzyme digestion
DNA chip technology

Table 2: Available methods for genotyping of hepatitis B viral strains.

	Lamivudine	**Emtricitabine**	**Adefovir dipivoxil**	**Entecavir**	**Telbivudine**
Resistance mutations	M204V M204I	M204V M204I	N236T A181V/T	S202G S202I T184G M250V	M204I
Compensatory mutations	V173L L180M				
Drugs active on resistant mutants	Adefovir Tenofovir Entecavir +/–	Adefovir Tenofovir Entecavir +/–	Lamivudine Emtricitabine Entecavir		Adefovir Tenofovir

Table 3: Main polymerase mutants responsible for drug resistance.

PHENOTYPING ASSAYS

The phenotype of HBV clinical isolates can now be analyzed. Rapid cloning of the entire HBV genome or the polymerase gene of the virus in appropriate vectors allows transfection of single or multiple HBV

clones in hepatoma cell lines to study their replication capacity and sensitivity to drugs [9, 10]. These assays can be used to show that the M204I and L180M+M204V polymerase mutants confer resistance to lamivudine and have a lower replication capacity than the wild type virus and to characterize the newly identified N236T polymerase mutant that causes adefovir resistance [11]. These assays may become critically important as more drugs become available and more resistant mutants are selected by therapy. They also show that combined therapy with compounds that do not have cross-resistance should prevent or significantly delay the selection of drug resistant viruses.

BIOCHEMICAL ASSAYS

Serum transaminases should be monitored as a marker of hepatic function. Elevated transaminases suggest that the immune system is no longer controlling the infection and that extensive lysis of hepatocytes is occurring. This is a signal that a new round of clinical symptoms may occur, since severe acute exacerbation of the disease can be observed in some patients in association with viral resistance. However, alanine aminotransferase (ALT) flares are usually preceded by an increase in viral DNA titers, suggesting that close monitoring of viral markers is required for optimal management of patients receiving antiviral therapy.

MONITORING PROTOCOLS

The choice of a monitoring protocol should take into account the clinical status and history of the patient (for example, HBeAg seroconversion, other risk factors for hepatic disease, immunological status) [12, 13]. The cost of monitoring is significant and will influence the public health policy on the type and frequency of tests when allocating resources. Treatment cost will also be considered in the future, but it should be weighed against the rate of drug resistance for each drug as well as the cost of management of drug resistant patients.

An optimal monitoring regime would be to follow the emergence of mutations using genotyping while measuring viral load, serological status and transaminases. This would provide information about the emergence of potentially resistant strains before intrahepatic spread of the mutant and viral breakthrough, and allow a suitable treatment

strategy to be begun. However, DNA sequencing is resource-intensive and may not be considered cost-effective for routine monitoring, although this may change if DNA chip technology could be used for automated screening. To guarantee consistency, DNA sequencing may also need to be performed by reference centers. The risk of emergence of resistant strains with currently available antiviral drugs may also affect the decision on the need for regular DNA sequencing. Although genomic assays are obviously of interest, there is no consensus on the optimal frequency of these tests.

The frequency of monitoring depends on the severity of hepatitis and the treatment duration. If treatment has been stopped, monitoring of biochemical and virological markers should be continued because of the risk of viral reactivation.

REFERENCES

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-1745.
2. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001;34:1225-1241.
3. EASL Consensus Conference Statement J Hepatol 2003;39:S3-S25.
4. Boni C, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavallo C, Cavalli A, Urbani S, Boehme R, Panebianco R, Fiaccadori F, Ferrari and C. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology 2001;33:963-971.
5. Werle B, Cinquin K, Marcellin P, Pol S, Maynard M, Trepo C, Zoulim F. Evolution of hepatitis B viral load and viral genome sequence during adefovir dipivoxil therapy. J Viral Hepat 2004;11:74-83.
6. Werle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C Goodman Z, Xiong S, Delaney W, Brosgart C , Wulfsohn M, Gibbs C, Zoulim F. Persistence of intrahepatic viral ccc DNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004 (in press).
7. Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, Johnson M, Merle P, Abidi H, Trepo C, Zoulim F. Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. Hepatology 2000, 32:1078-1088.
8. 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 2003;125:1714-1722.
9. Durantel D, Durantel S, Pichoud C, Werle B, Brunelle MN, Trépo C, Zoulim F. A New strategy for assessing drug susceptibility and studying replication capacity of clinical isolates of human hepatitis B virus. Hepatology 2004;(in press).
10. Yang H, Westland C, Xiong S, Delaney WE 4th. In vitro antiviral susceptibility of full-length clinical hepatitis B virus isolates cloned with a novel expression vector. Antiviral Res 2004;61:27-36.
11. Villeneuve JP, Durantel D, Durantel S, Westland C, Xiong S, Brosgart CL, Gibbs CS, Parvaz P, Werle B, Trepo C, Zoulim F. Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. J Hepatol 2003;39:1085-1089.
12. Lok AS, Zoulim F, Locarnini S, Mangia A, Niro G, Decraemer H, Maertens G, Hulstaert F, De Vreese K, Sablon E. Monitoring drug resistance in chronic hepatitis B virus (HBV)-infected patients during lamivudine therapy:

evaluation of performance of INNO-LiPA HBV DR assay. J Clin Microbiol 2002;40:3729-3734.

13. Zoulim F. Hepatitis B virus resistance to antivirals: clinical implications and management. J Hepatol 2003;39:S133-S138.

Natural History of Hepatitis B and Prognostic Factors of Disease Progression

Giovanna Fattovich, Irene Zagni, Chiara Scattolini

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global health problem responsible for between 500,000 and 1.2 million deaths annually from cirrhosis and hepatocellular carcinoma (HCC) [1]. Although the HBV vaccination has significantly reduced the number of new infections, a large reservoir of HBV infected individuals remains and it has been estimated that worldwide 360 million people are chronic carriers of the virus [1]. The clinical course and outcome of HBV infection is greatly influenced by age at infection, the level of HBV replication and host immune status. Thus hepatitis B is a heterogeneous disease that may either resolve spontaneously or progress to various forms of chronic infection, including the inactive hepatitis B surface antigen (HBsAg) carrier state, chronic hepatitis, cirrhosis and HCC [1-3]. Knowledge of the natural history of hepatitis B and the risk factors of disease progression is important for developing strategies for management and therapy.

PHASES OF HBV INFECTION

The individual course of HBV infection is determined by the interaction between virus replication and the host immune response

and can be divided into four phases: immune tolerance, immune clearance, low or non-replicative, and reactivation.

In the initial immunotolerant phase, patients are hepatitis Be antigen (HBeAg)-positive. They have high serum levels of HBV DNA, but normal or minimally elevated serum alanine aminotransferase (ALT) levels and normal liver or minimal histological activity, thus indicating that the host immune response against the infected hepatocytes is lacking or minimal. After a variable period of HBeAg positivity, depending on the age at acquisition of HBV infection, immune tolerance to the virus is lost and patients may enter the immunoactive phase, characterized by fluctuating, but progressively decreasing HBV DNA levels and increased ALT and histologic activity, reflecting immune-mediated histologic damage. The third low or non-replicative phase involves seroconversion from HBeAg to its antibody (anti-HBe). This is usually preceded by a marked decrease in serum HBV DNA that is not detectable by hybridization techniques, although low levels of HBV DNA can be detected with sensitive polymerase chain reaction (PCR) assays. During this phase, referred to as the inactive HBsAg carrier state, liver disease is inactive with normal ALT and there is a subsidence of hepatic necroinflammation [1-3]. The inactive carrier state can last for decades or even for life, but in a number of patients, reactivation of HBV replication with high levels of HBV DNA and a recrudescence of liver disease occurs either spontaneously or after discontinuation of immunosuppressive therapy [4]. Reactivation of viral replication may occur due to reactivation with the wild type virus (with HBeAg seroreversion) or when replication-competent HBV variants cannot produce HBeAg because of mutations in the pre-core or core promoter regions of the core gene [5].

The low or non-replicative phase may lead to resolution of hepatitis with HBsAg loss and development of neutralizing HBs antibodies (anti-HBs) [1-3]. After HBsAg seroclearance, HBV DNA may still be detectable by PCR in serum and liver biopsy specimens, suggesting that viral eradication is seldom achieved [6]. Immunosuppression in these patients, such as during cancer chemotherapy or after organ transplantation, can lead to reactivation of hepatitis B.

CLINICAL COURSE OF CHRONIC HEPATITIS B

Persistence of HBsAg, HBeAg and high levels of HBV DNA in serum for more than 6 months after the primary infection is defined as chronic HBV infection. The proportion of patients who develop chronic HBV infection varies with age at infection; ranging from more than 90% of infants born to highly infectious HBeAg-positive mothers to less than 1% in adults, with an intermediate frequency of approximately 30% in children infected after the neonatal period but before the age of 5 [7] (Figure 1).

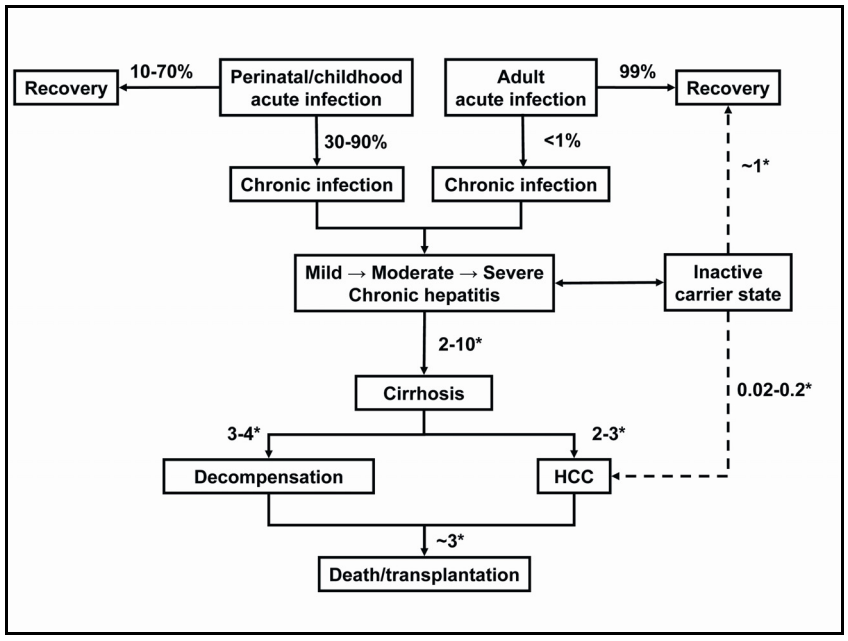

Figure 1: A diagrammatic representation of the clinical spectrum and potential outcomes of hepatitis B virus infection.
Hepatocellular carcinoma (HCC)
* incidence per 100 person years.

HBEAG-POSITIVE CHRONIC HEPATITIS B

The natural history of chronic hepatitis B is influenced by the mode of transmission and varies with regional endemicity. Perinatal transmission or infection during early childhood predominates in highly endemic areas such as Africa and Asia, where most HBsAg-positive mothers have circulating HBeAg. In low prevalence regions, such as Western Europe and North America, transmission is primarily horizontal in adolescents and adults through sexual contact and intravenous drug use.

Most Asian children with perinatally acquired chronic HBV infection, are in the immunotolerant phase when they present, with HBeAg positivity, very high serum levels of HBV DNA and mild or no liver disease (HBeAg-positive chronic hepatitis with normal ALT). The immunotolerant phase lasts 10 to 30 years and there is a very low rate of spontaneous HBeAg clearance [7]. In contrast, individuals who acquire HBV infection in late childhood, during adolescence or adulthood and become chronic carriers, usually present in the immunoactive phase with liver disease activity (HBeAg-positive chronic hepatitis).

Adult patients with HBeAg-positive chronic hepatitis usually present in the third or fourth decade of life and are more frequently males [8]. The spectrum of liver damage ranges from mild (approximately 20 to 40%) to moderate or severe chronic hepatitis (approximately 40 to 60%) or active cirrhosis (approximately 10 to 25%) [8]. Children usually show milder chronic hepatitis than adults.

Seroconversion from HBeAg to anti-HBe is a very important event in the natural history of chronic HBV infection as it is usually followed by a reduction of HBV DNA replication, biochemical and histological remission of hepatitis and a good prognosis [8-11]. The average rate of spontaneous HBeAg seroconversion has been reported to be between 8% and 15% of patients per year [8]. Factors that can affect the probability of HBeAg seroconversion include gender, age and the degree of liver disease activity. Older carriers and female carriers are more likely to clear HBeAg [12-14].

Patients with ALT levels more than 5 times the upper limit of normal (ULN) show a spontaneous HBeAg seroconversion rate of over 50% at the end of 1 year follow-up compared to less than 10% in patients with ALT levels less than 5 times the ULN [15]. Often the disappearance of HBeAg is preceded or accompanied by a transient

rise in ALT levels, known as a flare, which is the expression of a vigorous HBV-specific immune response. Although a sudden increase in serum ALT may be a favorable prognostic sign in chronic hepatitis B, some patients experience repeated episodes of flares without HBeAg loss. The clinical spectrum associated with these acute exacerbations varies greatly; patients may remain asymptomatic or develop symptoms of acute hepatitis that in some cases may progress to hepatic decompensation [13].

Recently the role of the HBV genotype on the rate of HBeAg seroconversion has been examined, but it is still uncertain. Studies from Asia have suggested that patients with genotype B tend to have a higher cumulative rate of HBeAg seroconversion than genotype C infected patients, but more recent data indicate that this difference disappears during long-term follow-up [16, 17]. There is only one longitudinal study in Western patients indicating that HBeAg seroconversion rate did not differ with different HBV genotypes [18].

HBeAg seroconversion associated with remission of liver disease reflects the transition from chronic hepatitis B to the inactive HBsAg carrier state, but longitudinal studies have shown that a proportion of HBeAg-negative patients retain or redevelop high levels of HBV DNA and persistent or intermittent elevation in ALT levels associated with liver necroinflammation and progressive fibrosis [5, 9-11]. These patients have HBeAg-negative chronic hepatitis.

HBEAG-NEGATIVE CHRONIC HEPATITIS B

The diagnosis of HBeAg-negative chronic hepatitis can be made in patients who (1) are HBsAg-positive for at least 6 months to establish chronic HBV infection, (2) HBeAg-negative and anti-HBe-positive, (3) have detectable serum HBV DNA levels by molecular hybridization techniques or by quantitative PCR assays (suggested threshold of 10^5 copies per mL), (4) elevated ALT, (5) liver necroinflammation at histology, and (6) no other concomitant or superimposed causes of liver disease [1-3, 5].

Most patients with HBeAg-negative chronic hepatitis harbor a variant virus that cannot produce HBeAg. The most common mutation preventing HBeAg production is a guanine (G) to adenine (A) change at nucleotide 1896 (G1896A) producing a stop codon (at pre-core codon 28) that prematurely terminates synthesis of HBeAg 5. Other pre-core changes as well as mutations in the basic core promoter

region which down-regulate HBeAg synthesis at the transcriptional level have been described [5]. The most common pre-core mutation (G1896A) is predominantly found in association with HBV genotype B, C and D with a thymidine (T) at pre-core position 1858. In contrast, in HBV genotype A the nucleotide 1858 is a cytosine (C) and, given the impaired base pairing between C and A, a G1896A mutation is not selected since it would diminish the replicative efficiency of HBV. Thus, HBeAg-negative chronic hepatitis is more common in Southern Europe, where genotype D predominates, and in Asia, where both genotype B and C are common. However available data suggest that HBeAg-negative chronic hepatitis is present worldwide with marked variations in the prevalence of pre-core and core promoter variants among HBeAg-negative patients in different part of the word [19].

Patients with HBeAg-negative chronic hepatitis are usually male and older than patients with HBeAg-positive chronic hepatitis (median 40, range 36-45 years) [5, 8, 20, 21]. Although the wide spectrum of histologic damage seen in HBeAg-positive chronic hepatitis may also be found in HBeAg-negative chronic hepatitis, HBeAg-negative patients are more likely to have severe necroinflammation (>50% of cases) or cirrhosis (approximately one third of cases) at the time of first clinical presentation [8, 20, 21].

HBeAg-negative chronic hepatitis has various profiles characterized by major fluctuations of both viremia and ALT (HBV DNA can fall below 10^5 copies per mL and ALT may normalize temporarily) in over 50% of patients [20]. Periods of completely normal ALT may be long lasting, but usually the disease recurs. Sustained spontaneous remissions of disease activity are rare and delayed spontaneous HBsAg loss occurs at a low rate of 0.5% [5, 11, 21].

INACTIVE HBSAG CARRIER STATE

The diagnosis of the inactive HBsAg carrier state is based on the following criteria: (1) HBeAg negativity and anti-HBe positivity, (2) undetectable or low levels of HBV DNA (suggested levels less than 10^5 copies per mL), (3) repeatedly normal ALT levels, (4) minimal or no necroinflammation, slight fibrosis or even normal liver on histology [1-3]. In patients who have already developed cirrhosis during the high replicative phase of infection, the picture in the inactive carrier state will be that of inactive cirrhosis.

Long-term follow-up studies (up to 29 years) of these carriers have shown that liver disease remains inactive in most patients, thus indicating a benign prognosis; patients rarely progress to cirrhosis or HCC [11, 22-24].

During follow-up an estimated 20 to 30% of all inactive HBsAg carriers experience spontaneous reactivation of hepatitis B with reappearance of ALT elevation and high serum levels of HBV DNA, with or without HBeAg seroreversion [4, 11]. Data indicate that HBeAg seroreversion occurs in approximately 4% of patients during 1 to 18 years of follow-up and it is often severe with a high risk of developing cirrhosis [11]. HBV reactivation is usually asymptomatic, although in some patients it may present as acute hepatitis, with or without jaundice. Intermittent or persistent reactivation of hepatitis B may be a major cause of progressive liver damage and in cirrhotic patients may lead to decompensation [4].

Spontaneous HBsAg clearance occurs during chronic HBV infection at an estimated annual incidence of 1 to 2% in Western carriers [14, 25], and even less (0.05 to 0.8%) in areas that are endemic for HBV where infection is usually acquired perinatally or in early childhood [12, 26]. The prognosis following HBsAg clearance is excellent, except in patients with cirrhosis or concomittant HCV or HDV infection before HBsAg clearance. In fact hepatic decompensation, HCC as well as liver-related mortality may still occur in patients who have already developed cirrhosis when spontaneous HBsAg loss occurs, with or without concurrent infections [25, 27].

RATES OF PROGRESSION TO CIRRHOSIS

In untreated carriers with predominantly HBeAg-positive chronic hepatitis referred to clinical centers, the reported incidence of cirrhosis ranges from 2 to 5.4 per 100 person years with a cumulative incidence of 8 to 20% over a 5 year period [8, 28, 29] (Figure 1). It has been suggested that the rate of cirrhosis is higher in patients with HBeAg-negative chronic hepatitis than in patients with HBeAg-positive chronic hepatitis, the incidence being 8 to 10 per 100 person years [11, 30, 31]. In a prospective study of patients with HBeAg-negative chronic hepatitis followed from early occurrence of the disease to after HBeAg seroconversion, the incidence of progression to biopsy proven cirrhosis was as high as 9.6 per 100 person years [11].

In adult patients with chronic hepatitis B, the average age at the time of diagnosis of cirrhosis was 41 to 52 years old (median 46) [8]. In general, progression to cirrhosis occurs insidiously and without symptoms, as indicated by a European study where only a minority (24%) of patients were symptomatic at diagnosis of compensated cirrhosis type B [32].

LIVER-RELATED COMPLICATIONS AND MORTALITY

Long-term liver-related complications of chronic HBV infection include the development of HCC, hepatic decompensation and death (Figure 1).

Hepatocellular carcinoma

The risk of HCC varies according to geographic factors, duration of HBV infection and the severity of liver disease. Indeed, there is a greater risk of HCC with chronic HBV infection from perinatal transmission in highly endemic areas than for infections acquired as an adult. In the presence of cirrhosis, the risk of acquiring HCC is correspondingly higher than inpatients without cirrhosis. In studies in countries where HBV is highly endemic (Taiwan, Singapore), the summary HCC incidence rate was 0.2 per 100 person years in inactive carriers, 1.0 in chronic hepatitis B without pre-existing cirrhosis at diagnosis and 3.2 in patients with compensated cirrhosis, with a 5-year HCC cumulative incidence of 15% in cirrhotics [33]. In Western countries where infection is slightly or moderately endemic the summary HCC incidence was 0.02 per 100 person years in inactive carriers, 0.1 in chronic hepatitis B without cirrhosis at diagnosis and 2.2 in compensated cirrhosis, with a 5-year HCC cumulative incidence of 10% in cirrhotics [33].

In a study analyzing the natural history of compensated cirrhosis type B in 161 Western European caucasian patients (EUROHEP cohort), who were delta-negative and remained untreated during a mean follow of 6 years, the 5-year cumulative HCC risk was 9% [34]. Most patients with HCC did not experience hepatic decompensation before or at the time of diagnosis of liver cancer, indicating that HCC usually develops in clinically silent cirrhosis [34].

Hepatic decompensation

The average age for the development of clinical cirrhosis is 55 years old [34, 35]. In longitudinal studies conducted in Europe [34] and Asia [36] including patients with early stages of cirrhosis (Child class A) the incidence of hepatic decompensation was 3 to 4 per 100 person years with a 5-year cumulative incidence of 16 to 20%. Overall these findings indicate that decompensation usually occurs several years after cirrhosis is diagnosed.

Mortality rates

Several longitudinal studies of the natural history of untreated chronic HBV carriers demonstrate that the mortality rate varies with the baseline clinical setting. In a series of untreated patients with chronic hepatitis B, both HBeAg-positive and HBeAg-negative, without pre-existing cirrhosis at baseline and without HDV infection, the incidence of liver-related death was low, ranging from 0 to 1.0 per 100 person years with a 5-year mortality rate of 0-1% [8, 28, 29].

The 5-year mortality rate is 14-20% in patients with compensated HBV-cirrhosis [32, 34, 37]. In the EUROHEP cohort of patients with compensated cirrhosis type B, the incidence of liver-related death was 3.5 and the 5-year mortality rate was 14% [34]. HCC and liver failure were the main causes of death. Once liver disease decompensation occurs, the prognosis is poor with the 5-year mortality rate ranging from 65%-85% [37, 38]. There is some correlation between the type of decompensation and the prognosis. The highest mortality was observed in patients with more than one complication and the lowest mortality rate in patients with ascites (62% at 5 years) [34].

PROGNOSTIC FACTORS OF DISEASE PROGRESSION

A number of viral-related, host-related and external factors may have an impact on the rate of disease progression (Table 1).

Viral-related
HBV replication status during follow-up
HBV variants
HBV genotype [a]
HDV co-infection
HCV co-infection
HIV co-infection [a]
Host-related
Age at diagnosis
Gender
Severity of liver disease at presentation
Recurrent flares of hepatitis
Sustained ALT normalization
External
Alcohol
Smoking [a]
Environmental contaminants (aflatoxin) [b]

Table 1: Factors affecting progression of chronic hepatitis and compensated cirrhosis due to hepatitis B virus (HBV).
Hepatitis delta virus (HDV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) [a] more research needed; [b] important in HBV endemic regions.

Virus-related factors

Ongoing "clinically" significant HBV replication, defined by the presence of detectable serum HBV DNA using a non-PCR assay ($>10^5$-10^6 copies per mL) or HBeAg, may accelerate the progression of chronic hepatitis to cirrhosis [20, 28, 39]. Once cirrhosis has

developed, patients with high levels of HBV replication are at increased risk of liver-related death, whereas those undergoing clearance of HBeAg, suppression of HBV DNA and ALT normalization have an improved survival rate [37, 40]. In the EUROHEP cohort of patients with compensated cirrhosis, the risk of decompensation and liver-related mortality in HBV DNA-positive patients compared to HBV DNA-negative patients was 4 fold and 5.9 fold respectively [34]. In the Dutch cohort of cirrhotic patients, loss of HBeAg and development of anti-HBe during follow-up was associated with a reduction in the likelihood of liver-related death by 2.2 fold [37]. Even in patients with decompensated cirrhosis, suppression of HBV replication and delayed HBsAg loss is an important event that may be associated with improved survival [35].

On the other hand, the prognostic role of active HBV replication at diagnosis in the prediction of HCC is still controversial. A population-based study in 11,893 Taiwanese men found that the risk of HCC increased 10-fold among men who were positive for HBsAg alone and 60-fold for those positive for both HBsAg and HBeAg at diagnosis compared to HBsAg-negative men [41]. In the EUROHEP cohort of patients with compensated cirrhosis type B, the risk of liver cancer did not differ among HBeAg-positive, HBeAg-negative/HBV DNA-positive or HBeAg-negative/HBV DNA-negative patients at diagnosis [34]. However, these negative results may be related in part to the small sample size in each group of patients and to the low overall incidence of HCC.

The influence of the HBV genotypes on the clinical outcome of chronic hepatitis B is still under investigation [42]. One study from Taiwan found that genotype C is associated with advanced fibrotic liver disease and genotype B is associated with an increased risk of HCC [43], but other studies from Japan and China reported that the life long risk of progression to cirrhosis and the development of HCC did not differ between HBV genotype B and C [17, 44] In India and the Mediterranean area, genotype D is associated with more severe liver disease than genotype A [18, 42]. Whether differences in the preferential occurrence of the pre-core and core promoter mutations in association with different HBV genotypes affect the clinical outcome of chronic liver disease has still not been clarified.

An important factor in disease progression is co-infection with similarly transmitted viral infections. The reported worldwide prevalence of serum anti-HCV in patients with chronic HBV or HDV

infection is greater than 10%, particularly among injecting drug users [45]. Co-infection with HCV and HBV or triple infection with HCV, HBV and HDV tend to aggravate the severity and the progression of liver disease to cirrhosis [45-47]. HCV or HDV co-infection in patients with cirrhosis has been linked to an increased risk of HCC [48-50].

In homosexual men with chronic HBV infection, HIV infection is associated with a lower rate of spontaneous HBeAg seroconversion and an increased incidence of cirrhosis in cases of low CD4 count ($<200/mm^3$) compared to HIV-positive patients with a CD4 count $>200/mm^3$ and to HIV-negative patients [51]. To our knowledge, no data are available on the risk of HCC in HBV/HIV co-infected persons.

Host-related factors

Older age at diagnosis appears to be an important determinant of progression to cirrhosis, HCC and increased mortality [32, 34, 37, 38, 49]. This may be because the aging immune system cannot contain the disease process or simply because of the longer duration of infection and liver disease. Fibrosis appears to progress more slowly in females than in males with chronic hepatitis B, suggesting that estrogens have a protective effect on fibrogenesis [52]. Contrasting data are available on the influence of gender on the risk of HCC [38, 53].

The biochemical and histological expression of the disease is significantly related to disease progression. The natural course of chronic hepatitis B is punctuated by spontaneous flares of hepatitis and recurrent episodes of severe necroinflammation and regeneration may increase the risk of fibrosis and cirrhosis [4, 39]. In addition, the severity of the fibrosis stage at presentation correlates with the risk of developing cirrhosis; in one study the rate of progression to cirrhosis was 0%, 6% and 17% after 5 years for stages F1, F2 and F3, respectively [29].

In patients with compensated HBV-cirrhosis, baseline clinical and biochemical characteristics that indicate decreased hepatocellular function (hypoalbuminemia, mild elevation in bilirubin levels (17-51μmol/L) and the presence of portal hypertension (decreased platelet counts, splenomegaly) correlate with an increased risk of hepatic decompensation and HCC and poor survival [32, 34, 37].

External factors

Chronic alcoholism plays a major role in increasing the rate of progression to both cirrhosis and HCC. Among patients with chronic hepatitis B, those with a history of heavy drinking have a 6 fold higher risk of progression to cirrhosis [29]. A case control study found that there was a synergy between alcohol drinking and HBV infection in the risk of liver cancer, with an increased risk of approximately 2 fold over that with alcohol alone for HBV infected subjects of both sexes who drank more than 60g/day [54]. Additional external factors that may increase the risk of liver cancer include smoking and dietary carcinogens such as aflatoxins which contaminate food stored in humid conditions [41, 55].

CONCLUSIONS

In conclusion, the difficulties of determining the natural history of chronic hepatitis B include (1) the absence of symptoms during the early stages, but also during progression to cirrhosis, (2) the slow and variable progression to cirrhosis and end-stage liver disease, (3) accompanying factors that can modify the course, including co-infections and co-morbid conditions, and (4) the now common use of antiviral treatments. At present there is no generally accepted way to predict the long-term outcome in an individual patient, although a combination of demographic, virological, biochemical, histological and environmental factors can provide useful information for describing the natural history of the disease. Further characterization of host, virus and external factors associated with rates of fibrosis progression and long-term complications of chronic hepatitis B would allow more efficient clinical management and treatment of this disease.

REFERENCES

1. EASL International Consensus Conference on Hepatitis B. J Hepatol 2003;39:S3-S25.
2. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000-Summary of a workshop. Gastroenterology 2001;120:1828-1853.
3. Lok ASF, McMahon BJ. Chronic hepatitis B. Hepatology 2001;34:1225-1241.
4. Perillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology 2001;120:1009-1022.
5. Hadziyannis SJ, Vassilopoulos D. Hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.
6. Chemin I, Zoulim F, Merle P, Arkhis A, Chevallier M, Kay A, Cova L, Chevallier P, Mandrand B, Trepo C. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. J Hepatol 2001;34:471-473.
7. Chang MH. Natural history of hepatitis B infection in children. J Gastroenterol Hepatol 2000;15:E11-19.
8. Fattovich G. Natural history and prognosis of hepatitis B. Semin Liver Dis 2003;23:47-58.
9. Bortolotti F, Jara P, Crivellaro C, Hierro L, Cadrobbi P, Frauca E, Camarena C, De La Vega A, Diaz C, De Moliner L, Noventa F. Outcome of chronic hepatitis B in Caucasian children during a 20-year observation period. J Hepatol 1998;29:184-190.
10. Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, Alberti A, Realdi G. Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology 1986;6:167-172.
11. Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, Liaw YF. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002;35:1522-1527.
12. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska natives chronically infected with hepatitis B virus. Ann Intern Med 2001;135:759-768.
13. Yuen MF, Yuan HJ, Hui CK, Wong DKH, Wong WM, Chan AOO, Wong BCY, Lai CL. A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut 2003;52:416-419.

14. Alward WLM, McMahon BJ, Hall DB, Heyward WL, Francis DP, Bender TR. The long-term serological course of asymptomatic hepatitis B virus carriers and the development of primary hepatocellular carcinoma. J Infect Dis 1985;151:604-609.

15. Liaw YF. Hepatitis flares and hepatitis B e antigen seroconversion: implication in anti-hepatitis B virus therapy. J Gastroenterol Hepatol 2003;18:246-252.

16. Chu CJ, Hussain M, Lok ASF. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared to hepatitis B virus genotype C. Gastroenterology 2002;122:1756-1762.

17. Yuen MF, Sablon E, Yuan HJ, Wong DKH, Hui CK, Wong BCY, Chan AOO, Lai CL. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology 2003;37:562-567.

18. Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodès J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in Western patients. Gastroenterology 2002;123:1848-1856.

19. Funk ML, Rosenberg DM, Lok ASF. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated pre-core and core promoter variants. J Viral Hepat 2002;9:52-61.

20. 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;36:263-270.

21. Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-α treated and untreated patients with HBeAg-negative chronic hepatitis B. J Hepatol 2001;34:306-313.

22. De Franchis R, Meucci G, Vecchi M, Tatarella M, Colombo M, Del Ninno E, Rumi MG, Donato MF, Ronchi G. The natural history of asymptomatic hepatitis B surface antigen carriers. Ann Intern Med 1993;118:191-194.

23. Bellentani S, Dal Molin G, Miglioli L, Crocè LS, Masutti F, Castiglione A, Campello C, Tiribelli C. Natural history of HBV infection: a 9 year follow-up of the Dionysos cohort. J Hepatol 2002;36:228.

24. Manno M, Grottola A, Ferretti I, Colantoni A, De Maria N, Vecchi C, De Palma M, Giannini F, Manenti F, Villa E. Natural history of chronic asymptomatic HBV infection: Survival analysis after 30 years. Gastroenterology 2002;122:A627.

25. Fattovich G, Giustina G, Sanchez-Tapias J, Quero C, Mas A, Olivotto PG, Solinas A, Almasio P, Hadziyannis S, Degos F, Carneiro de Moura M, Krogsgaard K, Pantalena M, Realdi G, Corrocher R, Schalm SW. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. Am J Gastroenterol 1998;93:896-900.

26. 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;13:627-631.

27. Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology 2002;123:1084-1089.

28. Fattovich G, Brollo L, Giustina G, Noventa F, Pontisso P, Alberti A, Realdi G, Ruol A. Natural history and prognostic factors of chronic hepatitis type B. Gut 1991;32:294-298.

29. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H. Disease progression and hepatocellular carcinogenensis in patients with chronic viral hepatitis: a prospective observation in 2215 patients. J Hepatol 1998;28:930-938.

30. Fattovich G, Brollo L, Alberti A, Pontisso P, Giustina G, Realdi G. Long-term follow-up of anti-HBe positive chronic active hepatitis B. Hepatology 1988;8:1651-1654.

31. Brunetto MR, Oliveri F, Rocca G, Criscuolo D, Chiaberge E, Capalbo M, David E, Verme G, Bonino F. Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis Be antigen. Hepatology 1989;10:198-202.

32. Realdi G, Fattovich G, Hadziyannis S, Schalm SW, Almasio P, Sanchez-Tapias J, Christensen E, Giustina G, Noventa F. Survival and prognostic factors in 366 patients with compensated cirrhosis type B: a multicenter study. J Hepatol 1994;21:656-666.

33. Fattovich G, Stroffolini T, Zagni I, Donato F. Cirrhosis and hepatocellular carcinoma: incidence and factors related to hepatocellular carcinoma development. Gastroenterology 2004 (in press).

34. Fattovich G, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. Am J Gastroenterol 2002;97:2886-2895.

35. Chung HT, Lai CL, Lok AS. Pathogenic role of hepatitis B virus in hepatitis B surface antigen-negative decompensated cirrhosis. Hepatology 1995;22:25-29.

36. Liaw YF, Lin DY, Chen TJ, Chu CM. Natural course after the development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Liver 1989;9:235-241.

37. De Jongh FE, Janssen HLA, De Man RA, Hop WCJ, Schalm SW, Van Blankestein M. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. Gastroenterology 1992;103:1630-1635.

38. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, Christensen E, Krogsgaard K, Degos F, Carneiro De Moura M, Solinas A, Noventa F, Realdi G. Occurrence of hepatocellular carcinoma and

decompensation in Western European patients with cirrhosis type B. Hepatology 1995;21:77-82.

39. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Hepatology 1988;8:493-496.

40. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis Be antigen-positive patients with compensated cirrhosis treated with interferon alfa. Hepatology 1997;26:1338-1342.

41. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis Be antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347:168-174.

42. Kao JH. Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J Gastroenterol Hepatol 2002;17:643-650.

43. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554-559.

44. Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, Kanda T, Fukai K, Kato M, Saisho H. Influence of hepatitis B virus genotypes on the progression of chronic liver disease. Hepatology 2003;37:19-26.

45. Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. Hepatology 1995;22:1101-1108.

46. Fattovich G, Boscaro S, Noventa F, Pornaro E, Stenico D, Alberti A, Ruol A, Realdi G. Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. J Infect Dis 1987;155:931-935.

47. Rosina F, Conoscitore P, Cuppone R, Rocca G, Giuliani A, Cozzolongo R, Niro G, Smedile A, Saracco G, Andriulli A, Manghisi OG, Pizzetto M. Changing pattern of chronic hepatitis D in Southern Europe. Gastroenterology 1999;117:161-166.

48. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, Schalm SW. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. Gut 2000:46:420-426.

49. Benvegnù L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, Alberti A. Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. Cancer 1994;74:2442-2448.

50. Tsai JF, Jeng JE, Ho MS, Chang WY, Hsieh MY, Lin ZY, Tsai JH. Effect of hepatitis C and B virus infection on risk of hepatocellular carcinoma: a prospective study. Br J Cancer 1997;76:968-974.

51. Di Martino V, Thevenot T, Colin JF, Boyer N, Martinot M, Degos F, Coulaud JP, Vilde JL, Vachon F, Degott C, Valla D. Marcellin P. Influence of HIV infection on the response to interferon therapy and the long-term outcome of chronic hepatitis B. Gastroenterology 2002;123:1812-1822.

52. Poynard T, Mathurin P, Lai CL, Guyader D, Poupon R, Tainturier MH, Myers RP, Muntenau M, Ratziu V, Manns M, Vogel A, Capron F, Chedid A, Bedossa P; PANFIBROSIS Group. A comparison of fibrosis progression in chronic liver diseases. J Hepatol 2003;38:257-265.

53. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61:1942-1956.

54. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, Decarli A, Travisi 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;155:323-331.

55. Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, Shao Y, Wu Z, Liu G, Wang X, Sun Z. Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology 2002;36:1214-1220.

New Nucleoside Analogs for the Treatment of Chronic Hepatitis B

Maria Buti, Rafael Esteban

Three agents have been approved for the treatment of chronic hepatitis B virus (HBV): interferon-alpha, lamivudine and adefovir. Each agent has certain limitations and none has an excellent efficacy. Lamivudine and adefovir have the advantages of oral administration and excellent safety profiles. However, optimal treatment of chronic hepatitis B is still under debate. The sustained response rates to these new therapies are still low, drug resistance to lamivudine limits its efficacy and new drugs are necessary for the treatment of chronic hepatitis B in different situations: immunocompromised and decompensated patients, patients with normal ALT levels, lamivudine-resistant patients and non-responders to lamivudine or interferon [1].

This review focuses on new antiviral agents such as entecavir, emtricitabine, clevudine and beta-L nucleosides. Some of them are still in phase II clinical studies; therefore available information remains limited.

ENTECAVIR

Entecavir, a cyclopentyl guanosine analog, is a potent inhibitor of HBV DNA polymerase, inhibiting both the priming and elongation steps of viral DNA replication [1, 2]. Entecavir is phosphorylated to its triphosphate, the active compound, by cellular kinases. It is a selective inhibitor of HBV DNA because it has little or no inhibitory effect on the replication of other DNA viruses such as herpes simplex, cytomegalovirus and RNA viruses such as HIV. Although entecavir is

also effective against lamivudine-resistant mutants, it is less effective than against wild-type HBV [2, 3].

In a randomized, double blind, escalating-dose, placebo-controlled phase II trial, four doses of entecavir (0.05, 0.1, 0.5 and 1.0mg once daily for 28 days) were evaluated. Serum HBV DNA levels decreased by 2-3 logs by day 28 and approx 25% of patients showed a decline in HBV DNA below the limit of detection of the Chiron HBV DNA assay (<0.7ME/mL). After stopping therapy, all patients showed a rebound of ALT levels and HBV DNA [3].

In a 24 week, double blind, randomized, multicenter clinical trial, the safety and efficacy of three different oral doses of entecavir (0.01, 0.1, or 0.5mg/day) were compared to lamivudine (100mg/day). One hundred and sixty-nine patients chronically infected with HBV (HBeAg-positive and HBeAg-negative) were treated for 24 weeks [4]. Both the 0.1 and 0.5mg/day doses of entecavir were more effective than lamivudine for viral load reduction, as measured by the Amplicor® polymerase chain reaction (PCR) assay. Compared to lamivudine, entecavir therapy reduced HBV DNA by an additional 0.97 $\log_{10}$ with the 0.1mg/day dose and an additional 1.28 $\log_{10}$ with the 0.5mg/day dose (P<0.0001 for both comparisons). A clear dose-response relationship was observed for entecavir with both the 0.1 and 0.5mg/day doses demonstrating significantly stronger viral suppression of HBV DNA than the 0.01mg/day dose (P<0.0001 for each). The 0.5mg/day dose of entecavir was more effective than the 0.1mg/day dose (P=0.018). 83.7% of the patients treated with entecavir 0.5mg/day had a decrease in HBV DNA levels to below the lower limit of detection of the Quantiplex® branched chain DNA (bDNA) assay, compared to 57.5% treated with 100mg/day of lamivudine and 62% treated with 0.1mg/day of entecavir. Entecavir was well tolerated at all dose levels; most adverse events were mild to moderate and transient with no significant differences observed between any of the different doses of entecavir and lamivudine. This study shows that entecavir has potent antiviral activity against HBV. The 0.1 and the 0.5mg/day entecavir doses were more effective than lamivudine in chronically HBV infected patients. Based on the results of this study, 0.5mg/day of entecavir can be recommended as the optimal dose for previously untreated patients. In addition, entecavir has antiviral activity in patients with lamivudine-resistant mutants. Results from a recent trial of entecavir against YMDD-variant HBV confirm that it is active in this setting [5]. Three doses of entecavir

(0.1, 0.5 and 1mg/day) were tested and compared with lamivudine in 181 patients who failed to respond to lamivudine therapy and had YMDD mutants. At week 24, the percentage of patients with undetectable HBV DNA by bDNA (Quantiplex assay) was 19% with 0.1mg, 53% with 0.5mg and 79% with 1mg entecavir daily while with lamivudine (100mg/day), only 13% had undetectable HBV DNA. The 0.5 and 1mg doses were more effective than lamivudine (P<0.0001). The mean $\log_{10}$ decrease in HBV DNA levels by PCR assay with entecavir was 1.95 with 0.1mg, 3.85 with 0.5mg and 4.36 with 1mg in contrast to 0.92 with lamivudine [5]. Therefore, in patients with lamivudine resistant YMDD mutants, entecavir significantly decreased hepatitis B viremia and 1mg of entecavir daily seems to be the optimal dose in these patients in contrast to the 0.5mg dose recommended for untreated patients.

Preliminary results do not show any emergence of entecavir resistance in lamivudine refractory patients treated for at least one year with entecavir. One hundred thirty two patients with lamivudine resistance were treated with entecavir for 48 weeks without the emergence of other reverse transcriptase sequences.

Different ongoing multicenter phase III studies are currently evaluating the efficacy and safety of entecavir in HBeAg positive and, HBeAg negative patients as well as in patients resistant to lamivudine. These studies are comparing entecavir vs. lamivudine for 48 weeks.

EMTRICITABINE

Emtricitabine (FTC) is a cytosine nucleoside analog with antiviral activity against both HBV and HIV. Unlike lamivudine, it has a fluorine at the 5-position of the nucleic acid. In a pilot study, 49 patients with HBeAg positive chronic hepatitis B received five different doses of emtricitabine: 25, 50, 100, 200, or 300mg/day for 8 weeks. At the end of treatment, serum HBV DNA levels decreased by 2-3 logs in patients receiving the higher doses [1].

In a second randomized, double blind study, three doses (25, 100, or 200mg/day) of emtricitabine were compared for 48 weeks in 98 Asian patients (77 HBeAg positive and 21 HBeAg negative) [6]. At week 48, HBeAg loss was observed in 40% of the 77 HBeAg positive patients (ranging from 32 to 50% depending on the dose group). For all patients, the median decrease in viral load was 2.59 $\log_{10}$ copies/mL for the 25mg dose, 3.12 $\log_{10}$ copies/mL for the 100mg

dose and 2.92 $\log_{10}$ copies/mL for the 200mg dose, with a range of up to 5.5 $\log_{10}$ copies/mL in patients receiving 100 or 200mg emtribitabine/day. The proportion of patients with undetectable HBV DNA at week 48 was 38%, 42% and 61% for the 25, 100, and 200mg dose groups, respectively. Genotypic analysis performed at week 48 showed that 12% of patients treated with 100mg of emtricitabine and 6% of those treated with 200mg had detectable viremia with phenotypic changes associated with HBV drug-resistance. The results of this study suggest that the optimal FTC dose is 200mg once daily. This dose is well tolerated, produces the highest rate of HBV suppression and is associated with the lowest incidence of drug resistant mutants.

HBV DNA loss occurred in a higher proportion (79%) of the 21 anti-HBe positive patients, than of the HBeAg positive patients. However, when HBV DNA results were adjusted for baseline viral load there was no difference between patients who were HBeAg positive and HBeAg negative in the proportion of patients with undetectable HBV DNA at week 48. Overall, ALT levels normalized in 95% of patients at week 48. These results suggest that emtricitabine has potent antiviral activity in HBeAg-negative, HBV DNA positive patients and it is an active therapeutic agent in this setting [7].

Phase III clinical trials are under way to determine the long-term safety and efficacy of emtricitabine. However, the role of emtricitabine in the treatment of chronic hepatitis B may be limited by its structural similarity to lamivudine and hence, the potential for cross-resistance and the development of mutations. For this reason some clinical trials comparing emtricitabine alone or in combination with adefovir are ongoing.

CLEVUDINE

Clevudine (L-FMAU;1-[2-fluoro-5methyl-β-L-arabinosyl uracil]) is a pyrimidine analog with marked “in vitro” activity against HBV but not HIV [1, 2]. The active triphosphate inhibits HBV DNA polymerase but is not an obligate chain terminator. “In vitro”, clevudine has an EC_{50} value ranging from 0.02 to 0.15μM with a mean of 0.08μM. “In vitro” studies suggest that it may also be effective against lamivudine-resistant HBV mutants. “In vivo” studies of the infected woodchuck model have demonstrated that a once daily dose of 10mg of clevudine resulted in as much as a 9 $\log_{10}$ decrease in viral load. An open labeled

phase I/II, non-randomized, dose–escalation study was performed in patients with chronic hepatitis B. Twenty-five patients were enrolled: 5 received 10mg daily of clevudine for 28 days, 10 received 50mg of clevudine/day and 10 were treated with 100mg of clevudine/day for the same period of time and were followed by a 24 week postreatment period [8]. All patients were HBV DNA positive (more that 3 x 10^6 copies/mL). At the end of the dosing period, the median reduction in serum HBV DNA was 2.48 $\log_{10}$, 2.74 $\log_{10}$ and 2.95 $\log_{10}$ in the 10mg, 50mg and 100mg/day cohorts, respectively. At the end of follow-up (20 weeks post-treatment), the median decrease in serum HBV DNA levels was 1.84 $\log_{10}$ and 2.38 $\log_{10}$ in the 10mg and the 50mg/day cohorts, respectively. No data was available for the 100mg dose cohort. Clevudine was well tolerated without associated adverse events. These preliminary results show that clevudine has potent antiviral activity at all three doses tested and maintains a sustained post-treatment antiviral effect for at least 6 months after the 28-day treatment period. More studies in patients with chronic hepatitis B are planned.

BETA-L-NUCLEOSIDES

The natural nucleosides in the beta-L-configuration (beta-L-thymidine [LdT], beta-L-2-deoxycytidine [L-dC] and beta-L-2-deoxyadenosine [L-dA]) represent a new class of compounds with potent, selective and specific activity against hepadnaviruses. “In vitro” studies have shown that these compounds are not active against other viruses such as herpes viruses or HIV, but these compounds have marked effects on HBV replication. It is not yet clear whether these compounds are active against lamivudine-resistant HBV mutants [1, 9, 10].

LdT is at the most developed stage of clinical investigation. A phase I/II, 4-week dose-escalation trial has been completed with 35 adults with chronic hepatitis B. All of them were HBeAg positive and HBV DNA positive. Subjects were randomized to receive five different oral doses of LdT: 25, 50, 100, 200, or 400mg/day. HBV DNA level reductions were dose dependent and were observed at all five doses tested. The dose-dependent antiviral effects of LdT were especially evident after the first week of treatment. The median HBV DNA reduction for the 400mg cohort, assessed by Roche polymerase chain reaction (PCR) assay, was 3.6–4.0 $\log_{10}$ by week 4. This reduction seems greater than those previously reported for other

antiviral drugs (lamivudine, adefovir, entecavir). The safety profile of LdT appeared similar to placebo [11]. A phase IIb study comparing five different therapeutic strategies for 1 year was recently completed. One hundred and four patients were randomized to receive LdT 400mg/day, LdT 600mg/day, LdT 400mg and lamivudine 100mg/day, LdT 600mg and lamivudine 100mg/day and lamivudine 100mg/day for one year. Median serum HBV DNA reductions at week 52 in $\log_{10}$ copies/mL for the five treatment groups were 4.66 for standard lamivudine therapy, 6.43 for LdT 400mg <7day, 6.09 for LdT 600mg/day, 6.40 for combination 400mg and 6.05 for combination 600mg/day. HbeAg loss was observed in 28% of the patients treated with lamivudine, 33% of those treated with LdT and 17% of those treated with Ldt plus lamivudine [12]. Therefore after one year of treatment, viral suppression, PCR non-delectability of serum HBV DNA and ALT normalization were significantly greater for LdT than lamivudine.

Another promising beta-L-nucleoside compound is val-LdC. It is in the middle of phase I/II testing and preliminary results indicate substantial antiviral activity with a good safety profile [1].

Combinations of beta-L-nucleosides appear to have additive or synergistic effects against HBV. “In vitro” studies and animal tests have shown that there is no evidence of cellular or mitochondrial toxicity. The combination of LdT and Val-LdC was analyzed in woodchucks. Over a 12-week treatment period, the combination of LdT and val-LdC cleared PCR detectable woodchuck hepatitis virus (WHV) DNA in 5 of the 5 animals tested with no safety problems noted. If similar antiviral activities are observed in humans, a relatively rapid clearance of HBV viremia could be a realistic goal in many patients.

SUMMARY AND CONCLUSIONS

Although many of the new antiviral agents discussed above are promising, it is unlikely that any of these compounds will result in a definitive answer to the treatment of chronic hepatitis B and the future of chronic hepatitis B therapy seems to be the combination of different drugs with two aims: to improve response to therapy and to avoid or reduce viral resistance.

REFERENCES

1. Lok AS, Heatchote EJ, Hoofnagle JH. Management of hepatitis B: 2000 summary of a workshop. Gastroenterology 2001;120:1828-1853.

2. Balfour HHJ. Antiviral drugs. N Engl J Med 1999;340:1255-1268.

3. De Man RA, Wolters LM, Nevens F, Chua D, Sherman M, Lai CL, Gadano A, Lee Y, Mazzotta F, Thomas N, DeHertogh D. Safety and efficacy of oral entecavir given for 28 days in patients with chronic hepatitis B. Hepatology 2001;34:578-582.

4. Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, Dehertogh D. Entecavir is superior in reducing HBV DNA in patients with chronic hepatitis B infection. Gastroenterology 2002:123:1831-1838.

5. Tassopoulos N, Hadziyannis S, Cianciara J, Rizzetto M, Schiff ER, Pastore G, et al. Entecavir is effective in treating patients with chronic hepatitis B who have failed lamivudine therapy. Hepatology 2001;34:340A.

6. Leung N, Gish RG, Wang C, Sacks S, Fried M, Wright T, et al. A randomized, double-blind comparison of 3 doses of emtricitabine in patients with chronic hepatitis B given 48 weeks of treatment. Hepatology 2001;34:349A.

7. Wang C, Corey L, Leung N, Gish R, Rigney A, Fang L et al. Antiviral activity of 48 weeks of emtricitabine treatment in patients with HBeAg negative/HBV DNA positive chronic hepatitis B. Hepatology 2001;34:323A.

8. Marcellin P, Mommeja-Marin H, Sacks SL, Lau GK, Sereni D, Bronowicki JP, Conway B, Trepo C, Blum MR, Yoo BC, Mondou E, Sorbel J, Snow A, Rousseau F, Lee HS. Anti-HBV activity and tolerability of clevudine, a novel nucleoside analogue. Initial results of a phase I/II 28 day study. Hepatology 2004;40:140-148.

9. Le Guehier F, Pichoud C, Guerret S, Chevallier M, Jamard C, Hantz O, Li XY, Chen SH, King I, Trepo C, Cheng YC, Zoulim F. Characterization of the antiviral effect of 2´3´-dideoxy-2´-3´didehydro-beta-L-5-fluorocytidine in the duck hepatitis B virus infection model. Antimicrob Agents Chemother 2000;44:111-122.

10. Pierra C, Imbach JL, De Clerq E, Balzarini J, Van Aerschot A, Herdewijn P, Faraj A, Loi AG, Sommadossi JP, Gosselin G. Synthesis and antiviral evaluation of some beta-L´-3´dideoxy-5-chloropyrimidine nucleosides and pronucleotides. Antiviral Res 2000;45:169-183.

11. Zhou X-J, Lim S-G, Lai C-L, Lam P-F, Murphy RL, Pow DM et al. Phase 1 dose-escalation pharmacokinetics of L-deoxythymidine in patients with chronic hepatitis B virus infection. Hepatology 2001;34:629A.

12. Han SH, Leung NWY, Teo EK, Tong M, Wong F, Ham HW et al. Results of a one-year international phase IIb trial of LdT, and LdT plus lamivudine in patients with chronic hepatitis B. J Hepatol 2004;40:16.

Treatment of HBeAg-Positive Chronic Hepatitis B with Nucleoside/Nucleotide Analogs

Geoffrey Dusheiko

INDICATIONS FOR THERAPY

There is no consensus on which patients should be treated for chronic hepatitis B and current treatments have limited long-term efficacy. In general, treatment of chronic hepatitis B should target patients with active disease and viral replication, preferably before there are the signs and symptoms of cirrhosis or significant injury has occurred [1]. Eradication of infection is only possible in a minority of patients. However, if HBV replication can be suppressed, the accompanying reduction in histological chronic active hepatitis reduces the risk of cirrhosis and hepatocellular carcinoma [2]. Patients with mild chronic hepatitis should be carefully monitored at appropriate intervals. Therapy should only be considered if there is evidence of moderate to severe activity. Hepatitis Be antigen (HBeAg)-positive patients should be followed for a few months to determine their status, and antiviral therapy should be considered if there is active hepatitis B virus (HBV) replication (HBV DNA above 10^5 copies/mL) and persistent elevation of aminotransferases after 3-6 months of observation. HBeAg-negative patients should be considered for antiviral therapy when serum aminotransferases are raised and if there is active viral replication (HBV DNA above 10^5 copies/mL). Many clinicians

consider a liver biopsy to be helpful in determining the degree of necroinflammation and fibrosis.

HBV/HIV co-infected patients whose immune status is preserved on highly active antiretroviral therapy (HAART) should be considered for anti-HBV therapy, with appropriate therapy for HIV infection to minimize resistance. If HAART is indicated in a patient with HIV co-infection, lamivudine can be administered since it is active against HIV and HBV. Adefovir dipivoxil (ADV) is also active against both viruses although a lower dose is used for HBV. Although tenofovir is active against HBV and HIV, its efficacy in hepatitis B infection has not been confirmed in large controlled trials.

Hepatitis B surface antigen (HBsAg)-positive patients with extra-hepatic manifestations and active HBV replication may respond to antiviral therapy. Patients with decompensated cirrhosis should be treated in specialist liver units, due to the complexity of antiviral therapy in these cases.

Prophylactic therapy is recommended in all patients undergoing liver transplantation for end-stage hepatitis B to lower HBV DNA levels to less than 10^5 copies/mL before transplantation. The optimal timing of transplantation has not been established, but the selection of resistant strains should be avoided before surgery. Lamivudine and ADV are suitable. Antiviral therapy for prophylaxis of post-transplantation recurrence will probably require life long treatment. The most promising prophylaxis includes lamivudine and lifelong hepatitis B immunoglobulin (HBIG) treatment after transplantation resulting in low rates of reinfection/reactivation. Shorter courses of HBIG and other forms of prophylaxis, including ADV combined with lamivudine, are being studied. The optimal treatments for hepatitis B, including suitable combination therapies, are being evaluated in different studies. Response rates in HBeAg-positive patients are better in patients with higher baseline alanine aminotransferase (ALT) for all currently licensed agents.

Interferon remains a benchmark therapy for chronic hepatitis B. Approximately 35-40% of HBeAg-positive patients are treated effectively by interferon, at a dose of 5-10mIU/three times weekly (5mIU daily in the USA) for 4-6 months. The efficacy of interferon alpha is discussed elsewhere in the present work. The rationale of first-line treatment with alpha interferon is to achieve loss of HBeAg (and even subsequent loss of HBsAg) after a short course of treatment.

Sustained loss of HBeAg is generally associated with a histological reduction in inflammation [3].

There are alternative options and strategies for treatment; new nucleoside analogs which act as chain terminators have influenced the treatment of hepatitis B. Lamivudine can be given (100mg/day for at least one year and maintained for 4-6 months after a virological response (loss of HBeAg) is achieved). Loss of HBsAg has been observed. If a virological response is not achieved within 1 year, the likelihood that continuation of treatment will produce a response is offset by the cumulative risk of developing drug resistance. Therapy remains useful if HBV DNA is suppressed (histological improvement has been documented). ADV 10mg/day is an effective alternative to lamivudine. As with lamivudine, most patients do not have a virological response after one year. ADV can be given at a dose of 10mg/day for at least one year, and maintained for 4-6 months after a virological response occurs (loss of HBeAg is achieved). Unlike lamivudine, the likelihood that continuation of treatment will produce a virological response is not clearly offset by the cumulative risk of developing drug resistance. Therapy also remains useful if HBV DNA is suppressed (histological improvement has been documented). Long-term use of ADV monotherapy (>2 years) will require monitoring for resistance and possible nephrotoxicity.

Patients with decompensated cirrhosis are not candidates for interferon-alpha therapy because of the risk of side-effects, but may be candidates for nucleoside or nucleotide therapy. Patients with moderate to severe chronic hepatitis (HBeAg-positive or -negative) whether treated or not, and patients with advanced liver disease should be monitored for the progression of liver disease and the development of complications, including hepatocellular carcinoma.

LAMIVUDINE

Lamivudine (2',3'-Dideoxy-3'-thiacytidine ((+) -SddC), 3TC or Epivir) is a potent inhibitor of HBV, as well as HIV (Figure 1). The drug acts by inhibiting DNA synthesis through chain termination. The (-)-form ((-)-SddC), which is resistant to deoxycytidine deaminase, is a more active antiviral stereoisomer than the (+)-form. The negative enantiomer (-)-SddC does not appear to affect mitochondrial DNA synthesis. Metabolic studies have shown that the drug is converted to the monophosphate, diphosphate, and triphosphate form. It is rapidly

absorbed after oral administration, with a bioavailability of >80%. Most of the drug is excreted unchanged in the urine. Lamivudine has been used in trials for the treatment of HIV infection since 1990, and this compound has been licensed as a component of HAART. Lamivudine is active *in vitro* against human hepatitis B transfected cell lines and in ducklings affected with duck hepatitis B virus (DHBV), as well as in chimpanzees infected with HBV.

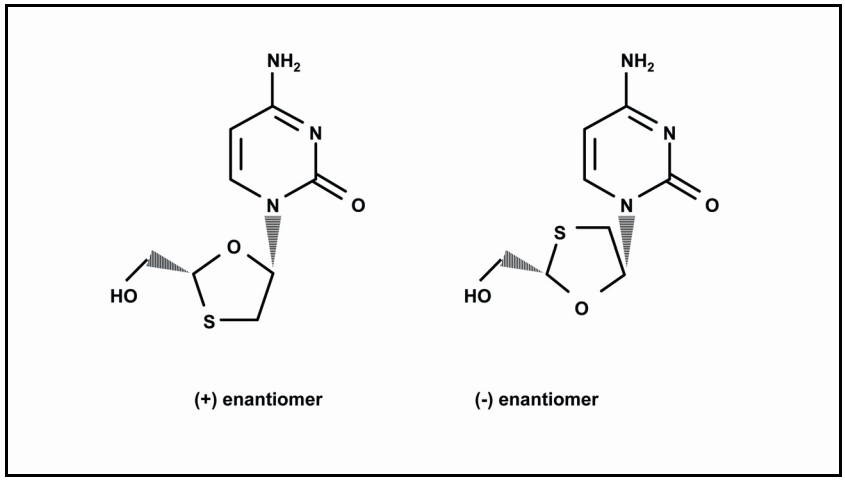

Figure 1: Lamivudine (GR109714x) Single (-) enantiomer of the racemic mixture 2' deoxy-3' thiacytidine.

Large phase III trials in patients with chronic hepatitis B have been completed. Doses above 25mg reproducibly decrease HBV DNA levels in serum. HBV DNA generally became undetectable (by hybridization assay) in more than 90% of patients who received 25mg-300mg/day. In most patients, HBV DNA reappears after therapy is completed. In large trials in Asia and the Western countries, approximately 15-20% of patients became HBeAg-negative after 12 months of treatment compared to 4% of placebo recipients. Histological improvement was noted after one year of treatment. Lamivudine therapy has consistently been associated with a highly

significant sustained reduction in levels of serum HBV DNA at the end of one year of therapy in up to 98% of patients. Undetectable levels of HBV DNA were sustained in 44% of treated patients compared to 16% on placebo. Loss of the HBe antigen with seroconversion to anti HBe was observed in 17% of patients after a year of treatment with lamivudine versus 6% on placebo. Histological improvement was the main outcome measured in the pivotal trial of lamivudine therapy in adults. Liver biopsies were scored according to the degree of necroinflammation and fibrosis and an improvement of 2 or more Human Awareness Institute (HAI) points. Significant differences in the total HAI score were observed in patients receiving lamivudine compared to those receiving placebo [4]. Lamivudine monotherapy reduces HBV DNA concentrations prior to liver transplant, but may be associated with subsequent resistance. Lamivudine and HBIG prophylaxis have been shown to be effective for the prevention of recurrent hepatitis B post-transplantation.

The drug seems to be well tolerated and relatively few serious side-effects have been reported. Serious side-effects have been observed in about 5% of patients; these include anemia, neutropenia, an increase in liver enzymes, nausea and neuropathy. Increased lipases may occur, but this is uncommon, and serious lactic acidosis has not been observed. Severe exacerbations of hepatitis accompanied by jaundice have been reported in patients whose HBV DNA became positive after stopping treatment, or after the development of resistance. Reactivation of hepatitis was observed in patients who developed a methionine to valine or isoleucine substitution in the highly conserved YMDD motif of the HBV polymerase [5]. This motif is part of the active site of the polymerase, and this mutation parallels the M184 mutation seen in resistant HIV where substitutions of valine and isoleucine for methionine have also been found. Lamivudine-resistance is conferred through acquired selection of HBV with mutations of the YMDD motif of the HBV DNA polymerase gene [5]. Four major patterns have been observed: L18OM + M204V; M204I; L180M + M204I; V173L + L180M + M204V; and occasionally L180M + M204V/I. The L18OM + M204V pattern occurs most frequently. Although viral “fitness” may be reduced, as lower levels of HBV DNA occur, recent studies have suggested that the disease may progress [6]. These changes cause a marked decrease in sensitivity to lamivudine *in vitro*. The incidence of lamivudine resistance in chronic hepatitis rises from 24% after one year of

treatment to 66% after 4 years. The incidence increases to 90% in HBV/HIV co-infected patients. The selection of antiviral resistance is a disadvantage of treatment with nucleoside analogs and is a fundamental disadvantage of treatment with long-term lamivudine therapy.

After lamivudine is stopped, HBV replication may reactivate and can sometimes be associated with severe "flares" or exacerbation of hepatitis as HBV DNA increases in serum. The pathogenesis of this injury is not fully understood. It is probably related in part to an immune response. The emergence of resistance could have a similar effect, as viral DNA increases. Combination studies with lamivudine and ADV are in progress.

ADEFOVIR DIPIVOXIL

Pharmacology

Adefovir dipivoxil 9-[2-[bis[(pivaloyloxy)methoxy]phosphinyl]meth oxy]-ethyl]adenine is an orally bioavailable prodrug of adefovir, a phosphonate nucleotide analog of adenosine monophosphate (Figure 2). It requires cellular nucleoside kinases for activation to ADV diphosphate and it then acts as a competitive inhibitor and chain-terminator of HBV replication mediated by HBV DNA polymerase. This drug inhibits viral polymerases and terminates the growing DNA chain by acting as a competitive inhibitor of deoxyadenosine 5'-triphosphate (dATP). Because ADV diphosphate lacks a 3' hydroxyl group, the compound causes premature termination of viral DNA synthesis upon its incorporation into the nascent DNA chain. ADV is active against HBV, DHBV and woodchuck hepatitis virus (WHV) in cell culture models and against chronically infected animals. This agent also has some immunomodulatory activity and stimulates natural killer activity.

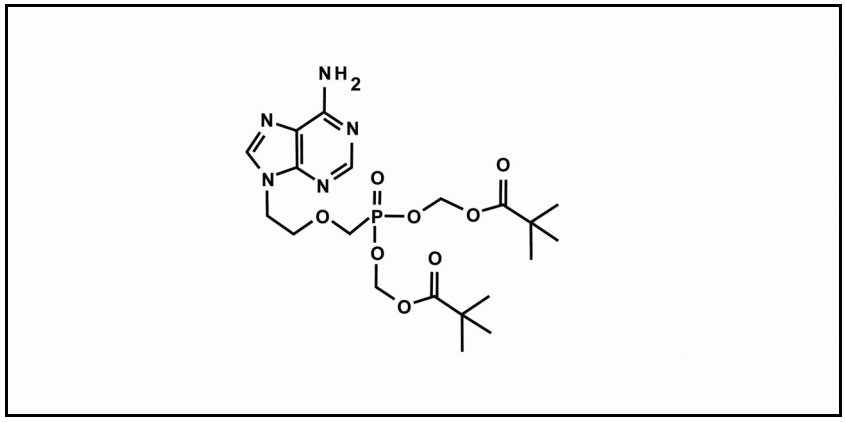

Figure 2: 9-[2-[bis[(pivaloyloxy)methoxy]phosphinyl]methoxy]-ethyl]adenine (ADV).

ADV is active *in vitro* against all known lamivudine, emtricitabine, famciclovir and HBIG resistant HBV, using both cell culture and enzyme assays. Resistance to ADV is remarkably delayed in patients with chronic hepatitis B. Recently, a novel N236T mutation was reported in two anti-HBe-positive patients after 96 weeks of treatment, which was not detectable after one year of treatment. This mutant showed lowered susceptibility to adefovir. The mutation does not share cross resistance with lamivudine. Sequencing of the RT domain of the HBV polymerase has suggested that two mutations, i.e. rtN236T and rtA181V confer resistance to ADV. These mutants remain sensitive to lamivudine, emtricitabine, telbuvidine, and entecavir [7, 8]. Life table analysis has suggested a cumulative incidence of 3.9-5.9% (in naive patients) after three years of treatment.

The limited development of resistance with ADV could be related to its close structural relationship with the natural substrate which limits the potential for steric hindrance as a mechanism of resistance. In addition, ADV contains a flexible acyclic linker that may allow it to bind to HBV polymerase with different conformations, and thus, further subvert steric hindrance [9, 10]. ADV also contains a phosphonate bond that is less susceptible to ATP-mediated chain

terminator excision, which has been recognized as a mechanism of HIV resistance.

Following oral administration of single doses of ADV 10mg in patients with chronic hepatitis B or healthy subjects, maximum ADV concentrations in plasma occur a median of 0.76-1.75 hours after administration. ADV may be taken once daily because of the long terminal elimination half-life. In preclinical studies, evidence of renal toxicity, characterized by renal tubular nephropathy, was noted in all species evaluated. The efficacy of ADV has been investigated in patients with compensated liver disease and evidence of HBV replication; in patients who did not respond to lamivudine therapy, including post-transplantation patients, patients with compensated and decompensated liver failure and patients co-infected with HIV.

Pivotal trials of ADV

Doses of 5-125mg/day have been assessed in the clinical development program. In Phase I/II clinical studies in both HBeAg-positive and HBeAg-negative patients with chronic hepatitis B, statistically significant decreases in serum HBV DNA concentrations were demonstrated within the first week of treatment, and were maintained for up to 136 weeks. Data from previous studies indicated that daily doses of 30mg/day after 24 weeks is associated with mild, reversible nephrotoxicity (seen at higher doses in HIV studies) after the drug is discontinued. Multinational double blind randomized placebo controlled trials, in both HBeAg-positive and negative patients with liver disease have been performed. The primary endpoint of these studies was the quantitative assessment of histological improvement after 48 weeks of treatment using the Knodell Histologic Activity Index (HAI/Knodell) scoring score. Both necro-inflammatory activity and fibrosis was more improved with ADV 10mg and 30mg than with placebo (P<0.001). The pivotal phase III studies examined both ADV 10mg and 30mg to determine the dose with the best risk-benefit profile. These studies were multinational, double blind, randomized, placebo controlled trials, in HBeAg-positive and HBeAg-negative patients with compensated liver disease, with evidence of active HBV replication, who were not undergoing current treatment [11, 12]. In the HBeAg-positive trial, 515 patients were randomized to one of three arms: ADV 30mg/day, ADV 10mg/day or placebo. The primary endpoint of this study was based on the quantitative assessment of

histological improvement after 48 weeks of treatment using the HAI/Knodell score [13]. Histological improvement was defined as a reduction of 2 points or more from baseline in the HAI/Knodell, with no worsening in the fibrosis score. Secondary endpoints in the study were based on established methods to determine the virological response (suppression of HBV replication based on the decrease of serum HBV DNA) and biochemical response (defined by reductions and normalization in ALT during therapy). HBeAg seroconversion, defined as loss of HBeAg and appearance of anti-HBe, was also a key secondary endpoint. Loss of HBeAg has been correlated with long-term clinical improvement [2]. A daily dose of 10mg of ADV was shown to have the best risk-benefit profile for long-term treatment. This dose resulted in significant improvement compared to placebo: improvement in liver histology (53% vs. 25%, $P<0.001$), reductions in HBV DNA (3.52 vs. 0.55 log copies/mL, $P<0.001$), normalization of ALT (48% vs. 16%, $P<0.001$), and HBeAg seroconversion (12% vs. 6%, $P=0.049$). There were no significant side-effects and no resistance was found. As a result, 10mg of ADV is the recommended and approved daily dose. Improved responses were seen in patients with increased ALT [11]. An effect of ADV on cccDNA was observed in treated patients, but the significance of these findings requires further study. 10mg is the preferred treatment dose because of the favourable risk-benefit ratio. In the large HIV trials an incidence of nephrotoxicity of between 17% and 60% was reported. However, in the two largest hepatitis B phase III trials involving 695 patients, no clinically significant renal toxicity was found at the 10mg dose.

ADV for lamivudine-resistant hepatitis B

ADV has been shown *in vitro* to be active against lamivudine-resistant HBV, [9, 14] and there are a number of reports of successful treatment of lamivudine-resistant patients with ADV, particularly for post – transplant recurrence of hepatitis B [15-18]. There does not appear to be an advantage in continuing lamivudine after starting ADV in patients with compensated liver disease if lamivudine resistance develops. Rapid reductions in HBV DNA were observed within 4 weeks in all recipients of ADV, but the median changes from baseline were not greater in those who continued lamivudine. Thus treatment with ADV alone seems to be most effective in these patients, and there is no long-term advantage of continuing lamivudine therapy in

patients with YMDD mutations [19, 20]. Although it is safe to change to ADV in patients with compensated liver disease, an overlapping period before discontinuing lamivudine seems advisable in these patients. However the effect of this strategy on subsequent emergence of ADV resistance requires further study [9, 15, 21].

ADV in liver transplant recipients

Recurrent HBV infection in the transplanted liver remains a major problem. A retrospective study of liver transplantation in Europe before lamivudine showed that patients with low levels of hepatitis B replication at transplantation and those given long-term immunoprophylaxis with HBIG had a reduced risk of recurrent HBV infection and reduced mortality [22]. Lamivudine has further improved these outcomes. Pre-transplant treatment with lamivudine resulted in suppression of HBV DNA levels in 12 of 19 treated patients [23, 24]. Currently both HBIG and lamivudine are used prophylactically and recurrent HBV is now rare [25-27]. However, cases associated with lamivudine-resistance are problematic, as patients with recurrent post-transplant hepatitis B may develop fibrosing cholestatic hepatitis, a manifestation of high levels of viral replication in immunosuppressed patients [28, 29]. A study of 10 patients treated with lamivudine pre-liver transplantation for HBV showed that there was a risk of lamivudine-resistant strains following transplant [30]. In a post liver transplant study, lamivudine-resistant patients all developed liver failure with liver dysfunction [31]. ADV has proved to be an important antiviral drug in patients with lamivudine resistance post-transplant. In an open label study 127 liver transplant patients with lamivudine-resistant HBV were treated with ADV 10mg [32]. Treatment resulted in a median 4 $\log_{10}$ drop in HBV DNA concentrations at 48 weeks indicating the important role of ADV as second-line therapy in patients who develop lamivudine resistance in the peri-transplant setting. Care should be taken in patients with pre-existing renal damage due to calcineurin inhibitors in liver transplant patients.

NEWER NUCLEOSIDE ANALOGS

Phase I and II trials with several new nucleoside analogs, including entecavir, emtricitabine, clevudine (L-FMAU), and L-dT are in progress.

a) Entecavir is a cyclopentyl guanine analog, which is an inhibitor of all HBV polymerase functions. The drug is readily phosphorylated to the active triphosphate form. It is a potent inhibitor of WHV, and in humans with HBV at doses of 0.05-1mg. In phase II trials, 84% of patients were negative for HBV DNA by bDNA assay after 24 weeks of treatment [33]. The drug is active against lamivudine resistant variants and phase III trials are in progress.
b) Emtricitabine (FTC) is a cytosine nucleoside analog, with fluorine at the 5 position. Pilot studies have shown that the drug causes a 2-3 log reduction in HBV DNA at doses of 300mg in patients treated for 8 weeks. In a 48 week phase II study, 61% of patients had undetectable HBV DNA. Drug resistant mutants were reported in 6% of treated patients. Phase III trials are in progress.
c) Clevudine (L-FMAU) is a pyrimidine nucleoside analog. Patients have been treated in phase II dose escalating studies, and up to 3 log reductions in HBV DNA have been observed [34, 35].
d) Beta-L thymidine (telbivudine), valtorcitabine (Val LdC) and beta-L-2'-deoxyadenosine (LdA) are small molecule inhibitors of HBV DNA polymerase. These agents induce marked viral load reduction in the woodchuck infected with WHV. Telbivudine (LdT) is a specific and potent inhibitor of hepatitis B and is not active against HIV or other viruses. Clinical trials are in progress in HBeAg-positive patients at doses ranging from 25-400mg [36]; phase I studies [37] have shown a dose dependent 2-4 log reduction in HBV DNA after 4 weeks of treatment. A phase II trial testing doses of LdT 400 or 600mg with or without lamivudine in HBeAg-positive patients is in progress. An interim analysis of the results at 24 weeks indicates that LdT 400 and LdT 600mg result in 6 log declines in HBV DNA (compared to a 4 log decline in HBV DNA in lamivudine treated patients). Similarly, a phase III trial of the efficacy of 600mg LdT vs. 100mg lamivudine in HBeAg and anti-HBe-positive patients is in progress.

At this time the long-term efficacy and safety of these new unlicensed drugs and their role in the management of patients with HBeAg-positive chronic hepatitis are being assessed. Patients with chronic type B hepatitis require relatively long courses of treatment, and viral resistance may emerge. The end-points of treatment must be carefully evaluated. Combination treatments may become necessary in some, but not all patients.

REFERENCES

1. Dusheiko G, Dibisceglie A, Bowyer S, Sachs E, Ritchie M, Schoub B, Kew M. Recombinant leukocyte interferon treatment of chronic hepatitis B. Hepatology 1985;5:556-560.

2. 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;334:1422-1427.

3. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002: Geneva, Switzerland. Consensus statement. J Hepatol 2003;39:S3-S25.

4. Dienstag JL, Schiff ER, Mitchell M, Casey DE Jr, Gitlin N, Lissoos T, Gelb LD, Condreay L, Crowther L, Rubin M, Brown N. Extended lamivudine retreatment for chronic hepatitis B: Maintenance of viral suppression after discontinuation of therapy. Hepatology 1999;30:1082-1087.

5. Ling R, Mutimer D, Ahmed M, Boxall EH, Elias E, Dusheiko GM, Harrison TJ. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 1996;24:711-713.

6. Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER. Histological outcome during long-term lamivudine therapy. Gastroenterology 2003;124:105-117.

7. Villeneuve JP, Durantel D, Durantel S, Westland C, Xiong S, Brosgart CL, Gibbs CS, Parvaz P, Werle B, Trepo C, Zoulim F. Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. J Hepatol 2003;39:1085-1089.

8. Westland CE, Yang H, Delaney WE 4th, Gibbs CS, Miller MD, Wulfsohn M, Fry J, Brosgart CL, Xiong S; 437 and 438 Study Teams. Week 48 resistance surveillance in two phase 3 clinical studies of adefovir dipivoxil for chronic hepatitis B. Hepatology 2003;38:96-103.

9. Xiong X, Flores C, Yang H, Toole JJ, Gibbs CS. Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. Hepatology 1998;28:1669-1673.

10. Xiong X, Yang H, Westland CE, Zou R, Gibbs CS. In vitro evaluation of hepatitis B virus polymerase mutations associated with famciclovir resistance. Hepatology 2000;31:219-224.

11. 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;348:808-816.

12. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart

CL; Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of hepatitis Be antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800-807.

13. 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;1:431-435.

14. Ono-Nita SK, Kato N, Shiratori Y, Lan KH, Yoshida H, Carrilho FJ, Omata M. Susceptibility of lamivudine-resistant hepatitis B virus to other reverse transcriptase inhibitors. J Clin Invest 1999;103:1635-1640.

15. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, Lamy P, Murray A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. Hepatology 2000;32:129-134.

16. Walsh KM, Woodall T, Lamy P, Wight DG, Bloor S, Alexander GJ. Successful treatment with adefovir dipivoxil in a patient with fibrosing cholestatic hepatitis and lamivudine resistant hepatitis B virus. Gut 2001;49:436-440.

17. Mutimer D, Feraz-Neto BH, Harrison R, O'Donnell K, Shaw J, Cane P, Pillay D. Acute liver graft failure due to emergence of lamivudine resistant hepatitis B virus: rapid resolution during treatment with adefovir. Gut 2001;49:860-863.

18. Tillmann HL, Bock CT, Bleck JS, Rosenau J, Boker KH, Barg-Hock H, Becker T, Trautwein C, Klempnauer J, Flemming P, Manns MP. Successful treatment of fibrosing cholestatic hepatitis using adefovir dipivoxil in a patient with cirrhosis and renal insufficiency. Liver Transpl 2003;9:191-196.

19. Liaw YF. Management of YMDD mutations during lamivudine therapy in patients with chronic hepatitis B. J Gastroenterol Hepatol 2002;17:S333-S337.

20. Peters MG, Hann Hw H, Martin P, Heathcote EJ, Buggisch P, Rubin R, Bourliere M, Kowdley K, Trepo C, Gray Df D, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosgart CL. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101.

21. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. Lancet 2001;358:718-723.

22. Samuel D, Muller R, Alexander G, Fassati L, Ducot B, Benhamou JP, Bismuth H. Liver transplantation in European patients with the hepatitis B surface antigen. N Engl J Med 1993;329:1842-1847.

23. Grellier L, Mutimer D, Ahmed M, Brown D, Burroughs AK, Rolles K, McMaster P, Beranek P, Kennedy F, Kibbler H, McPhillips P, Elias E,

Dusheiko G. Lamivudine prophylaxis against reinfection in liver transplantation for hepatitis B cirrhosis. Lancet 1996;348:1212-1215.

24. Mutimer D, Dusheiko G, Barrett C, Grellier L, Ahmed M, Anschuetz G, Burroughs A, Hubscher S, Dhillon AP, Rolles K, Elias E. Lamivudine without HBIg for prevention of graft reinfection by hepatitis B: Long-term follow-up. Transplantation 2000;70:809-815.

25. Han SH, Kinkhabwala M, Martin P, Holt C, Murray N, Seu P, Rudich S, Hiserodt D, Imagawa D, Busuttil RW. Resolution of recurrent hepatitis B in two liver transplant recipients treated with famciclovir. Am J Gastroenterol 1998;93:2245-2247.

26. Markowitz JS, Martin P, Conrad AJ, Markmann JF, Seu P, Yersiz H, Goss JA, Schmidt P, Pakrasi A, Artinian L, Murray NG, Imagawa DK, Holt C, Goldstein LI, Stribling R, Busuttil RW. Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. Hepatology 1998;28:585-589.

27. Sousa JM, Pareja F, Serrano J, Gomez MA, Garcia I, Tamayo MJ, Diaz C, Martin C, Pascasio JM, Hinojosa R, Perez-Bernal JB, Canas E, Sayago M, Bernardos A. Comparison between levels of anti-HBS with a fixed administration dose of HBIG and a combination of HBIG and lamivudine for the prophylaxis of hepatitis B after liver transplantation. Transplant Proc 2003;35:723-724.

28. Davies SE, Portmann BC, O'Grady JG, Aldis PM, Chaggar K, Alexander GJ, Williams R. Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. Hepatology 1991;13:150-157.

29. Lau JY, Bain VG, Davies SE, O'Grady JG, Alberti A, Alexander GJ, Williams R. High-level expression of hepatitis B viral antigens in fibrosing cholestatic hepatitis. Gastroenterology 1992;102:956-962.

30. Mutimer D, Pillay D, Dragon E, Tang H, Ahmed M, O'Donnell K, Shaw J, Burroughs N, Rand D, Cane P, Martin B, Buchan S, Boxall E, Barmat S, Gutekunst K, McMaster P, Elias E. High pre-treatment serum hepatitis B virus titre predicts failure of lamivudine prophylaxis and graft re-infection after liver transplantation. J Hepatol 1999;30:715-721.

31. Mutimer D, Pillay D, Shields P, Cane P, Ratcliffe D, Martin B, Buchan S, Boxall L, O'Donnell K, Shaw J, Hubscher S, Elias E. Outcome of lamivudine resistant hepatitis B virus infection in the liver transplant recipient. Gut 2000;46:107-113.

32. Schiff ER, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann HL, Samuel D, Zeuzem S, Lilly L, Rendina M, Villeneuve JP, Lama N, James C, Wulfsohn MS, Namini H, Westland C, Xiong S, Choy GS, Van Doren S, Fry J, Brosgart CL; Behalf of the Adefovir Dipovoxil Study 435 International Investigators Group. Adefovir dipivoxil therapy for lamivudine-

resistant hepatitis B in pre- and post-liver transplantation patients. Hepatology 2003;38:1419-1427.

33. Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, Dehertogh D. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. Gastroenterology 2002;123:1831-1838.

34. Chong Y, Chu CK. Understanding the unique mechanism of L-FMAU (Clevudine) against hepatitis B virus: molecular dynamics studies. Bioorg Med Chem Lett 2002;12:3459-3462.

35. Marcellin P, Mommeja-Marin H, Sacks SL, Lau GK, Sereni D, Bronowicki JP, Conway B, Trepo C, Blum MR, Yoo BC, Mondou E, Sorbel J, Snow A, Rousseau F, Lee HS. Anti-HBV activity and tolerability of clevudine, a novel nucleoside analogue. Initial results of a phase I/II 28 day study. Hepatology 2004;40:140-148.

36. Standring DN, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Cretton-Scott E, Schinazi RF, Myers M, Bryant ML, Sommadossi JP. Antiviral beta-L-nucleosides specific for hepatitis B virus infection. Antivir Chem Chemother 2001;12:119-129.

37. Bryant ML, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Marion P, Cretton-Scott E, Schinazi RF, Sommadossi JP. Antiviral L-nucleosides specific for hepatitis B virus infection. Antimicrob Agents Chemother 2001;45:229-235.

Treatment of HBeAg-Positive Chronic Hepatitis B with Conventional or Pegylated Interferon

Solko W. Schalm, Harry L.A. Janssen

INTRODUCTION

Despite the introduction of an effective hepatitis B vaccine in the early 1980s, infection with hepatitis B virus (HBV) is not a problem to be consigned to the past. Although the prevalence of chronic HBV is relatively low in Western Europe and the United States, it is 2-7% in southern and eastern Europe and 8-15% in Africa, some parts of Asia, and the western Pacific. Chronic HBV infection currently affects an estimated 400 million people, making it one of the world's most common infectious diseases, and it is among the world's 10 leading causes of death [1]. Unfortunately, the hepatitis B vaccine is not an option for patients who are already chronically infected with HBV; currently antiviral therapy is the only recourse [1].

The aim of this review is to provide practicing physicians with a brief, pragmatic overview of current concepts regarding treatment strategies in hepatitis Be antigen (HBeAg)-positive patients, the role of interferon in the strategy and the potential of pegylated interferon which is likely to be available for use in the near future.

NATURAL HISTORY AND PATIENT CLASSIFICATION

Chronic HBV infection is defined by the persistence of serum hepatitis B surface antigen (HBsAg) for 6 months or longer and accounts for most HBV-related morbidity and mortality. The onset of chronic HBV is characterized by persistent HBV DNA, HBsAg, and HBeAg in serum following infection. Patients with chronic HBV can be classified according to several phases of viral replication and immunologic responsiveness. When acquired in childhood, chronic HBV starts with the "immune tolerance" phase. In this high-replicative phase, HBeAg and high levels of HBV DNA are detectable, but ALT is normal, histological activity is minimal, and the patient is asymptomatic [2]. This phase may persist for 20-30 years. The second phase, which typically occurs between ages 15-35, is termed the "immune clearance" phase and is characterized by declining rates of HBV replication, transient alanine aminotransferase (ALT) elevation (hepatic flares), and hepatic necroinflammatory disease and fibrosis. Transition to the third phase is marked by seroconversion of HBeAg to HBeAg antibody (anti-HBe). Following seroconversion, ALT levels usually normalize, and hepatic damage is repaired. This third phase of "immune control" is characterized by persistent serum HBsAg, undetectable HBeAg, HBV DNA $<10^5$ copies/mL and normal serum ALT and is now widely called the "inactive HBsAg carrier state" [3].

The "inactive HBsAg carrier state" is usually associated with disease remission for decades, but, according to recent observations, mainly in patients with genotype A. In contrast, patients, particularly those living in Asia and southern Europe and with HBV genotype B, C or D, may develop reactivation of hepatitis with persistent or intermittent ALT elevations; they remain HBeAg-negative while simultaneously showing elevated levels of serum HBV DNA. This fourth phase due to "immune escape" is known as chronic HBeAg-negative HBV, this is the second of the two major forms of the disease and is also potentially progressive [4].

It is also noteworthy that each year, 5-15% of patients with chronic HBV seroconvert spontaneously [3]. By the time seroconversion occurs, however, the disease will already have progressed to cirrhosis in a proportion of patients [5], a fact that provides an implicit rationale for intervention.

DESIRABLE END-POINTS OF THERAPY AND TREATMENT OPTIONS

Ideally, the objective of treatment in chronic HBV is complete viral eradication. However, due to the difficulty of eliminating viral DNA from affected hepatocytes, it is unclear whether this is an attainable goal [6]. From a practical perspective, the main objective of therapeutic intervention is to stop progression of disease-related liver injury. This objective will be nearly achieved when the patient is brought into and maintained in the "inactive HBsAg carrier state", characterized by persistent serum HBsAg, undetectable HBeAg, HBV DNA $<10^5$ copies/mL and normal serum ALT. The increasing awareness that the disease may reactivate is changing the desired goal of treatment to complete serologic resolution of chronic hepatitis B, characterized by the additional clearance of serum HBsAg. After HBsAg seroconversion reactivation is rare and the long-term outcome is further improved [3, 6].

Various antiviral and immunomodulatory agents have been evaluated for the treatment of chronic HBV. However, until 2004, only interferon, lamivudine and adefovir have been judged to be sufficiently safe and effective to warrant approval in most countries. In the last year large randomized trials have been completed that document the safety and effectiveness of pegylated interferon and submission for regulatory approval of two forms of pegylated interferon is currently ongoing. For the practicing physician it is important to summarize the results of the trials performed in HBeAg-positive chronic hepatitis B and to describe the advantages and disadvantages of interferon-based therapy versus treatment with nucleoside analogs.

INTERFERON

Interferons are potent, naturally occurring cytokines that have antiviral, immunomodulating, and antiproliferative effects. Their complex scope of activity is mediated through a variety of inhibitory mechanisms that may affect most steps of viral replication. For many viruses, inhibition of protein synthesis appears to be the major inhibitory mechanism.

The predominant cause of viral persistence during HBV infection is generally thought to involve a weak immune response to viral

antigens. Immune tolerance to high viral burdens almost certainly plays a key role in chronicity following neonatal acquisition of HBV, but the specific basis for inhibited viral clearance in adult-acquired disease is less well-defined. One of several abnormalities found in the immunologic profile of patients with chronic HBV infection is deficient production or attenuated response to interferon [7]. This, coupled with the fact that some patients with chronic HBV respond to exogenous interferon, provides the basis for clinical use of the agent. The effects of natural interferon in the treatment of chronic HBV infection were first reported in small studies in the late 1970s. The recombinant product interferon has received extensive clinical use in the treatment of chronic hepatitis B for at least a decade.

EFFICACY FOR SEROLOGIC END-POINTS

A 1993 meta-analysis of 15 well-controlled studies [8] is the most frequently cited report on interferon in the treatment of chronic HBV. The aggregate population described included 837 HBeAg-positive adults with compensated liver disease who were treated with doses of 5-10MU three times weekly for 4-6 months. The primary indices of efficacy were loss of HBsAg and HBeAg. In this meta-analysis, interferon was associated with significant treatment effects on both efficacy indices. A significant treatment effect was also shown for normalization of ALT. Overall, loss of HBsAg and HBeAg in interferon-treated patients was about 6% and 20% respectively more frequent than that occurring spontaneously in the control group.

There is evidence that elevated ALT at baseline is an important predictor of favorable response to interferon (Table 1).

Baseline ALT	Placebo (n=196)	Interferon† (n=68)
>1 x ULN	20/171 (11%)	14/66 (21%)
>2 x ULN	16/112 (14%)	11/42 (26%)
>3 x ULN	7/28 (25%)	4/10 (40%)

Table 1: Rates of seroconversion (loss of HBeAg + anti-HBe) with interferon-alpha at week 52.
†Treatment duration=16 weeks
From Perrillo RP et al. [20].

Although interferon has been widely used in the treatment of chronic HBV for more than a decade, the optimal treatment duration remains uncertain. The typical recommendation is for a 16-week course of 10MU three times per week. However, a recent European Concerted Action on Viral Hepatitis (EUROHEP) study found that a second 16-week course in patients who remained HBeAg-positive at the conclusion of the first course produced a higher rate of seroconversion compared to a control group who received no additional treatment (28% vs. 12%, respectively; *P*=0.04) [9].

EFFICACY FOR CLINICAL END-POINTS

The long-term effects of interferon therapy on morbidity and mortality are less well defined and are more controversial. Most studies compared the survival of interferon treated patients with that of untreated controls [10-14]. Three studies [10, 11, 13] found prolonged survival and a decreased incidence of hepatocellular carcinoma or complications in general after HBeAg clearance; in two studies [12, 14] this effect was not demonstrated. The difference between the results of these studies may be explained by the limited follow-up or the low rate of complications in general due to the inclusion of mainly young patients with less advanced disease. In a recent study of 165 HBeAg-positive patients responders to interferon therapy were compared to non-responders [15]. Response to treatment was defined as HBeAg loss within 12 months after the end of therapy, thus

separating interferon-induced responses from later spontaneous HBeAg loss. Median follow-up was 8.8 years. Fifty-four patients (33%) responded to interferon treatment. Relapse (HBeAg reactivation) was observed in 7 (13%) responders. Loss of HBsAg occurred in 52% of responders compared to 9% of the initial 111 non-responders (Figure 1). Hepatocellular carcinoma was found in 8 patients, 6 of whom were non-responders and one relapser. Multivariate analysis showed significantly improved survival and reduced risk of developing hepatocellular carcinoma in responders. These results provide further support that the response to interferon therapy increases survival and reduces the risk of developing hepatocellular carcinoma.

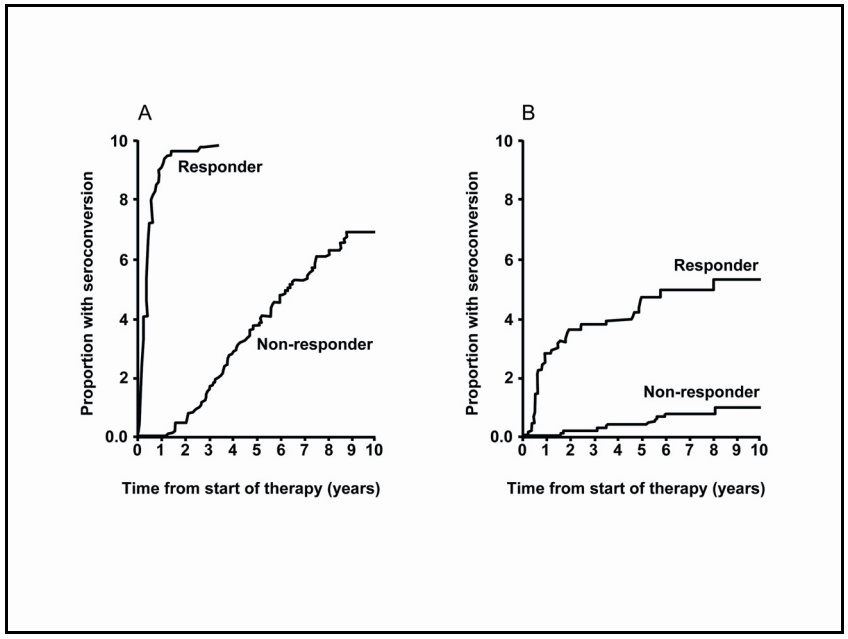

Figure 1: Time to HBeAg loss (A) and HBsAg loss (B) of responders to interferon therapy compared to non-responders. Response was defined as loss of HBeAg within 12 months after the end of interferon therapy.

STRENGTHS AND DRAWBACKS

The advantages of interferon are that treatment is given for a limited time, viral resistance does not occur, and drug-induced seroconversion is sustained in 80-90% of patients. Most importantly, response to interferon is associated with clinically proven reductions in HCC and increased survival [10, 11, 13, 15].

Interferon therapy is not without drawbacks. Interferon can only be administered by subcutaneous injection. Moreover, doses of 5-10MU are usually associated with an acute influenza-like syndrome that may include fever, chills, headache, myalgia, and gastrointestinal disturbances. Tolerance to these effects develops in most patients, but additional toxicities, such as depression and other central nervous system (CNS) disturbances, thyroid dysfunction, and blood cytopenias, may also occur. A large meta-analysis found that dose reduction of interferon was required in approximately 20% of patients; however, adverse events necessitated treatment withdrawal in only about 5% [8].

Some evidence suggests that Chinese patients may have lower rates of response to interferon than other groups [14]. However, differences in variables predictive of response to interferon probably play a role in the between-group differences attributed to race. For example, HBeAg-positive Asians with high baseline levels of ALT respond to interferon as well as other ethnic groups, but patients with low baseline ALT tend to respond poorly to interferon regardless of ethnicity [4]. However, in Asia many patients who acquire HBV during the perinatal period have low ALT during the protracted, immune-tolerance phase of the disease.

PEGYLATED INTERFERON

Pegylated versions of interferon were recently developed to address the short plasma half-life of interferon. The covalently attached polyethylene glycol moiety improved the pharmacokinetic properties of the molecule [16], resulting in greater efficacy, a more convenient once-weekly dosing schedule, and a similar safety profile in patients with chronic HCV infections [17]. Pegylated interferon has already been approved for the treatment of hepatitis C.

Preliminary studies in patients with chronic hepatitis B infections suggest that pegylated interferon will be more effective than interferon in these patients as well.

In the first large randomized controlled trial published in 2003 [18], 194 patients with chronic HBeAg-positive hepatitis, who had not been previously treated with interferon, were randomized to 3 doses of pegylated interferon-alpha-2a given weekly by subcutaneous injection or to conventional interferon-alpha-2a given three times weekly. The treatment duration was 24 weeks, with a 24 week follow-up. At the end of follow-up HBeAg was no longer detectable in 37%, 35% and 29% of patients receiving pegylated interferon 90μg, 180μg and 270μg, respectively, compared to 25% of patients on standard interferon. Two patients on pegylated interferon cleared HBsAg during treatment and remained negative for HBsAg to the end of follow-up. ALT normalization at the end of follow-up was observed in 43%, 35%, and 31% of patients on pegylated interferon 90μg, 180μg and 270μg, respectively, and in 26% of patients on standard interferon.

Additional favorable outcomes were the low proportion of patients who prematurely discontinued study medication (2% and 4% for pegylated interferon and standard interferon, respectively). The incidence of pyrexia, myalgia, severe fatigue, anorexia, insomnia and dizziness was similar for pegylated interferon and standard interferon, whereas headache, alopecia, nausea and diarrhoea were reported more frequently in patients receiving pegylated interferon. Dose modifications for laboratory abnormalities (usually neutropenia or elevated ALT values) occurred in 22-30% of patients receiving pegylated interferon vs. 10% of patients on standard interferon.

In patients with cirrhosis, adverse effects were not more prevalent, whereas 54% of 13 patients receiving pegylated interferon lost HBeAg (none of 4 cirrhotic patients on standard interferon had an HBeAg response). Other baseline factors known to be predictive of nonresponse (low ALT, high HBV DNA, genotype C) may also be less important with pegylated interferon therapy, since the 10% difference in response rates between pegylated interferon and conventional interferon was maintained in all subgroups, according to preliminary findings.

The conclusion that pegylated interferon is more advantageous than conventional interferon is not generally accepted in view of the,

according to the critics, low dose of conventional interferon used. It should be noted that licensed dose regimens differ for different interferons and different parts of the world; also, in retrospect, the superiority of the commonly used schedule of 10MU t.i.w. is hardly based on robust dose-response studies. When pharmocodynamics are investigated, pegylated interferon appears to have a greater antiviral effect than conventional interferon, at 4.5 or 10MU t.i.w. (Figure 2).

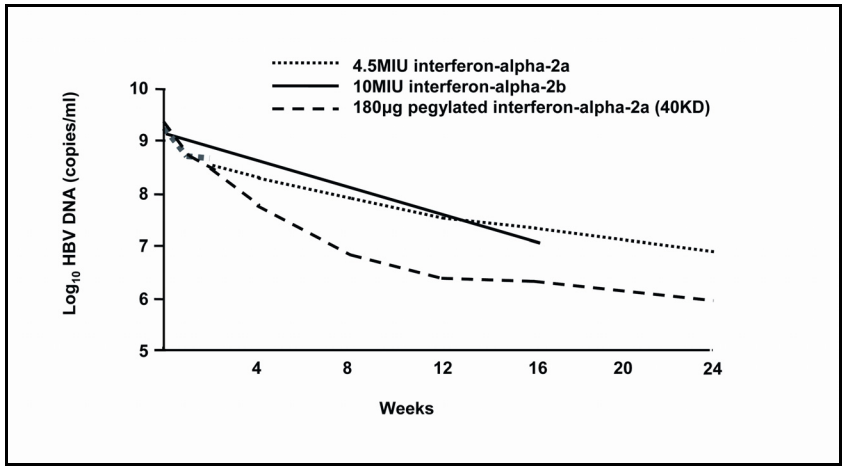

Figure 2: Pharmocodynamics of 180µg of pegylated interferon-alpha-2a compared to two standard regimens of conventional interferon-alpha-2a and alpha-2b in chronic hepatitis B. Note the steeper decline in serum HBV DNA levels in patients receiving pegylated interferon (data derived from Cooksley [18] and the EUROHEP study reported by Janssen [9]).

In a preliminary report from a second large trial [19], 266 patients with HBeAg-positive chronic hepatitis B and serum ALT levels at least twice the upper limit of normal, were randomized to pegylated interferon-alpha-2b 100µg/week and lamivudine 100mg/day or pegylated interferon-alpha-2b and placebo. Treatment was given for 52 weeks; however, the dose of pegylated interferon was reduced to 50µg at 32 weeks.

About 80% of patients completed therapy on the full dose; and dose reduction was about 10% in the first 8 weeks and another 10% between week 8 and week 26; thereafter dose reduction was rare. Ten percent of patients prematurely discontinued pegylated interferon, mainly between weeks 0 and 32; and this percentage was similar to the 9% discontinuation rate with conventional interferon for 16-32 weeks in 162 control patients participating in a multicenter randomized trial comparing short- and prolonged interferon therapy between 1994-1998. Psychiatric disturbances (3%: depression, psychosis), cytopenias (1%: anemia, neutropenia, thrombocytopenia) and flu-like syndrome (1%) were the causes with multiple discontinuations.

Thirty-five percent of patients receiving pegylated interferon lost HBeAg after the end of follow-up and there was no difference between the group receiving pegylated interferon alone and that receiving pegylated interferon plus lamivudine. Response rates (loss of HBeAg) varied by genotype: genotype A, 47%; genotype B, 44%; genotype C, 28%; and genotype D, 25%.

Together, these studies in 501 patients with chronic hepatitis B from both Asia and Europe suggest excellent tolerance of pegylated interferon therapy up to 1 year. The verdict on increased efficacy in comparison to conventional interferon awaits further study with stratification for genotype.

DISCUSSION

Effective management of the millions of patients chronically infected with HBV remains an important clinical objective. The introduction of nucleoside analogs such as lamivudine and adefovir represents an important advancement for tolerance, but questions persist about its long-term efficacy compared to interferon. Given the controversy about the durability of response and the significance of emergent drug resistance with nucleoside analogs, it seems premature to dismiss interferon-based treatment as an outmoded therapy. In fact, for many patients interferon or pegylated interferon may be the first choice of treatment.

Despite the success with interferon-based and nucleoside analogue monotherapy in a proportion of patients with chronic HBV, clinical and epidemiologic realities raise the question of how this problem can be managed more effectively. From a long-term perspective, one can speculate that the definitive treatment approach

will be based on the discovery of the reason(s) why 5% of immunocompetent adults exposed to HBV develop chronic infection, whereas 95% do not. Further investigation is needed to determine how immune control can be induced and - as important - can be maintained in patients with genotypes B, C, and D. Given the high prevalence of chronic HBV, clinicians who address this important disease need to make the most of the various options at hand.

REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.

2. Lok ASF. Chronic hepatitis B. N Engl J Med 2002;30;346:1706-1713.

3. Lok ASF, Heathcote J, Hoofnagle J. Management of hepatitis B: 2000-summary of a workshop. Gastroenterology 2001;120:1828-1853.

4. Hadziyannis SJ, Vassilopoulos D. Hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.

5. Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, Alberti A, Realdi G. Clinical, virological and histologic outcome following seroconversion from HbeAg to anti-Hbe in chronic hepatitis type B. Hepatology 1986;6:167-172.

6. Malik A, Lee W. Chronic hepatitis B virus infection: treatment strategies for the next millenium. Ann Intern Med 2000;132:723-731.

7. Ikeda T, Lever A, Thomas H. Evidence for a deficiency of interferon production in patients with chronic hepatitis B virus infection in adult life. Hepatology 1986;6:962-965.

8. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. Ann Intern Med 1993;119:312-323.

9. Janssen HL, Gerken G, Carreno V, Marchellin P, Naoumov NV, Craxi A, Ring-Larsen H, Kitis G, van Hattum J, de Vries RA, Michielsen PP, ten Kate FJ, Hop WC, Heijtink RA, Honkoop P, Schalm SW. Interferon-alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. Hepatology 1999;30:238-243.

10. Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, Haussinger D. Long-term follow-up of patients with chronic hepatitis B treated with interferon-alfa. Gastroenterol 1997;113:1660-1667.

11. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon-alfa. Hepatology 1997;26:1338-1342.

12. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow Up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-397.

13. Lin S, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-975.

14. Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: the effect on hepatitis Be antigen seroconversion and the development of cirrhosis-related complications. Hepatology 2001;43:139-145.

15. Van Zonneveld M, Honkoop P, Hansen BE, Niesters HGM, Darwish Murad S, de Man RA, Schalm SW, Janssen HLA. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis. B Hepatology 2004;39:804-810.

16. Glue P, Fang JW, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, Salfi M, Jacobs S. Pegylated interferon-alpha-2b: pharmacokinetics, pharmaco-dynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. Clin Pharmacol Ther 2000;68:556-567.

17. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J, Brunda MJ. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343:1666-1672.

18. Cooksley WGE, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, Yu Chang W, 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;10:298-305.

19. Janssen HLA, Senturk H, Zeuzem S, Akarca U, Cakaloglu Y, Simon K, So Man Kit T, Gerken G, Schalm SW. Peginterferon alfa-2b and lamivudine combination therapy compared with peginterferon alfa-2b for chronic HBeAg-positive hepatitis B: a randomized controlled trial in 307 patients. Hepatology 2003;38.246A.

20. Perrillo RP, Lai CH, Liaw YF, Dienstag JL, Schiff ER, Schalm SW, Heathcote J, Brown NA, Atkins M, Woessner M, Gardner SD. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36:186-194.

Case Study in the Management of Patients with HBV-Related Decompensated Cirrhosis

Robert P. Perrillo

CASE

A 60 year-old Middle Eastern male with no prior history of chronic hepatitis B is admitted to the intensive care unit in grade II encephalopathy. He is found to be hepatitis B surface antigen (HBsAg)-positive and hepatitis B Core antigen, antibody (Anti-HBc) IgM-negative. His aspartate aminotransferase (AST) is 153, alanine aminotransferase (ALT) is 90IU/L; bilirubin is 1.4mg/dL, and international normalized ratio (INR) is 1.3. He has mild to moderate ascites. He is hepatitis Be antigen (HBeAg)-negative but his hepatitis B virus (HBV) DNA is pending. His blood urea nitrogen (BUN) is 34 and his serum creatinine is 1.5 (upper limit of normal 1.4). What would you do?

(a) No treatment, wait for serum HBV DNA results
(b) Start lamivudine
(c) Start adefovir dipivoxil
(d) Begin evaluation for liver transplantation and start lamivudine
(e) Begin evaluation for liver transplantation and start adefovir

Further studies become available. The patient's HBV DNA is found to be 3.2 x 10^7 copies/mL by a commercially available polymerase chain reaction (PCR). He is Anti-HBe-positive. His INR is repeated (next day) and it is now 1.7. His serum creatinine is 1.6 despite hydration and colloid expansion. Would this change your thinking about how to approach management?

(a) I would start lamivudine because of concerns about nephrotoxicity with adefovir
(b) I would start adefovir as soon as the patient's renal dysfunction improves
(c) I would use adefovir knowing that I could adjust the dose according to creatinine clearance as listed in the package literature
(d) I would not worry about starting adefovir since he is unlikely to have severe significant renal dysfunction

The patient is started on lamivudine. Six weeks later, he no longer has encephalopathy, AST, ALT and serum bilirubin have decreased to normal range, and serum HBV DNA has declined to 1.2 x 10^4 copies/mL. His serum creatinine has stabilized at 1.4, and the patient's INR is now 1.2.

The patient is maintained on lamivudine and does well for 11 months when his AST and ALT increase to 80 and 63, respectively. Serum HBV DNA is now 2.5 x 10^6 copies/mL. Genotyping results show lamivudine resistance (double mutant at positions 180 and 204). His serum creatinine is 1.7. Creatinine clearance is 40mL/minute. Now what would you do?

(a) Stop the lamivudine and start adefovir
(b) Add adefovir to lamivudine maintenance
(c) Add adefovir and consider stopping the lamivudine after two to three months
(d) Maintain the patient on lamivudine alone

NUCLEOSIDE ANALOGS FOR PATIENTS WITH ADVANCED CHRONIC HEPATITIS B

Nucleos(t)ide analogs reduce viral replication by competitive inhibition of HBV DNA polymerase and are the preferred treatment for patients with decompensated cirrhosis. They do not have any direct immunologic activity and only rarely have been associated with flares of aminotransferase levels [1]. Unlike interferon, these agents do not suppress bone marrow function.

Lamivudine use in stable cirrhosis

Long-term data on lamivudine maintenance therapy for advanced hepatitis B have recently been reported [2]. In a study with more than 600 patients with clinically compensated stage 4 or greater fibrosis (Ishak score), lamivudine was compared to placebo in the ability to prevent disease progression. In this study, lamivudine therapy (median treatment exposure 32 months) was shown to significantly reduce disease progression and the risk of hepatocellular carcinoma. In addition, the presence of YMDD mutant HBV reduced, but did not totally nullify the benefit of treatment.

Lamivudine use in decompensated cirrhosis

Lamivudine therapy has been a major breakthrough in the management of patients with decompensated HBV-related cirrhosis due to its potent inhibitory effects on HBV replication and its excellent safety record [3]. Treatment with lamivudine monotherapy before and after liver transplantation has provided proof of concept that viral suppression often improves the clinical status of patients and reduces the risk of recurrent infection after liver transplantation [4]. Lamivudine may also increase the likelihood of transplant-free survival. Investigators at the University of California, San Francisco, for example, have demonstrated that time to death or transplantation was significantly longer (P<001) and transplantation was less frequent (35% vs. 74%) in lamivudine treated patients compared to a historical untreated cohort that was matched for age, sex, and baseline Child Pugh status [5].

The major downside of lamivudine has been the high rate of resistance when used for longer than one year, and this appears to have greater clinical consequences in patients with diminished parenchymal reserve. Accordingly, the timing of initiation of lamivudine treatment

was a key issue in the past because patients with long waiting times for liver transplantation might develop resistance while awaiting surgery. Fortunately, this is no longer the case because patients with lamivudine resistance can be treated with adefovir dipivoxil or tenofovir isofumarate [6, 7]. HBV DNA and serum aminotransferase levels often remain lower than baseline after the emergence of lamivudine resistance, but without specific treatment histological responses have been shown to eventually become blunted in clinically stable patients [8]. Some reports suggest that progressive liver injury and liver failure occur more commonly when drug resistance occurs after liver transplantation, and this may be due to the viral enhancing effects of anti-rejection therapy [9].

Adefovir dipivoxil use in stable or decompensated cirrhosis

This is an oral prodrug of adefovir, a nucleotide analog with antiviral activity against both wild-type and YMDD mutant HBV. The remarkable thing about this drug is that resistance is rare, occurring in 2% of patients after two years of continuous use and approximately 4% of patients after 3 years [10]. Adefovir (10mg) can be used safely and effectively in patients with YMDD mutant HBV irrespective of whether the patient has clinically stable cirrhosis, decompensated cirrhosis or recurrent hepatitis B after liver transplantation (Figure 1) [6, 11, 12]. The decrease in HBV DNA levels has been associated with a favorable effect on Child Pugh status as well as improvement in biochemical parameters of liver function.

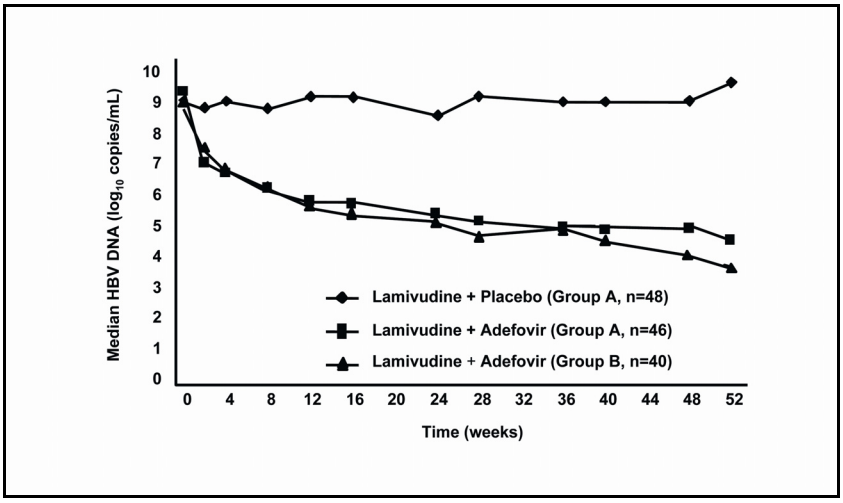

Figure 1: Virological and biochemical profiles of three groups of patients with YMDD mutant HBV who were either maintained with lamivudine alone (♦) or lamivudine and adefovir (■ and ▲). Group B patients (n=40) either had decompensated cirrhosis or recurrent hepatitis B after liver transplantation. Group A patients (n=94) were clinically stable.

Nephrotoxicity has been observed when adefovir is used in higher doses (30mg or more) than is currently recommended for hepatitis B (10mg). Thus, there may be some concern about the long-term administration of this agent in patients with concomitant renal dysfunction, particularly in patients who are treated after liver transplantation. While dose reduction according to the package insert based on creatinine clearance is advised, it is not known if this interferes with the ability of the drug to maintain viral suppression. In a recently reported study in which 324 patients were treated with adefovir before or after liver transplantation, changes in renal status were confirmed in 13% of patients [6]. Most of these patients had pre-existing renal dysfunction or were taking potentially nephrotoxic medications, however, making it difficult to attribute the change in renal function to adefovir therapy.

Other agents

There are several nucleoside analog drugs under development that have antiviral activity against YMDD mutant as well as wild type HBV [13]. Early clinical trials with entecavir look promising [14]. As this nucleoside has no known nephrotoxicity, it may play a particularly important role in the future management of renally compromised patients with lamivudine resistant HBV. Tenofovir is a drug which is chemically related to adefovir but is considerably less likely to be nephrotoxic at the effective dose (300mg). It may have even greater antiviral efficacy against lamivudine resistant HBV than adefovir [7]. Recently, the author treated a decompensated cirrhotic patient with tenofovir. The patient had marked improvement (Child Pugh C conversion to A) after just 4 months of therapy, and continued to do well with maintenance therapy (Figure 2). It is clear that in the relatively near future a number of therapeutic options will become available for lamivudine resistant patients with decompensated cirrhosis.

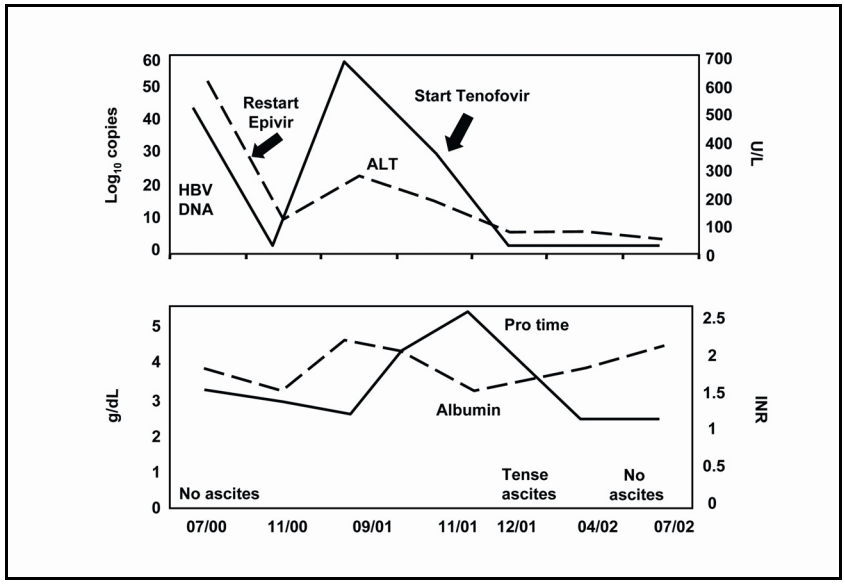

Figure 2: Virological and biochemical events in a 39 year-old nurse with decompensated cirrhosis due to HBeAg-positive chronic hepatitis B. The patient had been formerly treated successfully with lamivudine for two years and had stopped taking this nine months earlier. At the time of the reactivated hepatitis, lamivudine was restarted unsuccessfully. The patient continued to worsen and was started on tenofovir because adefovir was not yet available. Within 4 months, the patient had dramatic virologic, biochemical, and clinical improvement, going from Child Pugh C to A status. The patient died of unrelated causes two years later.

CASE DISCUSSION

There are several reasons to have instituted nucleoside analog therapy in the current case. First and foremost, is the potential for clinical stabilization. Despite the patient's severe encephalopathy he had a relatively low MELD score (15) upon presentation and was likely to be transplanted several months or more in the future. This interval provided time for continued viral suppression and clinical improvement. The literature supports the concept that the degree of

clinical improvement may reduce the need for transplantation [4, 6]. The second reason for treatment with a nucleoside analog is reduction in risk of recurrent hepatitis B. Lamivudine has been shown, for example, to reduce the risk of recurrent hepatitis B infection by approximately 40% [4]. Thus, the key question in the current case is not whether to use nucleoside analog therapy but which one to use as initial therapy? The emerging renal dysfunction in this patient makes lamivudine a better initial choice than adefovir. Tenofovir would also have been a reasonable (although more expensive) choice for first line therapy due to a lack of nephotoxicity at the 300mg dose, low resistance profile, and the probable need for long-term antiviral suppression in this patient with HBeAg-negative chronic hepatitis B.

Once resistance to lamivudine develops, as in this case, lamivudine should be continued in combination with a second nucleotide like adefovir or tenofovir. This is based upon the observation that a significant number of such individuals will have an ALT flare which is probably due to rapid emergence of wild type HBV. In one study in which lamivudine resistant patients received adefovir monotherapy, 37% of patients had an ALT flare of 5-10 times the upper limit of normal soon after discontinuing lamivudine [15].

Such flares have not been observed when patients are maintained on adefovir in combination with ongoing lamivudine maintenance therapy [12, 15].

CONCLUSIONS AND PERSPECTIVES

Cirrhosis due to infection with HBV is associated with decreased survival, but interferon can be dangerous in patients with relatively mild hepatic decompensation and should not be used once liver failure has emerged. Treatment of patients with advanced hepatitis B has been made simpler and safer with nucleoside analog therapy. Lamivudine resistance can be overcome with adefovir, but the potential for nephrotoxicity may make it difficult to use this agent in patients who are renally compromised. In the future, the development of alternative nucleoside analogs with greater antiviral potency, a low rate of viral resistance, and even better safety profiles will further improve our options in treating these seriously ill patients.

REFERENCES

1. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated disease. Gastroenterology 2001;120:1009-1022.

2. Liaw YF, Sung JJY, Chow WC, Shue K, Keene O, Farrell G. Effects of lamivudine on disease progression and development of liver cancer in advanced chronic hepatitis B: a prospective double-blind placebo-controlled clinical trial. 54th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, October 24-28,2003.

3. Perrillo RP. Management of the patient with hepatitis B virus-related cirrhosis. J Hepatol 2003;39:S177-S180.

4. Perrillo R, Wright T, Rakela J, Levy G, Schiff E, Gish R, Martin P, Dienstag J, Adams P, Dickson R, Anschuetz G, Bell S, Condreay L, Brown N, and the Lamivudine North American Transplant Group. A multicenter United States-Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. Hepatology 2001;33:424-432.

5. Yao F, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. Hepatology 2001;34:411-416.

6. Schiff ER, Lai C-L, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann HL, Samuel D, Zeuzem S, Lilly L, Rendina M, Villeneuve JP, Lama N, James C, Wulfsohn MS, Namini H, Westland C, Xiong S, Choy GS, Van Doren S, Fry J, Brosgart CL. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post-liver transplantation patients. Hepatology 2003;38:1419-1427.

7. Van Bommel F, Wunsche T, Schurmann D, Bergk A, Wiedenmann B, Hopf U, Berg T. A comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus (HBV) infection. 54th Annual Meeting of the American Association for the Study of Liver Diseases, Boston Massachusetts, October 24-28, 2003.

8. Dienstag JL, Goldin RD, Heathcote JE, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER. Histological outcome during long-term lamivudine therapy. Gastroenterology 2003;124:105-117.

9. Mutimer D, Pillay D, Shields P, Cane P, Ratcliffe D, Martin B, Buchan S, Boxall L, O'Donnell K, Shaw J, Hubscher S, Elias E. Outcome of lamivudine resistant hepatitis B virus infection in the liver transplant recipient. Gut 2000;46:107-113.

10. Qi X, Snow A, Thibault V, Zhu Y, Curtis M, Hadziyannis SJ, Brosgart CL, Currie G, Arterburn S, Gibbs CS, Miller MD, Xiong S. Long-term incidence of adefovir dipivoxil (ADV) resistance in chronic hepatitis B (CHB) patients after 144 weeks of therapy. 39th Annual Meeting of the European Association for the Study of the Liver, Berlin, Germany, April 11-14, 2004.

11. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, Lamy P, Murray A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. Hepatology 2000;32:129-134.

12. Perrillo R, Hann HW, Mutimer D, Willems B, Leung N, Lee WM, Moorat A, Gardner S, Woessner M, Bourne E, Brosgart CL, Schiff E. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology 2004;126:81-90.

13. Buti M, Esteban R. Entecavir, FTC, L-FMAU, LdT and others. J Hepatol 2003;39:S139-S142.

14. Chang T-T, Hadziyannis S, Cianciara J, Rizzeto M, Schiff E, Pastore G, Klesczewski K, Denisky G, DeHertogh D, Hindes R. Sustained viral load and ALT reduction following 48 weeks of entecavir treatment in subjects with chronic hepatitis B who have failed lamivudine. 53rd Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, November 1-5, 2002.

15. Peters MG, Hann HW, Martin P, Heathcote J, Buggisch P, Rubin R, Bourliere M, Kowdley K, Trepo C, Gray DF, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosgart CL. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101.

Treatment of HBeAg-Negative Chronic Hepatitis B with Nucleoside/Nucleotide Analogs

Stephanos J. Hadziyannis

INTRODUCTION AND HISTORICAL VIEW

Hepatitis Be antigen-negative/hepatitis Be antigen, antibody-positive (HBeAg-negative/anti-HBe-positive) chronic hepatitis B (CHB) was first identified in the early 1980s [1-3]. At that time, interferon-alpha was not yet available, while several other drugs were tried in CHB and proved to have little if any therapeutic benefit. In the mid 1980s, when conventional interferon-alpha became available for HBeAg-positive CHB, its efficacy was also evaluated in HBeAg-negative liver disease [4]. Four small randomized controlled trials (RCTs) of a short duration were performed in Greece and Italy, where this form of CHB prevails [3-7]. Although high end-of-treatment (EOT) response rates were achieved in these early studies, most patients who responded during therapy, relapsed soon after interferon was stopped. Thus the overall sustained response (SR) rate was not promising [3, 4]. These early "negative" treatment results in a disease that was generally viewed as atypical and rare [5, 6], discouraged pharmaceutical companies and most - but not all - clinical investigators from further studies on interferon-based therapies in HBeAg-negative CHB [8]. At the same time molecular biology made an impressive entry in the field of viral hepatitis and in 1989 replication competent hepatitis B virus (HBV)

mutants harboring a novel pre-core stop-codon which abolishes HBeAg production were discovered in patients with HBeAg-negative CHB [9-11] and explained, on a molecular basis, how HBeAg (a marker of HBV replication) can be negative despite ongoing HBV replication. These observations stimulated further research on several aspects of HBeAg-negative CHB, including epidemiology and treatment [12-15]. At present its frequency appears to be increasing worldwide [12-14] and in the Mediterranean area of Europe as well as in France and Germany HBeAg-negative type CHB represents 65-95% of newly diagnosed cases of CHB [12, 14, Hadziyannis. (in press), M. Manns and P. Marcellin, personal communication].

Treatment of HBeAg-negative CHB with conventional interferon alpha has continued in the 1990s in certain centers in Greece and Italy and longer treatment periods and higher interferon doses have resulted in an increase in the SR rate, in HBsAg loss in a number of patients, and improvement in the life threatening complications of the disease over the years [16-18]. Thus interferon-alpha was recently recommended as a first line therapy for HBeAg-negative CHB by the European Association for the Study of the Liver (EASL) and Asian Pacific Association for the study of the Liver (APASL). Pegasys, a more potent pegylated compound of interferon-alpha-2a [19], is now under evaluation for efficacy, safety and tolerability in HBeAg-negative CHB in a large, multicenter, one year phase III trial (see chapters in this volume by P. Marcellin and F. Bonino). However, interferon-based therapies may be only applicable in clinical practice in patients with compensated liver disease and, even with the best results, a high SR is not expected [8]. On the other hand the practicing physician now has access to oral nucleos(t)ide analogs, a new class of potent antiviral agents, [6, 8]. These agents have already been given to most subsets of patients with CHB, saving lives and revolutionizing the treatment of many individuals particularly with severe and advanced forms of viral B liver disease both in the pre- and post-transplant setting.

The nucleoside analog era in HBeAg-negative CHB started with lamivudine in 1999 [20], adefovir dipivoxil was introduced in 2003 [21], now includes entecavir [22], telbivudine [23-24] and other compounds (see chapter by R. Esteban). The first two nucleos(t)ides have already been approved for the treatment of CHB in the USA, Europe and in most other parts of the world; entecavir has completed Phase III registration trials and telbivudine is currently under

evaluation in phase III pre-registration trials. The aim of all analogs is the suppression of HBV replication by treatment courses of finite duration (6 months to 2 years). However short-term treatment courses with nucleoside analogs in HBeAg-negative CHB have been extended to maintain effective HBV suppression by long-term and possibly indefinite administration without the development of viral resistance [26-27].

In this article the efficacy, safety, tolerance and other aspects of short- and long-term anti-viral therapy with the two approved (lamivudine and adefovir dipivoxil) and other non-yet approved nucleos(t)ide analogs in the various subsets of patients with HBeAg-negative chronic viral B liver disease will be critically reviewed. Some evidence-based recommendations for therapeutic decision making in clinical practice and of treatment strategies in the various settings of HBeAg-negative chronic HBV infection and/or of liver disease are also mentioned.

GENERAL REMARKS ON NUCLEOS(T)IDE ANALOGS IN CHRONIC HEPATITIS B AND THEIR MODE OF ACTION

In the past few years several agents mimicking the structure of natural nucleosides have been synthesized chemically. They are generally referred to as nucleoside analogs but some are already phosphorylated to their triphosphate counterparts and are therefore now specifically defined as nucleotide analogs. Nucleoside analogs can be produced in their natural D- but also in the unnatural L-configuration e.g. L-deoxythymidine (LDT), and therefore they are also referred to as enantiomers [6].

When nucleos(t)ides are incorporated into newly synthesized DNA of HBV, they cause chain termination, thus inhibiting viral replication. Some nucleoside analogs competitively inhibit the polymerase of HBV either in its reverse transcriptase activity (synthesis of HBV DNA from the pregenomic HBV RNA transcript) or in its DNA-dependent DNA-directed activity (synthesis of the positive DNA strand within core particles). Theoretically, nucleoside analogs could also inhibit the amplification and replenishment of the pool of covalently closed circular DNA (cccDNA) in the nuclei of already infected hepatocytes as well as its formation in newly infected cells. This activity has recently been well documented in congenitally infected ducks treated by a combination of lamivudine and a

deoxyguanosine pro-drug and has been found to be dependent on the cell cycle phase [6, 25].

Despite the potency of several oral nucleotide analogs against HBV and the supposedly low rate of viral resistance even after long-term administration, none has been found to posses the properties and clinical characteristics of an ideal anti-HBV agent. In fact few, if any, HBeAg-negative CHB patients treated with finite courses of nucleoside achieve sustained virologic and biochemical remission [12, 27]. Thus, the present goal of treatment of HBeAg-negative chronic viral B liver disease with already approved and newer nucleoside analogs is effective and continuous suppression of HBV replication without the development of viral resistance based on various long lasting or even indefinite regimens [12, 26-28].

PREREQUISITES FOR INITIATING TREATMENT IN HBEAG-NEGATIVE CHRONIC HEPATITIS B

A number of criteria must be met concerning the diagnosis, differential diagnosis and severity of liver disease before initiating treatment of HBeAg-negative chronic viral B liver disease.

Safe diagnosis

For a diagnosis of HBeAg-negative CHB to be made, patients must have chronic HBV infection with documented HBsAg seropositivity and HBeAg negativity (usually positivity of anti-HBe as well) for at least 6 months, preferably one year; persistent or intermittent elevations in alanine aminotransferase/aspartate aminotransferase (ALT/AST) activity, detectable serum HBV DNA exceeding 105 copies/mL at least once in the last 3 month period and ≥grade 3 necroinflammation on liver biopsy [3, 4, 12, 13, 29]. Other concomitant or superimposed causes of liver disease should also be absent.

Differentiation from the inactive HBsAg carrier state

The serological profile of HBeAg-negative CHB i.e. **"HBsAg(+)/HBeAg(-)/anti-HBe(+)**" is identical to that of the inactive HBsAg carrier state. Moreover, several patients with HBeAg-negative CHB exhibit an intermittent rather than a continuous pattern of biochemical activity, sometimes with quite long-lasting intervening

periods of biochemical and even virologic remission [30, 31] mimicking the inactive HBsAg carrier state. Therefore, differentiation between these two conditions may require follow–up [30-32]. In studies with frequent assays of ALT/AST and of serum HBV DNA levels, major fluctuations in viremia and serum aminotransferase levels have been observed in more than 40% of untreated HBeAg-negative CHB cases [10, 30-31]. In this context an arbitrary serum HBV DNA level of 10^5 cP/mL has been suggested as a cut-off point for differentiation between patients with HBeAg-negative CHB and inactive HBsAg carriers [7]. However, several patients with HBeAg-negative CHB may have serum HBV DNA levels below 10^5 cP/mL at certain moments [32, 33]. According to our data, 20-30% of patients with histologically documented HBeAg-negative CHB, first present with normal ALT levels and low HBV DNA values below the cut-off level of 10^5 and even of 10^4 copies/mL, and may initially be misclassified as inactive HBsAg carriers [29, 30]. Thus, HBsAg(+)/HBeAg(-) individuals should be followed-up clinically, have frequent ALT assays and in case of increasing ALT levels be further tested for serum HBV DNA levels and considered as possible candidates for anti-viral therapy [26].

Severity of liver disease

Untreated HBeAg-negative CHB often runs a progressive course frequently terminating in cirrhosis and portal hypertension, liver failure and/or hepatocellular carcinoma [3, 34, 35]. On the other hand, patients with histologically minimal or mild HBeAg-negative liver disease and usually minimal ALT elevations may run a non-deteriorating or a very slowly progressive course, never reaching cirrhosis. Thus, considering the cost of long-term nucleos(t)ide analog therapy and the frequent development of viral resistance, at least with lamivudine treatment [21, 29, 36-38], the decision to treat or not to treat patients with HBeAg-negative CHB should be based on liver histology [26, 27, 29, 39]. Thus, in patients with minimal or mild liver disease, initiation of treatment is not usually recommended unless serum chemistries and liver necroinflammation/fibrosis deteriorate.

Previous treatment with interferon

In the absence of overt cirrhosis, patients with HBeAg-negative CHB may first be treated with an interferon course of a finite duration [7, 8,

31], probably pegylated interferon-alpha-2a for one year [41], thus providing a chance for sustained remission. If patients fail to respond to a first or second course of interferon treatment, if they are reluctant to be treated or retreated by interferon, if their liver disease has already advanced to de-compensated or clinically overt cirrhosis or if interferon is contraindicated for any reason, then long-term therapy with nucleoside analogs unequivocally becomes the treatment of choice.

GOALS AND END-POINTS OF THERAPY IN HBEAG-NEGATIVE CHRONIC HEPATITIS B

In HBeAg-positive CHB, sustained seroconversion of HBeAg to anti-HBe is considered to be a reliable end-point [7]. However, loss of HBeAg and seroconversion to anti-HBe are not applicable to HBeAg-negative/anti-HBe-positive CHB. Biochemical and virologic remission during a course of therapy that is sustained after stopping treatment, is considered to be a viable goal in approximately 25% of HBeAg-negative patients treated by interferon [16-18]. In several such responders, loss of HBsAg may also follow [16, 31]. Moreover, in observational studies with long-term follow-up, a reduced risk of the development of liver de-compensation and/or HCC, as well as improved survival have been reported [17, 35]. However, the goal of a sustained response is rarely if ever achieved with finite courses of nucleoside analog therapy lasting for 1, 2 or even 3 years [13, 38]. Thus, very long-term or even indefinite nucleoside analog treatment to maintain effective HBV suppression without viral resistance appears to be the next therapeutic alternative in HBeAg-negative CHB [16, 26-28]. It remains to be seen whether or not, at what frequency and with which regimens and compounds, sustained virologic responses can be achieved after stopping successful very long-term (for 5 and more years) nucleoside analog therapy.

A NOTE ON THE DEFINITIONS AND VOCABULARY OF RESPONSE TO TREATMENT

In both HBeAg-positive and HBeAg-negative CHB, virologic and biochemical responses can be evaluated during therapy (on-therapy responses) or after discontinuation of therapy (off-therapy or sustained responses) [13, 42]. In particular, on-therapy responses may be

subdivided into initial (achieved within the first months of therapy), maintained (persisting throughout the course of therapy), and end-of-therapy (evaluated at the end of a course of therapy with defined duration) [42]. Biochemical responses are defined by decreases in ALT/AST to the normal range, while virologic responses, preferably evaluated by qualitative polymerase chain reaction (PCR) assays, are arbitrarily considered to be achieved when serum HBV DNA levels fall below 10^5 cP/mL [5, 7, 13]. In particular in HBeAg-positive CHB, a virologic response also requires loss of HBeAg and seroconversion to anti-HBe. Although serum HBV DNA levels of 10^5cP/mL are currently used in many definitions of a virologic response [7], effective HBV suppression is probably achieved when serum HBV DNA levels drop below 400cP/mL or even 200cP/mL which are the cut-off levels of the Roche Monitor assay or of most in-house real time PCR assays [43]. A response is defined as complete when a biochemical and virologic response is accompanied by loss of HBsAg [16, 42]. When virologic and biochemical responses are maintained for several months, histologic improvement can also be demonstrated and is usually being defined as a reduction of the necroinflammatory Histology Activity Index (HAI/Knodell) score by ≥2 points without worsening in fibrosis [7, 13].

TREATMENT OF HBEAG-NEGATIVE CHRONIC HEPATITIS B WITH LAMIVUDINE (LAM)

Lamivudine (3TC or (-)-2′,3′-dideoxy-3′-thiacytidine), which was begun for the treatment of CHB in the late nineties, is a safe drug with rare and generally mild side-effects [44].

In patients with HBeAg-negative CHB, a 12-month course of LAM at a daily dose of 100-150mg has been shown to provide initial biochemical and virologic responses even by sensitive PCR assays in more than two thirds of patients [20, 37]. Unfortunately, biochemical and virologic relapses are observed in the vast majority of patients after stopping a 12-24 month lamivudine course [45, 46]. Given its excellent tolerability and safety profile, long-term treatment with lamivudine could be an acceptable, beneficial maintenance therapy in HBeAg-negative CHB. Unfortunately in clinical practice only 30-40% of patients remain in remission after the third year of lamivudine monotherapy without developing viral resistance [37, 38, 42].

Virologic breakthroughs under LAM monotherapy in CHB usually develop after the first 6 months of treatment [45, 47] and if therapy is extended their rate increases progressively, reaching approximately 60% at the end of the 3rd year in both HBeAg–positive and HBeAg–negative CHB [38, 45, 47]. Although several questions were initially raised about the clinical significance of LAM resistance, it is now quite clear that in patients with HBeAg-negative CHB its development is almost invariably followed by increasing viremia levels ending in biochemical breakthroughs [37], which ultimately have an adverse effect on liver histology [47]. Breakthroughs may be quite severe and this becomes of particular concern in patients with advanced liver disease and cirrhosis since it may lead to liver decompensation and death regardless of HBeAg status [38]. Finally it should be noted that the LAM resistant mutation M204I, which develops quite frequently in HBeAg-negative CHB patients of genotype D [37, 47], also appears to be resistant to treatment with some newer nucleoside analogs that are active against other LAM resistant HBV strains [6, 22-24]; but fortunately it remains sensitive to ADV (Hadziyannis S., unpublished).

Another disadvantage of lamivudine therapy for HBeAg-negative CHB is that, as previously mentioned, no course of finite duration has been shown to achieve sustained off-therapy responses in a sizeable proportion of patients and that the optimal duration of therapy remains currently unknown [38]. For example, it is noteworthy that most Greek patients with HBeAg-negative CHB, who have discontinued effective LAM therapy after 3-5 years duration have virologic and biochemical relapses [SJ Hadziyannis. unpublished].

TREATMENT OF HBEAG–NEGATIVE CHRONIC HEPATITIS B WITH ADEFOVIR DIPIVOXIL

Adefovir is an acyclic nucleotide analog with strong inhibitory activity for HBV replication and other viruses [6, 48]. It is administered orally in the form of its prodrug adefovir dipivoxil (ADV) and is commercially available under the trade name Hepsera. ADV is adefovir esterified with two pivalic acid molecules and has good oral availability [49]. It is rapidly converted to adefovir in plasma and tissues, has a plasma half-life of 5-7 hours and 90% of the drug is excreted in urine within 24 hours [6]. After being transported intracellularly by a receptor-based mechanism, adefovir is

phosphorylated to its diphosphate form, which is an analog of deoxyadenosine-5'-triphosphate but without a 3'-hydroxylic root. Therefore, it results in competitive inhibition of DNA synthesis by DNA polymerases and reverse transcriptases [50-51]. ADV has been shown to have no significant interaction with other drugs [6, 51] and the 10mg/day dose is very well tolerated with a safety profile similar to placebo [21, 52]. A higher daily dose of 30mg was found to be associated with an increased risk of renal damage without a significant increase in efficacy [52]. Thus, the approved ADV dose for CHB is 10mg/day, which can be safely administered even in patients with hepatic or mild renal impairment. However, dosing interval adjustments are recommended for patients with creatinine clearance of <50mL/min and in patients requiring hemodialysis [53-54].

ADV therapy was approved in 2002 for the treatment of all subgroups of chronically HBV infected patients with active viral replication and evidence of liver damage, who require therapeutic intervention: patients with CHB or decompensated HBV cirrhosis or HBV transplant patients, irrespective of HBeAg status and of the development of LAM resistance.

Efficacy, safety and tolerance

ADV monotherapy, at a dose of 10mg once a day, has been evaluated in a multicenter, randomized, double-blind, placebo-controlled trial including 185 patients with HBeAg-negative [21]. ADV was significantly more effective than placebo for all end-points. At 48 weeks, histologic improvement occurred in 64% of patients treated with ADV compared to 33% of those treated with placebo (P<0.001). ADV was also found to effectively suppress virologic and biochemical activity, reducing HBV DNA levels by approximately 4 logs and normalizing ALT in 72% of patients during the first 48 weeks of therapy [21].

Long-term extension of this trial has provided further information on the safety and efficacy of ADV therapy in HBeAg-negative CHB [28, 39]. During the second and third year on-therapy biochemical and virologic responses were maintained without any significant toxicity [28, 39] and with infrequent and late development of viral resistance. At the end of the second and third years of therapy, serum HBV DNA levels were undetectable by PCR in 71% and 79% of ADV treated patients respectively and ALT remained normal in 73% and 69% [28,

39]. Liver biopsies performed at week 96 in a subset of the overall cohort of this study suggested that an additional histological benefit is obtained if the duration of ADV treatment is extended [28].

This ongoing trial on the course of HBeAg-negative CHB also provided important information concerning results after stopping ADV treatment. Approximately one fourth of the total patient population in the trial (one third of the patients initially treated with ADV) switched to placebo after the first year of therapy. Most of them experienced biochemical and virologic relapses as well as reversal of the histologic improvement obtained during the first year of active treatment. Moreover, post-treatment flares in serum ALT levels were seen in some patients after stopping ADV [28]. Although these events were probably self-limiting and not associated with hepatic decompensation, they nevertheless stress the need for careful monitoring of patients who stop ADV therapy.

HBV resistance

There are two recognized ADV resistant HBV mutants, the rtN236T and the rtA181V [40, 56]. The rtN236T mutation is the most frequent and is associated with a selection of a novel asparagine to threonine substitution at residue rt236 in domain D of the HBV polymerase. It should be noted, however, that the incidence of ADV resistant mutations is delayed and infrequent, with a cumulative probability of 0% after 48 weeks, 2% after 96 weeks and 3.9% after 144 weeks of ADV therapy [39-40].

Patients developing the rtN236T mutation experience rebound in HBV DNA of >1 $\log_{10}$ from nadir with an increase in ALT activity [40]. The model structure of the reverse transcriptase of HBV suggests that the side chain of the rtN236T mutant may have a more favourable interaction with the gamma-phosphate of dATP compared to adefovir diphosphate, thus providing selectivity against adefovir diphosphate versus the natural substrate. The rtN236T mutation results in >60% reduction in the replicative capacity of HBV, but it is susceptible to L-deoxythymidine (LdT) and entecavir *in vitro* and fully susceptible to lamivudine both *in vitro* and *in vivo*. The significance of the rtA181V mutation is not clear and needs to be further characterized.

Efficacy of ADV in patients with LAM resistance

ADV monotherapy is effective in all subsets of patients with resistance to LAM [42, 57, 58] with similar antiviral efficacy against all LAM resistant HBV mutants, as well as in cirrhotic and transplant patients irrespective of their HBeAg status [59-63]. In 95 LAM resistant CHB patients who continued to take this drug, virologic responses at 48 weeks (defined as reduction in serum HBV DNA levels to $<10^5$cP/mL or >2 $\log_{10}$) were observed in 85% (39/46) of those who received additional ADV therapy and in only 11% of those who received placebo (P<0.001). Normalization of ALT was achieved in 31% and 6% of the cases respectively (P=0.002) [62]. In a large study including 324 HBV decompensated cirrhotics (n=128) or transplant patients (n=196) with resistance to LAM, the addition of ADV resulted in a significant reduction in serum HBV DNA levels, frequent normalization of liver function tests and improvement in Child-Pugh score (>90% of patients in both cohorts) [61]. Similar findings have also been reported in studies including fewer patients [59, 60, 62, 63]. These observations have been further analysed and commented on in relation to combination therapy both as an initial and rescue treatment to optimize efficacy and avoid the problem of multiple drug resistance [64-69].

The question of whether CHB patients with resistance to lamivudine should be switched to ADV monotherapy (immediately or after a period of concurrent LAM therapy) or receive long-term combination therapy with ADV and LAM is still debatable, primarily because of the high cost of combination therapy with oral nucleoside analogs. However, in patients with severe liver disease the financial cost should not be a criterion since the benefit can mean saving a life [69].

TREATMENT OF HBEAG-NEGATIVE CHRONIC HEPATITIS B WITH NEWER NUCLEOSIDE ANALOGS

Several nucleoside analogs are currently under evaluation for efficacy, safety and tolerance in phase II and III trials either alone or in combination in various types of chronic HBV infection including HBeAg-negative CHB. Those in advanced pre-registration stages and/or the most promising drugs appear to be entecavir, telbivudine, emtricitabine, clevudine, and tenofovir [43]. Advantages of these

newer compounds over LAM and ADV in the short- and long-term treatment of CHB have been claimed on the basis of more profound and more rapid [6, 55] suppression of HBV replication. However, the actual frequency of HBV resistance during long-term administration of the newer nucleoside analogs is still unknown; but a resistance threshold as high as that of adefovir cannot be really expected. Moreover the resistance profile of most new nucleoside analogs does not appear to be much different from that of LAM, and, therefore, cross-resistance in the treatment of lamivudune failures should be anticipated. Finally the antiviral efficacy of entecavir in LAM resistant mutants has been reported to be associated with baseline YMDD mutations [22] while telbivudine has been found to be inactive against rt M204I which is the predominant LAM mutant in HBeAg-negative CHB genotype D [47].

CONCLUDING REMARKS

The combination of a high rate of HBV replication with a slow death rate of HBV infected hepatocytes suggests that in chronic HBV infection, long-term treatment with potent antiviral agents will usually be required. This seems to be particularly true for HBeAg-negative chronic hepatitis B where the success of finite courses of interferon-alpha therapy has been limited [12, 13, 55] Treatment with the currently available nucleoside analogs has clearly confirmed that this is indeed the case. However, long-term nucleoside analog treatment, though initially quite effective in most HBeAg-negative patients, now faces the problem of a progressive decrease in efficacy because of the development of high rates of HBV resistance. In HBeAg-negative CHB patients, long-term monotherapy with lamivudine the first oral nucleoside analog approved for the treatment of HBV infection, only remains effective for more than 3 years in 30-40% of treated patients. Virologic and biochemical breakthroughs during long-term LAM treatment in HBeAg-negative CHB, destroy the benefit gained during the earlier periods of treatment. Moreover, such breakthroughs and relapses can be quite severe and even life threatening, particularly in patients with advanced liver disease and overt cirrhosis, thus raising major concerns on the suitability of long-term LAM therapy in such clinical settings. Long-term monotherapy with adefovir dipivoxil (ADV, Hepsera), the second nucleoside analog approved for the treatment of hepatitis B, is effective in more than two thirds of

HBeAg-negative patients with CHB at least for the first 3-4 years with rare and delayed appearance of HBV resistance . Moreover it has been shown to be effective against both wild type and LAM-resistant HBV strains as well as against all HBV genotypes either with a positive or negative HBeAg phenotype [65]. Currently the high cost of ADV remains its major drawback, otherwise it would already have completely replaced the use of LAM in most parts of the world and in all settings of HBeAg-negative chronic viral B liver disease. However, based on the lessons learnt for the treatment of HIV infection, practicing clinicians know that the use of "monotherapy" with any nucleoside analog is not the best way for a long-term/indefinite suppression of HBV replication. Combinations of drugs with complementary mechanisms of antiviral activity and different HBV resistance profiles [66, 67] (such as adefovir or tenofovir in combination with telbivudine or entecavir or lamivudine or emtricitabine) may soon prevent the development of HBV resistance and/or induce sustained virologic responses.

REFERENCES

1. Hadziyannis SJ, Lieberman HM, Karvountzis GG, Shafritz DA. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg vs. anti-HBe positive carriers of hepatitis B virus. Hepatology 1983;3:656-662.

2. Bonino F, Rosina F, Rizzetto M, Rizzi R, Chiaberge E, Tardanico R, Callea F, Verme G. Chronic hepatitis in HBsAg carriers with serum HBV-DNA and anti-HBe. Gastroenterology 1986;90:1268-1273.

3. Hadziyannis SJ. Hepatitis Be antigen negative chronic hepatitis B. From clinical recognition to pathogenesis and treatment. Vir Hep Rev 1995;1:7-36.

4. Brunetto MR, Oliveri F, Colombatto P, Capalbo M, Barbera C, Bonino F. Treatment of chronic anti-HBe-positive hepatitis B with interferon-alpha. J Hepatol 1995;22:S42-S44.

5. Hoofnagle JH, di Bisceglie AM. The treatment of chronic viral hepatitis. N Engl J Med 1997;336:347-356.

6. Karayiannis P. Hepatitis B virus: old, new and future approaches to antiviral treatment. J Antimicrob Chemother 2003;51:761-785.

7. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000-summary of a workshop. Gastroenterology 2001;120:1828-1853.

8. Hadziyannis SJ. Interferon alpha therapy in HBeAg-negative chronic hepatitis B: new data in support of long-term efficacy. J Hepatol 2002;36:280-282.

9. Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. Mutation preventing formation of hepatitis Be antigen in patients with chronic hepatitis B infection. Lancet 1989;2:588-591.

10. Brunetto MR, Giarin MM, Oliveri F, Chiaberge E, Baldi M, Alfarano A, Serra A, Saracco G, Verme G, Will H. Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. Proc Natl Acad Sci USA 1991;88:4186-4190.

11. Okamoto H, Tsuda F, Akahane Y, Sugai Y, Yoshiba M, Moriyama K, Tanaka T, Miyakawa Y, Mayumi M. Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. J Virol 1994;68:8102-8110.

12. Hadziyannis SJ, Vassilopoulos D. Hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.

13. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001;34:1225-1241.

14. Funk ML, Rosenberg DM, Lok AQS. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat 2002;9:52-61.

15. Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus-large-scale analysis using a new genotyping method. J Infect Dis 1997;175:1285-1293.

16. Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis Be antigen-negative chronic hepatitis B. Gastroenterology 2001; 121:101-109.

17. 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;36:263-270.

18. Lampertico P, Del Ninno E, Vigano M, Romeo R, Donato MF, Sablon E, Morabito A, Colombo M. Long-term suppression of hepatitis Be antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003;37:756-763.

19. 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 Be antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.

20. Tassopoulos NC, Volpes R, Pastore G, Heathcote J, Buti M, Goldin RD, Hawley S, Barber J, Condreay L, Gray DF. Efficacy of lamivudine in patients with hepatitis Be antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. Hepatology 1999;29:889-896.

21. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart CL; Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of hepatitis Be antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800-807.

22. Chang TT, Hadziyannis S, Cianciara J, Rizzetto M, Schiff E, Pastore G, Klesczenski K, Danisky G, DeHertogh D, Hindes R. Sustained viral load and ALT reduction following 48 weeks of entecavir treatment in subjects with chronic hepatitis B who have failed lamivudine. Hepatology 2002;36:300A.

23. Lai CL, Leung NWY, Teo EK, Tong M, Wongt F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown N. Results of a one-year international phase IIb comparative trial of telbivudine, lamivudine and the combination in patients with chronic hepatitis B. Hepatology 2003;38:262A.

24. Bryant ML, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Marion P, Cretton-Scott E, Schinazi RF, Sommadossi JP. Antiviral L-

nucleosides specific for hepatitis B virus infection. Antimicrob Agents Chemother 2001;45:229-235.

25. Addison WR, Walters KA, Wong WW, Wilson JS, Madej D, Jewell LD, Tyrrell DL. Half-life of the duck hepatitis B virus covalently closed circular DNA pool in vivo following inhibition of viral replication. J Virol 2002;76:6356-6363.

26. Papatheodoridis GV, Hadziyannis SJ. Review article: current management of chronic hepatitis B. Aliment Pharmacol Ther 2004;19:25-37.

27. Keeffe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, Tobias H, Wright TL. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States. Clin Gastroenterol Hepatol 2004;2:87-106.

28. Hadziyannis S, Tassopoulos N, Heathcote E, Chang TT, Kittis G, Rizzetto M, Marcellin P, Lim SG, Chen SS, Wulfsohn M, Wollman M, Frei J, Brosgart C. Two year results from a double-blind, randomized, placebo-controlled study of adefovir dipivoxil (ADV) for presumed precore mutant chronic hepatitis B. J Hepatol 2003;38:143A.

29. Conjeevaram HS, Lok AS. Management of chronic hepatitis B. J Hepatol 2003;38:S90-S103.

30. Hadziyannis SJ, Bramou T, Alexopoulou A, Makris A. Immunopathogenesis and natural course of anti-HBe positive chronic hepatitis with replicating B virus. In: Hollinger FB, Lemon SM, Margolis HS, editors. Viral hepatitis and liver disease. Baltimore, MD: Williams & Wilkins 1991:673-676.

31. Brunetto MR, Oliveri F, Colombatto P, Coco B, Ciccorossi P, Bonino F. Treatment of HBeAg-negative chronic hepatitis B with interferon or pegylated interferon. J Hepatol 2003;39:S164-S167.

32. Manesis EK, Papatheodoridis GV, Sevastianos V, Cholongitas E, Papaioannou C, Hadziyannis SJ. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis Be antigen-negative chronic hepatitis B virus infection. Am J Gastroenterol 2003;98:2261-2267.

33. Chu CJ, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. Hepatology 2002;36:1408-1415.

34. Naoumov NV, Schneider R, Grotzinger T, Jung MC, Miska S, Pape GR, Will H. Precore mutant hepatitis B virus infection and liver disease. Gastroenterology 1992;102:538-543.

35. 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;34:306-313.

36. Santantonio T, Mazzola M, Iacovazzi T, Miglietta A, Guastadisegni A, Pastore G. Long-term follow-up of patients with anti-HBe/HBV DNA-

positive chronic hepatitis B treated for 12 months with lamivudine. J Hepatol 2000;32:300-306.

37. Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2000;32:847-851.

38. Rizzetto M, Marzano A, Lagget M. Treatment of hepatitis Be antigen-negative chronic hepatitis B with lamivudine. J Hepatol 2003;39:S168-S171.

39. Hadziyannis S, Tassopoulos N, Chang TT, Heathcote J, Kittis G, Rizzetto M, Marcellin P, Lim SG, Chen SS, Arterburn S, Ma J, Xiong S, Wollman M, James C, Currie G, Brosgart CL. Three year study of adefovir dipivoxil (ADV) demonstrates sustained efficacy in presumed precore mutant chronic hepatitis B (CHB) patients in a long-term safety and efficacy study (LTSES). J Hepatol 2004;40:17.

40. Qi X, Snow A, Thibault V, Zhu Y, Curtis M, Hadziyannis SJ, Brosgart C, Currie G, Arterburn S, Gibbs CS, Miller MD, Xiong S. Long-term incidence of adefovir dipivoxil (ADV) resistance in chronic hepatitis B (CHB) patients after 144 weeks of therapy. J Hepatol 2004;40:20.

41. Marcellin P, Lau GKK, Bonino F, Farci P, Hadziyannis S, Jin R Piratvisuth T, Germanidis G, Pluck N. Peginterferon alfa-2A (40KD) (PEGASYS®) monotherapy is more effective than lamivudine monotherapy in the treatment of HBeAg-negative chronic hepatitis B: 72-week results from a phase III, partially double-blind study of PEGASYS® alone vs. PEGASYS® plus lamivudine vs. lamivudine. J Hepatol 2004;40:34.

42. Hadziyannis SJ, Papatheodoridis GV, Vassilopoulos D. Treatment of HBeAg-negative chronic hepatitis B. Semin Liver Dis 2003;121:101-109.

43. Pawlotsky JM. Molecular diagnosis of viral hepatitis. Gastroenterology 2002;122:1554-1568.

44. Jarvis B, Faulds D. Lamivudine: A review of its therapeutic poential in chronic hepatitis B. Drugs 1999;58:101-141.

45. Tassopoulos N, Volpes R, Pastore G, Heathcote J, Buti M, Gray DF, Barber J, Hawley S. Post lamivudine treatment follow-up of patients with HBeAg-negative chronic hepatitis. Br J Hepatol 1999;30:117.

46. Santantonio T, Mazzola M, Iacovazzi T, Miglietta A, Guastadisegni A, Pastore G. Long-term follow-up of patients with anti-HBe/HBV DNA - positive chronic hepatitis B treated for 12 months with lamivudine. J Hepatol 2000;32:300-306

47. Papatheodoridis GV, Dimou E, Laras A, Papadimitropoulos V, Hadziyannis SJ. Course of virologic breakthroughs under long-term lamivudine in HBeAg-negative HBV liver disease. Hepatology 2002;36:219-126

48. Nicoll AJ, Colledge DL, Toole JJ, Angus PW, Smallwood RA, Locarnini SA. Inhibition of duck hepatitis B virus replication by 9-(2-

phosphonylmethoxyethyl) adenine, an acyclic phosphonate nucleoside analogue. Antimicrobial Agents and Chemotherapy 1998;42:3130-3135.

49. Cullen JM, Li DH, Brown C, Eisenberg EJ, Cundy KC, Wolfe J, Toole J, Gibbs C. Antiviral efficacy and pharmacokinetics of oral adefovir dipivoxil in chronically woodchuck hepatitis virus-infected woodchucks. Antimicrobial Agents and Chemotherapy 2001;45:2740-2745.

50. Yu AS, Keeffe EB. Nucleoside analogues and other antivirals for treatment of hepatitis B in the peritransplant period. Clin Liver Dis 2003;7:551-572.

51. Holy A, Votruba I, Metra A, Cerny J, Vesely J, Vlach J, Sediva P, Rosenberg I, Otmar M, Hrebabecky H. Acyclic nucleotide analogues: synthesis, antiviral activity and inhibitory effects on some cellular and virus-encoded enzymes in vitro. Antiviral Res 1990;23:295-311.

52. 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 antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.

53. Mailliard ME, Gollan JL. Suppressing hepatitis B without resistance - so far, so good. New Engl J Med 2003;348:848-850.

54. Knight W, Hayashi S, Benhamou Y, Currie G, Ebrahimi R, Gill S, Fry J, Kearney B, Brosgart C. Dosing guidelines for adefovir dipivoxil in the treatment of HBV infected patients with renal or hepatic impairment. J Hepatol 2002;36:136.

55. Dusheiko G. Adefovir dipivoxil for the treatment of HBeAg-positive chronic hepatitis B: a review of the major clinical studies. J Hepatol 2003;39:S116-S123.

56. Xiong S, Yang H, Westland CE, Delaney WE, Colledge D, Bartholomeusz A, Thibault V, Benhamou Y, Anguo P, Wulfsohn M, Gibbs CS, Fry J, Brosgart C, Locarnini S. Resistance surveillance of HBeAg- chronic hepatitis B (CHB) patients treated for two years with adefovir dipivoxil (ADV). J Hepatol 2003;38:182.

57. Zoulim F, Trepo C, Poynard T, Pol S, Zarski JP, Marcellin P, Beaugrand M, Stitruk V, Trylesinski A, Kleber K, Van Doren S, Brosgart C. Adefovir dipivoxil (ADV) for the treatment of patients with chronic hepatitis B (CHB) failing lamivudine (LAM) therapy. J Hepatol 2003;38:184.

58. Westland CE, Yang H, Namimi H, Lama N, Gibbs CS, Miller MD, Fry J, Brosgart C, Xiong S. Comparison of anti-HBV activity of adefovir against different lamivudine-resistant HBV strains in vitro and in liver transplant patients. Hepatology 2001;34:446A.

59. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, Lamy P, Murray A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. Hepatology 2000;32:129-134.

60. Ahmad J, Dodson SF, Balan V, Vargas HE, Fung JJ, Rakela J, Shakil AD. Adefovir dipivoxil suppresses lamivudine resistant hepatitis B virus in liver transplant recipients. Hepatology 2000;32:292A.

61. Schiff ER, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann HL, Samuel D, Zeuzem S, Lilly L, Rendina M, Villeneuve JP, Lama N, James C, Wulfsohn MS, Namini H, Westland C, Xiong S, Choy GS, Van Doren S, Fry J, Brosgart CL; Behalf of the Adefovir Dipovoxil Study 435 International Investigators Group. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post-liver transplantation patients. Hepatology 38:1419-1427.

62. Perrillo R, Hann HW, Mutimer D, Willems B, Leung N, Lee WM, Moorat A, Gardner S, Woessner M, Bourne E, Brosgart CL, Schiff E. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology 2004;126:81-90.

63. Kuwahara R, Kumashiro R, Inoue H, Tanabe R, Tanaka E, Hino T, Ide T, Koga Y, Sata M. Adefovir dipivoxil as a treatment for hepatic failure caused by lamivudine-resistant HBV strains. Dig Dis Sci 2004;49:300-303.

64. Peters MG, Hann HH, Martin P, Heathcote EJ, Buggisch P, Rubin R, Bourliere M, Kleber K, Ebrahimi R, Xiong S, Brosgart C. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101.

65. 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;125:107-116.

66. Shaw T, Locarnini S. Combination chemotherapy for hepatitis B virus: the final solution? Hepatology 2000;32:430-432.

67. Shaw T, Bowden S, Locarnini S. Rescue therapy for drug resistant hepatitis B: another argument for combination chemotherapy? Gastroenterology 2004;126:343-347.

68. EASL International consensus conference on hepatitis B. J Hepatol 2003;39:S3-S25.

69. Zoulim F. Treatment of pre- and post-liver transplantation HBV infection: Should we aim at combination therapy? Hepatology 2003;38:1353-1355.

Treatment of HBeAg-Negative Chronic Hepatitis B with Conventional or Pegylated Interferon

Maurizia Rossana Brunetto, Filippo Oliveri, Piero Colombatto, Barbara Coco, Pietro Ciccorossi, Ferruccio Bonino

Hepatitis Be antigen, antibody (Anti-HBe)-positive chronic hepatitis B was first described and characterized in patients in the Mediterranean basin, where about 20% of hepatitis B surface antigen (HBsAg) carriers with antibody to hepatitis B "e" antigen (HBeAg) showed detectable serum levels of hepatitis B virus (HBV) DNA by hybridization assays and intrahepatic necro-inflammation [1, 2]. These patients are infected with HBV variants with mutations in the pre-core region which hamper HBeAg production. The G to A mutation at nucleotide 1896 of the pre-core region is the most commonly described [3, 4]. Liver disease runs an indolent course for 3-4 decades reaching the stage of histological cirrhosis at a median age of 45 years [5]; thereafter about 25% of patients progress to end-stage complications within 10 years [5].

In recent years several reports have suggested a worldwide increasing incidence of HBeAg-negative chronic hepatitis B [6, 7]. However, further virological and clinical studies are needed before the disease is completely characterized and to understand whether it shares common features with the anti-HBe-positive chronic hepatitis B observed in Mediterranean patients, who are infected with HBV genotype D, bearing the stop codon at nucleotide 1896 [8]. Because of the progressive course of HBeAg-negative/anti-HBe-positive chronic

hepatitis B, interferon-alpha treatment was attempted as soon as the drug became available for treatment of chronic HBV infection [9-12]. Most studies on interferon-alpha treatment of anti-HBe-positive chronic hepatitis B were performed in Southern Europe [9-14] and more recently a few reports have been published from Asia [15-17].

AIMS OF ANTIVIRAL TREATMENT AND MONITORING CRITERIA

In chronic hepatitis B the aim of antiviral treatment is to cure liver disease by sustained control of HBV infection. This occurs in steps: inhibition of viral replication, HBeAg to anti-HBe serocoversion and eventually clearance of HBsAg and seroconversion to anti-HBs [6, 18]. Since HBsAg to anti-HBs seroconversion occurs months or years after control of HBV replication it cannot be used as a short-term marker of response to treatment [19, 20]. Therefore, in HBeAg-positive patients, HBeAg to anti-HBe seroconversion is considered to be the hallmark of successful control of HBV replication and it is used to monitor response to antiviral treatment. In anti-HBe-positive chronic hepatitis B, however, HBeAg to anti-HBe seroconversion has already occurred and a general consensus on alternative criteria to monitor treatment has never been reached.

In addition, anti-HBe-positive chronic hepatitis B has various profiles characterized by major fluctuations of both viremia and alanine aminotransferase (ALT) levels (HBV DNA can fall below the 105 genomes/mL and ALT may be temporarily normal) in >50% of patients [5, 6]. This prevents a precise diagnosis of the disease or disease relapse when the detection limits for HBV DNA are 105-106 genomes/mL, unless surrogate markers of HBV induced liver damage (anti-HBc IgM) and stringent monitoring criteria are used [5]. These factors have significantly contributed to the different response rates to interferon-alpha therapy reported in the literature [21].

INTERFERON-ALPHA SCHEDULES AND EVALUATION OF EFFICACY

Two different treatment strategies have been used for anti-HBe-positive chronic hepatitis B. Between 1986 and 1990, when interferon-alpha was first introduced for treatment of chronic hepatitis B, medium-high doses (5-10MU) of recombinant or lymphoblastoid

interferon-alpha were given for 16-24 weeks, at the same schedules as for chronic HBeAg-positive hepatitis B [9-13, 21]. Thereafter, longer treatment courses (12-24 months) were attempted with medium interferon-alpha doses (5-6MU) [14, 22-24]. Therefore, to assess the efficacy of interferon-alpha and its impact on disease outcome, the duration of treatment must be considered in addition to factors such as disease variability and the different monitoring criteria in different studies.

On-treatment-response (OTR)

OTR has been described as being associated, independently of the interferon-alpha schedule, with a progressive decrease in serum HBV DNA levels, in parallel with a slower ALT decrease [9-11, 22]. ALT flares, such as those described in HBeAg-positive responders during month 2-4 of treatment, have only been described in one study, where serum HBV DNA clearance in 42% of responders was preceded by an ALT flare at least 2 times above the median pre-treatment value [12].

End-of-treatment-response (EOTR)

EOTR (Figure 1) occurs in 57-90% of treated patients, when defined as a decrease in HBV DNA levels to below 1-10pg/mL and normal ALT at the end of treatment [9-13]. A lower EOTR rate (38%) was only described in one study that had more stringent criteria (HBV DNA <1pg/mL and normal ALT in the last 6 months) despite the longer treatment course of 24 months [14].

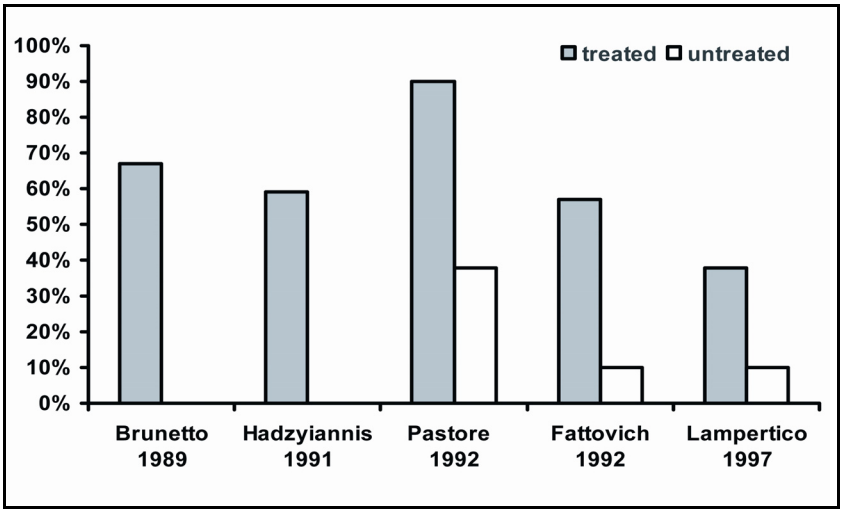

Figure 1: End of treatment response in 5 randomized controlled studies [9-12, 14].

At 12-24 months post-treatment relapse rates of 25-89% were observed [9-14, 21]. This marked variability appears to be influenced by at least 3 factors: 1) criteria used in the post treatment follow-up; 2) duration of treatment; and, 3) heterogeneity of the population.

As mentioned above, there is no general consensus for monitoring criteria (parameters and frequency) during post-treatment follow-up.

This issue was addressed in a multicenter study in 72 patients who were followed for 18 months after treatment by monthly monitoring of ALT, HBV DNA and anti-HBc IgM [21]. The authors showed that the most pertinent criteria was the monthly monitoring of ALT and detection of anti-HBc IgM every 3 months. Measurement of ALT and anti-HBc IgM every 3 months also had an acceptable diagnostic accuracy, but was slightly less sensitive (Figure 2). In comparison, HBV DNA was less useful in the identification of relapse patients, mainly due to the detection limits of hybridization assays. Future studies should investigate the diagnostic accuracy of

polymerase chain reaction (PCR) based assays, possibly defining clinically relevant viremia levels.

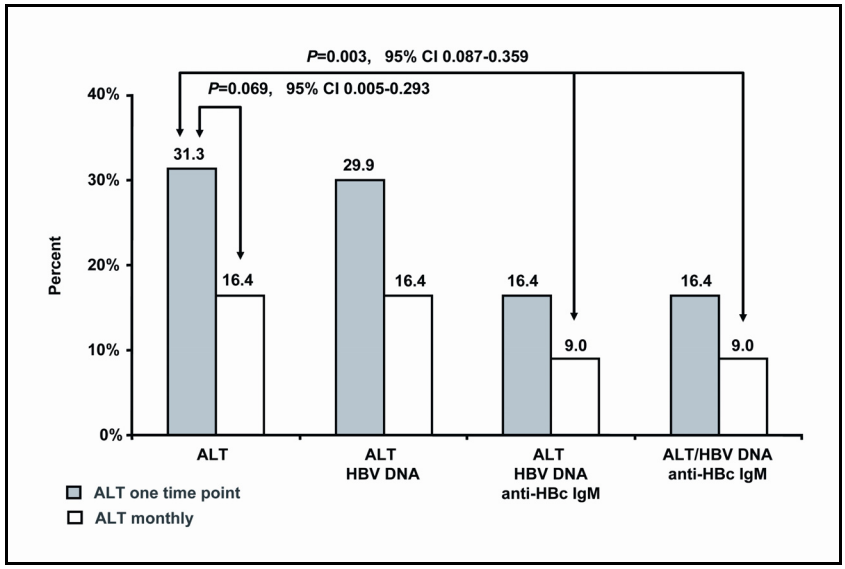

Figure 2: Rate of SR after 12-month post treatment follow-up according to different monitoring criteria [21] HBV DNA and anti-HBc IgM were measured every 3 months.

Duration of treatment

Available data suggest that the longer the treatment, the higher the sustained response (SR) rate. Sixteen to twenty four weeks of treatment is associated with relapse rates of 50-89%. The mean time until relapse was 6 months, with >90% of relapses occurring within the first year of follow-up, when monthly monitoring was begun.

A hepatitis relapse was associated with an ALT flare-up in 50-86% of patients [9-11, 13, 21]. Three studies report persistent clearance of viral replication markers and biochemical remission after

the hepatitis relapse, suggesting a possible "second intention" disease resolution [9, 11, 13, 21].

Interestingly, the only study with a short course of interferon-alpha that resulted in a lower relapse rate (37%), was also the only study to report the unusual association of ALT flares with treatment response [12]. This suggests a possible patient selection bias as well as the heterogeneity of the anti-HBe-positive patient population.

Data from a study with 24 months of treatment show a relapse rate of only 25% at 22 months median follow-up [14]. A longer follow-up (54 months) of 101 patients treated with the same schedule showed a slightly higher relapse rate of 35% [23]. These data confirm the results of a multivariate analysis of 216 patients showing a 1.64 times greater probability of sustained remission in 12-month courses compared to shorter treatment [22]. All results suggest that longer treatment increases the remission time and the rate of sustained response.

Overall the sustained response rate after 2-4 years of follow-up is 10-15% in patients treated for 4-6 months (usually with 9-10MU of interferon-alpha), 22% and 30% when 5MU of interferon-alpha were given for 12 and 24 months. Despite frequent temporary remissions, untreated patients very rarely resolve their liver disease. In the only study where a significant number of untreated patients showed a sustained remission (10% after 6 months, 17% after 18 months), a 6 month treatment also resulted in an unusually high rate of sustained response of 53% [12].

During post-treatment follow-up (median 4.5-7 years) 31.6-66.6% of sustained responders lost serum HBsAg, followed by anti-HBs seroconversion in 50-77% [5, 22-24]. Interestingly, >50% of patients who cleared serum HBsAg had HBV DNA levels <400 copies/mL compared to 25% of sustained responders who did not clear HBsAg [22], suggesting a better control of HBV replication in patients who clear HBsAg.

LONG-TERM IMPACT OF INTERFERON-ALPHA TREATMENT

Long-term studies to evaluate the effect of interferon-alpha therapy on disease outcome should analyze the early and late phase of the disease separately, as many factors, including treatment, may vary during the prolonged course of the disease. In addition, the heterogeneity of

criteria used to monitor patients and to define response to therapy may make it difficult to compare results from different studies. Furthermore, all existing studies with an untreated control group were not randomized; thus the data should be interpreted with caution. Thus far the impact of interferon-alpha on the progression of anti-HBe-positive chronic hepatitis B has been analyzed in 3 studies that followed 669 patients (413 treated, 256 untreated) for 4.5-6 years [5, 23-24].

Disease progression and terminal events were reduced 2.5 times in one study that defined a sustained response as persistently undetectable HBV DNA (<10pg/mL) and normal anti-HBc IgM as well as normal ALT after interferon-alpha treatment, independent from the response [5]. None of the long-term responders showed disease progression, which did occur in 20% of relapsers or non-responders. This difference was not statistically significant, because of the low rate of SR (14.6%). interferon-alpha helped reduce the progression of chronic hepatitis to cirrhosis and the occurrence of end-stage complications in patients with cirrhosis [5]. Improved long-term outcome and reduction of liver-related morbidity has been confirmed in biochemical [24] as well as biochemical and virological [23] sustained responders in the other 2 studies. The impact of treatment on the development of hepatocellular carcinoma (HCC) is unclear [23, 24]. The cumulative analysis of available data [5, 23, 24] indicates that 3/102 sustained responders (2.9%) developed HCC compared to 27/311 (8.7%) patients with relapse or no response (P=0.086, chi squared test 2.954). Therefore, studies in larger cohorts of patients with adequate follow-up and stratification by diagnosis at baseline (chronic hepatitis and cirrhosis) are needed to determine if interferon-alpha reduces the incidence of HCC by slowing the progression of chronic hepatitis to cirrhosis or by interfering with oncogenic mechanisms in patients with cirrhosis.

FACTORS INFLUENCING DISEASE OUTCOME AND PREDICTING RESPONSE TO INTERFERON-ALPHA

When factors influencing the progression of disease were evaluated by multivariate analysis, older age was predictive of the worst outcome in treated and untreated patients [5, 24]. In addition, when chronic hepatitis and patients with cirrhosis were analyzed separately, high levels of viral replication (more frequently observed in patients with a

persistent biochemical disease profile) and steatosis were associated with progression of chronic hepatitis to cirrhosis. Flares of anti-hepatitis B Core antigen, antibody (HBc) IgM levels, a hallmark of hepatitis B exacerbation, were associated with progression of cirrhosis to end-stage complications [5].

Factors predicting an interferon-alpha response are not well defined. In a pilot study, starting therapy when levels of Anti-HBc increase were associated with a higher sustained response rate [25]. Recently, a larger study showed a correlation between a high sustained response, high anti-HBc IgM levels and undetectable HBV DNA [23]. In addition, when baseline HBV DNA levels were measured by quantitative PCR, median baseline levels were significantly lower in sustained responders [22]. These findings suggest that starting treatment immediately after a hepatitis flare may increase the chance of a sustained response. During treatment an early virological and biochemical response was associated with a 3.45 times higher probability of a sustained response [22].

TREATMENT WITH PEGYLATED INTERFERON

A recent, large, multicenter study that investigated both the efficacy and safety of pegylated interferon-alpha-2a (40kd) with and without lamivudine versus lamivudine alone resulted in significantly higher post-treatment response rates in patients treated with pegylated interferon-alpha-2a than in those treated with lamivudine [26, 27]. Patients received pegylated interferon-alpha alpha-2a 180µg once-weekly plus oral placebo (n=177); pegylated interferon-alpha-2a 180µg once-weekly plus lamivudine 100mg daily (n=179); or lamivudine 100mg daily (n=181) for 48 weeks, with a 24-week, treatment-free follow-up. After 24 weeks of follow-up, the percentage of patients with either ALT normalization or HBV DNA <20,000 copies/mL was significantly higher with pegylated interferon-alpha alpha-2a monotherapy (59% and 43%, respectively) and pegylated interferon-alpha-2a plus lamivudine (60% and 44%) than with lamivudine monotherapy (44% and 29%; $P<0.01$). HBsAg loss was seen in 12 patients who received pegylated interferon-alpha-2a (alone or in combination) and in none of those receiving lamivudine. Reported adverse events were as to be expected for lamivudine and interferon-alpha-based therapy. The addition of lamivudine to pegylated interferon-alpha-2a did not alter the pegylated interferon-

alpha-2a safety profile over the 48-week treatment period. Interestingly, compliance (particularly depression rates) was much better than previously reported for pegylated interferon-alpha-2a (40kd) in patients with chronic hepatitis C. Pegylated interferon-alpha alone or in combination with antiviral treatments could improve the suppression of HBV replication or may result in a more effective control of HBV infection.

CONCLUSIONS AND PERSPECTIVES

In conclusion, interferon-alpha is the standard treatment for anti-HBe-positive chronic hepatitis B. However, optimization of treatment schedules is necessary.

Early treatment appears to be indicated in patients with disease profiles associated with faster progression. Interferon-alpha also appears to reduce end-stage complications in patients with cirrhosis. It is unclear at present whether interferon-alpha reduces the incidence of HCC. Prospective studies are needed to answer this question.

Future studies should investigate the kinetics of both viral replication and immune response during combination therapy to assess the antiviral and the immune-modulatory activity of interferon-alpha [27]. In addition further studies are needed to investigate whether treatment initiation after flares of transaminases increases the sustained response rate and whether subgroups of patients (i.e. infected with certain genotype) respond better to combination therapy [27].

REFERENCES

1. Bonino F, Hoyer B, Nelson J, Engle R, Verme G, Gerin J. Hepatitis B virus DNA in the sera of HBsAg carriers - a marker of active hepatitis B virus replication in the liver. Hepatology 1981;1:386-391.
2. Hadziyannis SJ, Lieberman HM, Karvountzis GG, Shafritz DA. Analysis of liver, nuclear HBcAg, viral replication and hepatitis B virus DNA in liver and serum of HBeAg versus anti-HBe positive carriers of hepatitis B virus. Hepatology 1983;3:656-662.
3. Brunetto MR, Stemler M, Schoedel F, et al. Identification of HBV variants which cannot produce precore derived HBeAg and may be responsible for severe hepatitis. Ital J Gastroenterol 1989;21:151-154.
4. Carman WF, Hadziyannis SJ, McGarvey MJ, Makris A, Thomas HC. Mutation preventing formation of hepatitis Be antigen in patients with chronic hepatitis B infection. Lancet 1989;2:588-591.
5. Brunetto MR, Oliveri F, Coco B, Leandro L, Colombatto P, Monti Gorin J, Bonino F. Outcome of anti-HBe positive chronic hepatitis in alpha-interferon treated and untreated patients: a long-term cohort study. J Hepatol 2002;36:263-270.
6. Lok AS, Heathcote J, Hoofnagle JH. Management of hepatitis B: 2000 - summary of a workshop. Gastroenterology 2001;120:1828-1853.
7. Chan HL, Leung NW, Hussain M, Wong ML, Lok AS. Hepatitis Be antigen-negative chronic hepatitis B in Hong Kong. Hepatology 2000;31:763-768.
8. Hadziyannis SJ. Interferon-alpha in HBeAg negative chronic hepatitis B: new data in support of long-term efficacy. J Hepatol 2002;36:280-282.
9. Brunetto MR, Oliveri F, Rocca G, Criscuolo D, Chiaberge E, Capalbo M, David E, Verme G, Bonino F. Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis Be antigen. Hepatology 1989;10:198-202.
10. Hadziyannis S, Bramou T, Makris A, Moussoulis G, Zignego L, Papaioannou C. Interferon alpha-2b treatment of HBeAg negative/serum HBV-DNA positive chronic hepatitis type B. J Hepatol 1990;11:S133-S136.
11. Pastore G, Santantonio T, Milella N, Monno L, Moschetta R, Pollice L. Anti-HBe positive chronic hepatitis B with HBV-DNA in the serum: response to a six month course of lymphoblastoid interferon. J Hepatol 1992;14:221-225.
12. Fattovich G, Farci P, Rugge M, Brollo L, Mandes A, Pontisso P, Giustina G, Lai ME, Belussi F, Busatto G. A randomized controlled trial of lymphoblastoid IFN-alpha in patients with chronic hepatitis B lacking HBeAg. Hepatology 1992;15:584-589.

13. Brunetto MR, Oliveri F, Colombatto P, Capalbo M, Barbera C, Bonino F. Treatment of chronic anti-HBe positive hepatitis B with interferon-alpha. J Hepatol 1995;22:42-44.

14. Lampertico P, Del Ninno E, Manzin A, Donato MR, Rumi MG, Lunghi G, Morabito A, Clementi M, Colombo M. A randomized, controlled trial of 24-month course of interferon alfa 2b in patients with chronic hepatitis B who had hepatitis virus DNA without hepatitis Be antigen in serum. Hepatology 1997;26:1621-1625.

15. Karasawa T, Aizawa Y, Zeniya M, Kuramoto A, Shirasawa T, Toda G. Genetic heterogeneity in the pre-core region of HBV in hepatitis Be antigen-negative chronic hepatitis B patients: spontaneous seroconversion and interferon-induced seroconversion. J Med Virol 1995;45:373-380.

16. Guptan RC, Thakur V, Malhotra V, Sarin SK. Low dose recombinant interferon therapy in anti-HBe positive chronic hepatitis B in Asian Indians. J Gastroenterol Hepatol 1998;13:675-679.

17. Lin CC, Wu JC, Chang TT, Huang YH, Wang YJ, Tsay SH, Chow NH, Chang FY, Lee SD. Long-term evaluation of recombinant interferon-alpha-2b in the treatment of patients with hepatitis Be antigen negative chronic hepatitis B in Taiwan. J Viral Hep 2001;8:438-446.

18. Hoofnagle JH, Di Bisceglie AM. The treatment of chronic viral hepatitis. N Engl J Med 1997;336;5:347-353.

19. Koreman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-634.

20. Wong DKH, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis Be antigen-positive chronic hepatitis B. Ann Intern Med 1993;119:312-323.

21. Oliveri F, Santantonio T, Bellati G, Colombatto P, Colloredo-Mels G, Carriero L, Dastoli G, Pastore G, Ideo G, Bonino F, Brunetto MR. Long-term response to therapy of chronic anti-HBe positive hepatitis B is poor independent of type and schedule of interferon. Am J Gastroent 1999;94;1366-1372.

22. Manesis EK, Hadziyannis SJ. Interferon-alpha treatment and retreatment of hepatitis Be antigen-negative chronic hepatitis B. Gastroenterology 2001;121:101-109.

23. Lampertico P, Del Ninno E, Viganò M, Romeo R, Donato MF, Sablon E, Morabito A, Colombo M. Extended interferon therapy increases hepatitis suppression and reduces liver-related morbidity of hepatitis Be antigen negative chronic active hepatitis B. Hepatology 2003;37:756-763.

24. 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;34:306-313.

25. Lobello S, Lorenzoni U, Vian A, Floreani A, Brunetto MR, Chiaramonte M. Interferon treatment in hepatitis B surface antigen-positive hepatitis e antibody-positive chronic hepatitis B: role of hepatitis B core antibody IgM titre in patients selection and treatment monitoring. J Viral Hep 1998;5:61-66.

26. Marcellin P, Lau GKK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piravisuth T, Germanidis G, Pluck N. Peginterferon alfa-2a (40KD) (Pegasys®) monotherapy is more effective than lamivudine monotherapy in the treatment of HBeAg negative chronic hepatitis B: 72-week results from a phase III, partially double-blind study of Pegasys® alone vs. Pegasys® plus lamivudine vs. lamivudine. J. Hepatol 2004;40:S34.

27. Colombatto P, Bizzarri R, Civitano L, Oliveri F, Bonino F, Germanidis G, Farci P, Kitis G, Hadziyannis S, Choudhury S, Gieschke R, Zahm F, Brunetto MR. A new bio-mathematical model to describe the dynamics of HBV-DNA and infected hepatocytes in HBeAg negative/anti-HBe positive chronic hepatitis B patients treated with peginterferon alfa 2a (40KD) (Pegasys®), lamivudine, or Pegasys® plus lamivudine combination. J Hepatol 2004;40:S125.

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