Management of Patients with Viral Hepatitis

This book contains the manuscripts of the presentations from the 2nd International Conference on the Management of Patients with Viral Hepatitis, January 2007, Paris, France.

Printed in January 2007
by the Nouvelle Imprimerie Laballery, 58500 Clamecy
Print number : 611205, copyright : January 2007, printed in France

Articles in this book can be cited as: In: Marcellin P, editor. Management of Patients with Viral Hepatitis. Paris: APHC; 2007.

This book is supported by an unrestricted educational grant from
F. Hoffmann-La Roche Ltd.

Management of Patients with Viral Hepatitis

Editor

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

CONTRIBUTING AUTHORS

Alfredo Alberti
Department of Clinical and Experimental Medicine and Venetian Institute of Molecular Medicine, University of Padova, Padova, Italy

Bruce Bacon
Division of Hepatology, St Louis University School of Medicine, St Louis, Missouri, USA

Jason Bratcher
Lenox Hill Hospital, New York, USA

Michel Beaugrand
Service d'Hépatologie, Hôpital Jean Verdier, Bondy, France

Yves Benhamou
Service d'Hépato-Gastroentérologie, Hôpital Pitié-Salpétrière, Paris, France

Maria Buti
Liver Unit, Hospital General Universitario Valle Hebron, Barcelona, Spain

Stéphane Chevaliez
French National Reference Center for Viral Hepatitis B, C and delta, Department of Virology & INSERM U635, Henri Mondor Hospital, University of Paris XII, Créteil, France

Gary L. Davis
Division of Hepatology, Baylor University Medical Center, Dallas, Texas, USA

Yves Deugnier
Clinique des Maladies du Foie, Hôpital Pontchaillou, University of Rennes, Rennes, France

Douglas Dieterich
Department of Medicine, Mount Sinai Medical Center, New York, USA

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

Rafael Esteban
Liver Unit, Hospital General Universitario Valle Hebron, Barcelona, Spain

Patrizia Farci
Department of Medical Sciences, University of Cagliari, Cagliari, Italy

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

Dominique Guyader
Service des Maladies du Foie, Inserm U 522 and CIC Inserm 0203
Hôpital Pontchaillou, 35033 Rennes, France

Chee-Kin Hui
Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong SAR, China

Stephanos J. Hadziyannis
Department of Medicine and Hepatology, Henry Dunant Hospital and Hepatitis Research Laboratory of Athens University, Evgenidion Hospital, Athens, Greece

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

George L. K. Lau
Division of Hepatology, Queen Mary Hospital, University of Hong Kong, Pokfulam, Hong Kong, China

Anna S. F. Lok
Division of Gastroenterology, University of Michigan Medical Center, Ann Arbor, Michigan, USA

Patrick Marcellin
Service d'Hépatologie and Inserm U 773, Hôpital Beaujon, University of Paris VII, Clichy, France

Francesco Negro
Division of Gastroenterology, University Hospital, Geneva, Switzerland

Nicola Olivari
Department of Surgical and Gastroenterological Science, Section of Gastroenterology, University of Verona, Italy

Michela Pasino
Department of Surgical and Gastroenterological Science, Section of Gastroenterology, University of Verona, Italy

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

Robert P. Perrillo
Division of Hepatology, Baylor University Medical Center, Dallas, Texas, USA

Bruno Roche
Centre Hépatobiliaire, Hôpital Paul Brousse, 14 Avenue Paul Vaillant Couturier, 94800 Villejuif, France

Patrick Saitta
The Mount Sinai Medical Center, New York, USA

Didier Samuel
Centre Hépatobiliaire, Hôpital Paul Brousse, Medical School of University of Paris XI, Villejuif, France

Christoph Sarrazin
Saarland University Hospital, Department of Internal Medicine II, Homburg, Germany

Silvia Zanni
Department of Surgical and Gastroenterological Science, Section of Gastroenterology, University of Verona, Italy

Stefan Zeuzem
Department of Internal Medicine II, Saarland University Hospital, Homburg, Germany

Fabien Zoulim
Service d'Hépato-Gastroentérologie and INSERM U271, Hôpital Hôtel Dieu, University of Lyon, Lyon, France

CONTENTS

Hepatitis C and Hepatitis B in 2007

P. Marcellin

INTRODUCTION

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]. Hepatitis B virus- 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.

In the last few years, knowledge of the epidemiology and natural history of HBV and HCV infection has markedly improved. Furthermore, considerable progress has been made 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

Approximately one third of the world's population has serological evidence of past or present infection with HBV and 350 million people are chronically infected. The prevalence of HBV infection is especially high in South-East Asia and Sub-Saharan Africa where more than 8% of the population are chronic hepatitis B surface antigen (HBsAg)-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 industrialized 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 to 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 healthcare 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 to 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 to 40%, with 40 to 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. Hepatitis B virus-related end-stage liver disease or HCC are responsible for over 1 million deaths per year and currently represent 5 to 10% of cases of liver transplantation [1,3,4,]. Hepatocellular carcinoma is one of the most common cancers worldwide and HBV is responsible for at least 75% of cases [6]. The availability of safe and effective vaccines allowed for large-scale 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, such as 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 precore and/or basic core promoter regions of the genome and represents a late phase of chronic HBV infection [12]. The latter form of the disease has been increasing in

many countries over the last decade and represents the majority of cases in many countries. HBeAg-negative chronic hepatitis B is generally associated with 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 to 20%. Morbidity and mortality in chronic hepatitis B are linked to evolution to cirrhosis or HCC. The 5-year cumulative incidence of hepatic decompensation is approximately 20% [15]. The 5-year probability of survival being approximately 80 to 86% in patients with compensated cirrhosis. Patients with decompensated cirrhosis have a poor prognosis (14 to 35% probability of survival at 5 years). Hepatitis B virus-related end-stage liver disease or HCC are responsible for at least 500,000 deaths per year. A recent study carried out in France showed that there were at least 1500 cases per year of HBV-related mortality due to decompensated cirrhosis or HCC [16]. Hepatocellular carcinoma is one of the most common cancers worldwide and approximately 75% of cases are related to chronic HBV infection. The worldwide incidence of HCC has increased and nowadays it constitutes the fifth most common cancer, representing around 5% of all cancers. The incidence of HCC appears to vary geographically and correlates with the underlying stage of liver disease. 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 alfa-fetoprotein determination [18]. This allows for effective therapy such as surgical resection, liver transplantation or percutaneous ablation to be carried out.

Therapy

Five drugs are currently available for the treatment of chronic hepatitis B: 1) conventional interferon alfa; 2) lamivudine (LAM); 3) adefovir dipivoxil (ADV); 4) pegylated interferon alfa-2a; and, 5) entecavir (ETV) [1,8,19]. Conventional interferon-alfa, administered for 4-6 months in HBeAg-positive patients and

12-24 months in HBeAg-negative patients, induces a sustained response in only a minority of patients (10 to 30%) and is associated with a poor tolerability which limits duration of therapy [20,21].

Lamivudine

Lamivudine was the first nucleoside analog used in the treatment of chronic hepatitis B that has the advantages of oral administration and excellent tolerance. Lamivudine administered for 12 months induces a sustained response in approximately 20% of HBeAg-positive and 5% of 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

Adefovir dipivoxil is the first nucleotide analog to be used in the treatment of chronic hepatitis C and similarly to LAM, has the advantages of oral administration and excellent tolerance. Adefovir 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 ADV is relatively low (29% at 5 years) [32]. Adefovir is effective in the treatment of LAM-resistant HBV [29]. It has been used successfully in patients with decompensated cirrhosis, in the pretransplant setting or in post-transplant patients who have developed resistance to LAM [33]. In patients with HBV/HIV co-infection with LAM-resistant HBV, treatment with ADV has a marked antiviral effect, similar to that observed in HIV-negative patients [34].

Pegylated interferon

A randomized controlled study of pegylated interferon alfa-2a vs. conventional interferon alfa-2a showed a trend towards better efficacy with pegylated interferon with HBeAg seroconversion rates of 37% and 25%, respectively [35]. Two randomized controlled studies of pegylated interferon alfa-2a in patients with HBeAg-positive or 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 (3% and 4%, respectively) as compared with less than 1% in patients treated with LAM.

Entecavir

Results of phase III trials of ETV in HBeAg-positive and HBeAg-negative chronic hepatitis B showed excellent efficacy and excellent safety. Mean serum HBV DNA decrease in HBeAg-positive and HBeAg-negative patients was 6.9 $\log_{10}$ copies/mL and 5.0 $\log_{10}$ copies/mL, respectively. Sixty-seven percent and 90% of patients had HBV DNA that was undetectable with polymerase chain reaction (PCR), respectively [38,39]. Despite the potent antiviral effect of ETV, the HBe seroconversion rate was relatively low (21% at 1 year). Interestingly, no resistance was observed in patients who were not previously treated with LAM.

Perspectives

Currently available drugs have limited long-term efficacy and new more potent drugs or therapeutic strategies are needed. The concept of combination therapy has been developed in order to increase efficacy and to decrease resistance. Combinations of pegylated interferon, with LAM and combination of ADV and LAM have been assessed.

Combination of adefovir and lamivudine

One randomized study evaluated the efficacy of the combination of ADV with LAM as compared to LAM alone or ADV alone in 59 patients with HBeAg-positive chronic hepatitis B with LAM-resistant HBV [40]. There was no significant difference in median serum HBV DNA reduction (-3.59 $\log_{10}$ copies/mL and -4.04 $\log_{10}$ copies/mL), rates of alanine aminotransferase (ALT) normalization (53% and 47%) and HBeAg loss (3 patients in each group) between the ADV with LAM combination group and the ADV monotherapy group. Another study compared the efficacy of the combination of ADV with LAM vs. LAM used in monotherapy in 112 treatment-naïve patients (107 HBeAg-positive) [41]. There was no significant difference in median serum HBV DNA reduction (-5.41 $\log_{10}$ copies/mL and -4.80 log copies/mL), rates of undetectable HBV DNA with PCR (39% and 41%) and HBeAg loss (19% and 20%) between the ADV with LAM combination group and the ADV monotherapy group. Finally, these 2 studies did not show superior

efficacy of combination therapy compared with either drug in monotherapy.

Combination of pegylated interferon and lamivudine

Two randomized controlled trials of combination therapy with pegylated interferon alfa-2a and LAM vs. pegylated interferon alone did not show a superiority of the combination treatment in terms of sustained response [36,37]. However, it is noteworthy that higher end-of-treatment response rates were observed with the combination therapy. Furthermore, in both studies, the combination of pegylated interferon alfa-2a and LAM decreased the incidence of LAM-resistance [36,37]. Different schedules of combination need to be assessed in order to improve the efficacy of the combination of pegylated interferon with potent nucleoside or nucleotide analogs.

New analogs

A number of nucleoside and nucleotide analogs, with favorable toxicity profiles and a promise of increased effectiveness against HBV, are at various stages of clinical development. The results of studies of telbivudine (LdT), tenofovir (TDF) and clevudine are promising [42,43]. Other interesting compounds are at an earlier phase of development (Table 1). These new analogs seem to be more potent than LAM and ADV and have a good safety profile. However, it could be expected that their use in monotherapy would not induce a high rate of sustained response and that long-term therapy or combination therapy would be needed to improve efficacy and/or reduce resistance.

Nucleos(t)ide analog	Stage of development
Lamivudine	Approved
Adefovir dipivoxil	Approved
Entecavir	Approved
Tenofovir	Phase III
Emtricitabine	Phase III
Telbivudine	Phase III
Clevudine	Phase III
Pradefovir	Phase II
Valtorcitabine	Phase II

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

HEPATITIS C

Epidemiology

Approximately 3% of the world's population, 170 million people, are chronically infected with HCV. The prevalence of chronic hepatitis C ranges from 0.1 to 5% in different countries [44-46]. It is estimated that there are 4 million HCV chronic carriers in the United States and 5 million 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 to 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 2 reasons: 1) transmission by blood products has been reduced to near zero; and, 2) 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 3759 deaths were attributed to HCV in 1999, although this is likely to be an underestimate [49]. A recent study in France found at least 3500 HCV-related deaths due to decompensated cirrhosis or HCC [16]. There was a 5-fold increase in the number of patients with HCV who underwent liver transplantation each year between 1990 and 2000. The total direct healthcare 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%, of whom 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 last few years, the natural history of chronic HCV infection has become increasingly 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 necro-inflammation and the 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 of progression of fibrosis 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 [50-51]. Viral load and genotype do not seem to significantly influence the progression rate. Progression of fibrosis is more rapid in immunocompromised patients [52]. Recently, the importance of hepatic steatosis, obesity and insulin resistance have been recognized and studies are being carried out to help our understanding of the relationship between metabolic disorders, HCV replication and liver steatosis, and progression of fibrosis [53]. There are no tests that reliably predict the rate of progression of fibrosis in an individual. High serum ALT levels are associated with a higher risk of fibrosis progression. The 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 in ALT level 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 about the stage of fibrosis and grade of necro-inflammation, both of which have prognostic significance. Repeating liver biopsy, 3-5 years after the initial biopsy, is the most accurate means of assessing the progression of fibrosis [2,47,51].

Therapy

Combination of pegylated interferon with ribavirin

The most impressive progress has been made in the efficacy of therapy. The combination of pegylated interferon with ribavirin (RBV) has become a reference therapy [2,56]. A sustained virological response (SVR) is observed in roughly 50 to 60% of patients [57-59]. The absence of detectable serum HCV RNA 6 months after therapy, which defines the SVR, may be considered nowadays as cure of HCV

infection since long-term follow-up studies have shown that 97 to 100% of patients maintain an undetectable level of 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]. Recently, 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 have confirmed the eradication of HCV infection in sustained responders [61]. 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 RBV (50%). The compliance with continuation of therapy with adequate dosing increases the response rates and studies on adjuvant treatments are needed to improve clinical and hematological tolerability (e.g. erythropoietin for RBV-related anemia) in order to improve tolerability, and increase compliance and the chance of response. Recent studies suggest that treatment regimens, in terms of RBV dosage and duration of therapy, may be adjusted according to genotype and viral load. Patients with genotypes 2 or 3 with low viral load (less than 400,000 IU/mL) could be treated for shorter periods (12-16 weeks) [62]; conversely, some patients with genotypes 3 and high viral load (more than 400,000 IU/mL) might benefit from longer-term (48 weeks) therapy. In addition, some patients with genotype 1, with slow response, might benefit from prolonged therapy (72 weeks). New algorithms with regard to treatment duration according to genotype, baseline viral load and rapid virological response (as assessed at 4 weeks of therapy) need to be confirmed.

Treatment of non-responders and relapsers

In patients who have received combination therapy with conventional interferon and RBV, the chance to achieve an SVR with retreatment with pegylated combination therapy depends mainly on the genotype and the presence or absence of cirrhosis. The noncirrhotic patients with genotype 2 or 3 have a 30 to 40% chance of achieving SVR while patients with genotype 1 and cirrhosis have almost 0% chance of sustained response [63]. In patients who relapsed after conventional interferon-RBV combination therapy, the chance of SVR with retreatment with the pegylated combination is as high as 50% [63].

Maintenance therapy

In non-responders to current pegylated combination therapy, the concept of maintenance therapy has been developed over 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 necro-inflammation 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

About half of all 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. New types of interferon (albuferon and more recently gene shuffled interferon) are promising. Furthermore, the use of toll-like receptor agonists (CPG), which enhance the cellular interferon pathways, are an interesting approach. Viramidine, an RBV analog has been shown to be associated with a lower incidence of anemia; however, at the dose used in recent trials, it is less effective than RBV. Further studies with weight-adjusted dosage are needed. 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 difficulty of reaching the target cells (hepatocytes). Indeed, the enzyme inhibitors appear to be the most promising strategy. In recent years, extensive research has been conducted to elucidate the structure of HCV enzymes in order to produce specific enzyme inhibitors. 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 therapeutic targets. The absence of cell culture models 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, represents a significant advance [64]. This model (replicon) is the best to date for

the study of HCV replication and the testing for antiviral molecules. The ability of an NS3 protease inhibitor (BILN 2061) to inhibit NS3 protease activity in the subgenomic HCV replicon cell model has been demonstrated [65]. In several recent phase I and II studies protease and polymerase inhibitors have shown their capacity to efficiently reduce HCV replication [67,68]. However, these compounds rapidly induce viral resistance and may be associated with non negligible side effects. Results of current trials of these drugs in combination with pegylated interferon and RBV will determine their long-term safety and efficacy, while future trials will determine the optimal schedules in terms of combination (with or without RBV; with 1 or 2 enzyme inhibitors). These drugs constitute a major step in the field of HCV therapy in patients with chronic hepatitis C.

CONCLUSION

Hepatitis B virus- and HCV-related liver diseases represent a major public health problem. In recent years, there has been considerable improvement in our understanding of the epidemiology, natural history, other factors influencing the course of the liver disease, and particularly of the efficacy of therapy. In order to improve the management of patients with chronic hepatic B or C, efforts to facilitate early diagnosis are still required. The spectacular progress over the last 5 years has led to the control of HBV replication in more than half of patients with chronic hepatitis B, and the eradication of HCV infection in more than half of patients with chronic hepatitis C. Taking into account the recent advances with new antiviral drugs, one may hope that the large majority of patients will be efficiently treated within the next 5 years, thus reducing the global burden of 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 YF, 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 Be antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.
13. Zarski JP, Marcellin P, Leroy V, Trépo C, Samuel D, Ganne-Carrie N, Barange K, Canva V, Doffoel M, Cales P, Fédération nationale des Pôles de Référence et des Réseaux Hépatiques. Characteristics of patients with chronic hepatitis B in France: predominant frequency of HBe antigen negative cases. J Hepatol 2006;45:355-360.
14. Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Gorin JM, Bonino F. Outcome of anti-HBe positive chronic hepatitis B in alfa-interferon treated and untreated patients: a long term cohort study. J Hepatol 2002;36:280-282.
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. Marcellin P, Pequignot F, Delaroque-Astagneau E, Ganne N, Hillon P, Bovet M, Mechain M, Antona D, Asselah T, Desenclos C, Jougla E. Evidence for high rates of HCV and HBV related mortality in France: a large-scale national survey. J Hepatol 2006;44:S174.

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 alfa-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 alfa 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 alfa 2b in patients with chronic hepatitis B who previously failed interferon therapy (abstract). Hepatology 1998;28:S388.

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 Be 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. Longterm 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. Marcellin P, Asselah T Resistance to adefovir: a new challenge in the treatment of chronic hepatitis B. J Hepatol 2005;43:920-923.

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 alfa 2a (40KDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.

36. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N; Peginterferon Alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005;352:2682-2695.

37. Marcellin M, Lau GKK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Kiago M, Gurel S, Lai MY, Button P, Pluck N, for the Peginterferon alfa-2a HBeAg-negative chronic hepatitis B Study Group. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206-1217.

38. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-1010.

39. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L; BEHoLD AI463027 Study Group. Entecavir versus lamivudine for patients with HbeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-1020.

40. 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.

41. 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.

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 JP, 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. Gastroenterol Clin Biol 2002;26:B312-B320.

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

50. 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.

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

52. 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.

53. Asselah T, Rubbia-Brandt L, Marcellin P, Negro F. Steatosis in chronic hepatitis C: why does it really matter? Gut 2006;55:123-130.

54. Marcellin P, Lévy S, Erlinger S. Therapy of hepatitis C: patients with normal aminotransferase levels. Hepatology 1997;26:S33-S37.

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 antihepatitis 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. N Engl 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. Ann Intern Med 1997;127:875-881.

61. Maylin S, Martinot-Peignoux M, Boyer N, Ripault MP, Féray C, Nicolas-Chanoine MH, Marcellin P. Evidence for eradication of HCV, assessed with transcription mediated amplification (TMA), in chronic hepatitis C patients with sustained virological response to therapy. J Hepatol 2006;44:S221.

62. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M. Zechini F, Sogari F, Spirito F, Andriulli A. Peginterferon alfa-z2b and ribavirin for 12 vs 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609-2617.

63. Moucari R. Ripault MP, Oules V, Martinot-Peignoux M, Asselah T, Boyer N, El Ray A, Cazals-Hatem D, Vidaud D, Valla D, Bourlière M, Marcellin M. High predictive value of early viral kinetics in retreatment with peginterferon and ribavirin of chronic hepatitis C patients non-responders to standard combination therapy. J Hepatol 2006 (in press).

64. 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.

65. 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.

66. Marcellin P, Asselah T, Boyer N. Treatment of chronic hepatitis B. J Viral Hepat 2005;12:333-345.

67. Neyts J. Selective inhibitors of hepatitis C virus replication. Antiviral Res 2006;71:363-371.

68. Del Vecchio AM, Sarisky RT. Small molecule and biologic inhibitors of hepatitis C virus: a symbiotic approach. Mini Rev Med Chem 2006;6:1263-1268.

How to Use Virological Tools for the Optimal Management of Chronic Hepatitis C

S. Chevaliez, J-M. Pawlotsky

INTRODUCTION

Hepatitis C virus (HCV) infects approximately 170 million individuals worldwide. Combination antiviral therapy with pegylated interferon alfa and ribavirin (RBV) is the basis to the prevention of complications associated with HCV infection. The use of serological and virological tests has become essential in the management of HCV infection in order to: 1) diagnose infection; 2) guide treatment decisions; and, 3) assess the virological response to therapy. Anti-HCV antibody testing and HCV RNA testing are used to diagnose acute and chronic hepatitis C. The HCV genotype should be systematically determined before treatment, to identify the indication, the duration of treatment, the dose of RBV and the virological monitoring procedure. HCV RNA monitoring during therapy is used to tailor treatment duration in HCV genotype 1 infection. Molecular assays are used to assess the end-of-treatment and, most importantly, the sustained virological response (SVR), i.e. the end-point of therapy.

The treatment of chronic HCV infection with pegylated interferon alfa and RBV results in the sustained eradication of infection in 40 to 50% of cases [1].

VIROLOGICAL TOOLS

Serological assays

Anti-HCV antibody detection

Third-generation enzyme immuno-assays (EIA) detect antibodies directed against various HCV epitopes in plasma or in serum. The specificity of third-generation EIAs for anti-HCV is more than 99% [2]. The sensitivity is more difficult to evaluate because there is no gold standard, but it is excellent in HCV-infected immunocompetent patients. Immunoblot tests are clinically obsolete thanks to the performance of third-generation anti-HCV EIAs [3].

Serological determination of the HCV genotype

The HCV genotype can be determined by seeking antibodies directed to genotype-specific HCV epitopes with a competitive EIA. The currently available assay (Murex HCV serotyping 1-6 HC02, Abbott Laboratories, North Chicago, Illinois) identifies the viral type (1 to 6), but not the subtype, and results can be interpreted in approximately 90% of chronically infected immunocompetent patients [4]. Mixed serological reactivity may occur and may be related to mixed infection. This may also be linked to cross-reactivity or recovery and thus persistent viremia from infection with another genotype.

Detection and quantification of HCV RNA

Qualitative, nonquantitative HCV RNA detection

Qualitative detection assays are based on target amplification with either "classic" polymerase chain reaction (PCR), "real-time" PCR or "transcription-mediated amplification" (TMA) [5-7]. Qualitative detection assays must detect HCV RNA ≤50 IU/mL, and be equally sensitive to all HCV genotypes. The low detection limit of the qualitative, nonquantitative reverse-transcriptase PCR-based assay Amplicor® HCV v2.0, or its semi-automated version Cobas® Amplicor® HCV v2.0 (Roche Molecular Systems, Pleasanton, California) is 50 IU/mL, whereas that of the TMA-based assay Versant® HCV RNA Qualitative Assay (Bayer HealthCare, Tarrytown, New York) is 10 IU/mL (Table 1). The low detection limits of real-time PCR assays, which also quantify HCV RNA, are 15 IU/mL (Cobas Ampliprep®-Cobas Taqman® [CAP-CTM] HCV

Assay	Manufacturer	Technique	Dynamic range of quantification (quantitative assay)
Amplicor HCV Monitor® v2.0	Roche Molecular Systems	Manual RT-PCR	600-500,000 IU/mL
Cobas® Amplicor HCV Monitor v2.0	Roche Molecular Systems	Semi-automated RT-PCR	600-500,000 IU/mL
LCx HCV RNA Quantitative Assay	Abbott Diagnostic	Semi-automated RT-PCR	25-2,630,000 IU/mL
Versant® HCV RNA 3.0 Assay	Bayer HealthCare	Semi-automated bDNA	615-7,700,000 IU/mL
Cobas® TaqMan HCV Test	Roche Molecular Systems	Semi-automated real-time PCR	43-69,000,000 IU/mL
Abbott RealTime	Abbott Diagnostic	Semi-automated real-time PCR	12-100,000,000 IU/mL

bDNA=branched DNA; HCV= hepatitis C virus; PCR=polymerase chain reaction; RT=reverse transcriptase

Table 1: Characteristics of current quantitative HCV RNA assays

Test, Roche Molecular Systems) and 12 to 30 IU/mL according to the amount of blood tested (Abbott RealTime™ HCV Assay, Abbott Diagnostic) when they are used as purely qualitative, nonquantitative assays. Qualitative, nonquantitative HCV RNA assays are being replaced in most settings by highly sensitive quantitative real-time PCR assays.

HCV RNA quantification

HCV RNA can be quantified by target (competitive PCR or real-time PCR) or signal amplification techniques (branched DNA [bDNA] assay) [5]. Five standardized assays are commercially available, 2 of which are based on competitive PCR: Amplicor HCV Monitor® v2.0 and its semi-automated version Cobas® Amplicor HCV Monitor® v2.0, and LCx® HCV RNA Quantitative Assay (Abbott Laboratories); another is based on bDNA technology, Versant® HCV RNA 3.0 Assay (Bayer Healthcare); and 2 others are based on real-time PCR amplification, Cobas® TaqMan HCV Test, which can be coupled with automated extraction in Cobas Ampliprep®, and Abbott RealTime™ HCV assay, which uses the Abbott *m*2000RT system and can also be coupled with an automated extraction procedure in *m*2000SP (*m*2000 Real-Time PCR System). HCV RNA levels above the upper limit of quantification of the assays are underestimated and the samples must

be diluted 1/10 to 1/100 then retested to obtain accurate quantification. The Cobas® TaqMan HCV Test has been shown to underestimate some HCV genotype 4 and, less often, HCV genotype 2 samples [8]. In addition, despite the use of international units for quantification, differences in calibration compared to the primary World Health Organization HCV RNA standard, cause slight differences in results using the same samples in different assays [8]. The most promising approach for the future is fully automated real-time PCR assays.

Molecular determination of the HCV genotype (genotyping)

The reference method to determine HCV genotype is direct sequencing of the NS5B or E1 regions of the HCV genome by "in-house" techniques, followed by sequence alignment with prototype sequences and phylogenetic analysis [9,10]. In clinical practice, the HCV genotype can be determined by various commercial kits, using direct sequence analysis of the 5' noncoding region (Trugene® 5'NC HCV Genotyping Kit, Bayer HealthCare) or reverse hybridization analysis using genotype-specific probes located in the 5' noncoding region (commercialized as INNO-LiPA HCV II, Innogenetics, Ghent, Belgium, or Versant® HCV Genotyping Assay, Bayer HealthCare) [11-14]. Mis-typing is rare with these techniques, but mis-subtyping may occur in 10 to 25% of cases, because of the region studied (5' noncoding region) rather than the technique. These errors have no clinical consequence, because only the type is used for therapeutic decision-making. An assay based on direct sequencing of the NS5B region is currently under development (Trugene® NS5B HCV Genotyping Kit, Bayer HealthCare).

DIAGNOSIS OF HCV INFECTION

Acute hepatitis C

Patients with suspected acute hepatitis C should be tested for both anti-HCV antibodies by EIA and HCV RNA with a sensitive technique, i.e. an HCV RNA assay with a low detection limit of 50 IU/mL or less [15]. Four marker profiles can be observed according to the presence or absence of markers. The presence of HCV RNA in the absence of anti-HCV antibodies strongly suggests acute HCV infection, which will be confirmed by seroconversion (i.e. the appearance of anti-HCV antibodies) a few days to weeks later.

Patients with acute infection can also have both HCV RNA and anti-HCV antibodies at diagnosis. In this case it is difficult to distinguish acute hepatitis C from an acute exacerbation of chronic hepatitis C or acute hepatitis from other causes.

Acute hepatitis C is very unlikely if both anti-HCV antibodies and HCV RNA are absent or if anti-HCV antibodies are present without HCV RNA. The latter should be retested a few weeks later because HCV RNA can be temporarily undetectable, due to transient, partial control of viral replication before the infection becomes chronic [16]. Except for these cases, the presence of anti-HCV antibodies in the absence of HCV RNA is generally seen in patients who have recovered from a past HCV infection. Nevertheless, this pattern cannot be differentiated from a false positive EIA result, the prevalence of which is unknown.

Chronic hepatitis C

In patients with clinical or biological signs of chronic liver disease, chronic hepatitis C is certain when both anti-HCV antibodies and HCV RNA are present [3,17]. Detectable HCV replication without anti-HCV antibodies is rare and almost only observed in profoundly immunodepressed patients, hemodialysis patients or agamma-globulinemic subjects [18,19].

In patients with no indication for therapy or contra-indications to antiviral drugs, virological tests have no prognostic value. They do not predict the natural course of infection or the onset of extrahepatic manifestations. In untreated patients, the severity of liver inflammation and fibrosis must be evaluated every 3-5 years by liver biopsy or non-invasive serological or ultrasound-based testing [1].

MANAGEMENT OF ANTIVIRAL THERAPY

The current standard treatment for chronic hepatitis C is a combination of pegylated interferon alfa and RBV [1]. The end-point for efficacy of hepatitis C treatment is an SVR, defined as the absence of detectable HCV RNA in serum assessed by an HCV RNA assay with a low detection limit of 50 IU/mL or less 24 weeks after the end of treatment [1]. As new, more sensitive assays with lower limits of HCV RNA detection become available, there may be some confusion as to whether patients have “undetectable” HCV RNA during therapy.

Initiation of therapy

Only patients with detectable HCV RNA should be considered for pegylated interferon alfa and RBV combination therapy [1]. The decision to treat patients with chronic hepatitis C depends on multiple parameters, including: 1) the precise assessment of the severity of liver disease and its outcome; 2) the presence of absolute or relative contra-indications to therapy; and, 3) the patient's willingness to be treated [20].

HCV genotype 1

As 40 to 50% of patients may have an SVR, treatment decision should be guided by a precise prognosis of liver disease by liver biopsy or a non-invasive method based on serological markers of fibrosis or ultrasound-based testing [21,22]. Patients with mild lesions should not be treated and their liver disease should be reassessed 3-5 years later. Patients with inflammation and/or fibrosis (METAVIR score A $\geq$2 and/or F $\geq$2) may be treated [1].

The approved dose of pegylated interferon alfa-2a is 180 µg per week, independent of body weight, whereas that of pegylated interferon alfa-2b is weight-adjusted at 1.5 µg/kg per week for all HCV genotypes. Patients infected with HCV genotype 1 should receive a high dose of RBV, i.e. 1000 to 1200 mg per day, based on body weight above or below 75 kg. Very overweight patients could benefit from a RBV dose of up to 1600 mg per day. Patients infected with HCV genotype 1 require 48 weeks of treatment (Figure 1) [1].

HCV RNA load decrease during therapy should be monitored to avoid treating patients who will not achieve an SVR [23,24]. Thus, HCV RNA quantification should be performed at baseline and after 12 weeks of treatment with the same technique and results compared [1]. Treatment must be continued when there is a 2 $\log_{10}$ drop in HCV RNA, i.e. when baseline HCV RNA levels decrease 100-fold or more, or when HCV RNA is undetectable at week 12 [1]. The presence of HCV RNA should be assessed in these patients with a sensitive technique (low detection limit: 50 IU/mL or less) at week 24. If HCV RNA is undetectable at week 24, treatment should be continued until week 48, with a high likelihood of SVR. It has been suggested that 24 weeks of therapy might suffice in patients with a low baseline viral load (<600,000 IU/mL) and undetectable HCV RNA at week 24 [25]. Rapid virological responders, defined as patients with no detectable HCV RNA at week 4 of therapy, could

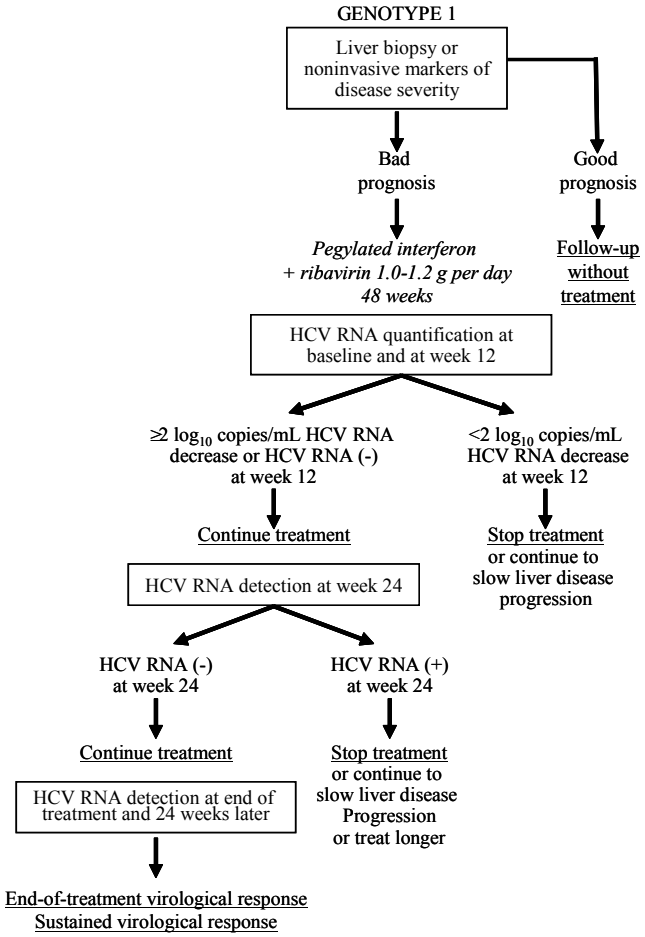


Figure 1: Treatment for patients infected with genotype 1

also benefit from shorter therapy. Ongoing trials will contribute information to future guidelines in this area.

In contrast, if HCV RNA is still detectable at week 24, there is virtually no chance of an SVR and treatment can be stopped, or continued to slow the progression of liver disease in patients with a severe prognosis [1,23]. Ongoing trials are studying whether prolonged antiviral treatment or maintenance therapy with pegylated interferon alfa monotherapy could be beneficial in these patients. When treatment is continued until week 48, the end-of-treatment and

SVRs should be assessed with a sensitive HCV RNA assay, with a low detection limit of ≤50 IU/mL [1]. The detection of HCV RNA at the end of therapy is highly predictive of a post-treatment relapse (relapse patients are identified earlier with more sensitive assays), whereas the absence of HCV RNA at the end of treatment indicates a virological response. The latter patients must be retested for HCV RNA with a sensitive method 24 weeks later to assess the SVR, i.e. the end-point of therapy [1,15]. Hepatitis C virus infection appears to be permanently cured in most patients with an SVR.

No virological response at 12 weeks (i.e. no change or an HCV RNA decrease of <2 $\log_{10}$ at week 12) is associated with a probability of a subsequent SVR of nearly zero [23,24]. Treatment can thus be stopped at week 12 in these patients, or continued in order to slow liver disease progression without clearing the virus. The benefits of maintenance therapy on the outcome of HCV-associated liver disease are under investigation. This "rule to stop", based on the monitoring of HCV RNA load reduction at week 12, has also been shown to apply to patients with HIV/HCV co-infection [26,28].

HCV genotypes 2 and 3

Patients infected with HCV genotypes 2 or 3 have a 70 to 80% chance of achieving an SVR with a low dose of RBV after 24 weeks of treatment [20,24,29]. Thus, if there are no contra-indications, these patients should be treated whatever the severity of liver disease. For these patients the recommended dose of pegylated interferon alfa-2a or -2b is the same as for HCV genotype 1. The fixed recommended dose of RBV is 800 mg per day [1]. Preliminary data suggest that lower doses of RBV and/or a shorter duration of treatment may be sufficient to achieve an SVR in certain subgroups of patients with genotypes 2 or 3, such as those with a low baseline viral load and no extensive fibrosis or cirrhosis [30]. Care should be taken when treating patients who have several baseline parameters of non-response, such as extensive fibrosis, older age and male gender; these patients may need 48 weeks of therapy to clear infection.

It is not necessary to monitor HCV RNA level during therapy in patients infected with HCV genotype 2 or 3, because most of them become HCV RNA-negative early during treatment. As with patients infected with HCV genotype 1, the virological response must be assessed with a sensitive HCV RNA assay at the end of therapy and 24 weeks later to determine whether the virological response is sustained (Figure 2) [1,15].

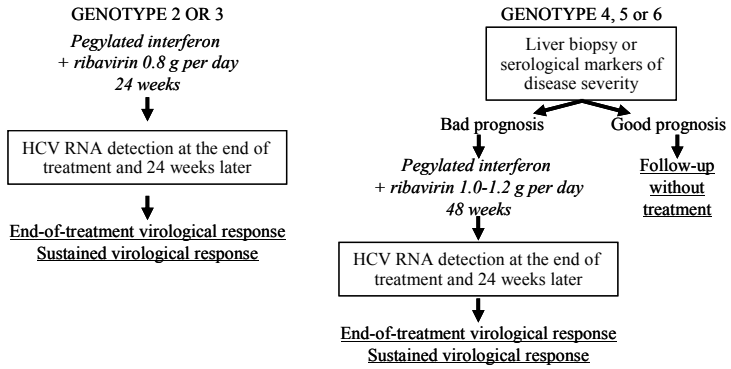


Figure 2: Treatment for patients infected with genotypes 2 to 6

HCV genotypes 4, 5 and 6

The likelihood of an SVR and the optimal treatment schedule remain unknown for patients infected with HCV genotypes 4, 5 or 6 because there have been no clinical trials with a sufficient number of patients. Thus, a treatment schedule similar to that for HCV genotype 1 is recommended (i.e. the usual dose of pegylated interferon alfa, combined with a high dose of RBV [1000 to 1200 mg per day, according to body weight more or less than 75 kg]). In the absence of published data, no rules have been defined for stopping treatment and a total duration of 48 weeks is recommended. The virological response must be assessed by a sensitive HCV RNA assay (low detection limit 50 IU/mL or less) at the end of therapy and 24 weeks later (Figure 2) [1,15].

CONCLUSION

Virological tools are now mandatory at every step in the treatment of HCV infection. Algorithms have been derived that allow clinicians to tailor treatment schedules to the individual patient and his/her virological response to therapy, to optimize pegylated interferon-RBV therapy results. This approach is cost-effective because treatment dose and duration are adapted to the patient's needs and administration can be stopped when there is no likelihood of an SVR. A number of ongoing studies are assessing whether viral load measurements at earlier points in time can predict an SVR or non-response. New algorithms will soon be developed based on viral load reductions at week 4 of therapy, or even earlier.

REFERENCES

1. NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements 2002;19:1-46.
2. 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.
3. 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.
4. 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.
5. Pawlotsky JM. Molecular diagnosis of viral hepatitis. Gastroenterology 2002;122:1554-1568.
6. Martell M, Gomez J, Esteban JI, Sauleda S, Quer J, Cabot B, Esteban R, Guardia J. High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. J Clin Microbiol 1999;37:327-332.
7. Komurian-Pradel F, Paranhos-Baccala G, Sodoyer M, Chevallier P, Mandrand B, Lotteau V, Andre P. Quantitation of HCV RNA using real-time PCR and fluorimetry. J Virol Methods 2001;95:111-119.
8. Chevaliez S, Bouvier-Alias M, Dameron G, Darthuy F, Remire J, Pawlotsky JM. Hepatitis C virus RNA quantification by automated Cobas Ampliprep-Cobas TaqMan 48 (CAP-CTM) real-time PCR assay: an evaluation of performance. J Hepatol 2006;44 (S2):S195-S196.
9. Simmonds P. Viral heterogeneity of the hepatitis C virus. J Hepatol 1999;31:54-60.
10. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, Halfon P, Inchauspe G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005;42:962-973.
11. Germer JJ, Vandenameele JN, Mitchell PS, Harmsen WS, Yao JD. Automated sample preparation for the TRUGENE HIV-1 genotyping kit using the MagNA pure LC instrument. Diagn Microbiol Infect Dis 2004;49:59-61.
12. 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.
13. Stuyver L, Wyseur A, van Arnhem W, Lunel F, Laurent-Puig P, Pawlotsky JM, Kleter B, Bassit L, Nkengasong J, van Doorn LJ, Maertens G. 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.

14. Zheng X, Pang M, Chan A, Roberto A, Warner D, Yen-Lieberman B. Direct comparison of hepatitis C virus genotypes tested by INNO-LiPA HCV II and TRUGENE HCV genotyping methods. J Clin Virol 2003;28:214-216.

15. Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36:S65-S73.

16. Lavillette D, Morice Y, Germanidis G, Donot P, Soulier A, Pagkalos E, Sakellariou G, Intrator L, Bartosch B, Pawlotsky JM, Cosset FL. Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. J Virol 2005;79:6023-6034.

17. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. J Hepatol 1999;30:956-961.

18. Lok AS, Chien D, Choo QL, Chan TM, Chiu EK, Cheng IK, Houghton M, Kuo G. Antibody response to core, envelope and nonstructural hepatitis C virus antigens: comparison of immunocompetent and immunosuppressed patients. Hepatology 1993;18:497-502.

19. 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.

20. 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.

21. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Ledinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-350.

22. Poynard T, Imbert-Bismut F, Munteanu M, Ratziu V. FibroTest-FibroSURE: towards a universal biomarker of liver fibrosis? Expert Rev Mol Diagn 2005;5:15-21.

23. 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.

24. 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.

25. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, Ibranyi E, Weiland O, Noviello S, Brass C, Albrecht J. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol 2006;44:97-103.

26. Chung RT, Andersen J, Volberding P, Robbins GK, Liu T, Sherman KE, Peters MG, Koziel MJ, Bhan AK, Alston B, Colquhoun D, Nevin T, Harb G, van der Horst C; AIDS Clinical Trials Group A5071 Study Team. Peginterferon alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. N Engl J Med 2004;351:451-459.

27. Pawlotsky JM. Treating hepatitis C in "difficult-to-treat" patients. N Engl J Med 2004;351:422-423.

28. Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, Carosi G, Sasadeusz J, Katlama C, Montaner J, Sette H Jr, Passe S, De Pamphilis J, Duff F, Schrenk UM, Dieterich DT; APRICOT Study Group. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. N Engl J Med 2004;351:438-450.

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

30. Dalgard O, Bjoro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Koury K, Ling M, Albrecht JK. Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 2004;40:1260-1265.

How to Predict the Outcome of Chronic Hepatitis C

A. Alberti

INTRODUCTION

Infection with the hepatitis C virus (HCV), the most common blood-borne viral infection in the Western world, is characterized by a silent onset, as acute infection is rarely identified, followed by a high rate of chronic disease. In most cases chronic hepatitis C also remains asymptomatic and silent for decades and its duration to clinically severe end-points is prolonged, ranging from 15-40 years [1-4].

Natural history studies indicate that around 20 to 30% of patients with chronic hepatitis C will eventually develop clinical sequelae, such as cirrhosis, liver decompensation and hepatocellular carcinoma (HCC) [5,6]. The physician must often explain to patients who have been diagnosed with asymptomatic HCV infection, how the disease will evolve and whether antiviral therapy should be started. However, the course of chronic hepatitis C is highly variable, ranging from an inexorably progressive disease, characterized by increasing fibrosis and progression towards cirrhosis and its complications, to a non-progressive or extremely slowly progressing disease that should not reduce life span and only marginally compromises quality of life [7]. Data indicate that a number of variables and cofactors influence the course and progression of chronic hepatitis C and can be used to reach a prognosis in the individual patient [8-10].

VIRAL FACTORS AND DISEASE PROGRESSION

There is little evidence that viral factors, such as viral load, genotype or quasispecies play a role in the severity and outcome of liver disease. However, some data suggest that patients with high HCV RNA levels and those infected by HCV genotype 1 may have a more rapid disease progression. Other reports have suggested that HCV genotype 3 is more pathogenic. These data remain controversial and at present few clinicians believe that these viral parameters are useful in predicting the outcome of hepatitis C outside of antiviral treatment.

On the other hand, there are strong and well established correlations between a series of host factors and parameters, and disease activity and progression.

LIVER DISEASE MARKERS OF PROGNOSTIC VALUE

Alanine aminotransferase (ALT), liver histology and biochemical and hematological parameters measuring the severity of liver disease are extremely useful for staging chronic hepatitis C and obtaining a prognosis in individual patients [11]. Alanine aminotransferase may be persistently normal, abnormal or fluctuating. Patients with persistently normal ALT (PNALT) have a markedly reduced risk of progressive liver disease compared to those with abnormal ALT [12]. The ALT profile over time is also somewhat predictive of how rapidly liver disease will progress, although this varies widely [13,14].

While a number of non-invasive markers are under evaluation and validation, liver histology is still the gold standard used to define the stage of fibrosis in compensated chronic hepatitis C. Liver biopsy also provides information about the type and level of necro-inflammatory activity, which correlates with how rapidly liver disease may progress. However, recent data indicate that these correlations are not perfect, due to variability in liver histology findings related to the timing and size of liver biopsies. Markers of advanced liver disease, such as low platelet count, reduced liver function and hypergammaglobulinemia are all indicators of a poor prognosis in chronic HCV infection.

HOST FACTORS

Age is an important host factor affecting the course of chronic hepatitis C, as several studies have indicated that older patients often have more progressive and advanced liver disease. Recent data indicate that in patients >65 years old chronic hepatitis C is: 1) more

severe; 2) presents with lower ALT; and, 3) is associated with more fibrosis and less necro-inflammatory activity than in younger patients. Nevertheless, older patients with HCV may also present with very mild and apparently non-progressive liver disease.

Gender may also play a role, as men are more likely than women to have progressive fibrosis. Race also seems to be an important factor, as several studies have indicated that fibrosis is more likely to progress in hepatitis C in Caucasians than in African-Americans [15].

Metabolic factors are major determinants of the outcome of liver disease in chronic hepatitis C. Many patients with HCV have evidence of metabolic syndrome and insulin resistance, and may or may not have overt diabetes type 2, be overweight and have liver steatosis. These patients and particularly those with HCV genotype 1, may have co-existing, nonalcoholic steatohepatitis and more rapid progression of liver fibrosis.

Iron overload has also been linked to more progressive liver disease in HCV. Genetic factors associated with certain class II human leukocyte antigens have been shown to influence disease expression and severity in HCV infected patients but they do not have practical implications [16].

EXOGENOUS COFACTORS

The complex interaction between alcohol and HCV has been shown to be both additive and synergistic.

Several studies have clearly indicated that alcohol affects the progression of chronic hepatitis C towards cirrhosis and HCC. Unfortunately most studies have evaluated high levels of alcohol intake and have not looked in to whether 1 to 2 drinks or <20 g of alcohol per day also has an effect. Therefore the lowest level of alcohol that can be taken without affecting liver disease in chronic hepatitis C has not been determined.

Smoking tobacco is also reported to have a deleterious effect on disease progression in patients with hepatitis C.

Many data and prospective studies have confirmed that HIV/HBV co-infection significantly increases the severity of chronic hepatitis C and the progression to cirrhosis, end-stage liver disease and HCC [17].

CONCLUSIONS

The clinical course and outcome of chronic hepatitis C is extremely heterogeneous. For this reason it is often difficult to predict disease

progression in the individual patient. Nevertheless age, race, ALT profile, liver histology, biochemical and hematological parameters that reflect liver disease activity and stage, and metabolic markers such as those present in metabolic syndrome, are very useful for assessing this risk.

Alcohol intake, as well as HBV/HIV co-infection should also be considered major risk factors for more severe and progressive liver disease.

REFERENCES

1. Kenny-Walsh E for the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. N Engl J Med 1999;340:1228-1233.
2. Wiese M, Berr F, Lafrenz M, Porst H, Oesen U. Low frequency of cirrhosis in a hepatitis C (genotype 1) single-source outbreak in Germany: a 20-year multicenter study. Hepatology 2000;32:91-96.
3. Alberti A, Benvegnù L, Boccato S, Ferrari A, Sebastiani G. Natural history of initially mild chronic hepatitis C. Dig Liver Dis 2004;36:646-654.
4. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002;36:S35-S46.
5. 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.
6. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, Nawrocki M, Kruska L, Hensel F, Petry W, Haussinger D. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687-1695.
7. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology 2002;36:S47-S56.
8. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol 2001;34:764-767.
9. Rai R, Wilson LE, Astemborski J, Anania F, Torbenson M, Spoler C, Vlahov D, Strathdee SA, Boitnott J, Nelson KE, Thomas DL. Severity and correlates of liver disease in hepatitis C virus-infected injection drug users. Hepatology 2002;35:1247-1255.
10. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, Nolt K, Nelson KE, Strathdee SA, Johnson L, Laeyendecker O, Boitnott J, Wilson LE, Vlahov D. The natural history of hepatitis c virus infection: host, viral, and environmental factors. JAMA 2000;284:450-456.
11. Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, Hashimoto E, Lefkowitch JH, Ludwig J, Okuda K. The long-term pathological evolution of chronic hepatitis C. Hepatology 1996;23:1334-1340.
12. Alberti A. Towards more individualised management of hepatitis C virus patients with initially or persistently normal alanine aminotransferase levels. J Hepatol 2005;42:266-274.
13. Boccato S, Pistis R, Noventa F, Guido M, Benvegnu L, Alberti A. Fibrosis progression in initially mild chronic hepatitis C. J Viral Hepat 2006;13:297-302.
14. 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.

15. Iley TE, Brown J, Chan J. Hepatitis C virus infection in African Americans: its natural history and histological progression. Am J Gastroenterol 2002;97:700-706.

16. Diepolder HM, Scholz S, Pape GR. Influence of HLA alleles on outcome of hepatitis C virus infection. Lancet 1999;354:2094-2095.

17. Thomas DL. Hepatitis C and human immunodeficiency virus infection. Hepatology 2002;36:S201-S209.

Fibroscan® in Patients with Chronic Viral Hepatitis

M. Beaugrand

INTRODUCTION

The Fibroscan® is a new device to measure liver elasticity or stiffness. Liver stiffness has been recognized as the hallmark of advanced liver disease since Hippocrates, and this parameter is used by clinicians to predict cirrhosis or advanced fibrosis. Transient elastography, the physical principle used by Fibroscan, makes it possible to record liver stiffness in a more sensitive, reliable and quantitative way. Measurement by Fibroscan relies on simple principles [1]. A probe positioned on the right part of the chest wall generates a mechanical shock wave transmitted to the liver parenchyma and records the velocity of the shear wave generated inside the liver using ultrasound. Liver elasticity or stiffness is related to the velocity of the shear wave by the following equation $E=rV^2$ (E=elasticity, V=velocity of the shear wave). Fibroscan provides a measurement of liver stiffness calculated by the software as the median value of repeated measurements (usually 10) called acquisitions. The software takes into account waves with a linear velocity, preventing the use of any measurements distorted by the encounter of heterogeneous structures such as large vessels or focal lesions. A success rate of more than 50% is also required to validate the final value called the liver stiffness measurement (LSM). The sampling effect is minimized by the number of acquisitions and the volume of the parenchyma explored: a 2 cm long and 1 cm in diameter cylinder which is much greater than the volume of a liver biopsy. As the need for new non-invasive tools to assess liver fibrosis has become recognized, the

Fibroscan has become more popular. The main reason for this success is that LSM is painless, well accepted by patients and easy to record at the bedside with immediate results. Furthermore, the link with liver fibrosis is direct and rational. Like any new technique, the Fibroscan must be evaluated and its limitations determined. This report will identify: 1) the results obtained in patients with chronic hepatitis, especially viral B or C; 2) the limitations of the method; and, 3) future developments of Fibroscan.

RESULTS IN PATIENTS WITH CHRONIC VIRAL HEPATITIS

There are many LSM data available for patients with chronic hepatitis, and studies from various countries have provided similar results. A French multicenter study enrolled more than 1000 patients with chronic viral hepatitis to compare LSM and stage of fibrosis according to the METAVIR classification. The study found that the failure rate of LSM (percentage of LSM that could not be obtained) was around 7%, which is less than the liver biopsy failure rate (the percentage of liver biopsy samples that are unsuitable for a reliable assessment of fibrosis grade) [2]. Furthermore, LSM values were closely correlated to the area of fibrosis assessed by morphometry, and the diagnostic accuracy of LSM in patients with chronic hepatitis C virus (HCV) was within the range of that with blood tests for F $\geq$2 (area under the receiver operating characteristic [AUROC] curve=0.79) but better than F $\geq$3 (AUROC=0.91) and F $\geq$4 (AUROC=0.97).

Similar results have been obtained in other studies of HCV patients. The results obtained in HIV/HCV co-infected patients and patients with hepatitis B virus (HBV) are also similar. Thus, Fibroscan appears to be a very reliable tool for the diagnosis of cirrhosis or advanced fibrosis [3].

Fibroscan has been shown to be as effective as or better than the combined blood tests (so called serum markers of fibrosis) in the same patients according to stage of fibrosis [4].

LIMITATIONS OF THE METHOD

Limitations are mostly technical but are also linked to the principle of transient elastography. Liver stiffness measurement cannot be recorded in patients with even minimal ascites because this prevents the transmission of the shock wave to the liver parenchyma. This has

little practical importance because in patients with chronic liver disease, ascites is the hallmark of advanced cirrhosis.

More importantly, in a European chronic hepatitis patient population, LSM failed in around 7% of cases of obese patients or patients with a fatty chest wall. In these patients, either no LSM could be recorded by Fibroscan or the failure rate was above 50% preventing any reliable measurement. There is a negligible failure rate in some cases when patients have narrow intercostal spaces, thoracic dysmorphia or interposition of the colon.

Intra- and inter-observer reproducibility of the Fibroscan is fair. Nevertheless, care should be taken to ensure that the probe is positioned perpendicular to the chest wall and that the correct intercostal space is chosen. As with liver biopsy, in certain cases an ultrasound examination of the liver can be useful. Finally, although the learning period is short, at least 50 to 100 examinations are needed before an operator can be considered experienced.

The interpretation of LSM may also be prone to error if the clinical background is not taken into account. Fibroscan values are correlated to the area of liver fibrosis, not to the degree of distortion of liver architecture. The amount of fibrosis in the liver is obviously different between the stages of fibrosis but some overlap may occur, for example patients with macronodular cirrhosis or those with inactive cirrhosis, who have been cured from viral disease, have a limited amount of liver fibrosis. In contrast, other conditions that cause dense perisinusoidal fibrosis may result in high LSM values despite the absence of nodular architecture. Therefore care should be taken not to systematically establish equivalence between LSM values and stage of fibrosis.

The effect of acute conditions such as extensive necrosis, massive steatosis, and liver congestion on LSM must be further evaluated. In patients with viral chronic hepatitis, steatosis or histological activity is not correlated with LSM. However, in acute conditions there are anecdotal reports suggesting that values may be increased, particularly in patients with extensive necrosis. Therefore LSM, like other liver tests, should be interpreted within the clinical context.

FUTURE DEVELOPMENTS

The high specificity and sensitivity of LSM for diagnosing of cirrhosis or extensive fibrosis suggests that Fibroscan could be a valuable screening tool for advanced liver diseases in populations that are identified as being “at risk”. Preliminary experience has shown that

the method is well accepted even in patients who are reluctant to have blood tests. Probably the most promising prospect is the monitoring of fibrosis in treated and untreated patients with chronic viral infections. Early data show a close relationship with virological response in patients with chronic HCV treated by pegylated interferon and ribavirin. Liver fibrosis is becoming an increasingly important end-point in clinical trials. If LSM is validated in this setting, it could become a valuable tool for assessing the efficacy of either antiviral or antifibrotic drugs in patients with chronic hepatitis, avoiding the need for repeated liver biopsies. Finally, in the more distant future, transient elastography might also allow characterization of liver lesions and provide a new non-invasive liver imaging technique.

REFERENCES

1. 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 non-invasive method for assessment method of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705-1713.

2. Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Ledinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Non-invasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis. Hepatology 2005;41:48-54.

3. Ganne-Carrié N, Ziol M, de Ledinghen V, Douvin C, Marcellin P, Castera L, Dhumeaux D, Beaugrand M. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. Hepatology 2006 (in press).

4. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Ledinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-350.

What is the Optimal Treatment for Naïve Patients with Chronic Hepatitis C?

S. Zeuzem, C. Sarrazin

INTRODUCTION

Hepatitis C Virus (HCV) is a small, enveloped RNA virus that belongs to the family *Flaviviridae*. Due to the lack of proofreading activity by the viral RNA-dependent RNA polymerase, HCV exhibits a highly variable genome and can be grouped into several distinct genotypes. Today, at least 6 different genotypes and more than 100 subtypes have been reported with different geographical distributions [1]. In western countries, genotypes 1, 2 and 3 are the most frequent with a predominance of genotype 1. Genotype 4 is found throughout North Africa and the Middle East whereas genotype 5 is found in South Africa. Genotype 6 is found in Hong Kong and more recently in Australia.

Therapy for HCV infection was first reported in the late 1980s when patients with so-called non-A, non-B hepatitis were treated with interferon [2]. After isolation and characterization of HCV, it became obvious that sustained virological response rates (SVR), defined as non-detectable HCV RNA 24 weeks after cessation of therapy, were markedly different for each HCV genotype. In large multicenter studies, SVR rates were 2 and 7% for patients infected with genotype 1 treated for 24 and 48 weeks, respectively. In contrast, in patients infected with genotypes 2 or 3, an SVR was achieved in 16% and in 29 to 33% of patients after 24 and 48 weeks of therapy, respectively [3,4].

The introduction of combination therapy with interferon alfa and the nucleoside analog ribavirin (RBV) for 48 weeks in the late 1990s

substantially improved treatment outcome with mean SVR rates of 41%. However, differences between patients infected with genotype 1 and genotypes 2 and 3 remained. Sustained virological response rates for patients with genotypes 1, 4, 5 and 6 were 28 to 36% compared with 61 to 79% for patients infected with genotypes 2 or 3 [3-6]. The development of pegylated interferons with a sustained absorption, a slower rate of clearance, and a half life that was longer than that of unmodified interferons, led to further improvement of sustained virological response rates, especially in patients infected with genotype 1 [5,6]. Again, in patients treated with pegylated interferon alfa combined with RBV, sustained virological response rates were significantly higher in patients infected with genotypes 2 and 3 (76 to 82%) than in those infected with genotype 1 (42 to 52%).

ANTIVIRAL THERAPY OF PATIENTS WITH HCV GENOTYPE 1

The standard treatment for patients with chronic hepatitis C genotype 1 infection as recommended in US and European guidelines is pegylated interferon alfa therapy in combination with RBV for 48 weeks. At present, 2 types of pegylated interferon alfa have been approved for the treatment of chronic hepatitis C: pegylated interferon alfa-2a and pegylated interferon alfa-2b, which differ in the size and form of the linked polyethylene glycol molecule (40 vs. 12 kDa, respectively). For pharmacokinetic reasons, the 40 kDa pegylated interferon alfa-2a is administered independent of body weight at a dose of 180 µg, whereas for the 12 kDa pegylated interferon alfa-2b, a dose of 1.0-1.5 µg/kg body weight is used. Both pegylated interferons are injected subcutaneously once a week. For HCV genotype 1-infected patients, RBV doses are adjusted according to body weight and range from 800 to 1400 mg/day.

Prediction of virological response at baseline is possible by different host- and virus-related factors (Figure 1). Host-related factors associated with a reduced virological response to pegylated interferon/RBV combination therapy are: older age, male gender, African-American ethnicity, elevated body mass index, advanced fibrosis or cirrhosis, liver steatosis, and elevated gamma glutamyltranspeptidase (GGT) levels [7]. As mentioned in the introduction, the most import viral factor for the prediction of a virological response is HCV genotype. HCV genotypes 2- or 3-infected patients generally have higher SVR rates than genotype 1-

infected patients. In addition, a high baseline viral load is also associated with a reduced probability of virological response [7]. Furthermore, different HCV proteins have been associated with inhibition of interferon alfa signal transduction pathways [8]. While the underlying mechanisms of HCV-related interferon alfa treatment resistance are still not fully understood, numerous clinical studies and a recent meta-analysis have shown a clear association between multiple mutations within the so-called interferon sensitivity determining region (ISDR) within the HCV NS5A protein and SVR [9-11].

During antiviral therapy, early discontinuation in virological non-responders is possible at certain time points based on HCV RNA kinetics. A high predictive value for virological non-responsiveness (98 to 100%) was found in several studies of patients infected with HCV genotypes 1, 4, 5 and 6 when the reduction in HCV RNA serum concentrations was <2 $\log_{10}$ scales between baseline and week 12 of pegylated interferon alfa/RBV combination therapy [5,12,13]. In addition, at week 24, discontinuation of treatment is recommended if there is HCV RNA in serum detectable by qualitative polymerase chain reaction (PCR)-based assays, which again has a predictive value of 98 to 100% for virological non-response [6,12-14].

Results of HCV RNA concentrations determined by different quantitative HCV RNA assays can differ at a magnitude of up to 0.5 $\log_{10}$ despite standardization of all commercially available test systems to IU [15]. Therefore, it is important to use the same assay at baseline and week 12 to make correct decisions about treatment discontinuation based on a 2 $\log_{10}$ decline. For assessment of detectable HCV RNA at week 24, a sensitive assay with a detection limit ≤50 IU/mL should be used [5,6,14]. For the moment it is unknown whether the presence of low viral titers at week 24 (between 5 to 10 and 50 IU/mL) can be used for selection of patients who may benefit from prolonged therapy.

INDIVIDUALIZED TREATMENT DURATION IN PATIENTS WITH HCV GENOTYPE 1

Most investigations on early viral decay and treatment response have focused on developing treatment algorithms for the discontinuation of therapy in patients with HCV genotype 1 infection and little or no chance of achieving an SVR.

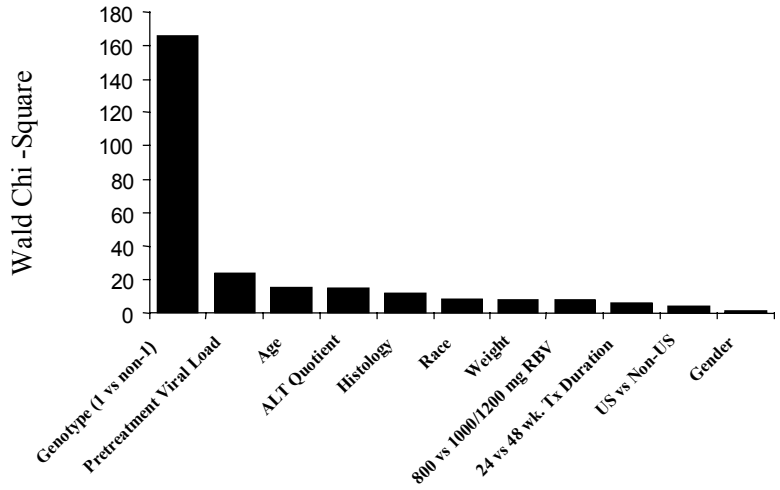


Figure 1: Relative importance of factors that influence virological response to therapy

However, the slopes of the initial decline in HCV RNA concentrations vary widely and HCV RNA may become undetectable in individual patients as early as week 4 or as late as week 24. Therefore, in different clinical trials, data on the early reduction in HCV RNA at week 4 was used to identify patients with a rapid virological response for whom the standard 48 weeks of therapy might be over-treatment and who might benefit from 24 weeks instead. One recent prospective clinical trial investigated shortening treatment from 48 to 24 weeks in patients with HCV genotype 1 infection and a low baseline viral load of ≤600,000 IU/mL. However, the overall SVR rate was 50% in this study compared to 71% in a matched historical control of patients with a baseline viral load ≤600,000 IU/mL but who were treated for 48 weeks. In a retrospective study it was shown that shortening the treatment duration in HCV genotype 1-infected patients with a low baseline viral load is only possible when there is a rapid virological response (RVR) defined as HCV RNA negativity (≤29 IU/mL) at week 4. In this patient subgroup, the sustained virological response rate after 24 weeks of therapy with pegylated interferon alfa-2b and RBV was 89% compared to 85% in the historical control of patients with a low baseline viral load and RVR who were treated for 48 weeks [16].

Recently, a retrospective analysis and an ongoing prospective clinical trial showed similar results for treatment with pegylated interferon alfa-2a and RBV [17,18]. Jensen *et al*. analyzed the different treatment durations within the trial by Hadziyannis *et al*. [22] for HCV genotype 1-infected patients with RVR defined as HCV RNA below 50 IU/mL at week 4. In this subgroup of patients, the SVR rates were similar after 24 and 48 weeks in 88% and 91% of patients, respectively [17]. Interestingly, flat dosing of RBV at 800mg/day in patients with RVR also led to high SVR rates (89%) after 24 weeks of therapy. Low baseline HCV RNA concentrations (<200,000 vs. >600,000 IU/mL) and HCV subtype (1b vs. 1a) were independent predictors of a rapid virological response [17]. In a prospective study by Ferenci *et al*., treatment duration was shortened from 48 to 24 weeks in HCV genotype 1-infected patients with RVR (<50 IU/mL HCV RNA at week 4) and recently an interim analysis of patients who had already completed therapy was presented. In this study, SVR rates were significantly higher in patients with a low baseline viral load (≤600,000 IU/mL) compared to those with a high baseline viral load (>600,000 IU/mL; 98% vs. 74%). Hepatitis C virus RNA at week 4 was assessed by both a standard HCV RNA assay with a lower detection limit of 50 IU/mL and a real-time PCR-based assay with a sensitivity of 10 IU/mL. Interestingly, up to 30% of patients with negative results from the standard assay (<50 IU/mL) were still HCV RNA-positive with the more sensitive assay [18]. It is possible that these differences will be important for the probability of later SVR and recommendations for shortening treatment duration must take into account the sensitivity of the assay used to detect HCV RNA.

For similar reasons, prolongation of therapy from 48-72 weeks may be suitable in patients with a very slow decline in HCV RNA levels. Two large multicenter studies have addressed this question [19,20]. Although prolonging treatment in all patients was not associated with an overall increase in SVR rates, both studies showed that a subgroup of patients with slow virological response could benefit from prolonging therapy from 48 to 72 weeks. In a study by Berg *et al*., a significant improvement in the sustained virological response was observed in patients with a 2 $\log_{10}$ decline. Patients with detectable HCV RNA at week 12 then became HCV RNA-negative at week 24. In this subgroup of patients, SVR rates increased from 17 to 29% when combination therapy was extended from 48-72 weeks [19]. Similar results were observed in the study by Sanchez-Tapias *et al*., showing a significant improvement in SVR rates in slow virological

responders (defined as patients with detectable HCV RNA at week 4) when they were treated for 72 instead of 48 weeks [20].

Taken together, in HCV genotype 1-infected patients, individualization of the treatment duration on the basis of HCV RNA concentrations at baseline and their decline during therapy seems to be beneficial, and prospective trials are under way to prove this concept with treatment durations of 24, 30, 36, 42, 48, 60 and 72 weeks. At present, shortening treatment with pegylated interferon alfa-2b plus RBV from 48 to 24 weeks has been approved in the EU for HCV genotype 1-infected patients with a low baseline viral load (<600 000 IU/mL) and RVR (HCV RNA <29 IU/mL at week 4).

ANTIVIRAL THERAPY IN PATIENTS INFECTED WITH HCV GENOTYPES 2 AND 3

In the first pivotal trials using pegylated interferon alfa combined with RBV, antiviral therapy was administered for 48 weeks in patients infected with all HCV genotypes. Patients with genotypes 2 or 3 infection treated with pegylated interferon alfa-2b/RBV or pegylated interferon alfa-2a/RBV achieved SVR rates of 82% and 76%, respectively, confirming the favorable results achieved by standard combination interferon/RBV treatment [5,6]. Subsequent studies showed that SVR rates (78 to 81%) obtained in patients treated for 24 weeks were similar to those treated for 48 weeks [21,22]. As a result, 24 weeks of treatment has become the standard for pegylated interferon alfa and RBV for first-line therapy in patients with HCV genotypes 2 or 3 infection.

A fixed dose of 800 mg RBV/day independent of body weight has been shown to be as effective as a body weight-adapted RBV schedule [22]. Current studies have even investigated lower RBV doses (e.g. 400 mg/day) in the treatment of HCV genotype 2- or 3-infected patients.

In previous studies, genotypes 2- and 3-infected patients were always grouped together. In recent studies, differences in the SVR rates have been well described between patients infected with HCV genotypes 2 and 3. In general, patients infected with genotype 2 have even more favorable SVR rates than those infected with HCV genotype 3 (93% vs. 79%) [21]. Analyses of patient characteristics before beginning antiviral therapy showed that apparent differences between genotypes 2 and 3 were mainly attributed to a subgroup of genotype 3-infected patients with high baseline HCV RNA concentrations of >600,000 IU/mL [21]. In this subgroup, a high

relapse rate after cessation of combination treatment was observed compared to relapse rates in genotype 2- and 3-infected patients with a low baseline viral load (23% vs. 5 to 8%) [21]. Furthermore, histologically-proven liver steatosis, which is frequently observed in genotype 3-infected patients, has been shown to be a significant negative prognostic factor for achieving an SVR. Although the fibrosis stage was not identified as a statistically-independent negative predictive factor for a sustained response in this study, it should be noted that patients with no fibrosis achieved higher SVR rates than those with bridging fibrosis or cirrhosis (97% vs. 75%) [21].

INDIVIDUALIZED TREATMENT OF PATIENTS WITH HCV GENOTYPES 2 AND 3

Recently, 4 independent studies have investigated whether the duration of treatment with pegylated interferon alfa-2a or -2b and RBV in patients with genotypes 2 and 3 infection could be further reduced from 24 weeks to 16, 14, and 12 weeks, respectively, without compromising the SVR rates [23-26].

In an initial non-randomized trial, 122 treatment-naïve Norwegian patients were treated with pegylated interferon alfa-2b at a dose of 1.5 μg/kg body weight and RBV (800 to 1400 mg adjusted to body weight) [24]. Patients with an early virological response defined as undetectable HCV RNA (<50 IU/mL) at weeks 4 and 8 after the initiation of treatment were treated for 14 weeks (n=95). The remaining patients received 24 weeks of treatment (n=27). A control group receiving a standard treatment of 24 weeks independent of the early virological response at weeks 4 and 8 was not included in this study. The overall SVR rate was comparable to previous studies (82%). Patients with an early virological response achieved a SVR rate of 90% whereas patients without an early virological response at week 4 showed sustained response rates of only 56%. As there was no control arm in this study, no data are available on SVR rates in patients with early virological responses who received combination therapy for a standard duration of 24 weeks. In this study, independent factors associated with a SVR rate included younger age, treatment according to protocol, undetectable HCV RNA at treatment week 4, and a lower viral load at baseline. Indeed, patients with genotype 3 and a baseline HCV RNA concentration of $<6x10^5$ IU/mL had higher SVR rates than those with higher HCV RNA concentrations (98% vs. 79%). There was no difference in SVR rates between patients infected

with HCV genotypes 2 or 3. However, only 23 patients infected with genotype 2 were enrolled in this trial.

In a second study, 283 patients were randomized to receive antiviral treatment with pegylated interferon alfa-2b at a dose of 1.0 µg/kg body weight and RBV (1000 to 1200 mg) for 24 weeks (standard duration group, n=70) or, depending on early virological response at week 4 (variable-duration group, n=213) for either 24 weeks (HCV RNA ≥50 IU/mL at week 4, n=80) or 12 weeks (HCV RNA <50 IU/mL at week 4, n=133) [23]. In the standard duration group the SVR rate was 76% compared to 77% in the variable duration group. Patients in the variable duration group who were HCV RNA-negative at week 4 achieved a sustained response rate of 85% compared to 64% in those without an early virological response. In the standard duration group, SVR rates were 91% and 48%, respectively, in patients with and without an early virological response. In this study, no independent baseline factor was significantly associated with early virological response or virological relapse. The overall rate of SVR was 80% in patients infected with genotype 2 and 66% in those infected with genotype 3 ($p<0.001$).

A third study reported virological response rates in 153 patients who were treated with pegylated interferon alfa-2a at 180 µg/week and 800 to 1200 mg/day RBV based on body weight [25]. Patients with undetectable HCV RNA by quantitative RT-PCR after 4 weeks of treatment (rapid virological responders, <600 IU/mL) were randomised for a total duration of 16 (n=71) or 24 weeks (n=71) of therapy. Patients without a rapid virological response at week 4 were treated for 24 weeks (n=11). The SVR rates in early responders who were treated for 16 or 24 weeks were similar (82% and 80%, respectively). However, only 36% of patients with detectable HCV RNA at week 4 cleared the virus. Generally, patients with HCV genotype 2 infection had higher SVR rates than patients with genotype 3 infection (92% vs. 73%). Infection with HCV genotype 2 was confirmed as an independent factor for an SVR in multivariate analysis. Other factors associated with SVR were low GGT levels at baseline and low pretreatment HCV RNA concentrations. Interestingly, when only those patients with an early virological response (n=142) were stratified by pretreatment HCV RNA concentrations (≤800,000 IU/mL vs. >800,000 IU/mL), it was shown that patients with HCV genotype 2 infection achieved SVR rates of 100% and 93%, respectively, whatever the treatment duration. However, in patients with HCV genotype 3 infection, treatment outcome was different in relation to pretreatment HCV RNA

concentrations. An SVR was observed in 85% of patients with low pretreatment HCV RNA concentrations whereas only 59% of patients with high HCV RNA concentrations became sustained virological responders (p=0.003).

Most recently, results of a large, international, multicenter trial on reducing the duration of treatment in HCV genotype 2- and 3-infected patients (ACCELERATE) were presented at the EASL meeting in Vienna in April 2006 [26]. In this study, patients were randomized to receive pegylated interferon alfa-2a (180 µg once weekly) in combination with a fixed dose of 800 mg RBV for 16 or 24 weeks. Overall, SVR rates were significantly lower in the 16 week treatment group compared to the 24 week group (62% vs. 70%, respectively). Also, in the subgroup of patients with a rapid virological response (<50 IU/mL at week 4) shortening treatment from 24 to 16 weeks was still associated with a significantly reduced SVR rate (82% vs. 90%) [26].

Several questions still need to be answered. While RBV was dosed according to body weight (800 to 1200 mg) in the 3 pilot studies, in the ACCELERATE trial a dose of 800 mg/day was given to all patients. Thus, to shorten treatment to <24 weeks, higher RBV doses may be required. This question should be prospectively addressed in future studies. In addition, HCV genotype 2- and 3-infected patients should be analyzed separately and grouped according to baseline HCV RNA concentrations. Furthermore, cut-off levels between low and high baseline viremia must be re-evaluated. Although results were not statistically significant, the relapse rates of patients who were treated for 12, 14 and 16 weeks, were always higher than the relapse rates of patients treated for 24 weeks including those who had an early virological response at week 4. The optimal duration of therapy must be determined according to HCV genotype, baseline viral load, initial virological response, and probably also according to liver fibrosis stage and other relevant host parameters for response prediction. Moreover, the extension of current standard therapy beyond 24 weeks needs to be investigated in future trials in patients without an early virological response at week 4 (Table 1).

INDIVIDUALIZED TREATMENT OF PATIENTS WITH HCV GENOTYPE 4, 5 AND 6

Data on individualized treatment durations in patients infected with HCV genotypes 4, 5 or 6 are scarce [32-35] and summarized in

Table 1. In general, these patients should be treated with pegylated interferon alfa plus RBV for 48 weeks.

FUTURE ANTIVIRAL STRATEGIES

New antiviral enzyme inhibitors directly targeting the HCV NS3/4A protease or the virus-encoded NS5B RNA-dependent RNA-polymerase are currently under clinical investigation in phase I/II studies. They are expected to markedly increase the number of treatment options for patients infected with chronic hepatitis C. However, due to variable amino acid sequences of the NS3 and NS5B proteins, the antiviral efficacy of enzyme inhibitors may differ among HCV genotypes. In a first proof-of-principle study of the NS3 protease inhibitor BILN-2061, a rapid and sharp decrease in HCV RNA levels was observed after 2 days of treatment in HCV genotype 1 infected patients. However, antiviral efficacy was less pronounced and more variable in patients with HCV genotypes 2 or 3 compared to that in patients with genotype 1 [27,28]. It has been suggested that a lower affinity of BILN-2061 for the NS3 protease of HCV genotypes 2 and 3 is the main reason for these findings. Further development of BILN-2061 was halted due to cardiotoxic side effects in monkeys. Two other HCV protease inhibitors are currently in phase II studies (VX-950 and SCH503034) and have resulted in a 2- to 5-$\log_{10}$ decline in HCV RNA levels after 2 weeks of treatment in patients with HCV genotype 1 [29,30]. As with other viruses (e.g. HBV, HIV) if an antiviral drug is extremely effective, this may be associated with a rapid selection of resistant isolates and subsequent virological break-through. The emergence of resistant isolates harboring specific mutations within the HCV NS3 protease gene has already been shown for the HCV protease inhibitor VX-950 [31]. However, optimal dosing, a sharp initial decline in HCV RNA levels, and combination with pegylated interferon alfa (and RBV) were associated with a continuous decline in HCV RNA without any apparent development of resistance. Future HCV treatment strategies will probably continue to be developed according to HCV genotype, baseline viremia and early virological response criteria.

HCV genotype	Pretreatment viral load	Definition of initial virological response	Data support following treatment duration	Reference
HCV-1	<600,000 IU/mL	<50 IU/mL (week 4)	24	Zeuzem *et al.* [16]
	Independent	<2 $\log_{10}$ (week 12)	48	Fried *et al.* [5] Davis [12]
	Independent	50-6000 IU/mL (week12)	72	Berg *et al.* [19]
HCV-2	Independent	<50 IU/mL (week 4)	14	Dalgard *et al.* [24]
	Independent	<50 IU/mL (week 4)	12	Mangia *et al.* [23]
	Independent	<600 IU/mL (week 4)	16	von Wagner *et al.* [25]
	<400,000 IU/mL	Independent	16	Shiffman *et al.* [26]
HCV-3	<800,000 IU/mL	<600 IU/mL (week 4)	16	von Wagner *et al.*[25]
	<400,000 IU/mL	Independent	16	Shiffmann *et al.* [26]
	>800,000 IU/mL		24	Hadziyannis *et al.* [22] Zeuzem *et al.* [16]
	Independent	>600 IU/mL	36-48(?)	No data available
HCV-4	<800,000 IU/mL	<50 IU/mL or 2 $\log_{10}$ decline (week 12)	36	Kamal *et al.* [32]
	>800,000 IU/mL		48	Diago *et al.* [33] Hasan *et al.* [34] Kamal *et al.* [32]
HCV-5	Independent	Independent	48	Bonny *et al.* [35]
HCV-6	Independent	Independent	48 (?)	No data available

Table 1: Individualized treatment duration in patients with chronic hepatitis C

REFERENCES

1. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, Halfon P, Inchauspe G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin I, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005;42:962-973.

2. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, Waggoner JG, Park Y, Jones EA. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986;315:1575-1578.

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

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 alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352:1426-1432.

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

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. Mihm U, Herrmann E, Sarrazin C, Zeuzem S: Review article: predicting response in hepatitis C virus therapy. Aliment Pharmacol Ther 2006;23:1043-1054.

8. Hofmann WP, Zeuzem S, Sarrazin C. Hepatitis C virus-related resistance mechanisms to interferon alpha-based antiviral therapy. J Clin Virol 2005;32:86-91.

9. Sarrazin C, Berg T, Lee JH, Teuber G, Dietrich CF, Roth WK, Zeuzem S. Improved correlation between multiple mutations within the NS5A region and al response in European patients chronically infected with hepatitis C virus type 1b undergoing combination therapy. J Hepatol 1999;30:1004-1013.

10. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77-81.

11. Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, Berg T. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. Gut 2004;53:1345-1351.

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

13. Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, Wiedenmann B, Hopf U, Zeuzem S. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. Hepatology 2003;37:600-609.

14. Mihm U, Kronenberger B, Hofmann WP, Zeuzem S, Sarrazin C. Highly sensitive HCV RNA detection assays for decision of treatment (dis)continuation in patients with chronic hepatitis C. J Hepatol 2005;42:605-606.

15. Sarrazin C, Gärtner B, Sizmann D, Babiel R, Mihm U, Hofmann WP, von Wagner M, Zeuzem S. Comparison of conventional PCR with real-time PCR and branched DNA-based assays for hepatitis C virus RNA quantification and clinical significance for genotypes 1 to 5. J Clin Microbiol 2006;44:729-737.

16. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, Ibranyi E, Weiland O, Noviello S, Brass C, Albrecht J. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol 2006;44:97-103.

17. Jensen DM, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, Ferenci P, Ackrill AM, Willems B. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. Hepatology 2006;43:954-960.

18. Ferenci P, Bergholz U, Laferl H, Scherzer TM, Maieron A, Gschwantler M, Brunner H, Hubmann RR, Datz C, Bischof M, Stauber R, Steindl-Munda P. 24 week treatment regimen with peginterferon alpha-2a (40KD) (Pegasys) plus ribavirin (Copegus) in HCV genotype 1 or 4 super-responders (abstract). J Hepatol 2006;44:S6.

19. Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Pape GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S. Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon alfa-2a plus ribavirin. Gastroenterology 2006;130:1086-1097.

20. Sanchez-Tapias JM, Diago M, Escartin P, Enriques J, Moreno R, Romero-Gomez M, Bercena R, Crespo J, Andrade R, Perez R, Suarez MJ, Planas R, Sola R, Garcia-Bengoechea M, Garcia-Samaniego J, Berenguer J, Montoro M, del Olmo J, Lopez G, Tome S, Casanovas T, Castellanos G, Castro A. Longer treatment duration with peginterferon alfa-2a (40KD) and ribavirin in naive patients with chronic hepatitis C and detectable HCV RNA by week 4 of therapy: final results of the randomized, multicenter TERAVIC-4 study (abstract). Hepatology 2004;40:S218.

21. 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.

22. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer Jr HC, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon alfa2a and ribavirin combination therapy in chronic hepatitis C. A randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-355.

23. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609-2617.

24. Dalgard O, Bjoro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Bell H. Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 2004;40:1260-1265.

25. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Haussinger D, Herrmann E, Zeuzem S. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129:522-527.

26. Shiffman ML, Pappas S, Nyberg L, Greenbloom S, Gibas A, Bacon B, Rasenack J, Suter F, Diago M, Lin A, Soman A, Zeuzem S. Peginterferon alpha-2a (Pegasys) plus ribavirin (Copegus) for 16 or 24 weeks in patients with HCV genotype 2 or 3. Final results of the ACCELERATE trial. J Hepatol 2006;44:S271A.

27. Hinrichsen H, Benhamou Y, Wedemeyer H, Reiser M, Sentjens RE, Calleja JL, Forns X, Erhardt A, Cronlein J, Chaves RL, Yong CL, Nehmiz G, Steinmann GG. Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients. Gastroenterology 2004;127:1347-1355.

28. Reiser M, Hinrichsen H, Benhamou JP, Reesink HW, Wedemeyer H, Avendano C, Riba N, Yong CL, Nehmiz G, Steinmann G. Antiviral efficacy of NS3-serine protease inhibitor BILN-2061 in patients with chronic genotype 2 and 3 hepatitis C. Hepatology 2005;41:832-835.

29. Zeuzem S, Sarrazin C, Wagner F, Rouzier R, Forestier N, Gupta S, Hussain M, Shah A, Cutler DL, Zhang J. Combination therapy with the HCV protease inhibitor, SCH 503034, plus PEG-Intron in hepatitis C genotype-1 PEG-Intron non-responders: phase I b results. Hepatology 2005;42:S276A.

30. Reesink H, Forestier N, Weegink C, Zeuzem S, McNair L, Purdy S, Chu H-M, Jansen PLM. Initial results of a 14-day study of the hepatitis C virus protesae inhibitor VX-950, in combination with peginterferon alfa-2a. J Hepatol 2006;44:S272A.

31. Sarrazin C, Kieffer T, Bartels D, Hanzelka B, Müh U, Welker M, Wincheringer D, Lin C, Grossman T, Purdy S, Weegink C, Reesink H, Zeuzem S, Kwong A. Characterization of viral variants in the HCV NS3 protease domain of genotype 1 patients that are selected during 14 days of dosing with VX-950. Hepatology 2005;42:S751A.

32. Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA, Saleh WA, Ismail A, Aziz AA, Madwar MA. Peginterferon α-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. Gut 2005;54:858-866.

33. Diago M, Hassanein T, Rodes J, Ackrill AM, Sedarati F. Optimized virologic response in hepatitis C virus genotype 4 with peginterferon-alpha2a and ribavirin. Ann Intern Med 2004;140:72-73.

34. Hasan F, Asker H, Al-Khaldi J, Siddique I, Al-Ajmi M, Owaid S, Varghese R, Al-Nakib B. Peginterferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C genotype 4. Am J Gastroenterol 2004;99:1733-1737.

35. Bonny C, Roche C, Fontaine H, Poynard T, Hezode C, Larrey D, Marcellin P, Bourliere M, Bronowicki JP, Merle P, Zarski JP, Nicolas C, Randl K, Bommelaer G, Abergel A. Efficacy of interferon (standard or pegylated) plus ribavirin in naive patients with hepatitis C virus genotype 5. A French national study. J Hepatol 2005;42:S200.

Clinical Case: Chronic Hepatitis C – Non-Responder to Pegylated Interferon and Ribavirin

J. Heathcote

CASE HISTORY

Mr R.H. first came to medical attention in July 2000 when during a routine check-up with his family physician he was found to have an elevated serum aminotransferase value. At that time he was a 54-year old employed man with no medical complaints. On routine questioning he volunteered a history of generalized psoriasis, first diagnosed 25 years ago. He had consumed 60 to 80 g of alcohol daily for the last 20 years. His body mass index was 27, and fat was centrally distributed. He was a non-smoker and had no psychiatric comorbitities.

He had received a blood transfusion in 1976, and from 1983 to 1985 used injection drugs and snorted cocaine. On examination no abnormalities were detected apart from his psoriasis. He is a First Nations Person (Canadian Aboriginal).

He was found positive for Hepatitis C, infected with genotype 1a and with a viral load of 3 x 100,000 copies/mL. A liver biopsy showed him to have minimal activity (A1), but to have cirrhosis (F4) with steatohepatitis. At that time he was treated with standard interferon alfa-2b 3 MU 2 times per week and 1000 mg of ribavirin (RBV) daily.

He failed to respond to this treatment and was referred to a tertiary referral centre in 2002 for consideration of retreatment. The results of his blood tests after reassessment are presented in Table 1.

Test parameter	Results
Hemoglobin (g/L)	146
White blood cell count (cells/L)	6×10^9
Platelets (/L)	158×10^9
Alanine aminotransferase (IU/mL)	110
Aspartate aminotransferase (IU/mL)	65
Alkaline phosphatase (IU/mL)	N
Fasting blood sugar (mmol/L)	5.2
Bilirubin (µmol/L)	11
Albumin (g/L)	41
International normalized ratio	1.03
Cholesterol (mmol/L)	3.10
Triglyceride (mmol/L)	1.23
Low-density lipoproteins (mmol/L)	1.60
High-density lipoproteins (mmol/L)	0.94
HCV RNA (copies/mL)	1,000,000
Cryoglobulins	Absent
Ultrasound	No focal lesion (fat)

Table 1: Results of blood test for Mr R.H.

Mr R.H. underwent retreatment with pegylated interferon alfa-2b 1.5 µg/kg plus RBV 1000 mg daily and achieved an early viral response with a >2 $\log_{10}$ decline in HCV RNA, however, HCV RNA remained detectable throughout the 48 weeks of treatment. At the end of treatment his viral load was 1000 copies/mL, and 6 months after discontinuation of treatment his viral load had risen to 3 x 1,000,000 copies/mL.

DISCUSSION OF THE CASE

Definition of response to antiviral therapy for chronic hepatitis C

Mr R.H. would be best described as being a slow responder, with an early virological response at 12 weeks with a >2 $\log_{10}$ drop in HCV RNA but with detectable virus at the end of 48 weeks of treatment (1000 copies/mL); Table 2. Had his antiviral treatment been continued for considerably longer he might eventually have cleared HCV RNA but he would have been at high risk of relapsing post cessation of therapy. Thus, relapsers are generally slow responders during treatment. Individuals who have no antiviral response to combination therapy, i.e. <1 $\log_{10}$ fall in HCV RNA at 12 weeks are described as "null responders" and the pathogenesis for "null" response is probably

Time	Response
Early virological response (12 week)	>2 $\log_{10}$ decline HCV RNA
End-of-treatment response (48 week)	HCV RNA 10^3 copies/mL
6 months post-treatment	HCV RNA 3 x 10^6 copies/mL

HCV=hepatitis C virus

Table 2: Response to therapy with pegylated interferon alfa-2b 1.5 μg/kg + ribavirin 1000 mg/day

very different from that for a "slow responder" or a relapser. Enhancing the sensitivity of the qualitative test used for measuring HCV RNA could potentially change the definition of a patient's response from "relapser" to a "non responder" should a relatively insensitive method be employed to measure HCV RNA at the end of treatment. It is, therefore, important to know the lower limit of detection of the qualitative test used [1]. If the virus is still detectable at the end of 24 weeks of therapy, the likelihood of achieving a sustained response is so low that it is probably inappropriate to continue therapy beyond this time.

Factors influencing response to therapy for chronic hepatitis C

Worldwide the biggest factor influencing success of antiviral therapy is the degree of adherence (Table 3). Maximal antiviral effect can only be achieved if the patient adheres to more than 80% of both treatments for at least 80% of the time prescribed [2]. Large community-based studies in North America have shown that only 20% of individuals are fully adherent for 48 weeks of therapy. Several studies have shown that both dose and the duration of therapy influence the final response. Different interferons have never been directly compared head-to-head. There have been reports that one interferon may give rise to a sustained virological response (SVR) in an individual who previously had not responded to a full course of therapy with another form of interferon (+ RBV) [3]. Tailoring the dose of antiviral therapy over time to the virological response has not been found to yield better SVR rates [4].

Viral factors, particularly genotype and viral load, probably have the strongest influence on response to antiviral therapy [5]. Co-infection with other viruses namely HIV or HBV also influence (negatively) the likelihood of an antiviral response.

Therapy	Virus	Host
Dose	Genotype	Hepatic fibrosis
Duration	Viral Load	Steatosis/high BMI
Adherence	HIV co-infection	Ethnicity
Type of pegylated interferon	Mutations	Age (adherence)
		Alcohol (adherence)

BMI=body mass index

Table 3: Factors affecting response to therapy for chronic hepatitis C

There are a number of host factors which influence the outcome of antiviral therapy, some of which may be a result of virus-host interaction while others are genetic. Regardless of genotype, the presence of cirrhosis reduces antiviral responsiveness particularly in those who are infected with a less interferon-sensitive genotype, i.e. genotype 1 [6]. It is not understood why cirrhosis negatively influences response to therapy. Obesity and/or hepatic steatosis is another independent marker of antiviral responsiveness [7]. It is thought that this is probably due to its effect on increasing suppressor of cytokine signalling-3 which induces insulin resistance and promotes a cytokine milieu interfering with the innate interferon response [8,9,10]. Insulin resistance may be modified and several studies have shown that exercise and weight loss lead to reduced hepatic steatosis and even loss of insulin resistance [11]. Studies have also shown that insulin resistance may be abrogated by successful antiviral therapy suggesting that this metabolic state is in part induced by chronic infection [12].

Clearly there are also inherited (genetic) factors that influence the likelihood of viral clearance. It is well recognized that African Americans have a significantly lower antiviral response than Caucasians, treated with the same drugs [13]. There are data to suggest that Alaskan natives may spontaneously clear hepatitis C even when they have been chronically infected [14]. Genetic factors which may influence antiviral responsiveness include major histocompatibility complex and specific interleukin (IL)-2 polymorphisms [15,16].

There are many observations that indicate that hepatitis C viral proteins may interfere with the innate interferon response by inhibiting STAT-1, TRIF, RIG-1, PKR, and IRF-3, TBK1 [17-21]. There are reports of up-regulation of the innate interferon pathway in liver tissue of non-responders because of up-regulation downstream of

the anti-ubiquitin gene USP18 [22]. What needs to be established is whether the new enzyme inhibitors will effectively abolish the effect of hepatitis C viral proteins at these multiple sites in the host innate interferon response allowing for viral clearance in patients such as Mr R.H. Clinical data suggest that effective immune control is required to sustain viral clearance. Hence, any new treatment with a small molecule will probably still require the use of interferon with or without RBV, or other immune stimulants, such as vaccines.

REFERENCES

1. Morishima C, Morgan TR, Everhart JE, Wright EC, Shiffman ML, Everson GT, Lindsay KL, Lok ASF, Bonkovsky HL, Di Bisceglie AM, Lee WM, Dienstag JL, Ghany MG, Cretch DR. HCV RNA Detection by TMA during the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (Halt-C) Trial. Hepatology 2006;44:360-367.
2. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mac C, Garaud JJ, Albrecht JK. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061.
3. Cornberg M, Hadem J, Schuppert F, Schmidt HH, Reiser M, Marschal O, Steffen M, Manns MP, Wedemeyer H. Treatment with daily consensus interferon (CIFN) plus ribavirin in non-responder patients with chronic hepatitis C: a randomized open-label pilot study. J Hepatol 2006;44:291-301.
4. Zeuzem S, Pawlotsky JM, Lukasiewicz E, van Wagner M, Goulis I, Lurie Y, Gianfranco E, Vrolijk JM, Esteban JI, Hezode C, Lagging M, Negro F, Soulier A, Verheij-Hart E, Hansen B, Tal R, Ferrari C, Schalm SW, Newmann AU; Ditto-HCV Study Group. International, multicenter, randomized, controlled study comparing dynamically individualized versus standard treatment in patients with chronic hepatitis C. J Hepatol 2005;43:250-257.
5. 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.
6. Heathcote EJ, Shiffman M, Cooksley G, Dusheiko G, Lee S, Balart L, Reindollar R, Reddy RK, Wright TL, Lin A, Hoffman J, De Pamphilis J. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673-1680.
7. Bressler B, Tomlison G, Guindi M, Heathcote J. High body mass index is an independent risk factor for non-response to anti-viral treatment in chronic hepatitis C. Hepatology 2003;38:639.
8. Walsh MJ, Jonsson JR, Richardson MM, Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. Gut 2006;55:529.
9. Ueki K, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol Cell Biol 2004;24:5434.
10. Hong F, Nguyen VA, Gao B. Tumor necrosis factor alpha attenuates interferon alpha signaling in the liver: involvement of SOCS3 and SHP2 and implication in resistance to interferon therapy. FASEB J 2001;15:1595.

11. Nobili V, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, Sartorelli MR, Anqulo P. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. Hepatology 2006;44:458-465.

12. Romero-Gomez M, Del Mar Viloria M, Andrade RJ, Salmeron J, Diago M, Fernandez-Rodriguez CM, Corpas R, Cruz M, Grande L, Vazquez L, Munoz-De-Rueda P, Lopez-Serrano P, Gila A, Gutierrez ML, Perez C, Ruiz-Extremera A, Suarez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology 2005;128:636.

13. Muir AJ, Bornstein JD, Killenberg PG; Atlantic Coast Hepatitis Treatment Group. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. N Engl J Med 2004;350:2265-2271.

14. Scott JD, McMahon BJ, Bruden D, Sullivan D, Homan C, Christensen C, Gretch DR. High rate of spontaneous negativity for hepatitis C virus RNA after establishment of chronic infection in Alaska Natives. Clin Infec Dis 2006;42:945-952.

15. Thursz M, Yallop R, Goldin R, Trepo, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. Lancet 1999;354:2119-2124.

16. Knapp S, Hennig BJ, Frodsham AJ, Zhang L, Hellier S, Wright M, Goldin R, Hill AV, Thomas HC, Thursz MR. Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. Immunogenetics 2003;55:362-369.

17. Lin W, Kim SS, Yeung E, Kameqava JT, Kim KA, Hotlzman MJ, Chung RT. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. J Virol 2006;80:9226-9235.

18. Ferreon JC, Ferreon AC, Li K, Lemon SM. Molecular determinants of TRIF proteolysis mediated by the hepatitis C virus NS3/4A protease. J Biol Chem 2005;280:20483-20492.

19. Breiman A, Grandvaux N, Lin R, Ottone C, Akira S, Yoneyama M, Fujita T, Hiscott J, Meurs EF. Inhibition of RIG-I-dependent signaling to the interferon pathway during hepatitis C virus expression and restoration of signaling by IK Kepsilon. J Virol 2005;79:3969-3978.

20. Francois C, Duverlie G, Rebouillat D, Khorsi H, Castelain S, Blum HE, Gatignol A, Wychowski C, Moradpour D, Meurs EF. Expression of hepatitis C virus proteins interferes with the antiviral action of interferon independently of PKR-mediated control of protein synthesis. J Virol 2000;74:5587-5596.

21. Foy E, Li K, Wang C, Sumpter R Jr, Ikeda M, Lemon SM, Gale M Jr. Inhibition of RIG-I-dependent signaling to the interferon pathway during hepatitis C virus expression and restoration of signaling by IK Kepsilon. Science 2003;300:1145-1148.

22. Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, Heathcote J, Edwards M, McGilvray ID. Hepatic gene expression profiling discriminates responders and non-responders in treatment of chronic Hepatitis C viral infection. Gastroenterology 2005;128:1437-1444.

How to Manage Patients with HIV/HCV Co-Infection

J. Bratcher, P. Saitta, D. Dieterich

INTRODUCTION

Over the past decade, HIV-related morbidity and mortality have dramatically decreased due to the development of antiretroviral therapy (ART) [1-3]. A recent analysis concluded that due to effective HIV treatment, at least 3 million life years have been saved in the United States, translating into an average survival after diagnosis of AIDS of more than 14 years [4]. Liver disease secondary to chronic hepatitis C virus (HCV) has become the greatest threat to patients with HIV/HCV co-infection [5]. Up to 30% of patients with HIV in the United States are also infected with HCV, resulting in a co-infected population of more than 300,000 patients [6,7]. Inner-city poor, active injection drug users and hemophiliacs have even higher co-infection rates, approaching 90% in some reports [8-11]. The progression of liver disease is accelerated by steatosis in co-infected patients [12,13] and the efficacy of HCV treatment is reduced if metabolic syndrome is present [14]. The focus of current research is on determining the optimal timing and duration of HCV treatment, along with managing medication interactions and adverse side effects. The goals of this chapter are to: 1) provide an overview of the evaluation of co-infected patients; 2) outline the major clinical trials regarding HCV treatment in HIV patients; and, 3) summarize the management of complications of treatment, including when to refer for liver transplantation.

DISEASE PROGRESSION IN HIV/HCV CO-INFECTED PATIENTS

End-stage liver disease is the leading cause of death in HIV/HCV co-infected patients and is mostly the result of HCV infection [15]. Admissions to hospitals due to liver disorders in HIV patients have increased 5-fold since the introduction of ART [16] and between 35 and 50% of deaths in patients with HIV are now attributed to complications from end-stage liver disease [17,18]. It is well-established that HIV hastens the progression of liver fibrosis in patients with HCV [19,20], but controversy remains regarding the influence of HCV on the control of HIV.

Effect of HIV on HCV progression

While progression to cirrhosis can take 30 years in patients with HCV mono-infection, in those with concomitant HIV this time is significantly decreased. Studies have shown that 15% of co-infected patients untreated for HCV develop cirrhosis within 10 years [21] and 25% within 15 years [22], compared with 6.5% and 2.6% in mono-infected patients, respectively. A meta-analysis of 8 studies with outcomes related to cirrhosis or decompensated cirrhosis found a significantly increased relative risk of progression to end-stage liver disease in co-infected patients compared with those with HCV mono-infection [23]. These data show that early treatment of HCV is critical to prevent complications of liver disease in patients with HIV. The treatment of HIV can accelerate liver damage, as all HIV medications are potentially hepatotoxic. This is often reported in patients (especially co-infected patients) treated with non-nucleoside inhibitors and protease inhibitors [24]. Of particular concern is the combination of didanosine and ribavirin (RBV), which increases the risk of severe and even fatal lactic acidosis and pancreatitis [25,26]. Substituting an alternative medication for didanosine in the ART regimen is recommended when HCV treatment is initiated in co-infected patients.

Effect of HCV on HIV control

A more complicated issue in the management of co-infected patients is the effect of chronic HCV on the progression of HIV. Several studies have found that co-infected patients have a more rapid progression to AIDS and death than HIV mono-infected patients [27-30]. Hepatitis C virus infection has also been attributed to an

increased risk of AIDS-defining events, a decreased response to antiretroviral therapy and a higher rate of toxicity to ART [31-33]. Other investigators have reported conflicting results. In the EuroSIDA cohort study, Rockstroh *et al.* found that while co-infected patients had a higher rate of liver-disease–related deaths, there was no association between HCV and the incidence of AIDS or HIV-related deaths [34]. Three studies have shown that in patients infected with hepatitis C ART had no effect on virological response [35-37]. In addition to liver toxicity caused by ART the impact of HCV infection may also affect HIV control [38-43].

Metabolic syndrome, insulin resistance and steatosis

The association between metabolic syndrome and insulin resistance in the development of nonalcoholic fatty liver disease or steatohepatitis is well documented [44-48]. Steatosis is found in up to 55% of HCV mono-infected patients [49]. Obesity and steatosis increase the risk for progression to fibrosis [14,50,51]. HCV induces insulin resistance and subsequently type 2 diabetes mellitus leading to steatosis [52-54]. Two types of steatosis are found in HCV patients. One type is caused by obesity, alcohol consumption and hyperlipidemia, and is seen in most patients with non-genotype 3 HCV. A second type is a virally induced steatosis mediated by HCV genotype 3 [60], which improves with viral eradication [61]. Genotype 3 is independently associated with a risk of steatosis and subsequent fibrosis [55-59]. The tendency for HCV patients to develop fatty liver actually decreases the response rate to standard HCV treatment with interferon and RBV [62-64].

Limited data exist regarding the association between HIV, HCV and fatty liver. Some evidence suggests that steatosis is more severe [65], can be seen in up to 69% of co-infected patients, and is more frequent with the use of the nucleoside analogs didanosine and stavudine [13,66]. One study determined that steatosis of any grade was more common and more severe in co-infected patients. In these patients a linear correlation between the progression to fibrosis and the grade of steatosis was found [12]. Recent data indicate that insulin-sensitization with thiazolidinediones reduces the progression to fibrosis in animal models [67,68]. These findings highlight the importance of addressing modifiable risk factors in this high-risk population. If indicated, weight reduction, lipid control, insulin-sensitizing medications and avoidance of alcohol should be part of the therapeutic plan for co-infected patients. Table 1 lists considerations and indications when treating hepatitis C in

HIV-infected patients and Table 2 lists liver toxicity associated with ART.

Diagnosis and Screening
– Hepatitis A, B, C antibody tests: If A and B negative, vaccinate
– Hepatitis C quantitative RNA (may also be needed in high-risk immunosuppressed patients who have negative antibody tests)
Indications for HCV Treatment
– Well-controlled HIV (ART or CD4 >350 cells/mm^3)
– Histological evidence of advanced hepatitis C-related liver disease (fibrosis or cirrhosis)
– HIV therapy interrupted by recurrent ART-induced hepatotoxicity

ART=antiretroviral therapy

Table 1: Considerations for the diagnosis and treatment of hepatitis C in HIV-infected patients

– HIV/HCV co-infected patients are at increased risk for hepatotoxicity
– All antiretroviral therapies are potentially hepatotoxic
– *In vivo*, RBV has not been shown to competitively inhibit phosphorylation of pyrimidine nucleoside analogs (AZT, d4T, ddC)
– Monitoring for lactic acidosis is recommended (NIH Consensus Panel on the Management of Hepatitis C: 2002)
– RBV may increase the risk of severe mitochondrial toxicity (i.e. pancreatitis and lactic acidosis) in patients taking ddI. Discontinuation of ddI prior to starting RBV should be strongly considered

AZT=zidovudine; d4t=stavudine; ddC=2',3'-dideoxycytidine; ddI=didanosine; HCV=hepatitis C virus; RBV=ribavirin

Table 2: Liver toxicity and antiretrovirals

TREATMENT: SUMMARY OF CLINICAL TRIALS

Current therapy for chronic hepatitis C consists of either standard interferon or pegylated interferon administered in subcutaneous (SC) injections, combined with daily oral RBV. Clinical trials have evaluated the effectiveness of various treatment regimens in

co-infected patients and have concluded that sustained viral response (SVR) is achievable, but at a lower rate than in patients without HIV.

APRICOT

The AIDS Pegasys Ribavirin International Co-Infection Trial (APRICOT) [69] is the largest international treatment trial for chronic hepatitis C in HIV co-infected patients. Eight hundred sixty-eight patients were randomized to receive 1 of 3 regimens: 1) standard interferon alfa-2a (3 million units SC 3 times per week) plus oral RBV (400 mg twice daily); 2) pegylated interferon alfa-2a (180 µg per week) plus oral placebo; or, 3) pegylated interferon alfa-2a plus oral RBV. Sustained virological response rate was 40% in the pegylated interferon alfa-2a plus RBV group, compared with 20% in patients on pegylated interferon alfa-2a monotherapy, and 12% in patients receiving standard interferon plus RBV. Genotypes 2 and 3 had higher SVR rates of 62%, 36% and 20%, while genotype 1 had SVR rates of 29%, 14% and 7%, respectively. Sustained virological response rate in patients with genotypes 2 and 3 was higher than in a prior study in which patients were treated for 24 weeks [70]. Results from the 48-week APRICOT study imply that a longer duration of therapy is appropriate in co-infected patients. Patients with genotype 1 and a baseline HCV RNA above 800,000 IU/mL were less likely to respond to any regimen, but this was not the case in genotypes 2 and 3. Although absolute CD4 counts decreased in all 3 arms, percentages did not change and there was no detrimental impact on HIV disease progression. Subgroup analysis of APRICOT reveals that the SVR for genotype 1 increased from 29 to 39% in patients who received at least 80% of the planned RBV dose, 80% of planned pegylated interferon alfa-2a dose, and 80% of planned total treatment duration (80/80/80). In patients with genotypes 2 and 3, the SVR increased from 59 to 69% in those with a total cumulative treatment exposure of 70% of planned RBV dose, 70% of planned pegylated interferon alfa-2a dose, and 70% of planned total treatment duration (70/70/70). Infected patients who demonstrated a rapid virological response (defined as HCV polymerase chain reaction-negative at week 4 had an SVR of 82% for genotype 1 and 94% for genotypes 2 and 3.

ACTG-A5071

The AIDS clinical trials group study A5071 randomized 133 patients to either: 1) standard interferon alfa-2a (6 million IU 3 times a week

for 12 weeks followed by 3 million IU 3 times per week for 36 weeks); or, 2) pegylated interferon alfa-2a (180 μg per week for 48 weeks) [71]. Both groups received RBV in escalating doses: 600 mg per day for 4 weeks, 800 mg per day for the following 4 weeks, then 1000 mg per day for the remaining study period. After 48 weeks of treatment, an SVR was attained at 72 weeks in 27% of patients with pegylated interferon alfa-2a and 12% of patients receiving standard interferon. Patients with genotype 2 or 3 had a 73% chance of attaining an SVR with pegylated interferon alfa-2a compared to 33% with standard interferon. The rates were 14% and 6% for genotype 1, respectively. Thirty-five percent of patients with no virological response at 24 weeks and 52% of those with virological response showed histological improvement on liver biopsy. Absolute CD4 counts decreased, but percentages remained constant, and no adverse effect on HIV disease progression was found. The higher relapse rate in this study may be attributed to the lower starting dose of RBV, emphasizing the necessity of early maximal RBV dosing.

Laguno

Ninety-five patients were randomized to receive either: 1) weekly SC injections of pegylated interferon alfa-2a (100 μg for body weight <75 kg, or 150 μg for >75 kg), plus weight adjusted RBV (800 mg if <60 kg, 1000 mg if 60 to 75 kg, and 1200 if >75 kg) split between 2 daily doses; or, 2) interferon 3 million IU SC 3 times per week plus weight adjusted RBV [72]. The duration of therapy was 48 weeks except for genotypes 2 and 3 and for those with HCV RNA below 800,000 IU/mL, who received therapy for 24 weeks. Overall, 34% of patients achieved an SVR, and this was significantly higher in the pegylated interferon alfa-2a group than the interferon group (44% vs. 21%). For genotypes 1 and 4, the pegylated interferon alfa-2a group reached an SVR of 38% compared with 7% in the interferon arm. There was a high incidence of depression, which improved with medical therapy, and mitochondrial toxicity was encountered in 12% of patients.

RIBAVIC

The RIBAVIC study is a randomized controlled trial of pegylated interferon alfa-2b plus RBV vs. interferon alfa-2b plus RBV for the initial treatment of chronic hepatitis C in HIV co-infected patients [73]. Four hundred twelve patients were randomized to

receive either: 1) weight-based weekly SC injections of pegylated interferon alfa-2b (1.5 µg/kg); or, 2) 3-times–weekly SC injections of standard interferon alfa-2b (3 million IU) for 48 weeks. All patients received 400 mg of oral RBV twice daily. Overall, an SVR was achieved in 27% of patients with pegylated interferon alfa-2b compared with 20% of patients receiving standard interferon. Genotypes 2 and 3 revealed SVRs of 44% and 43%, whereas genotypes 1 and 4 had SVRs of 17% and 6%, respectively. Those patients who received at least 80% of the total treatment dose had a much higher chance of reaching an SVR (40% vs. 29%), and those with an SVR showed significant histological improvement on liver biopsy.

PRESCO

The PRESCO study (pegylated interferon alfa-2a plus RBV for the treatment of chronic hepatitis C in patients co-infected with HIV in Spain) is an ongoing study by Soriano *et al.* evaluating the effect of longer therapy for HCV in co-infected patients to determine if the SVR can be enhanced by longer treatment duration and higher doses. Preliminary reports suggest that a high early viral response can be observed in genotypes 2 and 3, but not in genotypes 1 and 4. Furthermore, a subset of patients with genotypes 1 and 4 appear to be refractory to optimal HCV therapy [74,75]. The difference between the APRICOT and PRESCO studies is the use of weight-based RBV doses in the PRESCO trial. Preliminary reports indicate that a higher RBV dose results in a better SVR [76].

Conclusion from Clinical Trials

Available data show that in the treatment of HIV/HCV co-infected patients, pegylated interferon is clearly superior to standard interferon, and higher doses of RBV improve the chance of SVR. Overall, genotypes 2 and 3 have a better response rate to treatment than genotypes 1 and 4 and ongoing studies should help to define the optimal duration and dosage of treatment with current therapy. Tables 3-5 list the weight-based dosing for RBV and pegylated interferon alfa-2b.

MANAGING SIDE EFFECTS

Several well-recognized side effects are associated with interferon and pegylated interferon therapy in HCV mono-infected and HIV/HCV co-infected patients. These include gastrointestinal disturbances (nausea, diarrhea, weight loss), influenza-like symptoms (fever, fatigue, myalgia, and rigors), dermatological symptoms (alopecia, dermatitis, pruritus), neuropsychiatric symptoms (especially depression), and hematologic abnormalities (neutropenia, thrombocytopenia, anemia) [77-79].

Weight (kg)	Ribavirin Daily Dose (mg)
<40	600
40-64	800
65-85	1000
86-105	1200
>105	1400

Table 3: Weight-based dosage of ribavirin with pegylated interferon alfa-2b

Weight (kg)	Redipen™ (µg/0.5 mL)	Volume (mL)
<40	50	0.5
41-50	80	0.4
51-64		0.5
65-75	120	0.4
76-86		0.5
>85	150	0.5

Table 4: Weight-based dosage of pegylated interferon alfa-2b

Weight (kg)	Ribavirin Daily Dose (mg)
<75	1000
>75	1200

Table 5: Weight-based dosage of ribavirin with pegylated interferon alfa-2a

Ribavirin is primarily associated with dose-related hemolytic anemia, which can result in treatment discontinuation in 10 to 14% of patients or dose reduction in 32 to 42% of patients [80,81], while

neutropenia and thrombocytopenia are more frequent causes of dose reduction in patients being treated with interferon-based therapies [69,77]. Depression, influenza-like symptoms and gastrointestinal symptoms may also lead to dose reduction or discontinuation of combination therapy [69,77,78]. Treatment dose and medication adherence are known to affect treatment outcome. The SVR is significantly higher in patients receiving larger doses of pegylated interferon alfa-2b (1.5 μg/kg per week), and the RBV dose is an independent predictor of the SVR. The highest response is achieved in patients taking the maximum dose of pegylated interferon with an RBV dose of >10.6 mg/kg per day [78].

Adherence to treatment has been studied in a recent retrospective study [78,82-84]. Patients who received at least 80% of their total interferon dose, at least 80% of their RBV dose and who were treated for at least 80% of the expected duration of therapy had an SVR of 72% (62% for genotype 1) compared with 57% (34% for genotype 1) for those requiring dose reduction or receiving <80% of the total treatment.

Management of the hematologic abnormalities that lead to dose reduction and/or treatment discontinuation can be challenging in co-infected patients. Both interferon and RBV can cause anemia, and combination therapy can cause hemoglobin levels to drop below 11 g/dL in 25 to 30% of patients [83,84]. The hemolytic anemia associated with RBV is dose-dependent and can cause a hemoglobin drop of 2 to 3 g/dL at doses ≥800 mg/day, usually within 4 weeks of initiation of therapy [85]. Symptomatic anemia necessitates RBV dose reduction to sub-optimal levels, which can decrease the likelihood of achieving an SVR [78].

Recent studies have investigated the use of growth factors to prevent medication-induced anemia associated with interferon and RBV treatment. One study using epoetin alfa (weekly SC injections of 40,000 IU) revealed that 83% of patients were able to maintain RBV dosages of at least 800 mg/day compared with 54% without epoetin therapy [86]. In patients whose hemoglobin dropped below 12 g/dL, 88% of those treated with epoetin were able to maintain their initial RBV dose compared with 60% in the placebo group. Quality of life scores were significantly improved in the epoetin group and mean hemoglobin levels increased by 2.2 g/dL compared with 0.1 g/dL in the placebo group [87].

Neutropenia can occur with combination therapy with interferon and RBV, is more frequent with pegylated interferon, and is the most common reason for dose reduction with pegylated interferon [77-79].

Neutrophil counts can decrease by 21% after 1 injection of pegylated interferon [88] usually within the first 4 weeks of treatment, and can drop by as much as 34% before stabilizing [89]. Current recommendations for pegylated interferon therapy recommend dose reduction at a neutrophil count of 750 cells/mm^3 [3,90] which is extrapolated from evidence in cancer patients undergoing chemotherapy who have been found to have an increased risk of infection with neutrophil levels below 500 cells/mm^3 [3,91,92]. Several clinical trials have found that the use of recombinant human granulocyte-colony stimulating factor (G-CSF, 300 μg SC 3 times per week, titrated to neutrophil count >750 cells/mm^3) as an adjunctive agent to interferon therapy can result in maintenance of higher neutrophil counts [93-96].

Thrombocytopenia has been rarely associated with dose reduction in patients treated with combination therapy [69,77,97]. Interferon-based therapy can result in a 10 to 50% decrease in platelet count [98-101] and is particularly problematic in cirrhotics receiving treatment. Recombinant human interleukin (IL)-11, the only approved agent for enhancing platelet production, stimulates megakaryocytopoiesis, and can safely increase platelet levels in HCV-infected patients with interferon-induced thrombocytopenia [102].

Patients can try and minimize treatment side effects by maintaining a mild to moderate exercise schedule to improve generalized fatigue, and by taking antipyretics and analgesics for symptomatic relief of the headaches, pyrexia, myalgias and arthralgias associated with treatment [79]. Depression occurs in 20 to 30% of patients taking interferon and it is a frequent cause of decreased quality of life leading to dose reduction and discontinuation [77,79]. Symptoms associated with depression such as fatigue, decreased concentration and sleep disturbances occur frequently in patients receiving interferon [103], and are related to the impact on serotonin metabolism. Interferon interferes with serotonin by increasing tryptophan breakdown and decreasing the conversion of tryptophan to serotonin. This occurs because of an increase in the levels of pro-inflammatory cytokines such as IL-2, IL-6 and interferon-γ, which then increase stimulation of 2,3-dioxygenase, a major tryptophan-catabolizing enzyme. A trial of 18 patients receiving interferon demonstrated a significant increase in depression scores and a significant decrease in serum levels of tryptophan and 5-hydroxytryptamine (5-HT) [104]. Interferon also increases the activity of the 5-HT transporter, resulting in increased re-uptake of serotonin and a decrease in the availability of serotonin in

the synapse [105]. In light of the serotonin effect, it is not surprising that selective serotonin re-uptake inhibitors are the primary agents used and studied in the treatment of interferon-induced depression. In a prospective study evaluating paroxetine in patients who developed major depression during the course of interferon treatment, 79% were able to complete the full course of treatment [106]. Another prospective study of patients receiving interferon alfa and RBV found that 73% completed treatment and 85% had significant improvement in their depression scores when treated with citalopram [107].

In conclusion, there are many side effects associated with interferon/RBV treatment of HIV/HCV co-infected patients. These effects can lead to dose reductions and discontinuation of treatment. Adherence to optimal dosing regimens is imperative to achieve higher rates of SVR, especially in genotype 1.

Patients should be counseled regarding conservative measures for symptom relief. The use of hematological growth factors can reduce the need for dose reduction or discontinuation, and selective serotonin re-uptake inhibitors are effective against treatment-induced depression. Table 6 outlines dosing reductions and growth factor recommendations.

Symptom	Dose reduction	Growth factor recommendations
Anemia	Reduce RBV for Hb <10g/dL	Epoetin alfa 40,000 IU SC weekly if Hb falls below 12 g/dL or >2 g/dL decrease
Neutropenia	Reduce interferon dose for ANC <750 cells/mm^3	G-CSF 300 µg SC, 3 times/week, titrate to maintain ANC >750

ANC=absolute neutrophil count; G-CSF=granulocyte-colony stimulating factor; Hb=hemoglobin; RBV=ribavirin; SC=subcutaneous

Table 6: Dose reductions

MANAGEMENT OF TREATMENT FAILURES

Since a minority of patients with HIV/HCV co-infection achieve an SVR, the management of treatment failures is becoming a more important issue. Several reports have indicated some success in retreatment with interferon and RBV in combination in mono-infected HCV patients who did not respond to an initial regimen of interferon

monotherapy [108-110], but there are no trials evaluating the effectiveness of retreatment in co-infected patients. One approach being investigated in the REPEAT trial is the use of double-dose pegylated interferon for either 48 or 72 weeks [111]. Initial results are promising and an SVR has been attained in 62% of those receiving the larger dose. Until studies are available that offer recommendations for management of these patients, the approach to treatment failures should begin with identifying any factors that may have led to treatment failure, such as patient adherence, intolerable side effects and medication interactions. Any correctable factors should be maximized before retreatment is considered. In overweight patients with or without metabolic syndrome, a weight-loss regimen should be initiated and insulin-sensitizing medications should be considered to decrease insulin resistance and improve chances for retreatment success. Data indicate that HIV-positive patients may have equivalent survival after solid organ transplantation as non-HIV–infected patients [112]. Patients infected with HIV who otherwise meet criteria for liver transplantation should be evaluated at centers where they may be considered for transplantation or for inclusion in clinical trials.

SUMMARY

Co-infection with HIV/HCV presents a clinical challenge due to poor response to treatment, potential medication interactions and troubling side effects that may necessitate cessation of therapy. The optimal dosing and duration of treatment for HCV in this population remains to be determined, but the current recommendation is treatment for 48 weeks with pegylated interferon in combination with weight-based RBV for all genotypes. Ongoing studies will provide more information as to how to identify those who are most likely to respond to therapy and how treatment failures should be optimally managed. In the meantime, adherence to current treatment guidelines offers the best hope for therapeutic response. Pretreatment optimization with a supportive patient-physician relationship is necessary to combat the complex medical, social and psychological issues that arise during treatment.

REFERENCES

1. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338:853-860.

2. Hogg RS, Yip B, Kully C, Craib, KJ, O'Shaughnessy M, Schechter M, Montaner JS. Improved survival among HIV-infected patients after initiation of triple-drug antiretroviral regimens. CMAJ 1999;160:659-665.

3. Henry K, Erice A, Tierney C, Balfour HH Jr., Fischl MA, Kmack A, Liou SH, Kenton A, Hirsch MS, Phair J, Martinez A, Kahn JO. A randomized, controlled, double-blind study comparing the survival benefit of four different reverse transcriptase inhibitor therapies (three-drug, two-drug, and alternating drug) for the treatment of advanced AIDS. AIDS Clinical Trial Group 193A Study Team. J Acquir Immune Defic Syndr Hum Retrovirol 1998;19:339-349.

4. Walensky RP, Paltiel AD, Losina E, Mercincavage LM, Schackman BR, Sax PE, Weinstein MC, Freedberg KA. The survival benefits of AIDS treatment in the United States. J Infect Dis 2006;194:11-19.

5. Sulkowski MS, Thomas DL. Hepatitis C in the HIV-infected person. Ann Intern Med 2003;138:197-207.

6. Sherman KE, Rouster SD, Chung RT, Rajicic N. Hepatitis C virus prevalence among patients infected with human immunodeficiency virus: a cross-sectional analysis of the US Adult AIDS Clinical Trials Group. Clin Infect Dis 2002;34:831-837.

7. Tedaldi EM, Hullsiek KH, Malvestutto CD, Arduino RC, Fisher EJ, Gaglio PJ, Jenny-Avital ER, McGowan JP, Perez G; Terry Beirn. Prevalence and characteristics of hepatitis C virus coinfection in a human immunodeficiency virus clinical trials group: the Terry Beirn Community Programs for Clinical Research on AIDS. Clin Infect Dis 2003;36:1313-1317.

8. Hall CS, Charlebois ED, Hahn JA, Moss AR, Bangsberg DR. Hepatitis C virus infection in San Francisco's HIV-infected urban poor. J Gen Intern Med 2004;19:357-365.

9. Patrick DM, Tyndall MW, Cornelisse PG, LI K, Sherlock CH, Rekart ML, Strathdee SA, Currie SL, Schechter MT, O'Shaughnessy MV. Incidence of hepatitis C virus infection among injection drug users during an outbreak of HIV infection. CMAJ 2001;165:889-895.

10. Garfein RS, Doherty MC, Monterroso ER, Thomas DL, Nelson KE, Vlahov D. Prevalence and incidence of hepatitis C virus infection among young adult injection drug users. J Acquir Immune Defic Syndr Hum Retrovirol 1998;18:S11-S19.

11. Yokozaki S, Takamatsu J, Nakano I, Katano Y, Toyoda H, Hayashi K, Hayakawa T, Fukuda Y. Immunologic dynamics in hemophiliac patients infected with hepatitis C virus and human immunodeficiency virus: influence of antiretroviral therapy. Blood 2000;96:4293-4299.

12. Gaslightwala I, Bini EJ. Impact of HIV on the prevalence and severity of steatosis in patients with chronic hepatitis C virus infection. J Hepatol 2006;44:1026-1032.

13. Bani-Sadr F, Carrat F, Bedossa P, Piroth L, Cacoub P, Perronne C, Degott C, Pol S; ANRS HC02 - RIBAVIC Study team. Hepatic steatosis in HIV-HCV coinfected patients: analysis of risk factors. AIDS 2006;20:525-531.

14. Charlton MR, Pockros PJ, Harrison SA. Impact of obesity on treatment of chronic hepatitis C. Hepatology 2006;43:1177-1186.

15. Bica I, McGovern B, Dhar R, Stone D, McGowan K, Scheib R, Snydman DR. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clin Infect Dis 2001;32:492-497.

16. Gebo KA, Diener-West M, Moore RD. Hospitalization rates differ by hepatitis C status in an urban HIV cohort. J Acquir Immune Defic Syndr 2003;34:165-173.

17. Soriano V, Garcia-Samaniego J, Valencia E, Rodriguez-Rosado R, Munoz F, Gonzalez-Lahoz J. Impact of chronic liver disease due to hepatitis viruses as cause of hospital admission and death in HIV-infected drug users. Eur J Epidemiol 1999;15:1-4.

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

19. Fabris P, Barnes E, Tositti G, Giordani MT, Grasso A, De Lalla F. Therapy of chronic hepatitis C virus infection in HIV co-infected people. Curr Pharm Des 2004;10:2111-2122.

20. Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, Vidaud M, Bricaire F, Opolon P, Katlama C, Poynard T. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. Hepatology 1999;30:1054-1058.

21. Soto B, Sanchez-Quijano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol 1997;26:1-5.

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

23. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, Koziel MJ. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis 2001;33:562-569.

24. Nunez M, Lana R, Mendoza JL, Martin-Carbonero L, Soriano V. Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2001;27:426-431.

25. Butt AA. Fatal lactic acidosis and pancreatitis associated with ribavirin and didanosine therapy. AIDS Read 2003;13:344-348.

26. Moreno A, Quereda C, Moreno L, Perez-Elias MJ, Muriel A, Casado JL, Antela A, Dronda F, Navas E, Barcena R, Moreno S. High rate of didanosine-related mitochondrial toxicity in HIV/HCV-coinfected patients receiving ribavirin. Antivir Ther 2004;9:133-138.

27. Miller MF, Haley C, Koziel MJ, Rowley CF. Impact of hepatitis C virus on immune restoration in HIV-infected patients who start highly active antiretroviral therapy: a meta-analysis. Clin Infect Dis 2005;41:713-720.

28. Backus LI, Phillips BR, Boothroyd DB, Mole LA, Burgess J, Rigsby MO, Chang SW. Effects of hepatitis C virus coinfection on survival in veterans with HIV treated with highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2005;39:613-619.

29. Anderson KB, Guest JL, Rimland D. Hepatitis C virus coinfection increases mortality in HIV-infected patients in the highly active antiretroviral therapy era: data from the HIV Atlanta VA Cohort Study. Clin Infect Dis 2004;39:1507-1513.

30. Greub G, Ledergerber B, Battegay M, Grob P, Perrin L, Furrer H, Burgisser P, Erb P, Boggian K, Piffaretti JC, Hirschel B, Janin P, Francioli P, Flepp M, Telenti A. Clinical progression, survival and immune recovery during antiretroviral therapy in patients with HIV-1 and HCV coinfection: the Swiss HIV Cohort Study. Lancet 2000;356:1800-1805.

31. Weis N, Lindhardt BO, Kronborg G, Hansen AB, Laursen AL, Christensen PB, Nielsen H, Moller A, Sorensen HT, Obel N. Impact of hepatitis C virus coinfection on response to highly active antiretroviral therapy and outcome in HIV-infected individuals: a nationwide cohort study. Clin Infect Dis 2006;42:1481-1487.

32. Mocroft A, Phillips AN, Soriano V, Rockstroh J, Blaxhult A, Katlama C, Boron-Kaczmarska A, Viksna L, Kirk O, Lundgren JD; EuroSIDA Study Group. Reasons for stopping antiretrovirals used in an initial highly active antiretroviral regimen: increased incidence of stopping due to toxicity or patient/physician choice in patients with hepatitis C coinfection. AIDS Res Hum Retroviruses 2005;21:743-752.

33. Stebbing J, Waters L, Mandalia S, Bower M, Nelson M, Gazzard B. Hepatitis C virus infection in HIV type 1-infected individuals does not accelerate a decrease in the CD4+ cell count but does increase the likelihood of AIDS-defining events. Clin Infect Dis 2005;41:906-911.

34. Rockstroh JK, Mocroft A, Soriano V, Tural C, Losso MH, Horban A, Kirk O, Phillips A, Ledergreber B, Lundgren JD. Influence of hepatitis C virus infection on HIV-1 disease progression and response to highly active antiretroviral therapy. J Infect Dis. 2005;192:992-1002.

35. Sullivan PS, Hanson DL, Teshale EH, Wotring LL, Brooks JT. Effect of hepatitis C infection on progression of HIV disease and early response to initial antiretroviral therapy. AIDS 2006;20:1171-1179.

36. Al-Harthi L, Voris J, Du W, Wright D, Nowicki M, Frederick T, Landay A, Kovacs A. Evaluating the impact of hepatitis C virus (HCV) on highly active antiretroviral therapy-mediated immune responses in HCV/HIV-coinfected

women: role of HCV on expression of primed/memory T cells. J Infect Dis 2006;193:1202-1210.

37. Mayor AM, Gomez MA, Fernandez DM, Rios-Olivares E, Thomas JC, Hunter RF. Morbidity and mortality profile of human immunodeficiency virus-infected patients with and without hepatitis C co-infection. Am J Trop Med Hyg 2006;74:239-245.

38. Macias J, Castellano V, Merchante N, Palacios RB, Mira JA, Saez C, Garcia-Garcia JA, Lozano F, Gomez-Mateos JM, Pineda JA. Effect of antiretroviral drugs on liver fibrosis in HIV-infected patients with chronic hepatitis C: harmful impact of nevirapine. AIDS 2004;18:767-774.

39. Melvin DC, Lee JK, Belsey E, Arnold J, Murphy RL. The impact of co-infection with hepatitis C virus and HIV on the tolerability of antiretroviral therapy. AIDS 2000; 14:463-465.

40. Bonacini M. Liver injury during highly active antiretroviral therapy: the effect of hepatitis C coinfection. Clin Infect Dis 2004;38:S104-S108.

41. Ena J, Amador C, Benito C, Fenoll V, Pasquau F. Risk and determinants of developing severe liver toxicity during therapy with nevirapine-and efavirenz-containing regimens in HIV-infected patients. Int J STD AIDS 2003;14:776-781.

42. Stone SF, Lee S, Keane NM, Price P, French MA. Association of increased hepatitis C virus (HCV)-specific IgG and soluble CD26 dipeptidyl peptidase IV enzyme activity with hepatotoxicity after highly active antiretroviral therapy in human immunodeficiency virus-HCV-coinfected patients. J Infect Dis 2002;186:1498-1502.

43. Martin-Carbonero L, Nunez M, Rios P, Perez-Olmeda M, Gonzalez-Lahoz J, Soriano V. Liver injury after beginning antiretroviral therapy in HIV/hepatitis C virus co-infected patients is not related to immune reconstitution. AIDS 2002;16:1423-1425.

44. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol 2005;16:421-427.

45. Loria P, Lonardo A, Carulli L, Verrone AM, Ricchi M, Lombardini S, Rudilosso A, Ballestri S, Carulli N. Review article: the metabolic syndrome and nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2005;22:S31-S36.

46. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;432:S99-S112.

47. Scheen AJ, Luyckx FH. Obesity and liver disease. Best Pract Res Clin Endocrinol Metab 2002;16:703-716.

48. Luyckx FH, Lefebvre PJ, Scheen AJ. Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss. Diabetes Metab 2000;26:98-106.

49. Lonardo A, Loria P, Adinolfi LE, Carulli N, Ruggiero G. Hepatitis C and steatosis: a reappraisal. J Viral Hepat 2006;13:73-80.

50. Leandro G, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 2006;130:1636-1642.

51. Sanyal AJ, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Stravitz RT, Mills AS. Nonalcoholic fatty liver disease in patients with hepatitis C is associated with features of the metabolic syndrome. Am J Gastroenterol 2003;98:2064-2071.

52. Koike K. Hepatitis C virus infections can present with metabolic disease by inducing insulin resistance. Intervirology 2006;49:51-57.

53. Noto H, Raskin P. Hepatitis C infection and diabetes. J Diabetes Complications 2006;20:113-120.

54. Koike K. Hepatitis C as a metabolic disease: Implication for the pathogenesis of NASH. Hepatol Res 2005;29:145-150.

55. Rubbia-Brandt L, Fabris P, Paganin S, Leandro G, Male PJ, Giostra E, Carlotto A, Bozzola L, Smedile A, Negro F. Steatosis affects chronic hepatitis C progression in a genotype specific way. Gut 2004;53:406-412.

56. 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.

57. Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. Hepatology 2002;36:729-736.

58. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33:1358-1364.

59. Castera L, Chouteau P, Hezode C, Zafrani ES, Dhumeaux D, Pawlotsky JM. Hepatitis C virus-induced hepatocellular steatosis. Am J Gastroenterol. 2005;100:711-715.

60. Yoon EJ, Hu KQ. Hepatitis C virus (HCV) infection and hepatic steatosis. Int J Med Sci 2006;3:53-56.

61. Castera L, Hezode C, Roudot-Thoraval F, Lonjon I, Zafrani ES, Pawlotsky JM, Dhumeaux D. Effect of antiviral treatment on evolution of liver steatosis in patients with chronic hepatitis C: indirect evidence of a role of hepatitis C virus genotype 3 in steatosis. Gut 2004;53:420-424.

62. Yaginuma R, Ikejima K, Okumura K, Kon K, Suzuki S, Takei Y, Sato N. Hepatic steatosis is a predictor of poor response to interferon alpha-2b and ribavirin combination therapy in Japanese patients with chronic hepatitis C. Hepatol Res 2006;35:19-25.

63. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Kobayashi M, Arase Y, Ikeda K, Kumada H. Predictive factors of virological non-response to interferon-ribavirin combination

therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol 2006;78:83-90.

64. Patton HM, Patel K, Behling C, Bylund D, Blatt LM, Vallee M, Heaton S, Coonrad A, Pockros PJ, McHutchinson JG. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J Hepatol 2004;40:484-490.

65. Marks KM, Petrovic LM, Talal AH, Murray MP, Gulick RM, Glesby MJ. Histological findings and clinical characteristics associated with hepatic steatosis in patients coinfected with HIV and hepatitis C virus. J Infect Dis 2005;192:1943-1949.

66. McGovern BH, Ditelberg JS, Taylor LE, Gandhi RT, Christopoulos KA, Chapman S, Schwarzapfel B, Rindler E, Fiorino AM, Zaman MT, Sax PE, Graeme-Cooke F, Hibberd PL. Hepatic steatosis is associated with fibrosis, nucleoside analogue use, and hepatitis C virus genotype 3 infection in HIV-seropositive patients. Clin Infect Dis 2006;43:365-372.

67. Galli A, Crabb DW, Ceni E, Salzano R, Mello T, Svegliati-Baroni G, Ridolfi F, Trozzi L, Surrenti C, Casini A. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. Gastroenterology 2002;122:1924-1940.

68. Marra F, DeFranco R, Robino G, Novo E, Efsen E, Pastacaldi S, Zamara E, Vercelli A, Lottini B, Spirli C, Strazzabosco M, Pinzani M, Parola M. Thiazolidinedione treatment inhibits bile duct proliferation and fibrosis in a rat model of chronic cholestasis. World J Gastroenterol 2005;11:4931-4938.

69. Torriani FJ, Rodriquez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, Carosi G, Sasadeusz J, Katlama C, Montaner J, Sette H Jr, Passe S, De Pamphilis J, Duff F, Schrenk UM, Dieterich DT; APRICOT Study Group. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. N Engl J Med 2004;351:438-450.

70. 70 Perez-Olmeda M, Nunez 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 IFN-alfa-2b plus ribavirin as therapy for chronic hepatitis C in HIV-infected patients. AIDS 2003;17:1023-1028.

71. Chung RT, Andersen J, Volberding P, Robbins GK, Liu T, Sherman KE, Peters MG, Koziel MJ, Bhan AK, Alston B, Colquhoun D, Nevin T, Harb G, van der Horst C; AIDS Clinical Trials Group A5071 Study Team. Peginterferon alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected patients. N Engl J Med 2004;351:451-459.

72. Laguno M, Murillas J, Blanco JL, Martinez E, Miquel R, Sanchez-Tapias JM, Bargallo X, Garcia-Criado A, de Lazzari E, Larrousse M, Leon A, Lonca M, Milinkovic A, Gatell JM, Mallolas J. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV coinfected patients. AIDS 2004;18:F27-36.

73. Carrat F, Bani-Sadr F, Pol S, Rosenthal E, Lunel-Fabiani F, Benzekri A, Morand P, Goujard C, Pialoux G, Piroth L, Salmon-Ceron D, Degott C, Cacoub P, Perronne C; ANRS HCO2 RIBAVIC Study Team. Pegylated interferon alfa-2b

vs standard interferon alfa-2b plus ribavirin for chronic hepatitis C in HIV-infected patients. JAMA 2004;292:2839-2848.

74. Rendon A, Nunez M, Romero M, Barreiro P, Martin-Carbonero L, Garcia-Samaniego J, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Early monitoring of ribavirin plasma concentrations may predict anemia and early virological response in HIV/hepatitis C virus coinfected patients. J Acquir Immune Defic Syndr 2005;39:401-405.

75. Nunez M, Camino N, Ramos B, Berdun MA, Barreiro P, Losada E, Santos I, Echevarria S, Ocampo A, Miralles C, Arazo P, Martin-Carbonero L, Romero M, Garcia-Samaniego J, Soriano V. Impact of ribavirin exposure on early virological response to hepatitis C therapy in HIV infected patients with cirrhotic hepatitis C. Antivir Ther 2005;10:657-662.

76. Ramos B, Nunez M, Rendon A, et al. High ribavirin doses and early virological response in HCV/HIV-coinfected patients. Program and abstracts of the 2nd International Workshop on HIV and Viral Hepatitis Coinfection (abstract 36) Amsterdam, The Netherlands 2006.

77. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, 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.

78. 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 randomized trial. The Lancet 2001;358:958-965.

79. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2002;365:S237-S244.

80. Bodenheimer HC, 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.

81. De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, Stanzial AM, Solero P, Corrocher R. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology 2000;31:997-1004.

82. McHutchison JG, Manns MP, Patel K, Poynard T, Lindsay KL, Trepo C. Adherence to combination therapy enhances sustained response in genotype-1 infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061-1069.

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

84. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zuzem S, Trepo C, Albrecht J. Randomized trial of interferon alpha 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha 2b plus

placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426-1432.

85. Rebetron (Rebetol [ribavirin]/Intron A [interferon alfa-2b, recombinant]). [package insert]. Kenilworth, NJ: Schering, 2002.

86. Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, Wright T, Younossi Z, Goon BL, Tang KL, Bowers PJ; Proactive Study Group. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. Gastroenterology 2004;126:1302-1311.

87. Dieterich DT, Wasserman R, Brau N, Hassanein TI, Bini EJ, Bowers PJ, Sulkowski S. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. Am J Gastroenterol 2003;98:2491-2499.

88. Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M, Kreil A, Hofer H, Jessner W, Gangl A, Ferenci P. Rapid suppression of hematopoiesis by standard or pegylated interferon alfa. Gastroenterology 2002;123:141-151.

89. Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, Park Y, Liang TJ, Hoofnagle JH. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. Hepatology 2002;36:1273-1279.

90. PEG-Intron (peginterferon alfa-2b) [package insert]. Kenilworth, NJ: Schering, 2002.

91. Viscoli C. The evolution of the empirical management of fever and neutropenia in cancer patients. J Antimicrob Chemother 1998;41:S65-S80.

92. Viscoli C. Management of infection in cancer patients. Studies of the EORTC International Antimicrobial Therapy Group (IATG). Eur J Cancer 2002;38:S82-S87.

93. Fukuda A, Kobayashi H, Teramura K, Yoshimoto S, Ohsawa N. Effects of interferon-alpha on peripheral neutrophil counts and serum granulocyte colony-stimulating factor levels in chronic hepatitis C patients. Cytokines Cell Mol Ther 2000;6:149-154.

94. Shiffman ML, Hoffmann CM, Luketic VA, Sanyal AJ. Use of granulocyte macrophage colony-stimulating factor alone or in combination with interferon alfa-2b for treatment of chronic hepatitis C. J Hepatol 1998;28:382-389.

95. Sood A, Reddy N, Russo MW, Brown RS, Jacobson IM. Use of granulocyte colony stimulating factor (GCSF) for interferon induced neutropenia in patients with chronic hepatitis C infection. Program and abstracts of the 52nd annual meeting of the American Association for the Study of Liver Diseases (Dallas, TX). Alexandria, VA: (abstract 1029) American Association for the Study of Liver Diseases 200:429A.

96. Hepatitis Resource Network. Management of side effects in hepatitis C treatment. 2001. Available at: http://www.h-r.org/provider/algorithims/ hcvalgo1011.pdf. Accessed 10 December 2002.

97. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, Reindollar R, Reddy K, Wright TL, Lin A, Hoffman J, De Pamphilis J.

Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673-1680.

98. Hoofnagle J. Thrombocytopenia during interferon alpha therapy. JAMA 1991;266:849.

99. Shrestha R, McKinley C, Bilir BM, Everson GT. Possible idiopathic thrombocytopenic purpura associated with natural alpha interferon therapy for chronic hepatitis C infection. Am J Gastroenterol 1995;90:1146-1147.

100. Pockros PJ, Duchini A, McMillan R, Nyberg LM, McHutchison J, Viernes E. Immune thrombocytopenic purpura in patients with chronic hepatitis C virus infection. Am J Gastroenterol 2002;97:2040-2045.

101. Peck-Radosavljevic M, Wichlas M, Pidlich J, Sims P, Meng G, Zacherl J, Garg S, Datz C, Gangl A, Ferenci P. Blunted thrombopoietin response to interferon alfa-induced thrombocytopenia during treatment for hepatitis C. Hepatology 1998;28:1424-1429.

102. Rustgi VK, Lee P, Finnegan S, Ershler W. Safety and efficacy of recombinant human IL-11 (oprelvekin) in combination with interferon/ribavirin therapy in hepatitis C patients with thrombocytopenia. Program and abstracts of the 53rd annual meeting of the American Association for the Study of Liver Diseases (Boston). Alexandria, VA: (abstract 791) American Association for the Study of Liver Diseases, 2002:361A.

103. Dieperink E, Willenbring M, Ho SB. Neuropsychiatric symptoms associated with hepatitis C and interferon alpha: A review. Am J Psychiatry 2000;157:867-876.

104. Zdilar D, Franco-Bronson K, Buchler N, Locala JA, Younossi ZM. Hepatitis C, interferon alfa, and depression. Hepatology 2000;31:1207-1211.

105. Morikawa O, Sakai N, Obara H, Saito N. Effects of interferon-a, interferon-gamma and cAMP on the transcriptional regulation of the serotonin transporter. Eur J Pharmacol 1998;349:317-324.

106. Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M. Paroxetine for the treatment of interferon-alpha-induced depression in chronic hepatitis C. Aliment Pharmacol Ther 2002;16:1091-1099.

107. Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J, Gulati M, Thornton AJ, Schultz RL, Valentine AD, Meyers CA, Howell CD. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry 2002;7:942-947.

108. Parise E, Cheinquer H, Crespo D, Meirelles A, Martinelli A, Sette H, Gallizi J, Silva R, Lacet C, Correa E, Cotrim H, Fonseca J, Parana R, Spinelli V, Amorim W, Tatsch F, Pessoa M. Peginterferon alfa-2a (40KD) (PEGASYS®) plus ribavirin (COPEGUS®) in retreatment of chronic hepatitis C patients, nonresponders and relapsers to previous conventional interferon plus ribavirin therapy. Braz J Infect Dis 2006;10:11-16.

109. Taliani G, Gemignani G, Ferrari C, Aceti A, Bartolozzi D, Blanc PL, Capanni M, Esperti F, Forte P, Guadagnino V, Mari T, Marino N, Milani S, Pasquazzi S, Rosina F, Danilo T, Toti M, Zignego AL, Messerni L, Stroffolini T. Pegylated

interferon alfa-2b plus ribavirin in the retreatment of interferon-ribavirin nonresponder patients. Gastroenterology 2006;130:1098-1106.

110. Jacobson IM, Gonzalez SA, Ahmed F, Lebovics E, Min AD, Bodenheimer HC Jr, Esposito SP, Brown RS Jr, Brau N, Klion FM, Tobias H, Bini EJ, Brodsky N, Cerulli MA, Aytaman A, Gardner PW, Geders JM, Spivack JE, Rahmin MG, Berman DH, Ehrlich J, Russo MW, Chait M, Rovner D, Edlin BR. A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. Am J Gastroenterol 2005;100:2453-2462.

111. Marcellin P, Teuber G, Canva V, Weiland O, Di Bisceglie A, Brandao-Mello C, et al. Efficacy of standard-dose and fixed-dose induction peginterferon alfa-2a (40kd) (Pegasys®) plus ribavirin (Copegus®) among pegylated interferon alfa-2b (12kd)/ribavirin non-responders: interim analysis of the repeat study (abstract 11). EASL 2006.

112. Ragni MV, Belle SH, Im K, Neff G, Roland M, Stock P, Heaton N, Humar A, Fung JF. Survival of human immunodeficiency virus-infected liver transplant recipients. J Infect Dis 2003;188:1412-1420.

Pre- and Post-Treatment of Liver Transplant Patients with Hepatitis C

D. Samuel, B. Roche

INTRODUCTION

Liver disease caused by hepatitis C virus (HCV) is the main indication for orthotopic 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 nearly always occurs. Although the long-term impact of hepatitis C following liver transplantation varies, recurrence of HCV leads to acute hepatitis or chronic active hepatitis in most patients and may lead to cirrhosis or cholestatic hepatitis in a minority of cases. Effective treatment for the recurrence of HCV is therefore essential. Combination therapy with pegylated interferon and ribavirin (RBV) preferably begun before the onset of late-stage fibrosis could have an antiviral effect in 30 to 40% of patients.

This review discusses the existing knowledge on treatment of HCV graft infection after liver transplantation.

LIVER TRANSPLANTATION FOR HCV CIRRHOSIS

The effect of HCV infection on patient and graft survival after liver transplantation is controversial. However, recent data confirm that HCV infection impairs patient and allograft survival [1]. HCV recurrence is almost universal and 90% of patients develop lesions of chronic hepatitis on the graft [1-3]. Cholestatic hepatitis occasionally results in progressive and rapid liver dysfunction (in 2 to 8% of cases). Fortunately this severe complication is rare and occurs mainly during

first 2 years after transplantation. Overall, the course of HCV graft disease is accelerated in liver transplant recipients, compared to immune-competent patients, with a 5-year duration of cirrhosis in approximately 10 to 30% [1,3-4] of cases, sometimes reaching as high as 40% [5]. When cirrhosis occurs on the graft, there is a high risk of decompensation and a 60% risk of death within the year following the first episode of decompensation [6]. The course of HCV on the graft varies although fibrosis often progresses rapidly. In addition, the progression of fibrosis is generally linear, while in some cases it only accelerates after several years. At least 10% of patients undergoing transplant due to HCV cirrhosis will require retransplantation for hepatitis C graft failure.

The factors which may influence disease severity and progression of graft injury or survival remain unclear. Factors clearly associated with the severity of recurrent hepatitis C are: 1) high pre-transplant and early high post-transplant serum HCV RNA levels [7,8]; 2) severe early histological recurrence [9]; 3) rejection episodes and treatment with more potent immunosuppression compounds (methylprednisone boluses, OKT3) [5,10-13]; and, 4) increased age of donors [14-16]. Strategies to reduce the impact of immunosuppression on recurrent HCV infection have included an overall reduction in immunosuppression, discontinuation of individual agents and the use of immunosuppressive agents with potential antiviral effects. Current data have failed to show any differences in the incidence or severity of HCV recurrence using tacrolimus or cyclosporin even though the latter has some antiviral effects *in vitro* [17-20]. Many studies have shown a strong correlation between multiple rejection episodes, exposure to pulse solumedrol, greater daily exposure to steroids, OKT3 and the incidence and severity of HCV recurrence. In contrast to what was previously thought, early steroid withdrawal is now associated with more severe recurrence probably as a result of immunological rebound [21,22]. Post-transplantation use of mycophenolate mofetil (MMF) has not been associated with clear beneficial or deleterious effects; however MMF has no antiviral effects [23]. The effects of immunosuppression induction such as with anti-interleukin (IL)-2 receptor antibodies in HCV-infected transplant recipients has not been determined, however the presence of polyclonal anti-lymphocyte antibodies have been associated with a poorer outcome.

As a result, antiviral therapy should be offered to transplant patients with recurrent chronic hepatitis C to halt disease progression on the graft. However, the treatment of choice, a combination of

interferon and RBV, is not well-tolerated in transplant patients and may cause serious side effects (such as hemolytic anemia and risk of graft rejection). The aim of antiviral therapy is to clear HCV, or at least to lower HCV viremia to reduce disease progression on the graft. Antiviral therapy could therefore be used: 1) before transplantation to suppress viral replication and reduce the risk of recurrence; 2) early after transplantation to prevent the progression of hepatitis; and, 3) when HCV recurs.

TREATMENT OF HCV INFECTION

Pre-transplantation antiviral therapy

Although interferon alone or in combination with RBV has been shown to reduce viral levels in patients with cirrhosis, interferon use is problematic in this setting because of the risk of severe decompensation, the development of cytopenia or uncontrolled sepsis [24]. Forns *et al*. evaluated the efficacy and safety of antiviral therapy in 30 patients with HCV cirrhosis waiting for OLT (Child A n=15, Child B/C n=15, genotype 1b n=25) [25]. Treatment with 3 MU/day interferon alfa-2b and 800 mg/day RBV was initiated when the expected time for OLT was <4 months (median duration of treatment 12 weeks). Virological response was observed in 9 patients (30%). After OLT, 6 of them (20%) remained free of infection after a median follow-up of 46 weeks and HCV infection recurred in 3 patients. A viral load decrease of >2 $\log_{10}$ copies/mL at week 4 of treatment was the strongest predictor of a virological response. Side effects were frequent and dose reduction was necessary in 63% of patients. Everson *et al*. reported data from 124 patients with HCV and cirrhosis treated with interferon and RBV for 1 year with a low accelerating dose regimen [26]. A mean Child score of 7.1 was obtained. The end-of-treatment virological response and the sustained virological response (SVR) were 46% and 22%, respectively. Factors associated with an SVR were non-1 genotype (50% vs. 13% for HCV genotype 1), Child A score, optimal dosing and duration of treatment. Hepatitis C virus infection only recurred in 3 of the 15 sustained responders who underwent OLT. Twenty-two severe complications occurred during treatment leading to death in 4 patients. There are no data on the safety and efficacy of pegylated interferon with or without RBV in patients with severe (Child C) decompensated HCV cirrhosis. In conclusion, antiviral therapy in patients awaiting OLT should be

considered in order to prevent the recurrence of HCV in patients with cirrhosis without severe hepatocellular insufficiency.

Pre- and post-operative treatment with anti-HCV immune globulins

Polyclonal anti-HCV immune globulins (HCIG) have been given at different doses during and immediately after surgery. For the prophylaxis of HCV re-infection, the preliminary results are disappointing [27]. Indeed all patients receiving HCIG developed HCV recurrence and HCV RNA only decreased by 1 $\log_{10}$ copies/mL compared to those who did not receive treatment. In addition, the decrease in HCV RNA levels was transient. The choice of donors for HCIG and the dosing of HCIG should be further studied. A monoclonal preparation of HCIG is currently under evaluation [28].

Pre-emptive therapy in the early post-transplantation period

Hepatitis C virus RNA is present in the serum of more than 95% of those who are HCV RNA-positive before transplantation, which is the majority of patients. Hepatitis C virus RNA is detected in serum several hours post-transplantion [29]. However, HCV RNA is at its lowest levels in serum in the first week post-transplantation, which suggests that treatment should be initiated early [29]. Treatment is considered prophylactic if it is begun within 3 weeks after transplantation. Indeed, acute hepatitis may occur on the graft around 3 weeks after transplantation, with a median occurrence at 4 months [3]. Few studies have been performed on prophylactic antiviral treatment. In one study, 86 patients were randomized within 2 weeks of transplantation to receive either interferon alone (n=38) or placebo (n=48) for 1 year [30]. Patient and graft survival at 2 years and HCV viremia were not affected by treatment. In a second trial, 24 patients were randomized 2 weeks after transplantation to receive interferon (n=12) or placebo (n=12) for 6 months [31]. No differences were observed in graft or patient survival, incidence or severity of histological recurrence or 6-month HCV RNA levels. 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 associated with interferon were observed in 50% of patients (leukopenia 17%, headache and/or fatigue 33%). In a non-randomized pilot study, treatment with interferon alfa-2b and RBV was started in 36 patients

within 3 weeks of transplantation and continued for a median of 4.5 years [32]. Hepatitis C virus 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 were infected with genotype 2 (100% response rate). Of the remaining 24 patients, only 7 developed recurrent hepatitis, with significant fibrosis occurring in 4 patients. Dose reduction because of drug toxicity was necessary in 25% of patients but treatment was not discontinued in any of the patients. A subsequent pilot study of combination interferon and RBV therapy failed to obtain these results because of high dropout rates (48% related to severe RBV-induced hemolysis and interferon-induced neutropenia). An SVR was only achieved in 16% of patients [33]. Results with pegylated interferon alone as prophylaxis against recurrent hepatitis C after liver transplantation were disappointing with only an 8% SVR [34]. In a recent study, although the SVR with combination therapy was 18%, only 20% of the screened patients could receive treatment during the first post-transplant week. Thus, the feasibility of pre-emptive treatment is low [35]. In conclusion, published studies suggest that combination therapy is probably more effective than monotherapy. The main drawbacks are the risk of hematological side effects, rejection and sepsis. The results of pre-emptive treatment using available drugs with the intention of treatment analysis are disappointing. Indeed most patients have contra-indications to treatment in the first post-transplant weeks.

Treatment of established infection

Patients with HCV graft re-infection must be treated when the disease is severe to prevent the progression of hepatitis. 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 who absolutely must be treated, such as those with cholestatic hepatitis, due to the poor short-term prognosis, and those with rapidly evolving fibrosis confirmed by successive biopsies. For the latter, routine annual or biannual biopsies are essential to determine the progression of fibrosis. At present, most of these patients receive antiviral treatment; indeed in our experience 75% of patients received treatment within the first 2 years after transplantation.

Interferon is an immunostimulating agent that enhances the expression of human leukocyte antigens (HLA) class I and II molecules on hepatocytes and that has been reported to facilitate rejection in transplant recipients [36-38]. In our experience, histological disappearance of the interlobular bile duct suggesting chronic rejection was observed in 5 transplanted patients, 3 of whom underwent retransplantation [36]. Interferon treatment at doses of 3 MU 3 times per week for 6 months had a sustained virological effect in 0 to 7% of patients and had a minor beneficial histological effect [36,39-41]. A biochemical improvement was observed with RBV in 44 to 93% of patients but virological clearance did not occur [40,42,43]. The main side effect was hemolysis and the dosage should be adapted to renal function since the incidence of hemolysis was associated with significantly higher serum creatinine and decreased creatinine clearance [44].

Combination therapy is more effective than treatment with interferon or RBV alone. In a non-randomized pilot study, 21 patients with early recurrent hepatitis received a combination of interferon and RBV for 6 months and then RBV alone for another 6 months [45]. After 6 months of combination therapy, all patients had normal alanine aminotransferase (ALT) and histological improvement. Ten patients (48%) cleared HCV RNA from serum. During maintenance RBV monotherapy, ALT remained normal in all but 1 patient and HCV RNA reappeared in 5. The main side effect was anemia requiring withdrawal from RBV therapy in 3 patients. None of the patients experienced graft rejection. In a randomized controlled trial we compared 12 months of combination therapy vs. no treatment in 52 patients with HCV re-infection [46]. Intent to treat analysis for loss of serum HCV RNA showed a 21% SVR in the treated group, vs. 0% in the control group (p=0.019). Twelve treated patients (43%) were withdrawn from the study due to anemia (n=7), chronic rejection (n=1), insomnia (n=1), depression (n=1) and irritability (n=2) patients. Lavezzo *et al*. reported on data from 57 patients treated with interferon and RBV for 6 or 12 months [47]. Six additional months of RBV monotherapy was given to virological responders who had tolerated the drug well during combination therapy (n=7).

	N	Treatment (duration)	Months since transplant	Biochemical response (%)[a]	VR (%)[a]	Sustained VR (%)	Histological improvement (%)[a]	Rejection (%)	Therapy halted/SE (%)
Wright [39]	18	Interferon alfa-2b 3MU 3 x per week (4 mo)	15	28	0	0	0	4	11
Feray [36]	14	Interferon alfa-2b 3MU 3 x per week (6 mo)	44	23	7	7	14	35	28
Cotler [41]	8	Interferon alfa-2a 3MU daily (12 mo)	34	14	12.5	12.5	0	12.5	25
Gane [40]	14	Interferon alfa-2b 3MU 3 x per week (6 mo)	6	43	46	NA	21	0	0
	16	RBV (6 mo)	7	93	17	NA	64	0	12.5
Gane [42]	7	RBV (6 mo)	10	57	0	0	57	0	0
Cattral [43]	9	RBV (6 mo)	6	44	0	0	22	0	0
Chalasani [34]	33	Pegylated interferon 180µg/mL (48 weeks)			30	12	ND	ND	

mo=months; NA=not available; ND=no data; VR=virological response; RBV=ribavirin; SE= side effects; a=end of therapy

Table 1: Treatment of hepatitis C virus recurrence: interferon or ribavirin monotherapy

	N	Treatment (duration)	Months since transplant	Biochemical response (%)[a]	VR (%)[a]	Sustained VR (%)	Histological improvement (%)[a]	Rejection (%)	Therapy halted/SE (%)
Bizollon [45]	21	Interferon 3 MU 3 x per week +RBV (6 mo) then RBV (6 mo)	9	100	48 (6 mo) 24 (12 mo)	NA	94	0	14
Fischer [48]	8	Interferon 3 MU 3 x per week +RBV (6 mo)	5.5	87	12.5	0	NA	0	37.5
Samuel [46]	28	Interferon 3 MU 3 x per week +RBV (12 mo)	56	NA	25	21.4	NA	3.5	43
Gopal [49]	12	Interferon 3 MU 3 x per week +RBV (1-17 mo)	9	75	50	8.3	NA	8	8
De Vera [52]	32	Interferon 1.5-3 MU 3 x per week +RBV (3-18 mo)	NA	77	9	9	0	0	46.8
Alberti [51]	18	Interferon 3 MU 3 x per week +RBV (12 mo) then RBV (18-73 mo)	9	83	44 (12 mo)	33	73	5.5	22.2
Ahmad [50]	40	Interferon 3-5 MU 3 x per week (6 mo)	24	20	15	2.5	0	0	25
	20	Interferon 3-5 MU 3 x per week +RBV (12 mo)	38	25	40	20	0	0	25
Lavezzo [47]	27	Interferon 3 MU 3 x per week +RBV (6 mo)[b]	9	66	33	22	52	1.7	2
	30	Interferon 3 MU 3 x per week +RBV (12 mo)[b]	3-60	53	23	17			
Narayanan Menon [53]	26	Interferon 3 MU 3 x per week +RBV (12 mo)	14.6	42	35	30.7	75	0	50
Shakil [54]	38	Interferon 3 MU 3 x per week +RBV (12 mo) then RBV (6 mo)	23	18	13	5	0	0	42
Firpi [55]	54	Interferon 3 MU 3 x per week +RBV (12 mo)	31.2	39	38	30	30	5.5	12.9
Dumortier [57]	20	Pegylated interferon 0.5-1 μ/kg per week +RBV (12 mo)	28	75	55	45	NA	25	20
Castells [58]	24	Pegylated interferon 1.5 μ/kg per week +RBV (48 weeks)				34			
Rodriguez-Luna [59]	37	Pegylated interferon 0.5-1.5 μ/kg per week +RBV (48 weeks)				26			

mo=months; NA: not available; VR=virological response; RBV=ribavirin; SE= side effects; a=end of therapy;b=add 6 mo RBV to virological responders tolerating drug well

Table 2: Treatment of hepatitis C virus recurrence: interferon plus ribavirin combination therapy

The end of treatment and 12 month post-therapy SVR were 33% and 22%, respectively, for 6 months of therapy and 23% and 17% for 12 months of therapy (p=0.4). Non-genotype 1 was a significant predictor of SVR compared to genotype 1 (43% vs. 12%, p=0.02) and HCV RNA levels below 2 Meq/mL correlated with a higher rate of end of treatment virological response. The main side effects were anemia and leukopenia which required dose reduction in 51% of patients. Several recent studies of combination therapy have shown that the SVR rate was between 8 and 33% (Table 2) [48-55]. Bizollon *et al*. described the virological and histological course of 14 transplanted patients with a sustained virological response to antiviral therapy [56]. A complete response was sustained in 93% of patients 3 years after the end of therapy and associated with an absence of detectable intrahepatic HCV RNA and a marked decrease in histological activity as well as stabilization of the stage of liver fibrosis in most improved patients. Absence of detectable graft HCV RNA at the end of treatment seems to be a reliable indicator of SVR.

The optimal duration of therapy is uncertain. The efficacy and duration of additional RBV monotherapy in patients with a sustained response to combined treatment with interferon and RBV should be determined. As in the non-transplant setting, patients with the non-genotype 1 HCV responded better than patients with genotype 1. Other factors such as the interval from transplantation to the beginning of therapy, the severity of liver fibrosis, and the type and amount of immunosuppression could influence the efficacy of therapy.

All these studies showed a high incidence of side effects compared to that in non-transplant patients. Between 20 and 50% of patients could not complete treatment because of side effects from drugs. The most important side effect of RBV was hemolysis, which resulted in dose reductions or cessation of therapy. Erythropoietin may be effective in the treatment of anemia during combination therapy. Common side effects of interferon such as neutropenia, thrombocytopenia or depression are more frequent in the transplant setting. In a randomized trial, liver transplant recipients were treated with pegylated interferon alfa-2a 180 µg/week for 48 weeks vs. no treatment and the SVR was only 12% [34]. The results of treatment with pegylated interferon plus RBV showed an improved SVR of around 30 to 45% in treatment-naïve patients. Sustained virological response was related to genotype (better in genotypes 2 and 3), early virological response and optimal treatment dosing and duration [57-60]. Rejection occurred in 5 to 20% of patients during interferon treatment. The risk of rejection seemed to be independent of

virological response, but may depend on the dosing of interferon, the post transplant interval and the level of immunosuppression.

Retransplantation

Recurrence of HCV infection may lead to graft failure and an indication of retransplantation in a minority of cases (5 to 10% of patients). Early reports suggested a worse outcome in patients following retransplantation for HCV re-infection than in patients undergoing retransplantation for other indications [64]. However, the natural history of recurrent HCV disease in the second graft seems to be unrelated to that in the first graft. Recent studies have reported an improved outcome when retransplantation is performed before the development of infectious and renal complications [65]. Due to organ shortage and uncertainty of the natural history of the recurrence of HCV, retransplantation is still the subject of debate and further studies are needed [66].

CONCLUSION

Most patients with HCV infection will develop recurrence after transplantation. Although recurrence of HCV in the liver graft does not significantly reduce medium-term survival of the patient or the graft, HCV infection impairs long-term patient and graft survival. Treatment of recurrent HCV infection with interferon or RBV as single agents has been disappointing but results from combination therapy with pegylated interferon plus RBV are encouraging with an SVR in about 30 to 40% of patients. Pre-emptive therapy early after transplantation is limited by the high rate of side effects. Treatment of established infection on the graft is mandatory. However several questions should be raised. What is the best treatment? What is the optimal timing and duration of antiviral therapy? Future research should also focus on improving tolerance to treatment and determining the role of erythropoetin, and the place of new emerging antiviral therapies.

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. 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.

3. 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.

4. Feray C, Caccamo L, Alexander GJ, 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.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. 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.

13. Gane EJ, Naoumov NV, Qian KP, 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.

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. Rifai K, Sebagh M, Karam, V, Saliba F, Azoulay D, Adam R, Castaing D, Bismuth H, Reynes M, Samuel D, Feray C. Donor age influences 10-year liver graft histology independently of hepatitis C virus infection. J Hepatol 2004;41:446-453.

17. Papatheodoridis GV, Davies S, Dhillon AP, 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. Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporine A suppresses replication of hepatitis C virus genome in cultured hepatocytes. Hepatology 2003;38:1282-1288.

20. Berenguer M, Aguilera V, Prieto M, San Juan F, Rayon JM, Benlloch S, Berenguer J. Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected transplant recipients. Liver Transplant 2006;12:762-767.

21. Samonakis DN, Triantos CK, Thalheimer U, Quaglia A, Leandro G, Teixeira R, et al. Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. Liver Transplant 2005;11:386-395.

22. McCaughan GW, Zekry A. Mechanisms of HCV reinfection and allograft damage after liver transplantation. J Hepatol 2004;40:368-374.

23. Zekry A, Gleeson M, Guney S, McCaughan GW. A prospective cross-over study comparing the effect of mycophenolate versus azathioprine on allograft function and viral load in liver transplant recipients with recurrent chronic HCV infection. Liver Transpl 2004;10:52-57.

24. 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.

25. 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.

26. Everson GT, Trotter J, Forman L, Kugelmas M, Halprin A, Fey B, Ray C. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. Hepatology 2005;42:255-262.

27. Davis GL, Nelson DR, Terrault N, Pruett TL, Schiano TD, Fletcher CV, Sapan CV, Riser LN, Li Y, Whitley RJ, Gnann JW Jr; Collaborative Antiviral Study Group. A randomized, open-label study to evaluate the safety and pharmakokinetics of human hepatitis C immune globulin (Cicavir) in liver transplant recipients. Liver Transplant 2005;11:941-949.

28. Eren R, Landstein D, Terkieltaub D, Nussbaum O, Zauberman A, Ben-Porath J, Gopher J, Buchnick R, Kovjazin R, Rosenthal-Galili Z, Aviel S, Ilan E, Shoshany Y, Neville L, Waisman T, Ben-Moshe O, Kischitsky A, Foung SK, Keck ZY, Pappo O, Eid A, Jurim O, Zamir G, Galun E, Dagan S. Preclinical evaluation of two neutralizing monclonal antibodies against hepatitis C virus (HCV): a potential treatment to prevent HCV reinfection in liver transplant patients. J Virol 2006;80:2654-2664.

29. 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.

30. 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.

31. 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.

32. 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.

33. Reddy R, Fried M, Dickson R et al. 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 (abstract). Gastroenterology 2002;122:199A.

34. Chalasani N, Manzarbeitia C, Ferenci P, Vogel W, Fontana RJ, Voigt M, Riely C, Martin P, Teperman L, Jiao J, Lopez-Talavera JC; Pegasys Transplant Study Group. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. Hepatology 2005;41:289-298.

35. Shergill AK, Khalili M, Straley S, Bollinger K, Roberts JP, Ascher NA, Terrault NA. Applicability, tolerability and efficacy of pre-emptive antiviral therapy in hepatitis C-infected patients undergoing liver transplantation. Am J Transplant 2005;5:118-124.

36. 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.

37. 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.

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

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

40. 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.

41. Cotler SJ, Ganger DR, 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.

42. 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.

43. 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.

44. Jain AB, 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.

45. 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.

46. 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.

47. 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 alfa 2b with ribavirin. J Hepatol 2002;37:247-252.

48. 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 alfa. Transplant Proc 1999;31:494-495.

49. 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.

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

51. Alberti AB, Belli LS, 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.

52. De Vera ME, Smallwood GA, 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.

53. Narayanan Menon KV, Poterucha JJ, 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.

54. Shakil AO, 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.

55. Firpi RJ, Abdelmalek MF, 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.

56. 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.

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

58. Castells L, Vargas V, Allende H, Bilbao I, Luis Lazaro J, Margarit C, Esteban R, Guardia J. Combined treatment with pegylated interferon (alfa-2b) and ribavirin in the acute phase of hepatitis C virus recurrence after liver transplantation. J Hepatol 2005;43:53-59.

59. Rodriguez-Luna H, Khatib A, Sharma P, De Petris G, Williams JW, Ortiz J, Hansen K, Mulligan D, Moss A, Douglas DD, Balan V, Rakela J, Vargas HE. Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alfa2b and ribavirin: an open-label series. Transplantation 2004;77:190-194.

60. Berenguer M, Palau A, Fernandez A, Benlloch S, Aguilera V, Prieto M, Rayon JM, Berenguer J. Efficacy predictors of response, and potential risks associated with antiviral therapy in liver transplant recipients with hepatitis C. Liver Transplant 2006;12:1067-1076.

61. 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.

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

63. Samuel D, Forns X, Berenguer M, Trautwein C, Burroughs A Rizzetto M, Trepo C. report of the monothematic EASL conference on liver transplantation for viral hepatitis (Paris, France). J Hepatol 2006;45:127-143.

Steatosis and HCV: Dangerous Liaisons?

F. Negro

A direct relationship between hepatitis C virus (HCV) replication and steatosis has been proven by both clinical and experimental data [1,2]. In addition, models have been developed that allow detailed study of the mechanisms underlying triglyceride accumulation in hepatocytes [3-5].

STEATOSIS INDUCED BY HCV GENOTYPE 3a

Steatosis, mostly associated with HCV genotype 3a, is apparently mediated by impaired very low density lipoprotein (VLDL) secretion via reduced microsomal triglyceride transfer protein activity [4,6]. Hepatitis C virus may also up-regulate the sterol regulatory element binding protein signaling pathway [7], a protein involved in up-regulation of *de novo* fatty acid synthesis and inhibition of fatty acid β-oxidation, 2 mechanisms favoring triglyceride accumulation.

STEATOSIS, HCV AND INSULIN RESISTANCE

Viral induced steatosis may co-exist with fatty liver from other causes. In chronic hepatitis C patients infected with genotypes other than 3a and not consuming alcohol, the most frequent cause of steatosis is insulin resistance associated with excess weight [8]. This type of steatosis correlates with the body mass index (BMI) [8], and is not or only slightly modified by antiviral treatment [9,10]. The relationship between HCV and insulin resistance is complex, since HCV may be – at least in part – directly responsible for interfering with insulin signaling.

Although diabetes is a known complication of all liver diseases, especially in the advanced stages, both clinical and experimental data suggest a direct role of HCV in glucose metabolism disturbances. For example, Hui *et al.* [11] found that 121 HCV-infected patients with stage 0 or 1 fibrosis had higher homeostasis model assessment (HOMA) scores than 137 healthy controls matched by sex, body mass index and waist-to-hip ratio. This showed that HCV might induce insulin resistance independent of the stage of liver disease. Another recent study seems to suggest a relationship between the severity of insulin resistance and HCV replication levels [12]. Finally, Romero-Gomez *et al.* [13] have shown that insulin sensitivity improves in patients who clear HCV after therapy, while it does not improve in non-responders despite a decrease in BMI. Some experimental data also suggest that HCV interferes directly with the insulin cascade via proteasomal degradation of the insulin receptor substrate-1 and-2 [14], and their functional impairment via pro-inflammatory cytokines [15] or another post-receptor defect [16]. In patients with genotype 3a, HCV may alter intrahepatic insulin signaling through down-regulation of peroxisome proliferator-activated receptor-γ [17]. It is interesting to note that insulin resistance has been reported in all HCV genotypes, although to different extents [18], suggesting that there is an evolutionary advantage to inducing an insulin resistant state.

OTHER CAUSES OF STEATOSIS IN HEPATITIS C

Up to 30% of chronic hepatitis C patients with fatty liver who are not infected with genotype 3a and do not consume alcohol may present with normal BMI and HOMA scores [18]. This suggests that other causes of steatosis may exist in hepatitis C. Genetic polymorphisms, such as those associated with hyperhomocysteinemia, may be important [19].

STEATOSIS AND FIBROSIS

Steatosis in chronic hepatitis C has been repeatedly associated with increased fibrosis [1,8,20,21]. Some studies suggest that there is a genotype-specific association between steatosis and fibrosis, but results are controversial [22-25]. Insulin resistance is also known to be an important pathogenic factor for fibrosis, but the relative contribution of steatosis and insulin resistance to fibrosis has not been determined. A recent meta-analysis using individual data from 3068

patients recruited from 10 centers in 5 countries suggests that steatosis and diabetes are both independent factors of fibrogenesis in patients with genotype 1 infection [26]. However, when insulin resistance, an earlier and more sensitive parameter of glucose metabolism disturbance, is included in the logistic regression the association between steatosis and fibrosis disappears [11]. Recent data suggest that diabetes and insulin resistance are risk factors *per se* of advanced fibrosis and more rapid disease progression in chronic hepatitis C [11,27,28]. It has been hypothesized that the association between insulin resistance and the stage of fibrosis may not be direct: factors promoting insulin resistance (and/or steatosis) may also be responsible for accelerating fibrosis, and the role of inflammation has been emphasized [26,29].

Finally, the role of circulating cytokines needs to be determined. Metabolic syndrome is a chronic inflammatory state, where the liver is exposed to pro-inflammatory cytokines released into the circulation by adipocytes. Among these, leptin seems to play an important role. Recent data suggest that leptin may directly stimulate hepatic stellate cells to produce connective tissue growth factor [30]. In addition, decreased serum levels of adiponectin may fail to protect the liver from fibrogenic stimuli [31]. Hypo-adiponectinemia has been reported in chronic hepatitis C, especially in patients with steatosis [32].

STEATOSIS, INSULIN RESISTANCE AND THE THERAPEUTIC RESPONSE TO INTERFERON ALFA

Data from large clinical trials [10] have repeatedly shown that steatosis negatively affects response to antiviral treatment. This effect is significant for steatosis in patients with non-3a genotype infection, suggesting that insulin resistance is the factor affecting responsiveness to interferon alfa. This hypothesis was confirmed in a recent study [13], where sustained virological response (SVR) rates inversely correlated with HOMA scores before treatment. The molecular reasons for the correlation between insulin resistance and interferon alfa resistance are the object of intense research. Patients who fail to respond to interferon alfa may have increased intrahepatic suppressor of cytokine signaling 3 (SOCS-3) levels, a factor promoting the proteasomal degradation of insulin receptor substrate-1 (IRS-1) [33].

For clinical management of patients with chronic hepatitis C and fatty liver, control of excess body weight and increased physical exercise are the mainstays of therapy. Weight loss has been reported to improve liver fibrosis in HCV-infected persons [34], probably

because of improved insulin sensitivity. Results of Hickmann *et al*. [34] need to be independently confirmed by prospective studies with more patients followed for longer periods of time. Whether the pharmacological reduction of insulin resistance will be accompanied by increased sensitivity to interferon alfa remains to be proven by ongoing trials using metformin and thiazolidinediones. Thus, the most important advice that can be given to patients with chronic hepatitis C and steatosis is to change their lifestyle in order to increase insulin sensitivity. The use of insulin sensitizers should be limited to clinical trials.

REFERENCES

1. Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, Negro F. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. J Hepatol 2000;33:106-115.
2. Asselah T, Rubbia-Brandt L, Marcellin P, Negro F. Steatosis in chronic hepatitis C: why does it really matter? Gut 2006;55:123-130.
3. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997;78:1527-1531.
4. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, Koike K, Pessayre D, Chapman J, Barba G, Brechot C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. FASEB J 2002;16:185-194.
5. Abid K, Pazienza V, de Gottardi A, Rubbia-Brandt L, Conne B, Pugnale P, Rossi C, Mangia A, Negro F. An in vitro model of hepatitis C virus genotype 3a-associated triglycerides accumulation. J Hepatol 2005;42:744-751.
6. Mirandola S, Realdon S, Iqbal J, Gerotto M, Dal Pero F, Bortoletto G, Marcolongo M, Vario A, Datz C, Hussain MM, Alberti A. Liver microsomal triglyceride transfer protein is involved in hepatitis C liver steatosis. Gastroenterology 2006;130:1661-1669.
7. Su AI, Pezacki JP, Wodicka L, Brideau AD, Supekova L, Thimme R, Wieland S, Bukh J, Purcell RH, Schultz PG, Chisari FV. Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci USA 2002;99:15669-15674.
8. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33:1358-1364.
9. Kumar D, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes. Genotype-specific reversal of hepatic steatosis after sustained response to antiviral therapy. Hepatology 2002;36:1266-1272.
10. Poynard T, Ratziu V, McHutchison J, Manns M, Goodman Z, Zeuzem S, Younossi Z, Albrecht J. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003;38:75-85.
11. Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. Gastroenterology 2003;125:1695-1704.
12. Harrison SA. Correlation between insulin resistance and hepatitis C viral load. Hepatology 2006;43:1168.

13. Romero-Gomez M, Del Mar Viloria M, Andrade RJ, Salmeron J, Diago M, Fernandez-Rodriguez CM, Corpas R, Cruz M, Grande L, Vazquez L, Munoz-De-Rueda P, Lopez-Serrano P, Gila A, Gutierrez ML, Perez C, Ruiz-Extremera A, Suarez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology 2005;128:636-641.

14. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004;165:1499-1508.

15. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004;126:840-848.

16. Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology 2003;38:1384-1392.

17. de Gottardi A, Pazienza V, Pugnale P, Bruttin F, Rubbia-Brandt L, Juge-Aubry CE, Meier CA, Hadengue A, Negro F. Peroxisome proliferator-activated receptor-alpha and -gamma mRNA levels are reduced in chronic hepatitis C with steatosis and genotype 3 infection. Aliment Pharmacol Ther 2006;23:107-114.

18. Muzzi A, Leandro G, Rubbia-Brandt L, James R, Keiser O, Malinverni R, Dufour JF, Helbling B, Hadengue A, Gonvers JJ, Mullhaupt B, Cerny A, Mondelli MU, Negro F; Swiss Hepatitis C Cohort Study. Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. J Hepatol 2005;42:39-44.

19. Adinolfi LE, Ingrosso D, Cesaro G, Cimmino A, D'Anto M, Capasso R, Zappia V, Ruggiero G. Hyperhomocysteinemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients. Hepatology 2005;41:995-1003.

20. Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215-1219.

21. Fartoux L, Poujol-Robert A, Guechot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut 2005;54:1003-1008.

22. Rubbia-Brandt L, Fabris P, Paganin S, Leandro G, Male PJ, Giostra E, Carlotto A, Bozzola L, Smedile A, Negro F. Steatosis affects chronic hepatitis C progression in a genotype-specific way. Gut 2004;53:406-412.

23. Castera L, Hezode C, Roudot-Thoraval F, Bastie A, Zafrani ES, Pawlotsky JM, Dhumeaux D. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 2003;52:288-292.

24. 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.

25. Patton HM, Patel K, Behling C, Bylund D, Blatt LM, Vallee M, Heaton S, Conrad A, Pockros PJ, McHutchison JG. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J Hepatol 2004;40:484-490.

26. Leandro G, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, Terrault N, Pazienza V, Giordani MT, Giostra E, Sonzogni A, Ruggiero G, Marcellin P, Powell EE, George J, Negro F. The relationship between hepatic steatosis, inflammation and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 2006;130:1636-1642.

27. Ratziu V, Munteanu M, Charlotte F, Bonyhay L, Poynard T. Fibrogenic impact of high serum glucose in chronic hepatitis C. J Hepatol 2003;39:1049-1055.

28. Hickman IJ, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, Jonsson JR. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. J Hepatol 2003;39:1042-1048.

29. Asselah T, Boyer N, Guimont MC, Cazals-Hatem D, Tubach F, Nahon K, Daikha H, Vidaud D, Martinot M, Vidaud M, Degott C, Valla D, Marcellin P. Liver fibrosis is not associated with steatosis but with necro-inflammation in French patients with chronic hepatitis C. Gut 2003;52:1638-1643.

30. Hora C, Negro F, Rubbia L, Gonvers JJ, Muellhaupt B, Helbling B, Malinverni R, Oneta C, Dufour JF. Is connective tissue growth factor (CTGF) the missing link between steatosis, insulin resistance and fibrosis in patients with chronic hepatitis C? J Hepatol 2006;44:S199.

31. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa J, Shinomura Y, Funahashi T, Matsuzawa Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003;125:1796-1807.

32. Petit JM, Minello A, Jooste V, Bour JB, Galland F, Duvillard L, Verges B, Olsson NO, Gambert P, Hillon P. Decreased plasma adiponectin concentrations are closely related to steatosis in hepatitis C virus-infected patients. J Clin Endocrinol Metab 2005;90:2240-2243.

33. Walsh MJ, Jonsson JR, Richardson MM, Lipka GM, Purdie DM, Clouston AD, Powell EE. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. Gut 2006;55:529-535.

34. Hickman IJ, Clouston AD, Macdonald GA, Purdie DM, Prins JB, Ash S, Jonsson JR, Powell EE. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. Gut 2002;51:89-94.

Alcohol in Chronic Hepatitis C: Legal or Prohibited?

G. L. Davis

INTRODUCTION

Approximately 1.5 to 2% of the American and European population are infected with hepatitis C virus (HCV). Nearly 60% of the population are regular alcohol users consuming at least 1 alcoholic drink a month. Between 4.8 and 5.9% of adults in the United States are heavy alcohol consumers, and 6.9% are considered to be alcohol dependent (data calculated by the Alcohol Epidemiologic Data System of the National Institute on Alcohol Abuse and Alcoholism, November 2005; heavy drinking is defined as on average having greater than 2 drinks per day for men, and 1 drink per day for women during the past month) [1,2]. Given the prevalence of HCV infection and alcohol use, it is not surprising that the 2 conditions occasionally co-exist. However, the association between hepatitis C and alcohol is much stronger than would be anticipated by chance. Furthermore, there is considerable evidence that alcohol consumption influences the rate of progression of fibrosis and liver failure in patients with chronic hepatitis C, and probably decreases the chance of these patients responding to antiviral therapy.

EPIDEMIOLOGIC ASSOCIATION OF ALCOHOL AND HCV

Hepatitis C virus infection is prevalent among alcoholic patients (14 to 36%) and those with alcoholic liver disease (up to 50%) [3]. Furthermore, up to 60% of patients with chronic hepatitis C have a history of prior heavy alcohol consumption [4] and a surprisingly high

proportion (28 to 37%) continue consuming alcohol despite the knowledge that this behavior is deleterious [5,6]. The high prevalence of heavy alcohol consumption among hepatitis C patients is often related to intravenous drug use. Currently intravenous drug use accounts for more than 60% of chronic hepatitis C cases [7]. Between 57 and 90% of the individuals who have used intravenous drugs are anti-HCV–positive [8-10].

EFFECTS OF ALCOHOL IN HCV

The potential impact of alcohol use in patients with chronic hepatitis C can be separated into impact on the progression of parenchymal liver disease *per se*, risk of hepatocellular carcinoma (HCC), and response to antiviral therapy.

Progression of liver disease

Several studies have reported that a substantial proportion of deaths related to chronic hepatitis C (30 to 71%) occur in patients with a history of heavy alcohol use [11,12]. Furthermore, alcohol use has been identified as an independent risk factor for death in liver disease among patients with chronic hepatitis C with an odds ratio of about 1.4 [13,14], although in certain populations such as intravenous drug users, the risk of death remains high regardless of alcohol use [15]. However, in patients with alcohol-related liver disease, the presence of HCV infection does not appear to significantly influence survival [16], or at least not to the same degree as continued alcohol use, Child-Pugh score, or alcoholic hepatitis [17,18]. In contrast a study in the United Kingdom suggested that the rise in mortality from alcohol-related cirrhosis in middle-aged men after 1990 was related to the presence of HCV infection [19].

If HCV infection is responsible for greater liver-related mortality, then it must result in more progressive liver injury. Indeed, heavy alcohol intake is associated with more severe periportal inflammation [20,21], more rapid progression of fibrosis [10,22-24], a higher risk of cirrhosis [24-26], and greater risk of decompensated liver disease [24,27-29]. These effects are not trivial. Patients with heavy alcohol intake have a 2- to 16-fold increased risk for cirrhosis [24,25,30-32]. Furthermore, they develop cirrhosis on average in half to two-thirds of the time than it takes for non-consumers [24,33]. Only the duration of HCV infection has a greater influence on cirrhosis progression than alcohol, particularly among women [34].

Interestingly, it also appears that past or current alcohol consumption may account for much of the cirrhosis and increased mortality reported in patients co-infected with HIV and HCV [33,35,36]. Alcohol may interfere with potential mechanisms leading to liver injury in addition to HCV. It also appears that obese patients are nearly twice as sensitive to the negative effects of alcohol as non-obese patients [37].

Hepatocellular carcinoma

Chronic HCV is the most common cause of HCC in the United States and Europe. In most reports, the risk of HCC appears to be higher in patients with chronic hepatitis C and heavy alcohol consumption [38-43]. This relationship remains following sustained viral response (SVR) to antiviral treatment [44]. However, direct data supporting alcohol use *per se* in the pathogenesis of HCC is lacking and the relationship, if any, is controversial [29,38,45]. If such an effect exists, it may relate to a higher propensity to develop cirrhosis. In the population survey of Donato *et al*. HCV infection was a much stronger risk factor for HCC than heavy alcohol intake (>80 g/day) [38]. The relative risk of HCV and heavy alcohol consumption for HCC was much higher than either one alone (66.3 for HCV and alcohol vs. 23.3 for HCV and 4.6 for alcohol). However, it is not clear whether this risk was distinct from the greater likelihood of developing cirrhosis in those hepatitis C patients with heavy alcohol intake. Indeed, Niederau *et al.* found that the duration of infection and the presence of cirrhosis, and not alcohol intake, were the major risk factors for HCC [29].

The observation that habitual or heavy drinkers with chronic hepatitis C developed HCC at a younger age than those who did not use alcohol appears to support these results [46,47]. The study by Noda *et al.* found that HCC occurred on average 5 years earlier in patients with more than 46 g/day of alcohol intake [47].

Response to antivirals

It has been relatively difficult to assess the impact of alcohol on the response to interferon-based antiviral therapy since current and recent users of even moderate amounts of alcohol have typically been excluded from large registration trials. Unfortunately, the entry criteria for such trials often become the criteria for selecting treatment candidates once the therapy is approved. Past or current alcohol use is

a commonly cited reason for excluding patients from therapy [48,49]. Nonetheless, several centers have recently reported their experiences with interferon-based therapy in such patients and these suggest that alcohol does indeed decrease the response to antiviral therapy [50-53]. This effect correlates with the amount of alcohol consumed. Tabone and others have found that even modest drinkers had a reduced response to interferon monotherapy compared to non-drinkers (20% vs. 33%), while heavy drinkers had a significant reduction in outcome (9%) [52,54]. On the other hand, Chang *et al.* reported that an alcohol intake of >30 g/day was associated with a reduced response to interferon-based antiviral therapy, though abstinence was not distinguished from an intake of <30 g/day [51]. Surprisingly, the impact on treatment response appears to be due mainly to a higher rate of early treatment discontinuation (up to 40%) [48,55]. In those who completed the full course of treatment, the SVR rate appears to be similar regardless of alcohol intake [48]. Several studies have suggested that 6 to 12 months of abstinence may be necessary to overcome the reduced response to interferon-based antiviral therapy [48,50,56].

While these data suggest that alcohol impairs the response to interferon-based treatment, they are limited because the treatment regimens explored in these studies are not in line with the current standard of care, i.e. pegylated interferon and ribavirin (RBV). Although it is not known whether the greater efficacy of pegylated interferon might improve the response rate, given the role of early discontinuation in the overall SVR rate, this seems unlikely.

POSSIBLE INTERACTION MECHANISMS

Several potential mechanisms for the combined deleterious effects of the hepatitis C virus and alcohol on the liver have been postulated, but it is likely that no single mechanism can explain the observed effects. Acute alcohol intake is well known to cause generalized immune suppression [57,58]. In case of chronic hepatitis C infection, alcohol compromises both the innate and adaptive immune response, including humoral and cellular immunity. Specifically, both alcohol and HCV depress dendritic cell function and this effect is additive [58]. This impairs viral antigen processing and subsequent activation of more diverse immune responses. Furthermore, aberrant cytokine expression is observed, including elevation of interleukin-10, which has been shown to result in increased HCV replication and inflammation [58,59]. CD4 and CD8 cytotoxic T cells are critical to

control HCV infection and immune-mediated clearance. Proliferation of these cells is depressed by alcohol and only CD4 T cells appear to be partially restored with short-term abstinence [60]. Alcohol may directly impair the ability of the host to mount an innate response by blocking the ability to induce endogenous interferons [61,62]. Although some questions remain about how this occurs, it may be due to either the inhibition of tyrosine kinase activation of the Stat signaling pathway, or to nuclear factor κB-triggered interferon-stimulated genes [62,63]. The same mechanisms might explain the increase in HCV replication and reduced response to interferon due to alcohol use.

Alcohol use in patients with HCV infection dramatically increases oxidative stress and lipid peroxidation in a dose-dependent manner [64]. This results in increased expression of the cytokines transforming growth factor β (TGF-β) and tumor necrosis factor α (TNF-α) [65]. These mechanisms sensitize hepatocytes to the effects of pro-inflammatory cytokines, increase hepatic inflammation, induce apoptosis, and trigger profibrotic processes [66].

CLINICAL IMPLICATIONS AND RECOMMENDATIONS: HOW MUCH IS TOO MUCH?

As highlighted at the National Institute of Health Consensus Conference by Peters and Terrault, it is important to distinguish alcohol abuse on its own merits and treat it appropriately regardless of its potential effect on chronic hepatitis C infection [50]. The larger question is whether there is a safe amount of alcohol intake below the level of abuse. This is quite difficult to determine from the published literature, and this derives from the variability in studies, setting of arbitrary, excessive or statistically-driven consumption limits, and inevitable reporting inaccuracies. It is certain that daily alcohol consumption of more than 40 to 60 g increases hepatic inflammation [13,20,21,27], fibrosis [26,34,67], cirrhosis [24,26,30,31,34,36,67], hepatic decompensation [24], HCC [38], and perhaps HCV RNA [62,68,69]. It also increases the likelihood that interferon-based therapy will fail [48,51,52,55]. Although the studies are limited, lower levels of alcohol intake are also detrimental. However, the magnitude of the effect is obviously less than that seen with extremely heavy alcohol intake [31,67]. Monto *et al.* found that the ability of alcohol to promote the progression of fibrosis was continuous, rather than dichotomous, and gradually increased in proportion to consumption [67]. Even consumption of <20 g/day was associated

with a small increase in fibrosis compared to non-drinkers. Similarly, Corrao and Arico found that the intake of 25 to 50 g/day alcohol (0 to 25 g/day was not analyzed) was associated with an increased rate of cirrhosis [31]. Anand *et al.* found that any alcohol consumption within the previous 12 months reduced response to interferon and RBV [48].

Is there a safe level of alcohol intake in patients with chronic hepatitis C? Probably not. One must assume that there is some risk with any amount of intake. Given the evidence, it would therefore seem prudent to avoid alcohol altogether. Thus, setting a threshold limit does not seem particularly wise and invites patients to "push the envelope". Despite personal resistance and cultural barriers, alcohol use should be prohibited in patients with HCV infection.

REFERENCES

1. National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services. Behavioral Risk Factors Surveillance System (BRFSS). CD-ROM 1984-1995 (Series1, No.1, 2nd Edition), 1996-1997 (Series 1, No.2a, Series 1,No.3), 1998 (Series 1, No.4), 1999 (Series 1, No.5); 2000-2004 data from the CDC web site (http://www.cdc.gov/brfss/).
2. Grant BF, Dawson DA, Stinson FS, Chou SP, Dufour MC, Pickering RP. The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence: United States, 1991-1992 and 2001-2002. Drug Alcohol Depend 2004;74:223-234.
3. Bhattacharya R, Shuhart MC. Hepatitis C and alcohol: interactions, outcomes, and implications. J Clin Gastroenterol 2003;36:242-252.
4. Jamal MM, Saadi Z, Morgan TR. Alcohol and hepatitis C. Dig Dis 2005;23:285-296.
5. Campbell JV, Hagan H, Latka MH, Garfein RS, Golub ET, Coady MH, Thomas DL, Strathdee SA; The STRIVE Project. High prevalence of alcohol use among hepatitis C virus antibody positive injection drug users in three US cities. Drug Alcohol Depend 2006;81:259-265.
6. Backmund M, Schutz CG, Meyer K, Eichenlaub D, Soyka M. Alcohol consumption in heroin users, methadone-substituted and codeine-substituted patients-frequency and correlates of use. Eur Addict Res 2003;9:45-50.
7. Alter MJ. The epidemiology of acute and chronic hepatitis C. Clin Liver Dis 1997;1:559-568.
8. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558-567.
9. Garfein RS, Vlahov D, Galai N, Doherty MC, Nelson KE. Viral infections in short-term injection drug users: the prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human T-lymphotropic viruses. Am J Pub Health 1996;86:655-661.
10. Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006;144:705-714.
11. Harris HE, Ramsay ME, Andrews NJ. Survival of a national cohort of hepatitis C virus infected patients, 16 years after exposure. Epidemiol Infect 2006;134:472-427.
12. Seeff LB, Buskell-Bales Z, Wright EC, Durako SJ, Alter HJ, Iber FL. Long-term mortality after transfusion-associated non-A, non-B hepatitis. The National Heart, Lung, and Blood Institute Study Group. N Engl J Med 1992;327:1906-1911.

13. Kobayashi M, Ikeda K, Hosaka T, Sezaki H, Someya T, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Miyakawa Y, Kumada H. Natural history of compensated cirrhosis in the Child-Pugh class A compared between 490 patients with hepatitis C and 167 with B virus infections. J Med Virol 2006;78:459-465.

14. Tsui JI, Pletcher MJ, Vittinghoff E, Seal K, Gonzales R. Hepatitis C and hospital outcomes in patients admitted with alcohol-related problems. J Hepatol 2006;44:262-266.

15. Hisada M, Chatterjee N, Kalaylioglu Z, Battjes RJ, Goedert JJ. Hepatitis C virus load and survival among injection drug users in the United States. Hepatology 2005;42:1446-1452.

16. Said A, Williams J, Holden J, Remington P, Musat A, Lucey MR. The prevalence of alcohol-induced liver disease and hepatitis C and their interaction in a tertiary care setting. Clin Gastroenterol Hepatol 2004;2:928-934.

17. Pessione F, Ramond MJ, Peters L, Pham BN, Batel P, Rueff B, Valla DC. Five-year survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. Liver Int 2003;23:45-53.

18. Serra MA, Escudero A, Rodriguez F, del Olmo JA, Rodrigo JM. Effect of hepatitis C virus infection and abstinence from alcohol on survival in patients with alcoholic cirrhosis. J Clin Gastroenterol 2003;36:170-174.

19. Henry JA, Moloney C, Rivas C, Goldin RD. Increase in alcohol related deaths: is hepatitis C a factor? J Clin Pathol 2002;55:704-707.

20. Hezode C, Lonjon I, Roudot-Thoraval F, Pawlotsky JM, Zafrani ES, Dhumeaux D. Impact of moderate alcohol consumption on histological activity and fibrosis in patients with chronic hepatitis C, and specific influence of steatosis: a prospective study. Aliment Pharmacol Ther 2003;17:1031-1037.

21. Gomes de Sa Ribeiro Mde F, da Costa Gayotto LC, de Alencar Fischer Chamone D, Strauss E. Alcoholic intake predisposes to more interface hepatitis in chronic hepatitis C. Ann Hepatol 2005;4:176-183.

22. Rigamonti C, Mottaran E, Reale E, Rolla R, Cipriani V, Capelli F, Boldorini R, Vidali M, Sartori M, Albano E. Moderate alcohol consumption increases oxidative stress in patients with chronic hepatitis C. Hepatology 2003;38:42-49.

23. Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis and virus infection fibrosis progression. Gastroenterology 2003;125:1695-1704.

24. Wiley TE, McCarthy M, Breidi L, McCarthy M, Layden TJ. Impact of alcohol on histological and clinical progression on hepatitis C infection. Hepatology 1998;28:805-809.

25. Harris DR, Gonin R, Alter HJ, Wright EC, Buskell ZJ, Hollinger FB, Seeff LB. The relationship of acute transfusion-associated hepatitis to the development of cirrhosis in the presence of alcohol abuse. Ann Intern Med 2001;134:120-124.

26. Boccato S, Pistis R, Noventa F, Guido M, Benvegnu L, Alberti A. Fibrosis progression in initially mild chronic hepatitis C. J Viral Hepat 2006;13:297-302.

27. Delarocque-Astagneau E, Roudot-Thoraval F, Campese C, Desenclos JC, The Hepatitis C Surveillance System Steering Committee. Past excessive alcohol consumption: a major determinant of severe liver disease among newly referred hepatitis C virus infected patients in hepatology reference centers, France, 2001. Ann Epidemiol 2005;15:551-557.

28. Saigal S, Kapoor D, Tandon N, Thakur V, Guptan RC, Agarwal SR, Sarin SK. High seroprevalence and clinical significance of hepatitis B and C infection in hospitalized patients with alcoholic cirrhosis. J Assoc Physicians India 2002;50:1002-1006.

29. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, Nawrocki M, Kruska L, Hensel F, Petry W, Haussiznger D. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687-1695.

30. Roudot-Thoraval F, Bastie A, Pawlotsky JM, Dhumeaux D. Epidemiological factors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6,664 patients. Hepatology 1997;26:485-490.

31. Corrao G, Arico S. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. Hepatology 1998;27:914-919.

32. Stroffllini T, Sagnelli E, Mariano A, Craxi A, Almasio P. Characteristics of HCV positive subjects referring to hospitals in Italy: a multicenter prevalence study of 6,999 cases. J Viral Hepat 2006;13:351-354.

33. 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.

34. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol 2001;34:730-739.

35. Salmon-Ceron D, Lewden C, Morlat P, Bevilacqua S, Jougla E, Bonnet F, Heripret L, Costagliola D, May T, Chene G; The Mortality 2000 study group. Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. J Hepatol 2005;42:799-805.

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

37. Ruhl CE, Everhart JE. Joint effects of body weight and alcohol on elevated serum alanine aminotransferase in the United States population. Clin Gastroenterol Hepatol 2005;3:1260-1268.

38. Donato F, Gelatti U, Chiesa R, Albertini A, Bucella E, Boffetta P, Tagger A, Ribero ML, Portera G, Fasola M, Nardi G. A case-control study on family history of liver cancer as a risk factor for hepatocellular carcinoma in North Italy. Brescia HCC Study. Cancer Causes Control 1999;10:417-421.

39. Santagostino E, Colombo M, Rivi M, Rumi MG, Rocino A, Linari S, Mannucci PM; Study Group of the Association of Italian Hemophilia Centers. A 6-month versus a 12-month surveillance for hepatocellular carcinoma in 559 hemophiliacs infected with the hepatitis C virus. Blood 2003;102:78-82.

40. Simonetti RG, Camma C, Fiorello F, Cottone M, Rapicetta M, Marino L, Fiorentino G, Craxi A, Ciccaglione A, Giuseppetti R. Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case-control study. Ann Intern Med 1992;116:97-102.

41. 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 carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930-938.

42. Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002;36:1206-1213.

43. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long-term follow-up study after histologic diagnosis. Cancer 2000;89:53-59.

44. Iwasaki Y, Takaguchi K, Ikeda H, Makino Y, Araki Y, Ando M, Kobashi H, Kobatake T, Tanaka R, Tomita M, Senoh T, Kawaguchi M, Shimoe T, Manabe K, Kita K, Shimamura J, Sakaguchi K, Shiratori Y. Risk factors for hepatocellular carcinoma in hepatitis C patients with sustained virologic response to interferon therapy. Liver Int 2004;24:603-610.

45. Fukushima W, Tanaka T, Ohfuji S, Habu D, Tamori A, Kawada N, Sakaguchi H, Takeda T, Nishiguchi S, Seki S, Shiomi S, Hirota Y. Does alcohol increase the risk of hepatocellular carcinoma among patients with hepatitis C virus infection? Hepatol Res 2006;34:141-149.

46. Ohishi W, Kitamoto M, Aikata H, Kamada K, Kawakami Y, Ishihara H. Impact of aging on the development of hepatocellular carcinoma in patients with hepatitis C virus infection in Japan. Scand J Gastroenterol 2003;38:894-900.

47. Noda K, Yoshihara H, Suzuki K, Yamada Y, Kasahara A, Hayashi N, Fusamoto H, Kamada T. Progression of type C chronic hepatitis to liver cirrhosis and hepatocellular carcinoma; its relationship to alcohol drinking and the age of transfusion. Alcohol Clin Exp Res 1996;20:S95-S100.

48. Anand BS, Currie S, Dieperink E, Bini EJ, Shen H, Ho SB, Wright T. Alcohol use and treatment of hepatitis C virus: results of a national multicenter study. Gastroenterology 2006;130:1607-1616.

49. Nguyen HA, Miller AI, Dieperink E, Willenbring ML, Tetrick LL, Durfee JM, Ewing SL, Ho SB. Spectrum of disease in U.S. veteran patients with hepatitis C. Am J Gastroenterol 2002;97:1813-1820.

50. Peters MG, Terrault NA. Alcohol use and hepatitis C. Hepatology 2002;36:S220-S225.

51. Chang A, Skole K, Gautam M, Schmutz J, Black M, Thomas R, Horwitz B, Friedenberg FK. The impact of past alcohol use on treatment response rates in patients with chronic hepatitis C. Aliment Pharmacol Ther 2005;22:701-706.

52. Tabone M, Sidoli L, Laudi C, Pellegrino S, Rocca G, Della Monica P, Fracchia M, Galatola G, Molinaro GC, Arico S, Pera A. Alcohol abstinence does not offset the strong negative effect of lifetime alcohol consumption on the outcome of interferon therapy. J Viral Hepat 2002;9:288-294.

53. Okazaki T, Yoshihara H, Suzuki K, Yamada Y, Tsujimura T, Kawano K, Yamada Y, Abe H. Efficacy of interferon therapy in patients with chronic hepatitis C. Comparison between non-drinkers and drinkers. Scand J Gastroenterol 1994;29:1039-1043.

54. 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.

55. Cheung RC, Currie S, Shen H, Ho SB, Bini EJ, Anand BS, Brau N, Wright TL; VA HCV-001 Study Group. Chronic hepatitis C in Latinos: natural history, treatment eligibility, acceptance, and outcomes. Am J Gastroenterol 2005;100:2186-2193.

56. Safdar K, Schiff ER. Alcohol and hepatitis C. Semin Liver Dis 2004;24:305-315.

57. Brown LA, Cook RT, Jerrells TR, Kolls JK, Nagy LE, Szabo G, Wands JR, Kovacs EJ. Acute and chronic alcohol abuse modulate immunity. Alcohol Clin Exp Res 2006;30:1624-1631.

58. Szabo G, Aloman C, Polyak SJ, Weinman SA, Wands J, Zakhari S. Hepatitis C infection and alcohol use: A dangerous mix for the liver and antiviral immunity. Alcohol Clin Exp Res 2006;30:709-719.

59. Nelson DR, Tu Z, Soldevila-Pico C, Abdelmalek M, Zhu H, Xu YL, Cabrera R, Liu C, Davis GL. Long-term interleukin 10 therapy in chronic hepatitis C patients with extensive fibrosis has a proviral and anti-inflammatory effect that is mediated by viral-specific T cell suppression. Hepatology 2003;38:859-868.

60. Encke J, Wands JR. Ethanol inhibition: the humoral and cellular immune response to hepatitis C virus NS5 protein after genetic immunization. Alcohol Clin Exp Res 2000;24:1063-1069.

61. Jerrells TR, Mitchell K, Pavlik J, Jerrells J, Hoerman D. Influence of ethanol consumption on experimental viral hepatitis. Alcohol Clin Exp Res 2002;26:1734-1746.

62. Zhang T, Li Y, Lai JP, Douglas SD, Metzger DS, O'Brien CP, Ho WZ. Alcohol potentiates hepatitis C virus replicon expression. Hepatology 2003;38:57-65.

63. Plumlee CR, Lazaro CA, Fausto N, Polyak SJ. Effect of ethanol on innate antiviral pathways and HCV replication in human liver cells. Virol J 2005;2:89.

64. Rigamonti C, Mottaran E, Reale E, Rolla R, Cipriani V, Capelli F, Boldorini R, Vidali M, Sartori M, Albano E. Moderate alcohol consumption increases oxidative stress in patients with chronic hepatitis C. Hepatology 2003;38:42-49.

65. Perlemuter G, Letteron P, Carnot F, Zavala F, Pessayre D, Nalpas B, Brechot C. Alcohol and hepatitis C virus core protein additively increase lipid peroxidation and synergistically trigger hepatic cytokine expression in a transgenic mouse model. J Hepatol 2003;39:1020-1027.

66. Gao B. Interaction of alcohol and hepatitis viral proteins: implication in synergistic effect of alcohol drinking and viral hepatitis on liver injury. Alcohol 2002;27:69-72.

67. Monto A, Patel K, Bostrom A, Pianko S, Pockros P, McHutchison JG, Wright TL. Risks of a range of alcohol intake on hepatitis C-related fibrosis. Hepatology 2004;39:826-834.

68. Anand BS, Thornby J. Alcohol has no effect on hepatitis C virus replication: a meta-analysis. Gut 2005;54:1468-1472.

69. Degos F. Hepatitis C and alcohol. J Hepatol 1999;31:S113-S118.

Iron and HCV: The Middle Age?

Y. Deugnier, D. Guyader

INTRODUCTION

Since Blumberg *et al.* [1] first described an association between increased serum iron levels and viral hepatitis, there has been considerable interest in the role of iron in the pathogenesis of chronic hepatitis C virus (HCV); not only with regards to its effect on the progression of HCV-associated fibrosis but also in the use of iron removal as an adjunctive treatment to antiviral therapy [2,3]. The present review summarizes: 1) our current knowledge of iron status; 2) the mechanisms of iron metabolism abnormalities; 3) the influence of iron on the course of liver damage; and, 4) the effect of iron reduction in HCV-infected patients.

IRON STATUS IN HCV-INFECTED PATIENTS

Serum iron-related indices

Several studies have shown that serum iron and ferritin levels are increased in 25 to 59% of patients, and transferrin saturation is elevated in 10 to 25% of patients with chronic HCV infection [4-8].

Hepatic iron

Hepatic iron concentrations are increased in 10 to 36% of patients with chronic HCV infection. It is noteworthy that this is a smaller percentage than suggested by serum iron-related indices. However, when present, excess hepatic iron is usually small in quantity

(<100 µmol/g dry liver, n<36) [4,6,9,10]. Except in cirrhosis, iron deposits occur predominately within the portal and sinusoidal macrophages [11,12].

Hemochromatosis gene mutations

A number of studies have shown no difference in the prevalence of C282Y and H63D mutations in patients with chronic hepatitis C and controls [2,3]. However, patients with chronic HCV infection were found to present hemochromatosis gene (HFE) mutations significantly more often than controls, when subjects bearing one of the 2 hemochromatosis-associated HFE genotypes (i.e. C282Y homozygosity and C282Y/H63D compound heterozygosity) were not excluded. This suggests that hemochromatosis and HCV play a synergistic role in damaging the liver [13].

MECHANISMS OF IRON METABOLISM ABNORMALITIES IN HCV PATIENTS

To date, no large studies have compared the serum and liver iron-related indices and HFE genotypes in cases of chronic liver disease with different causes. Thus, it is difficult to know whether abnormalities of iron metabolism are, as suggested by some authors, really more frequent and more pronounced in hepatitis C patients than in subjects with chronic liver diseases unrelated to the hepatitis C virus [5,14]. Two mechanisms are considered with regard to increased serum iron-related indices: 1) cell necrosis – there is a good correlation between serum transaminases and ferritin levels in patients with chronic hepatitis C [4]; and, 2) the amount of liver iron – serum ferritin:transaminases ratio is well correlated with liver iron concentrations in patients with chronic hepatitis C [4]. The role of HCV-related inflammation is probably only of secondary importance as suggested by the lack of decrease in either serum iron or transferrin saturation in patients with increased serum ferritin levels. Other uncontrolled or poorly controlled factors that may have been involved in the increase in serum iron-related indices found in many series include alcohol consumption, metabolic syndrome, thyroiditis, and porphyria cutanea tarda, which are frequently associated with HCV infection and are known to impair iron metabolism.

The mechanisms involved in liver siderosis depend on whether or not cirrhosis is present. In the noncirrhotic liver, the main mechanism leading to excess hepatic iron is probably necro-inflammatory activity

because: 1) liver siderosis is usually slight or mild (<100 μmol/g, n<35); 2) iron deposits occur predominately within portal and lobular macrophages; and, 3) there is a good correlation between disease activity as assessed by the METAVIR score and iron within portal and sinusoidal cells (mesenchymal iron) [11]. Other factors may be involved in the development of excess hepatic iron in the cirrhotic liver, especially in end-stage disease. These may be related to: 1) cirrhosis itself, such as hepatocellular insufficiency, and portocaval shunts; or, 2) an associated cause of chronic liver damage, such as alcohol consumption, metabolic syndrome, and hemochromatosis [15]. Of these, hepatocellular insufficiency is probably the most important factor. Through the decreased synthesis of both transferrin and hepcidin [16] it results in hypersaturation of transferrin and in increased delivery of non-transferrin–bound iron to parenchymal cells.

The role of HFE mutations is still under debate [2]. Most studies have found evidence of an association between non-wild HFE genotypes and increased serum iron-related indices [17-20], even for the H63D heterozygous state [21]. In contrast, no clear conclusion can be drawn from studies assessing hepatic iron in relation to the HFE genotype in HCV-infected patients. Whilst some studies found a positive correlation between the presence of HFE mutations (C282Y and/or H63D) and liver iron deposits [17,18,21,22], others did not [10,19,20,23-25]. These contradictory results could be related to several biases in the studies including: 1) inclusion bias (most positive studies were performed in referral centers for hemochromatosis); 2) low statistical power due to small sample sizes; 3) mixing of cirrhotic and noncirrhotic patients; and, 4) insufficient attention paid to other common causes of hepatic siderosis, such as excess alcohol consumption and metabolic syndrome [26].

INFLUENCE OF IRON ON THE COURSE OF CHRONIC HEPATITIS C

Fibrosis

Experimental iron overload has been shown to enhance HCV pathogenicity in chimpanzees infected with HCV [27]. In humans the frequency and degree of excess hepatic iron increases with the stage of fibrosis, culminating in end-stage cirrhosis. This suggests that iron might promote the progression of fibrosis, possibly through activating the generation of free radicals [7]. Results of studies performed to test

this hypothesis have been contradictory. Most studies concluded that there was a positive association between the amount of hepatic iron and the progression of fibrosis [17-20]. In contrast, some studies found that the presence of the C282Y and/or the H63D mutation was associated with an increased rate in the progression of fibrosis [17,18,21,28] while others did not [10,19,20,23,24].

The reasons for these discrepancies include differences in study populations and failure to control confounding variables. Indeed, most variables positively associated with the progression of fibrosis (male sex, duration of infection, age at contamination, daily consumption of alcohol, degree of hepatocellular necrosis and features of metabolic syndrome) can also influence iron metabolism. Serum ferritin levels are higher in males than in females and increase with age. Iron metabolism disturbances are common in alcoholic liver disease and nonalcoholic steatohepatitis, conditions frequently associated with HCV infection. In a recent, large study of 586 patients with chronic HCV infection, the link between hepatic iron and the stage of fibrosis was no longer found after adjusting for all these confounding factors, especially alcohol intake and metabolic features [12]. Furthermore, no relationship was found between the presence of HFE mutations and the stage of fibrosis. This suggests that the roles of iron and HFE mutations in the development of fibrosis in patients with chronic hepatitis C are limited at best.

Such discrepancies suggest that HFE mutations play a more subtle role in the development of fibrosis in patients with chronic hepatitis C. As recently reviewed by Pietrangelo [2], HFE mutations may act by: 1) producing excess iron; 2) favoring the pathogenicity of HCV by impairing iron metabolism in Kupffer cells; and/or, 3) causing immunological abnormalities leading to an immuno-evasion strategy of HCV. The recent and unexpected *in vitro* demonstration of HCV replication impaired by iron [29], and the surprising homology between HCV and iron protein biology that suggests a synergy and/or competition between HFE and HCV [2], further imply that iron may play a complex role in the biology of HCV.

Liver cancer

Experimental and clinical studies support a (co)carcinogenic role of iron overload in cirrhosis (even mild iron overload) [30]. Whether iron overload contributes specifically to the development of hepatocellular carcinoma (HCC) in HCV infected patients has not been proven. However, Kato *et al*. reported that the decrease in hepatic

8-hydroxy-2–deoxyguanosine content secondary to phlebotomy therapy was associated with a lowered risk of HCC in patients with chronic HCV infection [31]. Loguercio *et al.* showed that there was a precocious expression of the p53 oncogene in patients with chronic liver damage that was related to HCV infection [32]. In addition, male C57BL/6 transgenic mice expressing the HCV polyprotein and fed a diet with excess iron were shown to develop oxidative stress, mitochondrial damage, reduced fatty acid oxidation, and marked steatosis in association with a high rate of hepatocellular proliferation and liver tumors [33]. If confirmed in other models, this would strongly suggest that iron plays a critical role in the development of HCV-related hepatocarcinogenesis.

IRON STORES AND TREATMENT OF CHRONIC HEPATITIS C

Iron tests as predictive factors of response to antiviral therapy

In early studies, high serum ferritin levels and hepatic iron concentrations were found to be associated with a poor response to standard interferons [34,35]. The predictive value of serum ferritin levels for a poor response to treatment was confirmed by most subsequent studies. However, the association of elevated hepatic iron concentrations was less often observed, especially in the most recent studies using pegylated interferon and ribavirin (RBV) [36-39].

Effect of iron reduction on response to antiviral therapy

Several prospective, randomized controlled trials have investigated whether the removal of iron improves the response rate to antiviral therapy [8,40-42]. All studies demonstrated that venesection had a beneficial effect on serum aminotransferase levels and hepatic inflammation. Some found an improved rate of virological response at the end of treatment [8], but all failed to demonstrate any improvement in sustained response to interferon, in treatment-naïve patients or in non-responders.

Consequence of antiviral therapy on hepatic iron stores

Boucher *et al*. demonstrated that hepatic iron concentration and, more precisely the histological mesenchymal iron score, decreased

significantly in patients treated with interferon whatever the final response to antiviral therapy [11]. Administration of RBV alone [43] or in association with interferon [44] is responsible for a mild increase in hepatic iron concentrations probably due to the hemolytic effect of this drug.

HYPERFERRITINEMIA IN PATIENTS WITH CHRONIC HEPATITIS C

Increased serum iron-related indices, including hyperferritinemia, do not clearly indicate that body iron stores are elevated. Failure to remember this basic rule has resulted in confusion about the relationship between HCV and iron. Therefore, direct assessment of liver iron content is necessary to confirm the presence of excess iron. This can be obtained by either: 1) magnetic resonance imaging that provides a reliable assessment of hepatic iron concentrations within a range of 50 to 350 µmol/g, n<36 [45]; 2) liver biopsy by semi-quantitative histological assessment; and/or, 3) biochemical measurement on either paraffin-embedded or fresh tissue [46].

Clinical, biological and histological data must be recorded to characterize abnormalities in iron metabolism. These include: 1) biometric data including blood pressure, body mass index and waist circumference; 2) symptoms of excess alcohol consumption, porphyria cutanea tarda, genetic hemochromatosis (arthropathy, hypogonadism, diabetes), and metabolic syndrome; 3) serum glucose, C reactive protein, cholesterol and triglycerides; 4) HFE testing if transferrin saturation is increased by >50%; and, 5) histological semi-quantitative assessment of iron load, and cellular and lobular distribution of iron deposits when liver biopsy is available (if liver biopsy is not available, magnetic resonance imaging must be considered in order to precisely assess hepatic iron stores).

There is currently no indicator of iron depletion in the absence of documented hepatic siderosis. Iron depletion should be considered before beginning antiviral treatment if significant hepatic iron overload is present and related to a well defined associated disorder such as genetic hemochromatosis or *porphyria cutanea tarda*. In all other cases of mild iron overload, venesection therapy can be discussed before beginning antiviral therapy since normalization of body iron stores has been shown to be associated with improvement in biological and histological features of hepatitis activity. The goal of this treatment is to obtain low serum ferritin levels (i.e. <100 ng/mL) in weekly or bimonthly phlebotomies. However, caution should be taken so as to avoid inducing anemia which would lower the tolerance

to antiviral therapy. Consequently, the volume of phlebotomies should not exceed 350 mL in females and 450 mL in males. Venesection therapy should be discontinued when antiviral therapy has been started.

CONCLUSION

In recent years there has been considerable clinical interest in the relationship between HCV and iron. Results of studies are difficult to interpret due to intrinsic biases related to inclusion criteria, small sample sizes, misinterpretation of serum iron-related indices, and underestimation of confounding variables associated with increased body iron stores. Some important questions remain unanswered: 1) is iron status specifically modified in chronic hepatitis C compared to chronic liver diseases resulting from other causes; 2) what is the exact clinical relevance of iron abnormalities and related protein/gene metabolism in hepatitis C patients, especially in relation to the development of fibrosis and cancer; and, 3) what is the place of iron removal in the management of patients with chronic hepatitis C? Only specifically designed clinical studies and basic research into the subtle interactions between HCV, iron-related proteins/genes and immunological processes can answer these questions.

REFERENCES

1. Blumberg BS, Lustbader ED, Whitford PL. Changes in serum iron levels due to infection with hepatitis B virus. Proc Natl Acad Sci USA 1981;78:3222-3224.
2. Pietrangelo A. Hemochromatosis gene modifies course of hepatitis C viral infection. Gastroenterology 2003;124:1509-1523.
3. Alla V, Bonkovsky HL. Iron in nonhemochromatotic liver disorders. Semin Liver Dis 2005;25:461-472.
4. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology 1992;102:2108-2113.
5. Arber N, Konikoff FM, Moshkowitz M, Baratz M, Hallak A, Santo M, Halpern Z, Weiss H, Gilat T. Increased serum iron and iron saturation without liver iron accumulation distinguish chronic hepatitis C from other chronic liver diseases. Dig Dis Sci 1994;39:2656-2659.
6. Piperno A, D'Alba R, Fargion S, Roffi L, Sampietro M, Parma S, Arosio V, Fare M, Fiorelli G. Liver iron concentration in chronic viral hepatitis: a study of 98 patients. Eur J Gastroenterol Hepatol 1995;7:1203-1208.
7. Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 1995;22:449-456.
8. Fontana RJ, Israel J, LeClair P, Banner BF, Tortorelli K, Grace N, Levine RA, Fiarman G, Thiim M, Tavill AS, Bonkovsky HL. Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. Hepatology 2000;31:730-736.
9. Riggio O, Montagnese F, Fiore P, Folino S, Giambartolomei S, Gandin C, Merli M, Quinti I, Violante N, Caroli S, Senofonte O, Capocaccia L. Iron overload in patients with chronic viral hepatitis: how common is it? Am J Gastroenterol 1997;92:1298-1301.
10. Hezode C, Cazeneuve C, Coue O, Roudot-Thoraval F, Lonjon I, Bastie A, Duvoux C, Pawlotsky JM, Zafrani ES, Amselem S, Dhumeaux D. Liver iron accumulation in patients with chronic active hepatitis C: prevalence and role of hemochromatosis gene mutations and relationship with hepatic histological lesions. J Hepatol 1999;31:979-984.
11. Boucher E, Bourienne A, Adams P, Turlin B, Brissot P, Deugnier Y. Liver iron concentration and distribution in chronic hepatitis C before and after interferon treatment. Gut 1997;41:115-120.
12. Guyader D, Thirouard A, Erdtmann L, Rakba N, Jacquelinet S, Daniélou H, et al. Liver iron is a surrogate marker of severe fibrosis in chronic hepatitis C. J Hepatol 2006 (in press).

13. Diwakaran HH, Befeler AS, Britton RS, Brunt EM, Bacon BR. Accelerated hepatic fibrosis in patients with combined hereditary hemochromatosis and chronic hepatitis C infection. J Hepatol 2002;36:687-691.

14. Uraz S, Aygun C, Sonsuz A, Ozbay G. Serum iron levels and hepatic iron overload in nonalcoholic steatohepatitis and chronic viral hepatitis. Dig Dis Sci 2005;50:964-969.

15. Deugnier Y, Turlin B, le Quilleuc D, Moirand R, Loreal O, Messner M, Meunier B, Brissot P, Launois B. A reappraisal of hepatic siderosis in patients with end-stage cirrhosis: practical implications for the diagnosis of hemochromatosis. Am J Surg Pathol 1997;21:669-675.

16. Detivaud L, Nemeth E, Boudjema K, Turlin B, Troadec MB, Leroyer P, Ropert M, Jacquelinet S, Courselaud B, Ganz T, Brissot P, Loreal O. Hepcidin levels in humans are correlated with hepatic iron stores, hemoglobin levels, and hepatic function. Blood 2005;106:746-748.

17. Piperno A, Vergani A, Malosio I, Parma L, Fossati L, Ricci A, Bovo G, Boari G, Mancia G. Hepatic iron overload in patients with chronic viral hepatitis: role of HFE gene mutations. Hepatology 1998;28:1105-1109.

18. Smith BC, Gorve J, Guzail MA, Day CP, Daly AK, Burt AD, Bassendine MF. Heterozygosity for hereditary hemochromatosis is associated with more fibrosis in chronic hepatitis C. Hepatology 1998;27:1695-1699.

19. Kazemi-Shirazi L, Datz C, Maier-Dobersberger T, Kaserer K, Hackl F, Polli C, Steindl PE, Penner E, Ferenci P. The relation of iron status and hemochromatosis gene mutations in patients with chronic hepatitis C. Gastroenterology 1999;116:127-34.

20. Martinelli AL, Franco RF, Villanova MG, Figueiredo JF, Secaf M, Tavella MH, Ramalho LN, Zucoloto S, Zago MA. Are haemochromatosis mutations related to the severity of liver disease in hepatitis C virus infection? Acta Haematol 2000;102:152-156.

21. Tung BY, Emond MJ, Bronner MP, Raaka SD, Cotler SJ, Kowdley KV. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 2003;124:318-326.

22. Bonkovsky HL, Troy N, McNeal K, Banner BF, Sharma A, Obando J, Mehta S, Koff RS, Liu Q, Hsieh CC. Iron and HFE or TfR1 mutations as comorbid factors for development and progression of chronic hepatitis C. J Hepatol 2002;37:848-854.

23. Pirisi M, Scott CA, Avellini C, Toniutto P, Fabris C, Soardo G, Beltrami CA, Bartoli E. Iron deposition and progression of disease in chronic hepatitis C. Role of interface hepatitis, portal inflammation, and HFE missense mutations. Am J Clin Pathol 2000;113:546-554.

24. Negro F, Samii K, Rubbia-Brandt L, Quadri R, Male PJ, Zarski JP, Baud M, Giostra E, Beris P, Hadengue A. Hemochromatosis gene mutations in chronic hepatitis C patients with and without liver siderosis. J Med Virol 2000;60:21-27.

25. Thorburn D, Curry G, Spooner R, Spence E, Oien K, Halls D, Fox R, McCruden EA, MacSween RN, Mills PR. The role of iron and haemo-

chromatosis gene mutations in the progression of liver disease in chronic hepatitis C. Gut 2002;50:248-252.

26. Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, Le Gall JY, Brissot P, David V, Deugnier Y. Insulin resistance-associated hepatic iron overload. Gastroenterology 1999;117:1155-1163.

27. Bassett SE, Di Bisceglie AM, Bacon BR, Sharp RM, Govindarajan S, Hubbard GB, Brasky KM, Lanford RE. Effects of iron loading on pathogenicity in hepatitis C virus-infected chimpanzees. Hepatology 1999;29:1884-1892.

28. Bonkovsky HL. Iron as a comorbid factor in chronic viral hepatitis. Am J Gastroenterol 2002;97:1-4.

29. Fillebeen C, Rivas-Estilla AM, Bisaillon M, Ponka P, Muckenthaler M, Hentze MW, Koromilas AE, Pantopoulos K. Iron inactivates the RNA polymerase NS5B and suppresses subgenomic replication of hepatitis C Virus. J Biol Chem 2005;280:9049-9057.

30. Deugnier Y. Iron and liver cancer. Alcohol 2003;30:145-150.

31. Kato A, Odamaki M, Nakamura H, Yodoi J, Hishida A. Elevation of blood thioredoxin in hemodialysis patients with hepatitis C virus infection. Kidney Int 2003;63:2262-2268.

32. Loguercio C, Cuomo A, Tuccillo C, Gazzerro P, Cioffi M, Molinari AM, Del Vecchio Blanco C. Liver p53 expression in patients with HCV-related chronic hepatitis. J Viral Hepat 2003;10:266-270.

33. Furutani T, Hino K, Okuda M, Gondo T, Nishina S, Kitase A, Korenaga M, Xiao SY, Weinman SA, Lemon SM, Sakaida I, Okita K. Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. Gastroenterology 2006;130:2087-2098.

34. Olynyk JK, Reddy KR, Di Bisceglie AM, Jeffers LJ, Parker TI, Radick JL, Schiff ER, Bacon BR. Hepatic iron concentration as a predictor of response to interferon alfa therapy in chronic hepatitis C. Gastroenterology 1995;108:1104-1109.

35. Bonkovsky HL, Banner BF, Rothman AL. Iron and chronic viral hepatitis. Hepatology 1997;25:759-768.

36. Pianko S, McHutchison JG, Gordon SC, Heaton S, Goodman ZD, Patel K, Cortese CM, Brunt EM, Bacon BR, Blatt LM. Hepatic iron concentration does not influence response to therapy with interferon plus ribavirin in chronic HCV infection. J Interferon Cytokine Res 2002;22:483-489.

37. Sievert W, Pianko S, Warner S, Bowden S, Simpson I, Bowden D, Locarnini S. Hepatic iron overload does not prevent a sustained virological response to interferon-alpha therapy: a long-term follow-up study in hepatitis C-infected patients with beta thalassemia major. Am J Gastroenterol 2002;97:982-987.

38. Hofer H, Osterreicher C, Jessner W, Penz M, Steindl-Munda P, Wrba F, Ferenci P. Hepatic iron concentration does not predict response to standard and pegylated-IFN/ribavirin therapy in patients with chronic hepatitis C. J Hepatol 2004;40:1018-1022.

39. Rulyak SJ, Eng SC, Patel K, McHutchison JG, Gordon SC, Kowdley KV. Relationships between hepatic iron content and virologic response in chronic hepatitis C patients treated with interferon and ribavirin. Am J Gastroenterol 2005;100:332-337.

40. Fong TL, Han SH, Tsai NC, Morgan TR, Mizokami M, Qian D, Phan C, Goad K, Redeker AG. A pilot randomized, controlled trial of the effect of iron depletion on long-term response to alpha-interferon in patients with chronic hepatitis C. J Hepatol 1998;28:369-374.

41. Di Bisceglie AM, Bonkovsky HL, Chopra S, Flamm S, Reddy RK, Grace N, Killenberg P, Hunt C, Tamburro C, Tavill AS, Ferguson R, Krawitt E, Banner B, Bacon BR. Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who have previously not responded to interferon: a multicenter, prospective, randomized, controlled trial. Hepatology 2000;32:135-138.

42. Fargion S, Fracanzani AL, Rossini A, Borzio M, Riggio O, Belloni G, Bissoli F, Ceriani R, Ballare M, Massari M, Trischitta C, Fiore P, Orlandi A, Morini L, Mattioli M, Oldani S, Cesana B, Fiorelli G. Iron reduction and sustained response to interferon-alpha therapy in patients with chronic hepatitis C: results of an Italian multicenter randomized study. Am J Gastroenterol 2002;97:1204-1210.

43. Di Bisceglie AM, Bacon BR, Kleiner DE, Hoofnagle JH. Increase in hepatic iron stores following prolonged therapy with ribavirin in patients with chronic hepatitis C. J Hepatol 1994;21:1109-1112.

44. Fiel MI, Schiano TD, Guido M, Thung SN, Lindsay KL, Davis GL, Lewis JH, Seeff LB, Bodenheimer HC Jr. Increased hepatic iron deposition resulting from treatment of chronic hepatitis C with ribavirin. Am J Clin Pathol 2000;113:35-39.

45. Gandon Y, Olivie D, Guyader D, Aube C, Oberti F, Sebille V, Deugnier Y. Non-invasive assessment of hepatic iron stores by MRI. Lancet 2004;363:357-362.

46. Turlin B, Deugnier Y. Iron overload disorders. Clin Liver Dis 2002;6:481-496.

Clinical Case: Management of Patients with Chronic Hepatitis C

B. Bacon

This clinical case illustrates a typical problem in the management of patients with chronic hepatitis C.

The patient is a 46-year-old Caucasian woman referred for further evaluation of elevated liver enzymes following a routine health evaluation. She had had symptoms of fatigue for several years. An evaluation for fatigue several years ago had shown normal complete blood count (CBC) and normal liver enzyme levels, with no further evaluation performed thereafter. The patient may have been exposed to hepatitis C from a blood transfusion received 25 years ago after a motor vehicle accident. She drinks 2 to 3 alcoholic beverages per day and a little more on weekends. She was diagnosed with glucose intolerance and diabetes that she had been trying to control through diet. She also has elevated total cholesterol and low-density lipoprotein cholesterol. There are some symptoms of gastroesophageal reflux disease for which the patient takes an over-the-counter proton pump inhibitor (PPI).

The patient is 5'6" (168 cm) and weighs 185 lbs (84 kg). Her body mass index (BMI) is 29.9 kg/m^2. Vital signs show a pulse of 88 beats/minute with a blood pressure of 145/88 mmHg. Respiratory rate is 16 breaths/minute. Heart and lungs are normal. An abdominal examination shows a liver edge palpable at the right costal margin. There is no splenomegaly or ascites. There are no extrahepatic stigmata of chronic liver disease.

Routine laboratory studies show a hematocrit of 41% and a platelet count of 180,000 $/mm^3$. Routine liver chemistries show levels of alanine aminotransferase (ALT) at 180 U/L, aspartate transaminase (AST) at 90 U/L, alkaline phosphatase at 85 U/L, total bilirubin at

0.9 mg/dL, and albumin at 4.2 g/dL. Further laboratory tests (ELISA) were positive for anti-hepatitis C virus (HCV) antibodies. Measurement of HCV RNA levels resulted in 2,350,000 IU/mL, and the patient was found to have HCV genotype 1a.

A liver biopsy was recommended and the patient agreed. The biopsy showed mild-to-moderately active chronic hepatitis C with periportal and bridging fibrosis (grade 2 to 3, stage 3). In addition, there was a moderate amount of macrovesicular steatosis and some perisinusoidal fibrosis consistent with a diagnosis of nonalcoholic steatohepatitis (Figure 1).

After receiving the liver biopsy results the patient consented to discuss therapeutic options.

In general, patients with HCV genotype 1 have about a 60% chance of virological cure with a 48-week course of pegylated interferon and ribavirin (RBV). Some clinicians suggest that the weight of the patient influences the virological response. Of additional concern in this patient is the presence of steatohepatitis. There are 3 concerns about steatosis and steatohepatitis in patients with chronic hepatitis C. In patients with features of the metabolic syndrome, steatosis and steatohepatitis may be present on the basis of co-existent nonalcoholic fatty liver disease (NAFLD) while patients who drink excess amounts of alcohol may have alcohol-related liver disease in conjunction with their hepatitis C. In this patient, alcohol consumption should be discontinued to eliminate this variable. In patients with chronic hepatitis C and HCV genotype 3, so called "virological steatosis" can be present. In these patients, the presence of steatosis does not seem to adversely affect the response to therapy. On the other hand, patients with metabolic syndrome and steatohepatitis do have a reduced response to therapy. In a study by Harrison *et al.* [1], there was a significant reduction in sustained virological response in patients with over 30% steatosis identified upon pretreatment liver biopsy compared to those without steatosis upon liver biopsy.

Proposals for managing these co-existent diseases include recommendations for aggressive weight loss and exercise prior to treatment; one protocol has proposed orlistat (Xenical®) as a pretreatment medication prior to antiviral therapy. The role of insulin resistance in response to therapy has not been clarified as it may not be possible to differentiate insulin resistance vs. steatosis in the liver without insulin resistance. Since insulin resistance is an important feature, the use of insulin-sensitizing agents should be considered to improve the virological response rate in patients with insulin resistance.

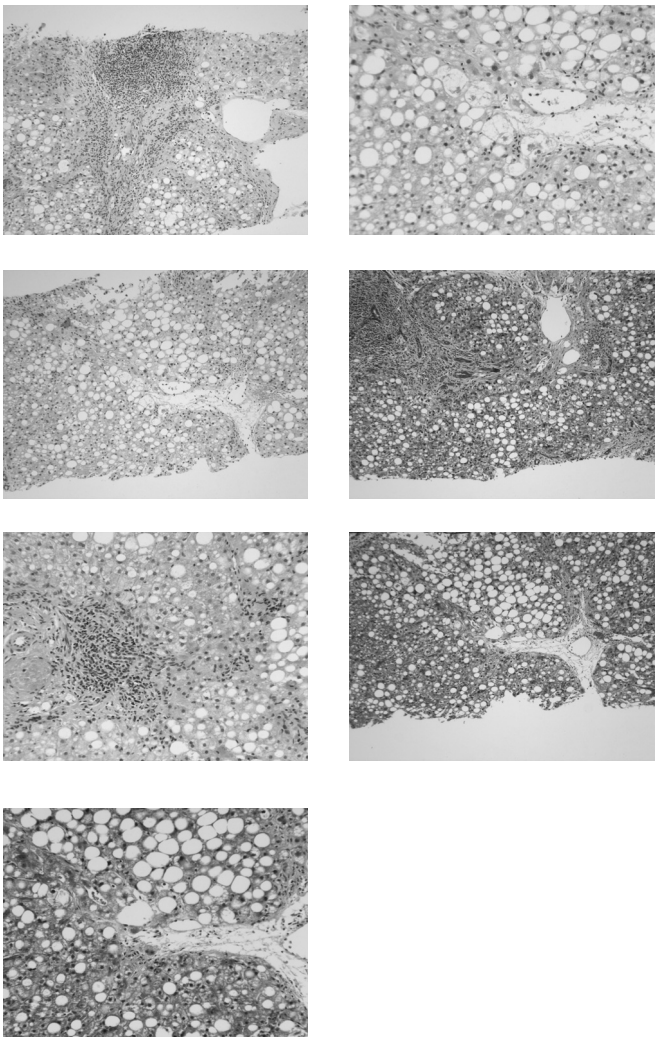


Figure 1: A liver biopsy from a patient showing mild-to-moderately active chronic hepatitis C with periportal and bridging fibrosis. There is a moderate amount of macrovesicular steatosis and some perisinusoidal fibrosis consistent with a diagnosis of nonalcoholic steatohepatitis

In the present patient, treatment was begun with pegylated interferon and RBV. At 12 weeks, the HCV RNA level was reduced to 800 IU/mL and the patient experienced an early virological response. At 24 weeks HCV RNA was negative and treatment was continued for a full 48 weeks. The patient remained HCV RNA-negative throughout treatment and did not relapse and was described as having had a virological cure. Interestingly, although she was HCV RNA-negative, liver enzymes remain elevated with ALT levels in the 70 to 120 U/L range and AST levels in the 50 to 75 U/L range throughout treatment. This suggests that liver enzymes remained elevated because of fatty liver disease despite a virological response.

The interaction between fat, steatohepatitis, insulin resistance, and chronic hepatitis C require further study and clarification as treatment for patients with chronic hepatitis C continues to improve.

REFERENCES

1. Harrison SA, Brunt EM, Qazi RA, Oliver DA, Neuschwander-Tetri BA, Di Bisceglie AM, Bacon BR. Effect of significant histologic steatosis or steatohepatitis on response to antiviral therapy in patients with chronic hepatitis C. Clinical Gastroenterology and Hepatology 2005;3:604-609.
2. 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.
3. McCullough AJ. Obesity and its nurturing effect on hepatitis C. Hepatology 2003;38:557-559.

How to Use Virological Tools for the Optimal Management of Chronic Hepatitis B

F. Zoulim

BACKGROUND

Hepatitis B virus (HBV) is a DNA virus which replicates its genome via a reverse transcriptase phase. It is usually noncytopathic and liver damage occurs from the immune attack of infected hepatocytes. Viral persistence is mainly due to a defective immune response against infected hepatocytes and viral covalently-closed circular (ccc) DNA which persists in the nucleus of infected cells [1]. Furthermore, as a result of spontaneous viral genome diversity, several genotypes have been described and viral mutants may be selected depending on selection pressure, i.e. the presence of a specific antiviral immune response or antiviral therapy.

For optimal management of chronic hepatitis B, virological tools should be used to define the stage of the disease and to monitor antiviral therapy. Two types of assays should be used: those measuring viral load and those analyzing viral genome sequence to detect genotypes and mutants.

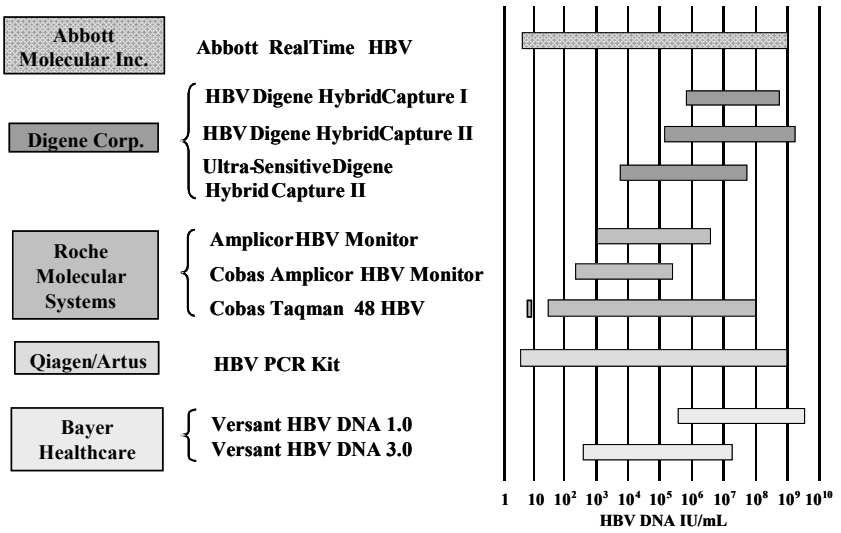
THE MAIN HEPATITIS B ASSAYS

Study of viral load

Except for occult HBV infection, viral load is correlated to the severity of liver damage in most cases, so that viral load assays are necessary for the diagnosis of chronic hepatitis B.

Quantification of serum HBV DNA

With the development of nucleoside analogs that inhibit viral replication and decrease viral load, more sensitive and quantitative assays for serum viral load monitoring became necessary for the treatment of chronic hepatitis B. The sensitivity limit of hybridization assays was approximately 1,000,000 copies/mL and decreased to 3000 copies/mL, and the development of real-time polymerase chain reaction (PCR) assays provided another major improvement, with a wide linear range of up to 10^9 copies/mL and a detection limit below 50 copies/mL (Figure 1) [2].



HBV=hepatitis B virus; PCR=polymerase chain reaction

Figure 1: Range of detection of viral load assays

Detection of intrahepatic cccDNA

Although ccc-DNA plays a crucial role during persistent infection and its clearance mechanisms need to be clarified, there are few clinical data on this subject [3,4]. Indeed, our current understanding of cccDNA has been obtained primarily through studies of woodchuck and duck HBV models [5-8]. The study of HBV cccDNA has been hindered by: 1) the need for liver biopsies, which are difficult to collect, especially from patients in quiescent natural history phases; and, 2) the lack of sensitive, specific and quantitative methods for the detection of cccDNA from biopsies.

With the development of PCR, assays were needed to detect and quantify cccDNA in the livers of patients using small quantities of liver tissue samples. Several teams have tried to design PCR methods to specifically amplify and quantify viral cccDNA by choosing primer pairs that preferentially amplify the ccc form rather than the replicative intermediates [9-12]. A novel real-time PCR assay that can quantify levels of cccDNA in biopsies collected from chronic hepatitis B patients was recently reported [13]. The specificity of this method is based on 2 major steps. Firstly, a plasmid-safe DNAse treatment is used to digest non-cccDNA, i.e. all replicative intermediates. This step was validated by experiments performed on woodchuck livers. Secondly, primers located on both sides of the gap of relaxed circular DNA are used to preferentially amplify cccDNA. The quantification of viral DNA was performed by a real-time PCR assay using labeled probes and results were normalized to the number of cells by quantifying the beta globin gene. The specificity of other PCR or non PCR-based (i.e. Invader Technology) assays has not been clearly demonstrated since some studies reported serum cccDNA levels from 10,000 copies /mL up to 1,000,000 copies/mL. This suggests that this assay may detect relaxed circular and double-stranded linear DNA instead of cccDNA in highly viremic samples [14]. It is therefore important to develop standardized assays to be used in clinical trials for the evaluation of new antiviral treatments.

Study of HBV genome variability

One of the major problems for anti-HBV therapy is HBV genome variability. Several studies have shown that HBV genotypes may affect the severity of liver disease and the outcome of interferon therapy. Genotypes A and B have been associated with a less severe evolution of chronic hepatitis and a better response to standard interferon and pegylated interferon therapy, compared to genotypes C

and D [15]. Moreover, during antiviral therapy with nucleoside analogs, the selection of HBV polymerase gene mutants responsible for drug resistance is an emerging clinical problem. Therefore, analysis of the HBV genome prior to and during therapy can help improve clinical management of chronic hepatitis B.

Sequencing of the HBV genome

Analysis of the viral genome sequence after PCR amplification of targeted viral genome regions by in-house or commercial assays (Trugene, Bayer) provides useful information about viral genotypes, precore and basal core promoter (BCP) mutations, and HBV polymerase mutations. This approach is still the gold standard when developing new assays. One major advantage of this method is that new mutations can be detected, which is especially important in patients receiving new antiviral drugs to which viral resistance mutations are unknown. For instance, a new mutation responsible for primary resistance to adefovir dipivoxil (ADV) was recently identified [16]. The drawback to these sequencing assays is that they are time consuming and that information on the genome sequences of all clinically relevant regions requires PCR amplification and sequence analysis of each region. Several assays have been or are being developed for easier analysis of the entire HBV genome.

Determination of HBV genotypes and detection of viral mutants by line probe assays

Line probe assays are based on a reverse hybridization method using probes specific for the mutations of interest or for the viral genotype to be analyzed, after PCR amplification of the target viral region. Thus, these assays detect HBV genotypes, precore and BCP mutations, as well as polymerase gene mutations [17, 18]. They have been shown to be specific and reproducible, as well as sensitive; they can detect a mutant representing as little as 5% of the viral population, which is an advantage over classical sequencing methods. For example using this assay, HBV drug-resistant mutants were detected in the serum of infected patients prior to the rise in viral load allowing early diagnosis of resistance [19]. However, this method only detects known mutations and therefore regular updates are needed with probes for new mutations when new resistant strains are identified.

Determination of HBV genotypes and detection of viral mutants with DNA chip technology

More powerful methods are being developed to analyze the polymorphism of the whole HBV genome with a high throughput system. One of them uses DNA chip technology [20]. The HBV chip assay is based on duplex amplification of the whole HBV genome and a high-density DNA chip designed to detect over 200 mutations at 151 positions and to determine the genotype of the virus in patient serum [21]. The assay has been evaluated with 170 samples, characterized by viral load and sequencing in the Pol, S, precore and BCP genes. One-hundred fifty-three samples (90%) could be amplified and analyzed by the chip. Only 2 samples with more than 1000 genome copies/mL could not be analyzed. Genotype had no impact on analytical sensitivity. Reproducibility studies showed no difference between repeats for codon and genotype determination. Genotype determination results were comparable between sequencing and the chip in 148/151 samples. Using both techniques, 12,161 codons were analyzed. Only 89.4% could be determined by sequencing and among the 11,335 remaining codons, 92.8% were identical by sequencing and the chip. The failure to identify an amino acid using the chip was mainly due to reduced hybridization efficiency linked to unexpected polymorphisms or polymorphisms with an unexpectedly high impact on hybridization. This method is still being developed and optimization of the chip-based reagent for the analysis of the HBV genome is ongoing, as well as its clinical validation for the study of HBV genotypes and antiviral drug resistance.

This method has the same drawback as the line probe assay because it can only detect known polymorphisms and needs to be updated when new, clinically relevant mutations are identified. On the other hand, its main advantage is the ability to analyze of the entire HBV genome sequence in one set of experiments.

Quantification of specific mutants

It has also been reported that real-time PCR-based assays may be useful for detecting and quantifying specific mutants, performing dynamic analysis of HBV mutants over time and studying their evolution within the viral quasi-species [22-24]. However, with the current limitations of real-time PCR assays, they can only be applied to 1 specific mutation, thus decreasing the relevance of this approach in chronic hepatitis B infection, due to viral genome variability in this disease.

Phenotypic assays

HBV resistance was initially studied by evaluating HBV replication in the presence of the antiviral drug following transfection of HBV clones harboring the suspected resistance mutations. The resistance mutations are introduced into the HBV genome either by site-directed mutagenesis or by exchange of viral genome fragments. Several alternative methods were then developed, relying either on vector-free or vector-mediated phenotypic assays. In the vector-free approach, the entire HBV genome is amplified by PCR and the PCR products transfected into hepatoma cell lines to analyze the *in vitro* drug susceptibility of HBV genomes from patients receiving antiviral therapy. The other approach requires cloning the entire HBV polymerase gene or HBV genome into plasmid vectors allowing cell transfection of single clones or mixtures of clones representing the viral quasi-species circulating in the patient, and the study of viral susceptibility to antiviral drugs. These methods are currently used in reference centers for clinical research to characterize the phenotype of new HBV mutants and to study the cross-resistance profile of available polymerase inhibitors as well as those in clinical development [25-28]. As these assays improve and become faster, they may become useful in clinical practice to monitor anti-HBV therapy and adapt drug regimens to the phenotype of the circulating viral quasi-species, as in the field of anti-HIV therapy.

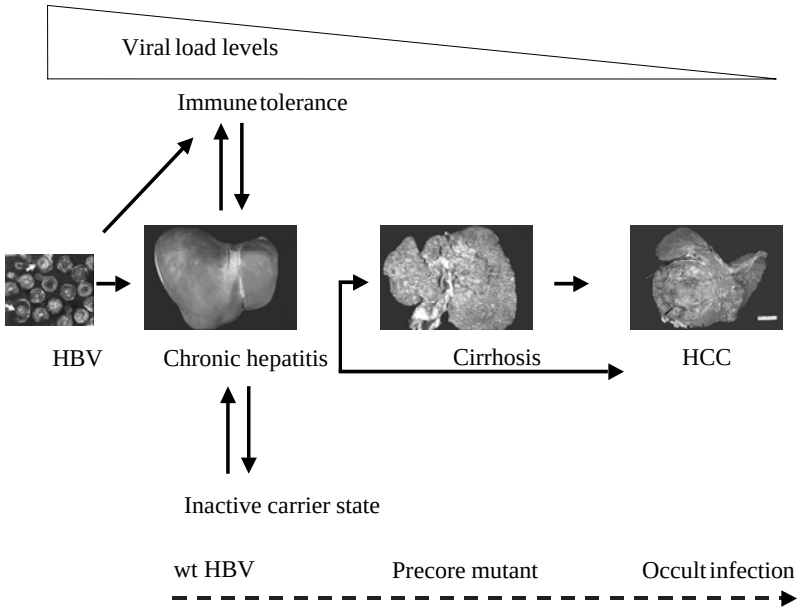
HOW VIROLOGICAL TOOLS HELP DEFINE THE CLINICAL FORM OF CHRONIC HEPATITIS B

Several forms of chronic hepatitis B have been characterized, and they may correspond to different stages of the natural history of the disease and to the balance between control of replication of the wild type virus and its mutants and the host immune response (Figure 2).

The immune tolerance phase is usually associated with a high viral load (>10^8 copies/mL) and normal transaminases. Usually liver histology shows minimal lesions, but several Asian studies have shown a correlation between viral load and the presence of liver fibrosis or the development of hepatocellular carcinoma (HCC).

The immune clearance phase associated with the wild type virus is characterized by the presence of viral replication levels between 10^5 copies/mL and 10^9 copies/mL, with elevated alanine aminotransferase (ALT) levels and signs of inflammation and necrosis on liver biopsy. Several studies have suggested that the severity of necro-inflammatory damage is associated with a viral load

$>10^5$ copies/mL. With wild-type HBV infection, hepatitis Be antigen (HBeAg) is positive, while HBeAg-negative chronic hepatitis B is associated with precore mutant or BCP infections.



HBV=hepatitis B virus; HCC=hepatocellular carcinoma; wt=wild-type

Figure 2: Natural history of chronic HBV infection with respect to viral load and viral genome sequence evolution

The inactive carrier state is characterized by HBe seroconversion, a decline in viremia to below 10,000 copies/mL, and normalization of ALT levels.

The hepatitis B surface antigen (HBsAg) clearance phase is characterized by persistent anti-HBc antibodies and minimal viral load levels which can only be detected in the liver. Occult HBV infection is characterized by HBsAg negativity but a persistent viral genome detectable only by ultrasensitive PCR in serum and/or in the liver [29]. It will be interesting to see if new real-time PCR assays improve the diagnosis for this form of chronic hepatitis B. This may have major

implications for the screening of donors in blood banks as well as for the etiologic diagnosis of cryptogenic chronic liver diseases.

IMPLICATION FOR MONITORING ANTIVIRAL THERAPY

Importance of monitoring serum viral load

With new assays the response to antiviral therapy and viral drug resistance can be defined in detail. The treatment response is also defined according to the timing of treatment. The initial response is characterized at week 12 of therapy by a decrease in viral load of at least 1 $\log_{10}$ copies/mL compared to baseline [30]. Several clinical trials have shown that the magnitude of the initial decrease in viral load is associated with subsequent HBe seroconversion. In contrast, the same studies have shown that a persistent viral load above 3 $\log_{10}$ copies/mL is associated with an increased risk of viral resistance [31]. Virological breakthrough is defined by an increase of at least 1 $\log_{10}$ copies/mL compared to the lowest value during treatment, associated with the presence of resistant mutations. These assays therefore provide very important clinical information for monitoring antiviral therapy.

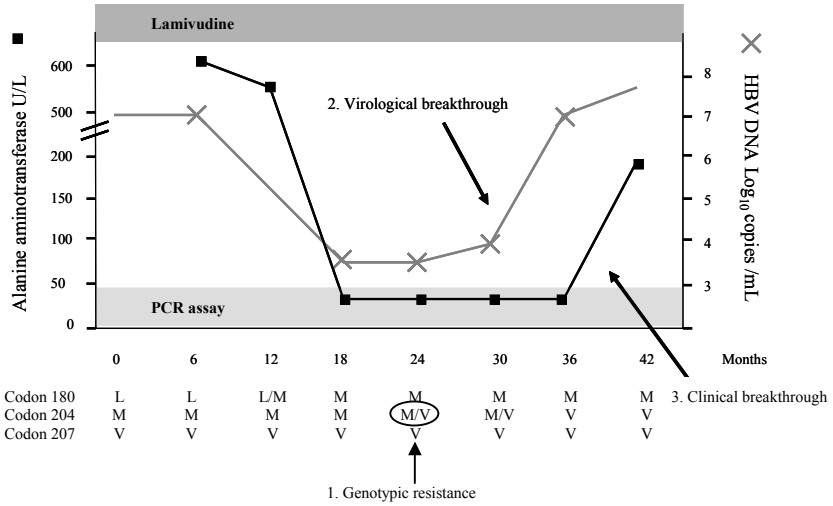
Insight from studies of intrahepatic viral DNA

Studies performed with PCR-based assays have shown that antiviral therapy with a potent HBV polymerase inhibitor significantly reduces cccDNA in chronic hepatitis B patients, but that the kinetics of viral clearance are slow, requiring long-term antiviral therapy to control viral infection [13,32]. It is important to note that reductions in cccDNA correlated with reductions in serum HBsAg titers. The parallel change in HBsAg further suggests that transcriptionally-active cccDNA is being depleted during therapy. Furthermore, since cccDNA cannot be easily measured during biopsies in routine clinical practice, HBsAg quantification in serum may represent a surrogate marker of the intrahepatic cccDNA pool [13]. However, this should be further evaluated in clinical studies.

Definition of drug resistance

More precise definitions of HBV drug resistance have been obtained using new assays for monitoring viral load and analyzing the viral

genome sequence (Figure 3) [33]. *Genotypic resistance* corresponds to the detection of mutation(s) in the HBV genome which are known to develop specifically during antiviral therapy and to confer resistance to the antiviral agent; with nucleoside analogs it currently corresponds



HBV=hepatitis B virus; PCR=polymerase chain reaction

Figure 3: Kinetics of emergence of antiviral drug resistance

to the detection of specific viral polymerase gene mutations. *Virological breakthrough* corresponds to the increase in serum HBV DNA levels during therapy, following the development of genotypic resistance. It is usually defined by a confirmed increase which is not due to a problem of compliance, of 1 $\log_{10}$ copies/mL compared to the lowest value during treatment. *Clinical breakthrough* is defined as a virological breakthrough with increased serum ALT levels and/or worsening of liver histology.

Monitoring antiviral therapy

When patients are treated with nucleos(t)ides, they should be monitored due to the risk of developing drug-resistance (Figure 4). The decision of when to monitor is based on the observation that the biochemical breakthrough usually occurs several weeks after the virological breakthrough and that the clinical impact is usually different in noncirrhotic than in cirrhotic patients. In the former, the

ALT breakthrough usually has no major clinical consequences; in the latter it may precipitate liver failure and death. Monitoring should

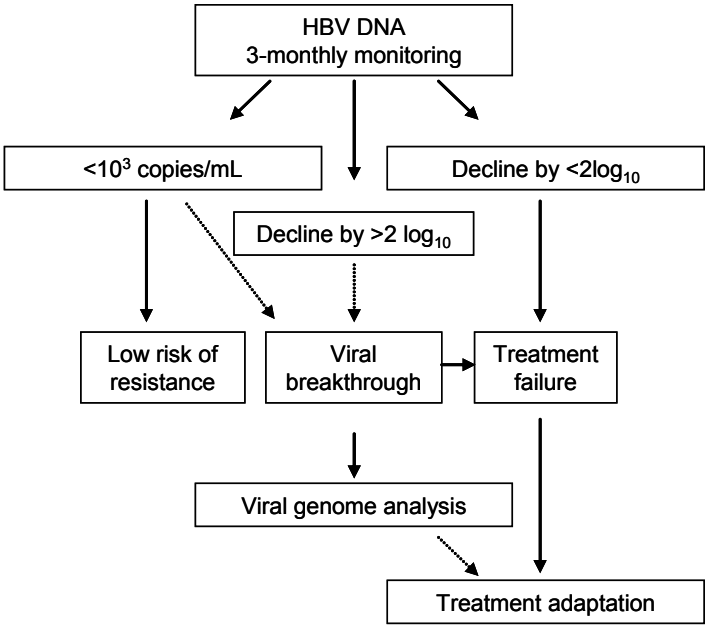


Figure 4: Monitoring of antiviral therapy

include measuring the viral load with quantitative HBV DNA testing whenever possible.

Early during therapy (week 8 or 12) viral load monitoring assesses the initial response which may predict treatment outcome. In HBeAg-positive patients treated with lamivudine (LAM) or adefovir (ADV), the magnitude of HBV DNA decline early in therapy correlates with the trend of subsequent HBe seroconversion [34]. The antiviral response at week 24 has also been found to be a predictor of subsequent efficacy (HBeAg loss, HBV DNA <200 copies/mL, ALT normalization, and viral breakthrough) in patients treated with LAM or telbivudine [35].

In a 5-year study of ADV administration in HBeAg-negative chronic hepatitis, patients with a viral load below 3 $\log_{10}$ copies/mL after 1 year of therapy had a significantly lower risk of developing resistance by year 3 of treatment (<3%) compared to a risk of 26%

and 66% in those with a viral load between 3 to 6 $\log_{10}$ copies, and >6 $\log_{10}$ copies/mL, respectively [36]. These results suggest that patients who do not achieve a significant decrease in viral load should be given rescue therapy before the development of true resistance.

During long-term treatment, viral load and serological markers should be assessed every 3 months to monitor antiviral treatment efficacy and to determine whether the response is maintained or drug resistance is likely to occur. The use of the most sensitive assays is therefore recommended; currently real-time PCR assays represent the best choice. Detection of polymerase mutations by sequencing, line probe assay, DNA chip technologies, or other tools will be important in the future to target new treatments corresponding to the profile of mutations in the polymerase gene [17,19]. Indeed the cross resistance profile is different from one mutant to another [26,27].

As described earlier, new tools may become available to monitor the efficacy of antiviral therapy, such as the quantification of intrahepatic cccDNA or of serum HBsAg as surrogate markers [13,32,37]. Furthermore, with the development of new drugs and the increasing complexity of the resistance profile, phenotypic assays to determine drug susceptibility of the clinical isolates may prove useful to tailor antiviral therapy to the virological situation of the patient, as already shown in HIV [26,27].

REFERENCES

1. Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev 2000;64:51-68.
2. Weiss J, Wu H, Farrenkopf B, Schultz T, Song G, Shah S, et al. Real time TaqMan PCR detection and quantitation of HBV genotypes A-G with the use of an internal quantitation standard. J Clin Virol 2004;30:86-93.
3. Miller RH, Robinson WS. Hepatitis B virus DNA in nuclear and cytoplasmic fractions of infected human liver. Virology 1984;137:390-399.
4. Yokosuka O, Omata M, Imazeki F, Okuda K, Summers J. Changes of hepatitis B virus DNA in liver and serum caused by recombinant leukocyte interferon treatment: analysis of intrahepatic replicative hepatitis B virus DNA. Hepatology 1985;5:728-734.
5. Fourel I, Cullen JM, Saputelli J, Aldrich CE, Schaffer P, Averett DR, Pugh J, Mason S. Evidence that hepatocyte turnover is required for rapid clearance of duck hepatitis B virus during antiviral therapy of chronically infected ducks. J Virol 1994;68:8321-8330.
6. Mason WS, Cullen J, Moraleda G, Saputelli J, Aldrich CE, Miller DS, Tennant B, Frick L, Averett D, Condreay LD, Jilbert AR. Lamivudine therapy of WHV-infected woodchucks. Virology 1998;245:18-32.
7. Dandri M, Burda MR, Will H, Petersen J. Increased hepatocyte turnover and inhibition of woodchuck hepatitis B virus replication by adefovir in vitro do not lead to reduction of the closed circular DNA. Hepatology 2000;32:139-146.
8. Kock J, Schlicht HG. Analysis of the earliest steps of hepadnavirus replication: genome repair after infectious entry into hepatocytes does not depend on viral polymerase activity. J Virol 1993;67:4867-4874.
9. Addison WR, Wong WW, Fischer KP, Tyrrell DL. A quantitative competitive PCR assay for the covalently closed circular form of the duck hepatitis B virus. Antiviral Res 2000;48:27-37.
10. He ML, Wu J, Chen Y, Lin MC, Lau GK, Kung HF. A new and sensitive method for the quantification of HBV cccDNA by real-time PCR. Biochem Biophys Res Comm 2002;295:1102-1107.
11. Kock J, Theilmann L, Galle P, Schlicht H. Hepatitis B virus nucleic acids associated with human peripheral blood mononuclear cells do not originate from replicating virus. Hepatology 1996;23:405-413.
12. Mason AL, Xu L, Guo L, Kuhns M, Perillo RP. Molecular basis for persistent hepatitis B virus infection in the liver after clearance of serum hepatitis B surface antigen. Hepatology 1998;27:1736-1742.

13. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C, Marcellin P, Goodman Z, Delaney WE 4th, Xiong S, Brosgart CL, Chen SS, Gibbs CS, Zoulim F. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004;126:1750-1758.

14. Wong DK, Yuen MF, Yuan H, Sum SS, Hui CK, Hall J, Lai CL. Quantitation of covalently closed circular hepatitis B virus DNA in chronic hepatitis B patients. Hepatology 2004;40:727-737.

15. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters HG, Zondervan P, Hansen B, Schalm SW; HBV 99-01 Study Group; Rotterdam Foundation for Liver Research. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005; 365:123-129.

16. Schildgen O, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, Helm M, Rockstroh JK, Willems WR, Will H, Gerlich WH. Variant of hepatitis B virus with primary resistance to adefovir. N Engl J Med. 2006;354:1807-1812.

17. 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.

18. Grandjacques C, Pradat P, Stuyver L, Chevallier M, Chevallier P, Pichoud C, Maisonnas M, Trepo C, Zoulim F. Rapid detection of genotypes and mutations in the precore promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity. J Hepatol 2000;33:430-439.

19. Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, et al. 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.

20. Wilson JW, Bean P, Robins T, Graziano F, Persing DH. Comparative evaluation of three human immunodeficiency virus genotyping systems: the HIV-GenotypR method, the HIV PRT GeneChip assay, and the HIV-1 RT line probe assay. J Clin Microbiol 2000;38:3022-3028.

21. Tran N, Berne R, Chann R, Gauthier M, Martin D, Armand MA, Ollivet A, Teo CG, Ijaz S, Flichman D, Brunetto M, Bielawski KP, Pichoud C, Zoulim F, Vernet G. European multicenter evaluation of high-density DNA probe arrays for detection of hepatitis B virus resistance mutations and identification of genotypes. J Clin Microbiol 2006;44:2792-2800.

22. Pang A, Yuen MF, Yuan HJ, Lai CL, Kwong YL. Real-time quantification of hepatitis B virus core-promoter and pre-core mutants during hepatitis E antigen seroconversion. J Hepatol 2004;40:1008-1017.

23. Zhang M, Gong Y, Osiowy C, Minuk GY. Rapid detection of hepatitis B virus mutations using real-time PCR and melting curve analysis. Hepatology 2002;36:723-728.

24. Whalley SA, Brown D, Teo CG, Dusheiko GM, Saunders NA. Monitoring the emergence of hepatitis B virus polymerase gene variants during lamivudine therapy using the LightCycler. J Clin Microbiol 2001;39:1456-1459.

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

26. Yang H, Westland C, Xiong S, Delaney WE. In vitro antiviral susceptibility of full-length clinical hepatitis B virus isolates cloned with a novel expression vector. Antiviral Res 2004;61:27-36.

27. Durantel D, Carrouee-Durantel S, Werle-Lapostolle B, Brunelle MN, Pichoud C, Trepo C, et al. A new strategy for studying in vitro the drug susceptibility of clinical isolates of human hepatitis B virus. Hepatology 2004;40:855-864.

28. Durantel D, Brunelle MN, Gros E, Carrouee-Durantel S, Pichoud C, Villet S, Trepo C, Zoulim F. Resistance of human hepatitis B virus to reverse transcriptase inhibitors: from genotypic to phenotypic testing. J Clin Virol 2005;34:S34-S43.

29. Minuk GY, Sun DF, Uhanova J, Zhang M, Caouette S, Nicolle LE, Gutkin A, Doucette K, Martin B, Giulivi A. Occult hepatitis B virus infection in a North American community-based population. J Hepatol 2005;42:480-485.

30. Locarnini S, Hatzakis A, Heathcote J, Keeffe EB, Liang TJ, Mutimer D, Pawlotsky JM, Zoulim F. Management of antiviral resistance in patients with chronic hepatitis B. Antivir Ther 2004;9:679-693.

31. Lai CL, Leung N, Teo EK, Tong M, Wong F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown NA; Telbivudine Phase II Investigator Group. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis Be antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528-536.

32. Sung JJ, Wong ML, Bowden S, Liew CT, Hui AY, Wong VW, et al. Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained response to therapy. Gastroenterology 2005;128:1890-1897.

33. Zoulim F. Antiviral therapy of chronic hepatitis B. Antiviral Res 2006; 25:Epub ahead of print.

34. Werle B, Cinquin K, Marcellin P, Pol S, Maynard M, Trepo C, et al. Evolution of hepatitis B viral load and viral genome sequence during adefovir dipivoxil therapy. J Viral Hepat 2004;11:74-83.

35. Lai C, Gane E, Liaw Y-F, et al. Telbivudine versus lamivudine for chronic hepatitis B: first year results from the international phase III globe trial. Hepatology 2005;42:S748A.

36. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart CL. Long-term adefovir dipivoxil treatment induces regression of liver fibrosis in patients with HBeAg-negative chronic hepatitis B: results after 5 years of therapy. Hepatology 2005;42:S745A.

37. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol 2005;42:302-308.

How to Predict the Outcome of Chronic Hepatitis B

G. Fattovich, N. Olivari, M. Pasino, S. Zanni

INTRODUCTION

Chronic hepatitis B virus (HBV) infection currently affects about 400 million people, particularly in developing countries, and is responsible for over 500,000 deaths annually worldwide from cirrhosis and hepatocellular carcinoma (HCC) [1]. The natural history of chronic HBV infection and disease is variable and complex, with severity and progression of the underlying liver disease determined by the interactions among several host-related, virus-related and environmental factors. Recent advances provide the basis for a clear understanding of the clinical outcomes and factors affecting disease progression that are key to the effective management of chronic HBV.

NATURAL HISTORY

The likelihood of chronic HBV infection is higher in individuals infected perinatally (90%) or during childhood (20 to 30%), when the immune system is thought to be immature, than in immunocompetent subjects infected during adulthood (<1%). The natural course of chronic HBV infection can be divided into 4 phases: 1) immune tolerance; 2) immune clearance; 3) low or nonreplicative; and, 4) reactivation.

The initial immunotolerant phase is characterized by the presence of: 1) the hepatitis Be antigen (HBeAg); 2) high serum levels of HBV DNA; 3) normal or minimally elevated serum alanine aminotransferase (ALT); and, 4) normal liver or only minimal

histological activity. Most Asian children, in whom chronic HBV infection is usually acquired perinatally, present in the immunotolerant phase with "HBeAg-positive chronic hepatitis with normal ALT". The immunotolerant phase may persist for 10-30 years in perinatally infected subjects, whereas it is generally short-lived or absent in childhood or adult-acquired HBV infection. After a variable period of HBeAg-positivity, depending on the age at acquisition of HBV infection, immune tolerance to the virus is lost and the immune system mounts an attack on infected hepatocytes. This second immuno-active phase is characterized by: 1) fluctuating, but progressively decreasing HBV DNA levels; 2) elevated ALT; and, 3) hepatic necro-inflammation. Patients with late childhood, adolescence or adult-acquired chronic HBV infection usually present in the immuno-active phase with "HBeAg-positive chronic hepatitis" with elevated serum ALT and active inflammation upon liver biopsy. Serum HBV DNA levels generally exceed 100,000 copies/mL (approximately 20,000 IU/mL) in patients with HBeAg-positive chronic hepatitis and may be as high as 10^{10} copies/mL.

An important outcome of the immuno-active phase is seroconversion from HBeAg to anti-HBe that is followed by a marked reduction of HBV DNA replication, biochemical remission and a return to inactive disease upon liver biopsy with a diminished risk of disease progression in most (65 to 95%) patients [2-4]. The rate of spontaneous HBeAg seroconversion averages between 10 and 15% per year in adults and children with elevated ALT, but is <5% per year in Asian children in the immunotolerant phase [2]. Factors associated with higher rates of spontaneous HBeAg seroconversion include older age, higher ALT levels, HBV genotype B (compared with C) and ethnicity (other than Asian) [2, 5].

The third low or nonreplicative phase (inactive hepatitis B surface antigen [HBsAg] carrier state) is characterized by: 1) HBeAg-negativity and anti-HBe–positivity; 2) undetectable or low levels of HBV DNA (suggested levels <1000 copies/mL); 3) persistently normal ALT levels; and, 4) absence of significant hepatitis with a necro-inflammation score of <4 [1,2,6]. In cases that have already developed cirrhosis during the high replicative phase of infection, the picture in the inactive HBsAg carrier state will be inactive cirrhosis. Most carriers remain in the inactive phase for life. However, as viral supercoiled DNA persists in the liver, a number of patients eventually develop HBV reactivation with recrudescence of liver disease either spontaneously or during immunosuppression. Reactivation of viral replication may occur due to reactivation with the wild-type virus with

reversion back to the HBeAg-positive state, or much more frequently with replication-competent HBV variants that bear mutations in the precore or core promoter regions of the core gene that prevent HBeAg expression (HBeAg-negative chronic hepatitis) [2,3,6,7].

The fourth reactivation phase is characterized by: 1) HBeAg-negativity with anti-HBe-positivity; 2) detectable serum HBV DNA levels (suggested threshold of 10,000 copies/mL); 3) ALT elevation; and, 4) liver necro-inflammation at histology (HBeAg-negative chronic hepatitis) [2,3,6,7]. In a long-term follow-up study of patients with spontaneous HBeAg seroconversion the cumulative incidence of HBeAg-negative chronic hepatitis was 25% after 16 years [3]. Over 50% of patients with HBeAg-negative chronic hepatitis have wide fluctuations in both serum ALT and HBV DNA levels and 20 to 30% of the patients with histologically documented chronic hepatitis have normal ALT at presentation [6,7]. Thus the differential diagnosis between the inactive HBsAg carrier state and HBeAg-negative chronic hepatitis requires serial testing of ALT and HBV DNA levels [1,6]. Available data indicate an increased prevalence of HBeAg-negative chronic hepatitis worldwide, which probably reflects the aging of existing HBsAg carriers [6].

During the inactive carrier state, spontaneous hepatitis B surface antigen (HBsAg) loss may occur at a rate of 1 to 2% per year in white carriers, and 0.05 to 0.8% in Chinese carriers [2]. Longitudinal studies have demonstrated that HBsAg seroclearance confers an excellent long-term prognosis, except in patients with cirrhosis or concurrent HCV or HDV infection before HBsAg clearance [8-10]. Indeed HCC may still develop, particularly in patients with cirrhosis who had HBsAg seroclearance at an older age [9].

Based on the knowledge of the natural history of chronic HBV infection, patients can be classified according to their immunological and serological status as shown in Table 1.

COMPLICATIONS OF CHRONIC HBV

The complications of chronic HBV include progression to cirrhosis, HCC, decompensation and liver-related death.

Incidence of cirrhosis

In HBeAg-positive chronic HBV the incidence of cirrhosis ranges from 2 to 6 per 100 person years with a cumulative incidence of

Phase	Serum ALT	HBeAg	Anti-HBe	HBV DNA (copies/mL)
Immuno-tolerant	Normal	Positive	Negative	>100,000
Immuno-active hepatitis	Elevated	Positive	Negative	>100,000
	Elevated	Negative	Positive	>10,000
Inactive carrier	Normal	Negative	Positive	<1000

ALT=alanine aminotransferase; HBeAg=hepatitis Be antigen; HBV=hepatitis B virus

Table 1: Serological profiles of chronic hepatitis B virus infection

8 to 20% over a 5-year period [2,3]. A longitudinal study of 240 asymptomatic HBeAg-positive patients with normal ALT from Taiwan has reported the occurrence of cirrhosis in 13 patients (5%) with an incidence of 0.5 per 100 person years and with a cumulative incidence of 12.6% after 17 years of follow-up [11]. Thus the estimated incidence of cirrhosis is much lower in this cohort of HBeAg-positive carriers presenting in the early immunotolerant phase of chronic HBV infection.

Progression to cirrhosis appears to occur faster in HBeAg-negative chronic hepatitis, with reported incidence rates of 8 to 10 per 100 person years [2,3,6,7].

Incidence of hepatocellular carcinoma

In patients with chronic HBV infection the risk of HCC appears to vary depending upon geographic area and the underlying stage of liver disease; in the presence of cirrhosis, the risk of developing HCC becomes correspondingly higher compared with the risk in patients without cirrhosis. In a review of published studies in East Asian countries, the summary HCC incidence rate increased from 0.2 per 100 person years among inactive carriers to 1.0 in persons with chronic HBV but with no cirrhosis and 3.2 in subjects with compensated cirrhosis; the 5-year HCC cumulative incidence was 15% in those with cirrhosis [12]. In contrast, in studies performed in Europe, where there is a low or intermediate rate of HBV endemicity, the summary HCC incidence was found to be 0.02 per 100 person years in inactive carriers, 0.1 in those with chronic HBV and no

cirrhosis, and 2.2 in those with compensated cirrhosis; the 5-year HCC cumulative incidence was 10% in the cirrhotic group [12].

Incidence of decompensation

The incidence of hepatic decompensation was found to be 3 to 4 per 100 person years in patients in the early stages of cirrhosis (child class A) from Europe [13] and Asia [14]. Approximately 20% of patients with cirrhosis for more than 5 years develop liver decompensation [13,14].

Liver-related mortality rates

In patients with chronic HBV infection, the mortality rate differs according to the study population. Liver-related mortality appears to be rare in inactive HBsAg carriers [3,4,15,16] (Table 2).

Author (reference)	Hsu (6)	De Franchis (15)	Manno (16)	Bortolotti (4)
Area	Asia	Europe	Europe	Europe
N° patients	189	68	296	80 [a]
Median follow-up (years)	8	10	30	14
Histologic deterioration*	0.06	0.15	NR	0
Hepatocellular carcinoma*	0.19	0	0.02†	0
Liver-related death*	0	0	0.03‡	0
HBsAg loss*	0.6	1.0	1.0	1.0

HBsAg=hepatitis B surface antigen; NR=not reported; a=children at enrolment; *=incidence per 100 person years; †=alcohol intake > 60g/day; ‡=2 hepatocellular carcinoma, 1 alcoholic cirrhosis

Table 2: Studies on the incidence of major events in inactive hepatitis B surface antigen carriers

An Italian study has reported that in a 30-year follow-up, healthy HBsAg-positive blood donors did not develop liver-related mortality at a higher rate than uninfected controls [16]. Among the 296 chronic HBV carriers, liver-related deaths occurred in 3 subjects. In 2 of these subjects, who were alcohol abusers, death was due to HCC whilst

alcohol induced cirrhosis was the cause of death in the remaining subject. This suggests that cofactors causing damage to the liver may be important in the progression of the disease in inactive HBsAg carriers [16].

In patients with compensated cirrhosis B the incidence of liver-related death was 3.5 and the 5-year mortality rate was 14 to 20% [13,17]. Once decompensation occurs, the mortality rate increases significantly at 5-year follow-up ranging from 65 to 85% in different studies [2,13,17].

FACTORS PREDICTING PROGRESSION TO CIRRHOSIS

Host-related, viral-related and environmental factors have been recognized as predictors of progression to cirrhosis (Table 3).

Factors	Comment
Host related	
Older age	Important
Male gender	Important
Severity of fibrosis stage at presentation (F3)	Important
Recurrent flares of hepatitis	Important
Viral related	
High levels of HBV replication during follow-up	Important
HBV genotype (C >B)	Increasing evidence
HBV variant (core promoter)	More research needed
HDV co-infection	Important
HCV co-infection	Important
HIV co-infection	More research needed
External	
Alcohol consumption	Important
Diabetes	More research needed
Obesity	More research needed

F3=stage 3 fibrosis; HBV=hepatitis B virus; HCV=hepatitis C virus; HDV=hepatitis D virus

Table 3: Factors associated with increased risk of progression to cirrhosis of chronic hepatitis B

Host-related factors

Host factors that appear to have an impact on the progression of chronic HBV to cirrhosis include older age, male gender and disease expression [2,18].

The severity of fibrosis at presentation correlates with the risk of cirrhosis [2]. Repeated severe acute exacerbations with failure to suppress HBV replication has been shown to predict higher rates of cirrhosis [19].

Virus-related factors

Viral load

Important evidence supports the association between sustained, high levels of HBV replication during hepatitis and the risk of cirrhosis. Clinical follow-up studies have reported that ongoing HBV replication, defined by serum HBV DNA detectable by hybridization assays (>100,000 to 1,000,000 copies/mL) or HBeAg, may accelerate the progression of chronic HBV to cirrhosis [2,3,7,19]. Older age at the time of HBeAg seroconversion, indicating prolonged viral replication and necro-inflammation, has been associated with an increased risk of cirrhosis [11]. In HBeAg-negative patients with chronic HBV, the progression to clinical cirrhosis was significantly associated with higher serum HBV DNA levels (always or frequently >10 pg/mL) [7]. More recently, a prospective, population-based cohort study of 3582 untreated HBsAg carriers from Taiwan found that the risk of cirrhosis increased significantly with increasing baseline serum HBV DNA levels detected by sensitive polymerase chain reaction (PCR) assays, independent of HBeAg status and serum ALT levels [18].

The adjusted relative risk of cirrhosis was 2.5, 5.6 and 6.5 when baseline HBV DNA levels were at least 10,000, 100,000 and ≥1,000,000 copies/mL, respectively [18]. The results of this study suggest that HBV DNA levels of 10,000 copies/mL or more are the strongest predictor of the risk of cirrhosis. On the other hand patients with spontaneous suppression of HBV replication early in the course of their disease are at a very low risk of progression to cirrhosis [2-4]. In a recent longitudinal study of 91 HBeAg-positive Italian children, 80 of 85 children (95%) without cirrhosis at enrollment, who underwent spontaneous HBeAg seroconversion remained inactive carriers after a 29-year follow-up [4]. Finally, long-term follow-up studies have shown that adult inactive HBsAg carriers rarely progress to cirrhosis (Table 2) [6,15].

HBV genotypes

HBV is currently classified into 8 genotypes; A through H. Increasing evidence suggests that different HBV genotypes play a role in determining the clinical outcome of liver disease. This has been shown with genotypes B and C, which are prevalent in Asia, and genotypes A and D, which are prevalent in Europe and the United States. Several cross-sectional studies have suggested that genotype C is more prevalent in patients with cirrhosis than genotype B [5]. In a longitudinal study of 202 patients with HBeAg-positive chronic hepatitis from Taiwan, genotype C was shown to be an independent predictive factor of cirrhosis [20]. Studies indicate that genotype A may be associated with a slower progression of liver disease than genotype D [5]. Some data suggest that the association between genotype C and a poor histology may be due to the close relationship between genotype C and core promoter mutations [21]. The relationship between HBV genotypes and core promoter and precore viral mutants in the progression of chronic hepatitis B merits further study.

Concurrent infection

Co-infection with HBV and hepatitis C virus (HCV) and/or hepatitis D virus (HDV) is not uncommon due to the shared route of parenteral transmission. Studies have shown that dual infection (HBV/HCV or HBV/HDV) or triple infection (HBV/HDV/HCV) is associated with more severe forms of chronic liver disease and with more rapid progression to cirrhosis than HBV infection alone [2,22,23]. A study has reported that during a follow-up period of 1-21 years, chronic HBV carriers with acute HCV superinfection had a significantly higher cumulative incidence of cirrhosis than carriers with acute HDV superinfection. This indicates that the long-term prognosis following acute HCV superinfection is much worse than that following acute HDV superinfection [23].

HIV-related immune deficiency modifies the natural history of chronic HBV infection with higher levels of HBV replication and a lower rate of spontaneous HBeAg seroconversion, leading to a more rapid progression towards cirrhosis [24].

External factors

Heavy alcohol intake can increase the risk of progression to cirrhosis 6-fold compared to abstinent patients chronically infected with HBV [2]. Diabetes and obesity may promote steatosis, which may be

a cofactor in the progression of liver disease. There are few data on the impact of diabetes or obesity on the progression to cirrhosis in HBV-infected patients and further research is needed in this area. One longitudinal study in Asian patients with chronic HBV showed that diabetes was an independent risk factor for cirrhosis in multivariate analysis (odds ratio [OR] 5.2, 95% CI, 2.0-13.5) [25]. Another longitudinal study of Italian patients with chronic HBV reported that steatosis (present in approximately 40% of patients) was associated with an increased risk of progression to clinical cirrhosis (OR 2.0, 95% CI, 1.1-3.7) [7].

FACTORS AFFECTING MORBIDITY AND MORTALITY

Several factors are associated with an increased risk of HCC (Table 4), decompensation and liver-related mortality.

Factors	Comment
Host related	
Older age	Important
Male gender	Important
Presence of cirrhosis	Important
Family history of HCC	Important in HBV-endemic regions
Race (Asian, African)	Important
Viral related	
High levels of HBV replication during follow-up	Important
HBV genotype (C>B)	Increasing evidence
HBV variant (core promoter)	More research needed
HDV co-infection	Important
HCV co-infection	Important
HIV co-infection	More research needed
External	
Alcohol consumption	Important
Environmental contaminants (aflatoxin)	Important in HBV endemic regions
Diabetes	More research needed
Obesity	More research needed
Smoking	More research needed

HCC=hepatocellular carcinoma; HBV=hepatitis B virus; HCV=hepatitis C virus; HDV=hepatitis D virus

Table 4: Factors associated with increased risk of hepatocellular carcinoma

Host-related factors

Older age, male gender and cirrhosis are the most recognizable host factors associated with HCC in chronic HBV infection [12,26]. Older age appears to be an important factor of progression to HCC and increased mortality, probably because it indicates a longer duration of HBV infection and liver disease. Being over 50 years old at diagnosis of compensated cirrhosis B increases the risk of HCC approximately 4-fold [12].

The risk of HCC is higher in persons with HBV infection from Asia than from European countries, possibly because of earlier acquisition of the virus infection and longer disease duration [12].

In patients with compensated HBV-cirrhosis, baseline biochemical characteristics indicating advancing cirrhosis are also significant predictors of HCC, decompensation and liver-related mortality [2,13].

Virus-related factors

Viral load

Studies conducted in tertiary care centers have shown that patients with compensated cirrhosis B and high levels of HBV replication, as indicated by HBeAg-positivity and/or serum HBV DNA detectable by hybridization assays, are at increased risk of decompensation and liver related death [13,17]. A population-based study in 11,893 Taiwanese men found that the risk of HCC was increased 10-fold in men positive for HBsAg alone and 60-fold for those positive for both HBsAg and HBeAg at diagnosis compared to the reference group of men negative for both markers [27]. The increased risk of HCC in individuals who are seropositive for HBeAg remained significant regardless of serum levels of ALT and the status of cirrhosis [28]. A recent prospective, population-based cohort study of 3653 HBsAg-positive individuals from Taiwan showed that the risk of HCC increased with increasing baseline serum HBV DNA levels detected by sensitive PCR assays, independent of HBeAg, serum ALT levels and cirrhosis [26]. The risk of HCC started to increase significantly at 10,000 copies/mL and was highest for patients with the highest baseline HBV DNA levels (>1,000,000 copies/mL) with hazard ratios of 2.3 and 6.1, respectively [26]. Another prospective population-based cohort study in 2763 HBsAg-positive Chinese adults has reported that high viral load at baseline (≥100,000 copies/mL) is significantly associated with increased mortality from HCC and chronic liver disease mortality over an 11-year period [29].

Overall clinical liver series and population-based studies suggest that the higher the level of HBV replication, the greater the risk of HCC, decompensation and liver related mortality. On the other hand, persistent suppression of HBV replication during follow-up predicts a favorable outcome. In a study of 1536 Alaskan natives with chronic HBV infection, 70% of those who were initially HBeAg-positive cleared HBeAg within the first 10 years of follow-up, and a higher HCC rate was observed among carriers who reverted from anti-HBe to HBeAg than in those with sustained HBeAg seroconversion [30]. In addition, it has been reported that cirrhotic patients who clear HBeAg with sustained suppression of HBV DNA, ALT normalization, and eventually, HBsAg loss, have a very low risk of developing HCC, decompensation and have increased survival compared to cirrhotic patients with persistent high levels of HBV replication [8,17].

HBV genotypes

Studies from Asia have shown that genotype C is associated with an increased risk of HCC compared to genotype B [5,31]. In a prospective cohort study of 426 chronic HBV patients from Hong Kong, clinical cirrhosis and genotype C were independently associated with the development of HCC with an adjusted relative risk of 10.24 and 2.84, respectively [31]. This may be related to the longer duration of high levels of HBV replication and a higher frequency of core promoter mutations in genotype C than B. However, the mechanism of carcinogenesis of these mutations warrants further investigation [12]. Data on the association of other HBV genotypes and HCC is scanty and controversial [5]. In one study patients infected with genotype F showed a higher mortality rate than those infected with genotype A or D [5].

Concurrent infection

Several studies (meta-analysis of case-control studies and cohort studies) demonstrated that HBV/HCV and HBV/HDV co-infections increase the risk of HCC (2- to 6-fold relative to each infection alone) [12,32].

A higher rate of decompensation, but not of HCC, has been reported in HIV/HBV co-infected individuals with cirrhosis [24]. In addition individuals co-infected with HIV and HBV are at greater risk of liver-related mortality than those infected with HIV or HBV alone [24].

Environmental factors

Alcohol abuse increases the risk of HCC 2- to 4-fold compared to abstinence in patients with chronic HBV infection [12,26,32]. To date, the adverse effects of low (<20 g/day) to moderate (20-50 g/day) alcohol consumption on the severity of HBV-related liver disease have not been clarified. Certain data support the role of diabetes, obesity and tobacco as single agents or cofactors in causing HCC [32]. Some studies have investigated the association between diabetes and HCC and found a 2- to 4-fold increase in the risk of HCC, taking into account the major risk factors for the disease, including HBV infection [32].

CONCLUSIONS

HBV DNA concentrations at enrollment and during follow-up are the best predictor of adverse clinical outcomes (cirrhosis, HCC, decompensation and liver related mortality). The higher the HBV DNA levels, the greater the risk of liver-related complications and mortality. If sustained suppression of HBV replication is obtained before the onset of cirrhosis, the prognosis is favorable and the survival rate is similar to that in uninfected individuals. Sustained suppression of viral replication in cirrhotic patients lowers the risk of HCC and improves survival. Older age, male gender, multiple ALT flares, severity of fibrosis and severity of compensated cirrhosis at presentation, concurrent infections (HBV/HCV and/or HBV/HDV) and alcohol abuse are additional important predictors of disease progression. There is increasing evidence that HBV genotype may play a role in determining the clinical outcome. Further studies are needed to investigate other viral factors (e.g. HBV mutant, HIV co-infection) and preventable or treatable comorbidities (e.g. obesity, diabetes) in the prognosis of chronic hepatitis B.

REFERENCES

1. EASL International Consensus Conference on Hepatitis B. Consensus statement. J Hepatol 2003;38:533-540.
2. Fattovich G. Natural history and prognosis of Hepatitis B. Semin Liver Dis 2003;23:47-58.
3. 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.
4. Bortolotti F, Guido M, Bartolacci S, Cadrobbi P, Crivellaro C. Noventa F, Morsica G, Moriondo M, Gatta A. Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. Hepatology 2006;43:556-562.
5. Kramvis A, Kew MC. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. J Viral Hepat 2005;12:456-464.
6. Hadziyannis S, Papatheodoridis GV. Hepatitis Be antigen-negative chronic hepatitis: natural history and treatment. Semin Liver Dis 2006;26:130-141.
7. Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Monti Gorin J, 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.
8. Fattovich G, Giustina G, Sanchez-Tapias J, Quero C, Mas A, Olivotto PG, Solinas A, Almasio P, Hadziyannis S, Degos F, De Moura MC, Krogsgaard K, Pantalena M, Realdi G, Corrocher R, Schlam 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.
9. Yuen MF, Wong DKH, Sablon E, Tse E, Ng IOL, Yuan HJ, Siu CW, Sander TJ, Bourne EJ, Hall JG, Condreay LD, Lai CL. HBsAg seroclearance in chronic hepatitis B in the Chinese: Virological, histological, and clinical aspects. Hepatology 2004;39:1694-1701.
10. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Long-term outcome after Hepatitis B surface antigen seroclearance in patients with chronic Hepatitis B. Am J Med 2006;119:719-716.
11. Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis Be antigen to antibody seroconversion in patients with normal serum aminotransferase levels. Am J Med 2004;116:829-834.
12. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127:S35-S50.
13. 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.

14. 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.

15. De Franchis R, Meucci G, Vecchi M, Tatarella M, Colombo M, Del Ninno E, Rumi MG, Donato MF, Rochi G. The natural history of asymptomatic hepatitis B surface antigen carriers. Ann Intern Med 1993;118:191-194.

16. Manno M, Cammà C, Schepis F, Bassi F, Gelmini R, Giannini F, Miselli F, Grottola A, Ferretti I, Vecchi C, De Palma M, Villa E. Natural history of chronic HBV carriers in Northern Italy: morbidity and mortality after 30 years. Gastroenterology 2004;127:756-763.

17. 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.

18. Iloeje UH, Yang HI, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-686.

19. 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.

20. Chu CM, Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis Be antigen-positive patients with normal aminotransferase levels at baseline. J Hepatol 2005;43:411-417.

21. Yuen MF, Tanaka Y, Ng IOL, Mizokami M, Yuen JCH, Wong KH, Yuan HJ, Sum SM, Chan AO, Lai CL. Hepatic necro-inflammation and fibrosis in patients with genotypes B and C, corepromoter and precore mutations. J Viral Hepat 2005;12:513-518.

22. Perumalswami PV, Bini EJ. Epidemiology, natural history and treatment of hepatitis B virus and hepatitis C virus co-infection. Minerva Gastroenterol Dietol 2006;52:145-155.

23. Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM. Impact of acute hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. Gastroenterology 2004;126:1024-1029.

24. Puoti M, Torti C, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co-infected patients. J Hepatol 2006;44:S65-S70.

25. Huo TI, Wu JC, Hwang SJ, Lai CR, Lee PC, Tsay SH, Chang FY, Lee SD. Factors predictive of liver cirrhosis in patients with chronic hepatitis B: a multivariate analysis in a longitudinal study. Eur J Gastroenterol Hepatol 2000;12:687-693.

26. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.

27. 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.

28. You SL, Yang HI, Chen CJ. Seropositivity of hepatitis Be antigen and hepatocellular carcinoma. Ann Med 2004;36:215-224.

29. Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. Am J Gastroenterol 2006;101:1-7.

30. 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.

31. Chan HLY, Hui AY, Wong ML, Tse AML, Hung LCT, Wong VWS, Sung JJY. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut 2004;53:1494-1498.

32. Donato F, Gelatti U, Limina RM, Fattovich G. Southern Europe as an example of interaction between various environmental factors: a systematic review of the epidemiological evidence. Oncogene 2006;25:3756-3770.

New Drugs for Chronic Hepatitis B

M. Buti, R. Esteban

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most common viral infections in humans. Approximately 2 billion people have been infected with HBV, and 350 million of them have become chronically infected. Around 25 to 40% will eventually die from liver disease. The ultimate goals of treatment are to achieve sustained suppression of HBV replication to below 100,000 copies/mL in patients positive for hepatitis Be antigen (HBeAg) and, undetectable levels in HBeAg-negative patients. The registered agents currently available for the treatment of chronic HBV infection are divided into 2 main groups: immunomodulators, which include interferon alfa, and pegylated interferon alfa; and nucleos(t)ide analogs such as, lamivudine (LAM), adefovir dipivoxil (ADV), and entecavir (ETV). However, not all patients with chronic hepatitis B infection respond to these treatments.

Most recently, telbivudine (LdT) has been approved by regulatory authorities in the United States. Agents in clinical development for the treatment of hepatitis B include tenofovir (TDF) and emtricitabine (FTC) which are already approved for the treatment of HIV, and clevudine and pradefovir. However, the potential for the emergence of viral resistance to these drugs particularly during prolonged therapy could be a major limitation to their use in clinical practice. The availability of multiple agents has fueled debate over whether the use of combination therapy might be associated with improved outcomes with, in the case of antiviral drugs, a reduced risk of viral resistance.

NUCLEOSIDE ANALOGS

Telbivudine

Telbivudine, β-L-2'-deoxythymidine, is 1 of 3 L-nucleosides that specifically inhibit HBV replication. The other 2 agents are β-L-2'-deoxycytidine and β-L-2'-deoxyadenosine. The anti-HBV activities are conferred by the common hydroxyl group in the 3'position of the β-L-2'-deoxyribose sugar of the molecules. In woodchuck models LdT has led to a reduction in HBV DNA levels of 8 $\log_{10}$ copies/mL. Phase I and II clinical trials with LdT showed a marked dose-proportional antiviral activity, with 4 weeks of 800 mg per day causing a reduction in median HBV DNA levels of 4 $\log_{10}$ copies/mL. No side effects have been observed and this may be related to the L-configuration of the molecule [1,2].

Five different therapeutic strategies for 1-year treatment have recently been compared in a phase IIb study [3]. One hundred four patients were randomized to receive: 1) LdT 400 mg daily; 2) LdT 600 mg daily; 3) LdT 400 mg and LAM 100 mg daily; 4) LdT 600 mg and LAM 100 mg daily; or, 5) LAM 100 mg daily. At week 52 reductions in median serum HBV DNA were 6.43 $\log_{10}$ copies/mL, 6.09 $\log_{10}$ copies/mL, 6.40 $\log_{10}$ copies/mL, 6.05 $\log_{10}$ copies/mL, and 4.66 $\log_{10}$ copies/mL, respectively. HBeAg loss was observed in 28% of patients treated with LAM, 33% of those treated with LdT and 17% of those treated with LdT in combination with LAM. Therefore after 1 year of treatment viral suppression, polymerase chain reaction (PCR)-nondetectability of serum HBV DNA and, alanine aminotransferase (ALT) normalization were significantly greater for patients treated with LdT compared with LAM.

The GLOBE study was designed to compare virologic and clinical efficacy of LdT vs. LAM in individuals with chronic HBV infection [4]. Large-scale, randomized controlled phase III trials of LdT (600 mg/day) have been performed with primary end-points of serologic and virological responses. Data from week 52 of the GLOBE trial showed that LdT led to a better virological response than LAM across nearly all patient subgroups that were analyzed. In a multivariate analysis, race, geographic region, and ALT levels were identified as key predictive factors of virological response to LdT. Greater HBV DNA suppression was observed with LdT in HBeAg-positive patients with baseline ALT >2.5 times the upper limit of normal (ULN) compared with patients having baseline ALT <2.5 times ULN ($p<0.0001$). This was particularly the case for

HBeAg-positive patients in Asia compared with those in North America ($p<0.001$) or other regions ($p<0.038$), and in HBeAg-negative Asians compared with HBeAg-negative patients of other races ($p=0.0145$). Telbivudine was also superior to LAM across all genotypes in both HBeAg-positive and HBeAg-negative patients.

Additional analyses of the GLOBE study evaluated the relationships between HBV DNA suppression at week 24 and patient outcomes at week 52 [5]. Levels of HBV DNA detectable by PCR assay at week 24 were predictive of the presence of resistance at week 52, while PCR negativity was predictive of no resistance at week 52. The second year GLOBE study results showed LdT antiviral efficacy and clinical measurements that are superior to LAM regardless of HBeAg status. Patients receiving LdT demonstrated less treatment failure, resistance, and viral breakthrough than those receiving LAM. The safety, HBeAg-loss durability and seroconversion results for LdT were equivalent to those obtained with LAM [6].

Emtricitabine

Emtricitabine has a similar molecular structure, antiviral potency and selectivity to LAM in the woodchuck model [7,8]. A recent double-blind phase III clinical trial randomized patients (72% men; 53% Asian, 47% Caucasian; 52% HBeAg-positive; median baseline HBV DNA of 6 $\log_{10}$ copies/mL) to either 200 mg/day FTC (n=167) or placebo (n=81) [9]. At the end of 48 weeks of treatment, 103 (62%) patients receiving FTC had improved liver histology (defined as ≥2-point reduction in the Knodell necro-inflammatory score with no worsening of fibrosis) compared with 20 (25%) of those receiving placebo. Serum HBV DNA was undetectable (<400 copies/mL) in 91 (56%) and 2 (2%) patients, respectively. Upon completion of treatment with FTC, 23% subsequently developed a flare of HBV viremia.

All of the 64 FTC-treated patients with detectable serum HBV DNA at the end of treatment were genotyped. Mutations associated with FTC resistance in the YMDD motif of the HBV polymerase were identified in 19 (30%) of these patients [10]. In the overall study population, the 48-week incidence of FTC resistance mutations was 12.6%. This is similar to what is seen historically in patients treated with LAM. Given the development of newer agents with high barriers to the development of viral resistance, these data suggest that this drug might have limited use as monotherapy for management of chronic HBV infection. It is unlikely that FTC will

play a significant role in the management of chronic HBV other than as part of a combination therapy.

In a double-blind, placebo controlled, phase II study of nucleoside-naïve, HBeAg-positive individuals FTC and ADV combination therapy was evaluated against ADV monotherapy [11]. The combination regimen was associated with a greater decline in HBV RNA by week 48; the median change was -5.44 $\log_{10}$ copies/mL for the combination therapy compared with -3.40 $\log_{10}$ copies/mL for the monotherapy arm (p=0.03).

Clevudine

Clevudine is a pyrimidine analog with potent anti-HBV activity. It has good bio-availability with no apparent toxicity in mice and woodchucks. 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 0.15 μM with a mean of 0.08 μM. *In vitro* studies suggest that it may also be effective against LAM-resistant HBV mutants. *In vitro* studies of the infected woodchuck model have demonstrated that a once daily dose of 10 mg of clevudine resulted in as much as a 9 $\log_{10}$ decrease in viral load. Clevudine was not found to be incorporated into mitochondrial DNA or to be associated with significant lactic acid production *in vitro*. The efficacy of clevudine was evaluated in a double-blind, randomized, phase II trial comparing 30 mg/day and 50 mg/day dosing regimens with a placebo. Clevudine was prescribed for 12 weeks and patients were followed-up for 24 weeks after the end of treatment [12]. At week 12 there was a median decrease in HBV DNA levels of 4.49 $\log_{10}$ copies/mL and 4.45 $\log_{10}$ copies/mL in the 30 mg and 50 mg groups, respectively. Unlike treatment with most nucleoside analogs, no rebound in HBV DNA levels was seen after the cessation of clevudine. Moreover, the reduction in HBV DNA was sustained so that 24 weeks after cessation of therapy, levels were 2.6 $\log_{10}$ copies/mL and 1.8 $\log_{10}$ copies/mL lower than at the start of treatment [12]. Another dose-escalating multicenter study of clevudine 10 mg, 50 mg, 100 mg and 200 mg for 28 days was performed in a population of 32 patients (88% Asian), of whom 81% were HBeAg-positive. After 28 days, patients demonstrated a reduction in median HBV DNA from baseline of 2.5 $\log_{10}$ copies/mL, 2.7 $\log_{10}$ copies/mL, 3.0 $\log_{10}$ copies/mL and 2.5 $\log_{10}$ copies/mL, respectively. At follow-up, 24 weeks after therapy the median reduction of HBV DNA was sustained at 1.2 $\log_{10}$ copies/mL, 1.4 $\log_{10}$ copies/mL, 2.7 $\log_{10}$ copies/mL and 1.7 $\log_{10}$

copies/mL for the 10 mg, 50 mg, 100 mg and 200 mg patients, respectively. Six of the 27 HBeAg-positive patients lost HBeAg and 3 of the 27 patients seroconverted to anti-HBe antibodies [13].

In a phase III study, Yoo and colleagues evaluated the use of clevudine in HBeAg-positive and HBeAg-negative patients treated for 24 weeks. Among HBeAg-positive patients, 59% had undetectable HBV DNA and 68% had normalized ALT levels. Ninety-two percent of HBeAg-negative patients were HBV DNA-negative at week 24 and 75% had normalized ALT levels [14].

A recent phase III trial in HBeAg-positive patients evaluated FTC monotherapy (200 mg/day; n=81) compared with FTC and clevudine in combination therapy (200 mg/day FTC + 10 mg/day clevudine; n=82) [15]. After 24 weeks of treatment, there were no statistically significant differences in response between the 2 arms; 65% vs. 74%, respectively had serum HBV DNA levels <4700 copies/mL (p=0.114). However at 24 weeks post-treatment, there were significant differences between FTC monotherapy and the combination therapy in undetectable HBV DNA (23% vs. 40%, respectively; $p \leq 0.025$) and normalized ALT (42% vs. 63% respectively; $p \leq 0.025$). This suggests that there may indeed be advantages to combination therapy with some agents but that HBV drug resistance is a limitation to some combinations.

NUCLEOTIDE ANALOGS

Tenofovir

Tenofovir disoproxil fumarate is a nucleotide analog similar in structure to ADV and is approved for treatment of HIV. Both drugs have a similar mechanism of action, so it was postulated that TDF would also have some degree of efficacy against HBV, and this has been confirmed in several preclinical studies. A mixed population of HBV mono-infected and HBV/HIV co-infected patients (n=106) with high levels of serum HBV DNA (>6 $\log_{10}$ copies/mL) and genotypic evidence of resistance to LAM were followed (median TDF follow-up: 35 ± 10 months; median ADV follow-up: 21 ± 5 months) after they switched to either ADV (n=68) or TDF (n=38) [16]. A greater proportion of patients treated with TDF (100%) had a negative viral load at week 24 when compared with patients treated with ADV (49%). In total, 49% of TDF-treated individuals had HBeAg loss compared with 13% of ADV-treated patients. In addition, 19% of

patients receiving TDF had hepatitis B surface antigen (HBsAg) loss compared with 6% of those receiving ADV. A study by van Bommel and colleagues evaluated the use of TDF in 20 LAM-refractory HBV mono-infected individuals having a suboptimal response to ADV [17]. In a median time of 3.5 months, 19 of 20 patients achieved undetectable HBV DNA (<400 copies/mL). Furthermore, by the end of follow-up (median 12 months, range 3-24 months), ALT levels normalized in 10 of 14 patients with elevated levels at baseline. Four patients lost HBeAg within 16 months and 1 patient seroconverted to anti-HBs after 16 months of TDF therapy.

Benhamou *et al.* [18] presented a retrospective analysis of HIV/HBV co-infected patients who had a baseline serum HBV DNA $\geq$2.3 $\log_{10}$ copies/mL (n=65). Many of these patients had been receiving LAM and had developed mutations conferring LAM-resistance (68.8%). They were then treated with TDF (300 mg/day) for at least 6 months. This analysis found a mean reduction from baseline of 4.56 $\log_{10}$ copies/mL of serum HBV DNA in HBeAg-positive patients and 2.53 $\log_{10}$ copies/mL in HBeAg-negative patients treated with TDF, with serum HBV DNA becoming undetectable in 29.6% and 81.6% of patients, respectively. Peters *et al.* have recently published a study comparing TDF and ADV in 52 patients with HIV/HBV co-infection showing that TDF or ADV were safe and efficacious in those patients [19]. At baseline, 73% of patients had plasma HIV-1 RNA <50 copies/mL, 86% were HBeAg-positive and 94% LAM-resistant. The mean time-weighted average change in serum HBV DNA from baseline to week 48 was -4.44 $\log_{10}$ copies/mL for TDF and -3.21 $\log_{10}$ copies/mL for ADV.

In vitro studies demonstrate that TDF has favorable metabolism, and activity against wild-type and several resistant forms of HBV [20]. Tenofovir had near-wild–type activity against the LAM-resistance patterns L180M/M204I and L180M/M204V. The drug was also active against ADV-resistant virus. However, the TDF concentration required to reduce HBV DNA activity by half (EC_{50}) was 3-fold higher when the virus was harboring the A181V ADV mutation, and 4.6-fold higher when the virus harbored the N236T ADV mutation.

In vitro data are supported by both retrospective and prospective trials in individuals co-infected with HIV and HBV indicating that TDF is active against HBV in this population. Collectively these results are promising for the treatment of HBV with TDF. Further studies from HBV-mono-infected patients are anticipated.

Pradefovir

Like ADV, pradefovir (formerly remofovir) is a phosphono methoxy ethyl adenine (PMEA) prodrug that is active against HBV. Unlike ADV, pradefovir is transported to the liver intact, and is activated there by cytochrome P450 enzyme CYP3A4. Animal studies show that while high levels of the active drug are found in the liver, no active drug is seen in the kidneys. This difference in metabolism is expected to alleviate some of the renal symptoms that can arise from ADV administration.

A recent phase I trial examined the safety and pharmacokinetics of pradefovir at varying doses after 28 days of treatment in chronic hepatitis B patients [21]. The results indicate that pradefovir is readily converted to PMEA and drug concentrations and clearance corresponded with the dose. All doses examined were superior to placebo, and dosing of 60 mg/day led to a 3 $\log_{10}$ copies/mL reduction in viral load. After promising virologic activity, and a safety analysis showing few adverse events, and no significant renal effects, phase II studies have been planned.

OTHER NUCLEOS(T)IDE ANALOGS

New antiviral agents such as valtorcitabine, LB 80380 (ANA 380) and elvucitabine (ACH-126,433; Beta-L-FD4C) and others are in early stages of development [22-24].

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 chronic hepatitis B. The future of chronic hepatitis B therapy is likely to involve the combination of different drugs in order to improve response to therapy and avoid or reduce viral resistance.

REFERENCES

1. Lai CL, Lim SG, Brown NA, Zhou XJ, Lloyd DM, Lee YM, Yuen MF, Chao GC, Myers MW. A dose-finding study of once-daily oral telbivudine in HBeAg-positive patients with chronic hepatitis B virus infection. Hepatology 2004;40:719-726.
2. Zhou XJ, Chao G, Brown N. Absence of pharmacokinetic drug-drug interaction between telbivudine and lamivudine or adefovir in healthy subjects. J Hepatol 2005;42:S197.
3. Lai CL, Leung N, Teo EK, Tong M, Wong F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown NA. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis Be antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528-536.
4. Thongsawat S, Lai CL, Gane E, Chao G, Fielman B, Brown N. Telbivudine displays consistent antiviral efficacy across patient subgroups for the treatment of chronic hepatitis B: results from the GLOBE study (abstract 110). J Hepatol 2006.
5. Zeuzem S, Lai CL, Gane E, et al. Optimal virologic and clinical efficacy at one year is associated with maximal early HBV suppression in nucleoside-treated hepatitis B patients (abstract 51). J Hepatol 2006.
6. Chan HLY, Lai CL, Cho M, et al. A randomized trial of telbivudine (LdT) vs. adefovir for HBeAg-positive chronic hepatitis B: results of the primary week 24 analysis (abstract 52). J Hepatol 2006.
7. Raffi F, Snow A, Borroto-Esoda K, Shaw A, Anderson J, Sorbel J, Quinn J, Mondou E, Rousseau F. Anti-HBV activity of emtricitabine (FTC) in patients co-infected with HIV and hepatitis B virus (abstract 215). Antivir Ther 2003.
8. Gish RG, Trinh H, Leung N, Chan FK, Fried MW, Wright TL, Wang C, Anderson J, Mondou E, Snow A, Sorbel J, Rousseau F, Corey L. Safety and antiviral activity of emtricitabine (FTC) for the treatment of chronic hepatitis B infection: a two-year study. J Hepatol 2005;43:60-66.
9. Lim SG, Ng TM, Kung N, Krastev Z, Volfova M, Husa P, Lee SS, Chan S, Shiffman ML, Washington MK, Rigney A, Anderson J, Mondou E, Snow A, Sorbel J, Guan R, Rousseau F; Emtricitabine FTCB-301 Study Group. A double-blind placebo-controlled study of emtricitabine in chronic hepatitis B. Arch Intern Med 2006;166:49-56.
10. Snow A, Krastev Z, Lim SG, NG TM, Kotzen IA, ChanS, Husa P, Šperl J, Mommeja-Marin H, Borroto-Esoda K, Anderson J, Mondou E, Sorbel, J, Rousseau F. Antiviral response and resistance surveillance for HBeAg positive patients enrolled in a combination study evaluating emtricitabine plus clevudine versus emtricitabine monotherapy for treatment of chronic hepatitis B infection. Hepatology 2005;S1004.
11. Lau G, Cooksley H, Ribeiro RM, et al. Randomized, double-blind study comparing adefovir dipivoxil (ADV) plus emtricitabine (FTC) combination

therapy versus ADV alone in HBeAg (+) chronic hepatitis B: Efficacy and mechanisms of treatment response (abstract). Hepatology 2004;40:272.

12. Lee HS, Chung YH, Lee K, Byun KS, Paik SW, Han JY, Yoo K, Yoo HW, Lee JH, Yoo BC. A 12-week clevudine therapy showed potent and durable antiviral activity in HBeAg-positive chronic hepatitis B. Hepatology 2006;43:982-988.

13. 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. A phase II dose-escalating trial of clevudine in patients with chronic hepatitis B. Hepatology 2004;40:140-148.

14. Yoo BC, Kim JH, Lee KS, et al. A 24-week clevudine monotherapy produced profound on-treatment viral suppression as well as sustained viral suppression and normalization of aminotransferase levels for 24 weeks off-treatment in HBeAg(+) chronic hepatitis B patients (abstract 186). Hepatology 2005;42:270A.

15. Lim SG, Krastev Z, Ng TM, Mechkov G, Kotzev IA, Chan S, Mondou E, Snow A, Sorbel J, Rousseau F; FTCB-204 Study Group. Randomized, double-blind study of emtricitabine (FTC) plus clevudine versus FTC alone in treatment of chronic hepatitis B. Antimicrob Agents Chemother 2006;50:1642-1648.

16. Villeneuve J, Willems B, Zoulim F, et al. Efficacy of tenofovir in patients with chronic hepatitis B and resistance or sub-optimal response to adefovir (abstract). Hepatology 2005;42:S997.

17. Van Bömmel, Zöllner B, Sarrazin C, Spengler U, Huppe D, Moller B, Feucht HH, Wiedenmann B, Berg T. Tenofovir for patients with lamivudine-resistant hepatitis B virus (HBV) infection and high HBV DNA level during adefovir therapy. Hepatology 2006;44:318-325.

18. Benhamou Y, Fleury H, Trimoulet P, Pellegrin I, Urbinelli R, Katlama C, Rozenbaum W, Le Teuff G, Trylesinski A, Piketty C; TECOVIR Study Group. Anti-hepatitis B virus efficacy of tenofovir disoproxil fumarate in HIV-infected patients. Hepatology 2006;43:548-55.

19. Peters M, Anderson J, Lynch P, Liu T, Alston-Smith B, Brosgart CL, Jacobson JM, Johnson VA, Pollard RB, Rooney JF, Sherman KE, Swindells S, Polsky B; ACTG Protocol A5127 Team. Randomized controlled study of tenofovir and adefovir in chronic hepatitis B virus and HIV infection: ACTG A5127. Hepatology 2006;44:1110-1116.

20. Lada O, Benhamou Y Cohour A, Katlama C, Poynard T, Thibault V. In vitro susceptibility of lamivudine-resistant hepatitis B virus to adefovir and tenofovir. Antivir Ther 2004;9:353-363.

21. Lee KS, Lim SG, Chuang WL, et al. Safety, tolerability and antiviral activity of pradefovir mesylate in patients with chronic hepatitis B virus infection: 48-week analysis of a phase 2 study. J Hepatol 2006;S741.

22. Tennant B, Menne S, Baldwin B, Juodawlkis A, Bryant M, Imbach JL, Gosselin G. Antiviral activities of beta-L-thymidine (LdT), val-beta-L-2'-deoxycytidine (val-LdC), and lamivudine alone and in combination

in a 12 week, oral dose study using the woodchuck model of chronic hepatitis B virus infection (abstract). Antiviral Res 2001.

23. 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 R, Myers M, Bryant M, Sommadossi JP. Antiviral beta-L-nucleosides specific for hepatitis B virus infection. Antiviral Chemistry Chemother 2001;12:S119-129.

24. Lim SG, Lai CL, Myers M, et al. Final results of a phase I/II dose escalation trial of valtorcitabine in patients with chronic hepatitis B (abstract 34). J Hepatol 2005;42:S185-186.

Treatment of HBeAg-Positive Chronic Hepatitis B with Interferon or Nucleos(t)ide Analogs

G. Dusheiko

INTRODUCTION

Hepatitis B has a complex natural history and causes a wide spectrum of disease. Treatment is indicated for chronic, progressive disease, and rapidly acting nucleoside analogs may be indicated in fulminant acute hepatitis or subacute hepatic necrosis. In most endemic countries in Asia and Africa the main route of acquisition of chronic infection is thought to be during the perinatal period or childhood. This explains the disease chronicity, and the overall prevalence of hepatitis Be antigen (HBeAg)-positive disease in these regions.

Several difficulties remain concerning the treatment of hepatitis B virus (HBV) infection and the optimal management of chronic hepatitis B is still under debate.

Choice of therapy is based on: 1) factors that predict treatment response; 2) the clinical picture and stage of disease; 3) the likelihood and consequences of resistance to treatment; and, 4) the personal choice of the patient and physician. Current guidelines must be constantly and rapidly reviewed as new therapies become available, although the licensing of new drugs for hepatitis B is often based upon protocol-specified treatments and the assessment of the efficacy of single agents. This paper focuses on the treatment of HBeAg-positive disease, as other authors will address anti-HBe-positive disease.

IMMUNOPATHOGENESIS OF HEPATITIS B

The key steps in the replication of HBV have been defined. An inadequate innate and adaptive host immune response accounts for persistent infection, and immunological tolerance is evident in HBeAg-positive patients with high viral loads. The host immune responses that characterize the chronic phase of active disease are not sufficient to control active viral replication (for reasons that are unclear). Hepatitis B virus-specific T cell responses are very weak or totally undetectable in the peripheral blood of patients with long-lasting chronic hepatitis B [1-3]. Weak, narrowly focused responses are directed to subdominant epitopes of HBV. Persistent, ineffective immune response appears to be responsible for liver damage. A complex pattern of genomic variability and selection of precore mutants and core promoter mutants occur during prolonged chronic infection, accounting for different serological disease patterns.

Chronic HBeAg-positive disease is often accompanied by acute exacerbations that lead to a decline in HBV DNA and seroconversion to anti-HBe. Such spontaneous seroconversions occur in 5 to 15% of patients per year, and are closely related to alanine aminotransferase (ALT) flares. Attaining viral "clearance" after antiviral therapy in chronic hepatitis B is difficult, and may in fact not be possible.

High expression of antigen in the liver may impair T cell effector functions, and in many patients the immune paresis is irreversible. However, T cell responsiveness can be restored. Lamivudine (LAM) and adefovir (ADV) have been shown to transiently restore or enhance the HBV-specific human leukocyte antigen (HLA) class II response to HBV antigens. Elevated ALT levels predict a higher probability of HBeAg loss in patients with chronic hepatitis B, indicating a "primed" immunological status that has not yet been elucidated. Treatment may need to be maintained until specific immunological control can be elicited.

CHRONIC HBeAg-POSITIVE HEPATITIS B

Chronic hepatitis B is defined as persistent hepatitis B surface antigens (HBsAg) in the circulation for over 6 months. The disease may cause liver damage varying from mild chronic hepatitis to severe, active hepatitis, cirrhosis and primary liver cancer. Chronic hepatitis B is more likely to occur when: 1) the infection is acquired during childhood than during adult life; and, 2) in patients with natural or acquired immune deficiencies, including HIV infection. In countries where hepatitis B infection is endemic the highest prevalence of

HBeAg is found in young children, with steadily declining rates among older age groups. Whereas the prevalence of anti-HBe increases with age, HBeAg is reportedly more common in young than in adult carriers of hepatitis B.

Although levels of serum aminotransferases are usually elevated in patients with HBeAg HBV DNA-positive chronic hepatitis, some patients may have normal or near normal values. Many patients develop moderate-to-severe HBeAg-positive chronic hepatitis with raised serum ALT after several decades of infection, which may ultimately progress to cirrhosis. Aminotransferases may fluctuate over time. As the disease progresses to cirrhosis, the aspartate aminotransferase (AST):ALT ratio may be reversed. Elevation of these enzymes may be the only abnormality found in individuals with asymptomatic and anicteric infections. A progressive decline in serum albumin concentrations and prolongation of prothrombin time are characteristic of decompensated cirrhosis. Single measurements of ALT are not useful in a disease which is as dynamic as hepatitis B, thus repeated measurements, over at least a few months may be required.

Hepatitis B virus genotypes have been correlated with spontaneous and interferon-induced HBeAg seroconversion, activity of liver disease, and progression to cirrhosis and hepatocellular carcinoma (HCC). Hepatitis B virus genotypes may also correlate with response to interferon therapy, and resistance to antiviral therapy. In China and Japan, where genotypes B and C predominate, there is evidence of increased pathogenicity and probability of developing HCC in genotype C compared to B patients but further study is required [4-7].

HBeAg-positive disease is typically associated with high levels of HBV replication for a prolonged period of time. This disease is found in young individuals with chronic hepatitis B and high levels of HBV DNA (usually $>10^7$ copies/mL) in serum. These patients may have normal ALT in the "immunotolerant phase" or have raised ALT in the later active phase of the disease ("immuno-active"). Spontaneous seroconversion rates are higher in patients with raised ALT and genotype B (vs. C) or genotype D (vs. A). After infection, patients may have normal or near normal ALT for decades, with little necro-inflammatory disease. It is important to note that patients with normal serum ALT and high circulating concentrations of HBV DNA display profound peripheral immunological tolerance. It is difficult to discern antigen-specific T cells in the blood or liver and treatment remains difficult, probably for this reason. These individuals are poor responders to interferon therapy, and poor short-term responders to

nucleoside or nucleotide antiviral drugs. Spontaneous seroconversion rates remain low in this group.

In HBeAg-positive patients, progression to cirrhosis occurs at an annual rate of 2.0 to 5.5%, with a cumulative 5-year incidence of progression of 8 to 20%. Recurrent exacerbations and bridging fibrosis with severe necro-inflammatory changes characterize patients who are more likely to progress to cirrhosis. The reported yearly incidence of hepatic decompensation is about 3%, with a 5-year cumulative incidence of 16%. In a European multicenter, longitudinal study to assess the survival of 366 cases of HBsAg-positive–compensated cirrhosis, death occurred in 23% of patients, mainly due to liver failure or HCC. The cumulative probability of survival in this cohort was 84% and 68% at 5 and 10 years, respectively. The worst survival rate was seen in HBeAg and HBV DNA-positive subjects [8]. HBeAg-positive Chinese patients were more likely to develop HCC [9-10]. However, seroconversion to anti-HBe, occurring relatively late in patients who acquire the disease early in life, is not necessarily a marker of remission.

ANTIVIRAL THERAPY FOR HEPATITIS B

Treatment of acute hepatitis B

Most icteric patients with acute hepatitis B resolve their infection and do not require treatment. Fulminant hepatitis B is a severe form of acute infection complicated by encephalopathy, bleeding and liver failure. Subacute hepatic necrosis is characterized by a more protracted acute course and transition to chronic hepatitis with ongoing HBV replication. Patients with fulminant hepatitis (including acute and subacute forms) should be considered for liver transplantation, if appropriate. Interferons are not used for the treatment of acute or fulminant hepatitis. There are no controlled trials of LAM or ADV for patients with acute fulminant or subacute fulminant hepatitis. However, uncontrolled reports suggest that LAM may be effective in these patients, and if there is evidence of ongoing HBV replication therapy can be administered carefully. Randomized, controlled clinical trials will probably not be performed in this group of patients [11-14].

Therapy of chronic hepatitis B

The spectrum of disease states associated with chronic hepatitis B has not yet been completely characterized. Treatment of hepatitis B remains complex, with somewhat unpredictable responses.

Existing antiviral agents either inhibit hepatitis B replication or invoke immune responses, which may be necessary but not sufficient to effect viral control.

Indications

Many clinicians consider a liver biopsy helpful in determining the degree of necro-inflammation and fibrosis in HBeAg-positive patients. In many centers a biopsy is used to assess the stage and grade of inflammation, as liver morphology can be a factor in the decision to treat. Progression of disease is often punctuated by episodes of activity which injure the liver. Patients with mild disease may not require immediate treatment and should be carefully and regularly monitored. Most clinicians recommend therapy only if there is evidence of moderate-to-severe activity. HBeAg-positive patients should be followed for several months to determine their status. Antiviral therapy should be considered if there is: 1) active HBV replication (HBV DNA above 1,000,000 copies/mL); 2) persistently elevated ALT (for 3-6 months); and, 3) biopsy shows active hepatitis i.e. inflammation, necrosis or accumulating fibrosis. HBeAg-positive patients with more disease activity may have a better chance of seroconverting to anti-HBe with treatment than those with less disease activity.

Patients should be carefully and regularly monitored to identify changes in the pattern of disease based on increasing ALT levels and a decline in viral load.

Goals of treatment

The major goals of treating for HBeAg-positive hepatitis B are the prevention of progression to cirrhosis, end-stage liver disease or HCC. Extrahepatic manifestations of hepatitis B such as glomerulonephritis or polyarteritis nodosa require treatment. The immediate treatment objectives depend upon the stage of disease. If the disease has not progressed to cirrhosis then the goal is the prevention of progression to advanced fibrosis or cirrhosis. If cirrhosis has developed then preventing decompensation, HCC or death is important. If HBV replication can be suppressed, the accompanying reduction in

histological chronic active hepatitis lessens the risk of cirrhosis and HCC [15]. It may be difficult to reduce the risk of HCC in the short-term. Studies in the Far East have shown that the cumulative incidence of cirrhosis, decompensation and HCC are dependent upon ongoing viral replication – and also, most probably, active disease.

If decompensated disease is already present, it is important to reduce viral load and stabilize the disease. In some patients this may prevent the need for liver transplantation. There is a reduced risk of recurrence if viral loads are lower. Suppression of HBV replication improves liver function and results in a decrease in the Child-Pugh score in patients with early decompensation.

End-points

The end-points of treatment for HBeAg-positive disease differ to those for patients with HBeAg-negative disease. If HBV replication is suppressed with an accompanying improvement in serum ALT and hepatic necro-inflammatory disease, it is reasonable to assume that disease outcome will be improved. Serological markers help determine treatment outcome in HBeAg-positive disease. At present the goal of antiviral therapy for HBeAg-positive disease is to obtain loss of HBeAg and long-lasting seroconversion to anti-HBe. In HBeAg-positive disease, reduction in HBV replication leads to a reduction in ALT. A reduction in HBV DNA concentrations to <10,000 copies/mL or to levels undetectable by sensitive polymerase chain reaction (PCR) of 50 IU/mL (<200 copies/mL), may become the benchmark for treatment. Histological improvement follows suppression of necro-inflammatory disease.

Loss of HBeAg and seroconversion to anti-HBe is a potential end-point in HBeAg-positive patients, although treatment with nucleoside analogs should be prolonged for at least 6 months after loss of HBeAg. Loss of HBeAg and associated viral suppression leads to biochemical remission, histological improvement, and in a small percentage of patients, loss of HBsAg. Histological improvement and declining concentrations of covalently closed circular DNA (cccDNA) within cells has been documented. Unfortunately a variable T cell response suggests that finite courses of treatment are only sufficient in a minority of HBeAg-positive patients, and that most patients still require long-term maintenance suppressive therapy. Categorical analysis has not clarified the relative or absolute reductions in ALT and cccDNA concentrations necessary to predict histological improvement and HBeAg seroconversion, although there are some

indications that this is the case. Thus, it is not clear whether marked reductions in HBV DNA (for example, 7 $\log_{10}$ copies/mL) are critical for long-term therapy, although the rapidity and efficacy of HBV DNA reduction is clearly important in the development of resistance.

Antiviral therapies

Two major groups of antiviral drugs are used in the treatment of HBV: 1) interferon alfa or pegylated interferon alfa; and, 2) nucleoside or nucleotide analogs including LAM, ADV, and entecavir (ETV). There are numerous new nucleosides and nucleotides in the pipeline. Thus available nucleosides may shortly include LAM, ADV, ETV, tenofovir (TDF), emtricitabine (FTC), telbivudine (LdT), clevudine, elvucitabine, valtorcitabine, amdoxovir, racivir, MIV 210, β-L-FddC, alamifovir and hepavir B.

The patterns of response with nucleosides are basically similar, although these agents have different structures and inhibit different phases of hepatitis B replication including: 1) the priming of reverse transcription; 2) elongation of (-) strand DNA; 3) DNA-dependent DNA polymerase activity; and, 4) (+) strand synthesis. Nucleosides and nucleotides have different mechanisms of action, and their pharmacokinetics, inhibitory capacity, onset of action, resistance patterns and rates of HBeAg seroconversion vary during the first year of treatment. Interferon alfa may have additional immunomodulatory properties. For many patients, longer durations of therapy are required to suppress viral replication and slow the disease process.

Approaches to therapy of hepatitis B

Therapy for hepatitis B may be a finite course, continuous or long-term (or indefinite suppressive therapy). For many patients, the treatment course is undefined at the start, and is dependent upon the initial response. It is difficult to predict whether monotherapy will suffice, or whether combination therapies are necessary or more beneficial. Thus, several treatment options exist for individual patients, making the choice of first-line and second-line treatment somewhat difficult [16]. Recent evidence from clinical trials, whose goals are to demonstrate short-term efficacy and safety, has not greatly improved prediction of outcome. Several guidelines have been published, but these require regular and frequent reassessment [17,18]. There is currently no clear consensus on the approach to therapy,

because newer agents with different efficacy and rates of resistance are still being evaluated.

TREATMENT RESPONSES WITH INTERFERONS

Interferons

Interferon alfa binds to cell receptors and activates secondary messengers to initiate production of multiple proteins which are pivotal for cell defense against viruses. The mechanisms of action are complex. The antiviral effects of interferon include degradation of viral mRNA, inhibition of viral protein synthesis, and prevention of viral infection. The immunomodulating effects of interferon include enhancement of antigen presentation by human leukocyte antigens (HLA) I and II to the immune system, activation of natural killer cells and other immune cells, and increased cytokine production.

The main advantages of interferon alfa compared with nucleoside analogs are the absence of resistance, and the possibility of a finite treatment course [19-21]. A meta-analysis of 15 randomized controlled trials in HBeAg-positive patients showed a 33% HBeAg seroconversion rate after 16 weeks of interferon alfa treatment compared with 12% in untreated control patients [22]. The incidence of HBsAg loss was 7.8% and 1.8%, respectively.

Pegylated interferon alfa-2a

Pegylated forms of interferon alfa with improved pharmacokinetic profiles and more convenient once-weekly administration are licensed for the treatment of hepatitis C, and pegylated interferon alfa-2a is licensed for the treatment of hepatitis B. Pegylated alfa interferons are replacing standard interferons for the treatment of chronic hepatitis C infection, and they will probably do the same for the treatment of chronic hepatitis B infection. The mechanisms of action are similar to those of standard interferons. The efficacy of pegylated interferon alfa-2a in the treatment of HBeAg-positive and -negative chronic hepatitis B has been demonstrated in 2 large pivotal trials: pegylated interferon alfa-2b has also been shown to be active against HBeAg-positive chronic hepatitis [23,24].

HBeAg-positive patients

A study in HBeAg-positive patients compared treatment for 48 weeks with: 1) pegylated interferon alfa-2a alone; 2) pegylated interferon

alfa-2a and LAM in combination; and, 3) LAM monotherapy [23]. After 24 weeks' follow-up, HBeAg seroconversion rates were 32%, 27% and 19%, respectively. Alanine aminotransferase normalization occurred in 41%, 39% and 28% of the same groups. HBeAg levels above 100 IU/mL at weeks 12 and 24 were highly predictive of failure to achieve seroconversion while low HBeAg levels at baseline, week 12, and week 24 correlated with improved rates of seroconversion.

The addition of LAM to pegylated interferon alfa-2a did not improve seroconversion rates compared to pegylated interferon alfa-2a alone. However, in HBeAg-positive patients who received pegylated interferon alfa-2a, a -7.2 $\log_{10}$ suppression of HBV DNA was found at the end of 48 weeks compared to -4.5 $\log_{10}$ in patients treated with pegylated interferon alfa-2a alone. These data suggest a possible additive effect during treatment. Resistance to LAM was reduced in combination therapy. These results during treatment did not lead to higher seroconversion rates during follow-up, but suggest that prolongation of treatment in these groups with an oral agent such as a nucleoside or nucleotide might consolidate the on-treatment response.

Pegylated interferon alfa-2b has also been shown to be active in HBeAg-positive patients, with similar seroconversion rates [24]. Genotype and other baseline factors may affect the response to pegylated interferon alfa-2a in HBeAg-positive chronic hepatitis B: patients with genotypes A or B tend to respond better than patients with genotypes C or D, for example. Thus the highest HBeAg seroconversion rates to date in HBeAg-positive patients after 1 year of treatment have been reported with standard and pegylated interferon alfa. Somewhat higher HBeAg (and HBsAg) seroconversion rates in HBeAg-positive patients suggest that a finite course of treatment may be sufficient in these patients. Relapse rates are high after 48 weeks of pegylated interferon alfa-2a treatment in anti-HBe–positive patients. Long-term treatment is necessary in most anti-HBe–positive patients, and the pertinence of long-term pegylated interferons is uncertain in this group

Interferon should be used with caution and with regular monitoring in patients with compensated cirrhosis, due to the risk of hepatic decompensation with prolonged treatment [25]. Moreover, serious bacterial infections have been reported in this group of patients [26].

Frequent side effects and the need for close monitoring are the main disadvantages of interferon alfa treatment. Interferon alfa is not used in the treatment of acute or fulminant hepatitis B. The use of interferon in patients with decompensated hepatitis B is difficult due

to the effect on platelets and neutrophils, and the pro-inflammatory effects of this drug.

TREATMENT RESPONSES WITH NUCLEOSIDE ANALOGS

Nucleoside analogs have structures that are similar to natural nucleotides and compete at the HBV polymerase catalytic site during viral DNA synthesis. They lack a hydroxyl group, preventing formation of a covalent bond with the adjoining nucleotide, causing chain termination of the elongation of DNA. Although all nucleotide analogs act on HBV polymerase, their mechanisms differ; thus ADV inhibits the priming of reverse transcription, while LAM and FTC inhibit the synthesis of the viral (-) strand DNA [27]. Entecavir inhibits 3 major stages of HBV replication [28-31]. Clevudine inhibits the elongation of the (+) strand DNA and has a weaker effect on priming. Nucleic acids cannot inhibit *de novo* cccDNA formation after viral entry into the hepatocyte, and thus residual viremia persists after antiviral treatment [32-34].

A strong intrahepatic T cell response occurs with immune restitution for example after highly active antiretroviral therapy in HIV-positive patients. Lamivudine may be more effective in HIV/HBV co-infected patients with higher numbers of CD4 cells, suggesting that the immune response plays a role in antiviral responses.

Lamivudine

Lamivudine (2',3'-dideoxy-3' thiacytidine [3TC]) is a cytidine analog that competes for cytosine in the synthesis of viral DNA. It is a (-) enantiomer and a phosphorylation step is required for the transformation to active drug. This drug has a strong efficacy and safety record, and reduces HBV DNA concentrations in serum by 2 to 4 $\log_{10}$ copies/mL. Patients with chronic hepatitis B and elevated serum ALT levels have a greater chance of HBeAg loss with LAM. Lamivudine is relatively inexpensive, and has few side effects making it a good choice in patients with advanced disease. It is often used as a first-line treatment for HBeAg and anti-HBe–positive disease. The major disadvantage of LAM is the high rate of resistance in both HBeAg and anti-HBe–positive patients. Resistance to LAM has been mapped to mutations in the tyrosine-methionine-aspartate-aspartate motif of the reverse transcriptase domain of HBV DNA polymerase.

Lowering the viral load may restore some cytotoxic T cell reactivity [35].

Lamivudine is mainly eliminated by the kidneys and dosages should be adapted to creatinine clearance. Lamivudine resistance can be managed by sequential treatment with ADV or ETV but the benefits of this strategy compared to combination therapy have not been clarified.

Lamivudine in acute hepatitis B

Although 95% of immune-competent adults clear HBsAg spontaneously, LAM may play a role in acute HBV infection by preventing progression to fulminant hepatic failure. In small studies of patients with acute severe HBV, with an international normalized ratio >1.5, elevated bilirubin levels and raised ALT levels, treatment with LAM 100 mg/day may have prevented death from fulminant hepatic failure.

Lamivudine for HBeAg-positive chronic hepatitis B

After more than a decade of use in the treatment of chronic hepatitis B LAM has clearly been shown to be effective. Important information has been obtained from the early controlled trials of LAM and its use as the control arm in trials of newer agents, as well as in longer-term studies. After 1 year of treatment in HBeAg-positive patients, reductions in HBV DNA concentrations, HBeAg seroconversion, ALT normalization and histological improvement reached 44%, 17%, 41% and 52%, respectively [36]. After 5 years of therapy HBeAg seroconversion rates reach 35%. Pretreatment factors predictive of response are high baseline serum ALT levels and a high degree of histologic necro-inflammation [37-38]. Several factors, including genotype and the presence of cirrhosis may predict the long-term resistance rates to LAM: higher rates of resistance have been reported in serotype adw (genotype A) than ayw (genotype D) (54% vs. 8%, respectively) [39]. Early viral suppression, in particular HBV DNA levels either below 200 copies/mL or <3 $\log_{10}$ after 6 months of treatment, predict a lower risk of resistance after 1 year of treatment [40,41]. Lamivudine has been extensively tested in the prevention of the exacerbation of hepatitis B. It is effective in preventing reactivation although this event can be unpredictable. The argument for “deferred” or “pre-emptive therapy” seems to favor early treatment and prolonged therapy.

	LAM 100 mg	Pegylated Interferon	LAM 100 mg	ETV 0.5 mg	LAM 100 mg	LdT 600 mg
Number	272	214	355	354	463	458
Histological response*	38%	34%	62%	72%	56%	64%
HBV DNA decline (log_{10})	-5.8	-4.5	-5.4	-6.9	-5.5	-6.5
DNA negative (PCR)†	40 (5)%	25 (14)%	38%	69%	40%	60%
ALT normal	39 (41)%[a]	62 (28)%	60%	68%	75%	72%
HBeAg seroconversion	20 (19)%[a]	27 (32)%	18%	21%	22%	21%
Resistance‡	27%	4%	18%	0-2%	10%	3%

ALT=alanine aminotransferase; ETV=entecavir; HBeAg=hepatitis Be Antigen; LAM=lamivudine; PCR=polymerase chain reaction; a=end of follow-up; *=histological response was measured by varying methods; †=PCR negativity was variously measured (typically <200-<400 copies/mL); ‡=resistance defined variously; pegylated interferon study may have received LAM previously
The same definitions were used in the investigational drug and lamivudine control arm

Table 1: One year studies of pegylated interferon, entecavir and telbivudine in hepatitis Be antigen-positive patients with lamivudine as the control arm [48,85]

Thus, the efficacy of LAM monotherapy is limited by the development of resistance, restricting its use as a first-line monotherapy although it may be used in some patients with low levels of replication. Lamivudine could be (like other future nucleosides with even lower rates of resistance) the backbone of maintenance combination therapies.

The 2 main clinical concerns during LAM monotherapy are the emergence of viral resistance and withdrawal hepatitis flares. Patients who remain HBeAg-positive can have flares as resistance develops. Once resistance emerges, the clinical benefit of continuing LAM is doubtful, and resistance implies treatment failure. Adefovir (and ETV or TDF) are active against LAM-resistant hepatitis B, but LAM and ADV should both be continued in these patients rather than ADV alone. The clinical course after the development of resistance is complex and variable. Hepatitis is common, but is not always severe. Most patients experience worsening of liver disease [42].

Adefovir dipivoxil

Adefovir dipivoxil is an orally bio-available prodrug of adefovir, a phosphonate acyclic nucleotide analog of adenosine monophosphate [43]. Adefovir diphosphate acts by selectively inhibiting the reverse transcriptase-DNA polymerase of HBV by direct binding in competition with the endogenous substrate deoxyadenosine triphosphate [44]. Adefovir lacks a 3' hydroxyl group and, after incorporation into the nascent viral DNA, results in premature termination of viral DNA synthesis. Unlike other nucleoside analogs such as LAM, ADV is monophosphorylated and is not dependent on initial phosphorylation by viral nucleoside kinases to exert its antiviral effect. Adefovir is cleared by renal excretion and its pharmacokinetics are substantially altered in subjects with moderate and severe renal impairment (creatinine clearance <50 mL/min) [45,46].

Nephrotoxicity is the major side effect of high doses of ADV. Although ADV can cause a proximal convoluted tubule lesion characterized by a rise in urea and creatinine, in the 2 largest hepatitis B phase III trials involving 695 patients, no renal toxicity was found at the 10 mg dose.

Adefovir in HBeAg-positive chronic hepatitis B

The efficacy of ADV has been assessed in patients with HBeAg-positive and -negative disease as well as in chronic hepatitis B

infection. Pivotal phase III studies examined both ADV 10 mg and 30 mg to determine the most favorable dose with the best risk:benefit profile. In the HBeAg-positive trial, 515 patients were randomized to 1 of 3 arms: 1) ADV 30 mg daily; 2) ADV 10 mg daily; or, 3) placebo. The primary end-point of this study was based on the quantitative assessment of histological improvement after 48 weeks using the Knodell HAI score [47,48]. Secondary end-points were suppression of HBV replication based on the decrease in serum HBV DNA and biochemical response (as defined by reductions and normalization in ALT during therapy). A daily dose of 10 mg of ADV resulted in significant improvement compared to placebo: 1) improvement in liver histology (53% vs. 25%); 2) reductions in HBV DNA (3.52 $\log_{10}$ copies/mL vs. 0.55 $\log_{10}$ copies/mL), normalization of ALT (48% vs. 16%); and, 3) HBeAg seroconversion (12% vs. 6%). There were no significant side effects and no resistance. As a result, 10 mg ADV is the recommended and approved daily dose. A pivotal phase III trial demonstrated a dose effect at 48 weeks, 10 mg ADV resulted in 3.5 $\log_{10}$ suppression of HBV DNA, whilst 30 mg ADV resulted in 4.5 $\log_{10}$ suppression. Although the 10 mg dose has been chosen because of the more favorable risk:benefit ratio, it may not be optimal for certain patients.

HBeAg loss and ALT normalization may increase over time: 40% HBeAg seroconversion rates have been reported after 3 years of treatment. These responses are satisfactory, and suggest that continuous treatment with an antiviral drug with low resistance rates in HBeAg-positive patients can result in good HBeAg seroconversion rates that increase over time [49]. However, many patients in this study received drug misallocations, with interrupted therapy and flares in serum aminotransferases after the first year of treatment; the presented data refer to a subset of 65 HBeAg-positive patients who continued long-term treatment.

Some patients, particularly HBeAg-positive patients with a high body mass index and viral load have slow and poor primary responses. In one study the lower quartile (25%) of patients had <2.2 $\log_{10}$ reduction; the third quartile had a 2.2 to 3.5 $\log_{10}$ reduction. These effects may be found in routine clinical practice where worse compliance and a higher body mass index affect sensitivity to ADV and result in poor primary responses [50].

Patients who will respond to ADV monotherapy need to be identified. In HBeAg-positive patients, or patients with decompensated cirrhosis or high viral loads, rapid suppression of

HBV DNA replication with a low risk of primary non-response or resistance is important, and combination therapies should be effective.

Adefovir for Lamivudine resistance

There is clear evidence that ADV is effective in patients with HBV who develop resistance to LAM therapy.

Entecavir

Entecavir, also known as BMS-200475, is a cyclopentyl guanosine analog. Early studies in animals and humans indicate that ETV is a very potent inhibitor of viral replication. No clinically relevant activity against HIV has been documented [51-54]. Trials in woodchucks (an animal model of chronic hepatitis B infection) indicated that cccDNA was undetectable in liver samples, for several months post-treatment. Entecavir has been licensed for the treatment of chronic hepatitis B.

Entecavir inhibits all 3 activities of the HBV polymerase/reverse transcriptase: 1) base priming; 2) reverse transcription of the (-) strand from the pregenomic messenger RNA; and, 3) synthesis of the (+) strand of HBV DNA. Phase III trials have been completed.

Entecavir for HBeAg-positive patients

Phase II trials showed ETV to be effective in the treatment of HBV [55]. A randomized study of ETV 0.5 mg daily vs. LAM 100 mg daily for 52 weeks in 715 treatment-naïve patients showed a histological improvement in 72% of patients with ETV compared with 62% of those who were treated with LAM. Hepatitis B virus DNA was suppressed to <300 copies/mL in 67% and 36% of patients with ETV and LAM, respectively. The mean change from baseline was -6.9 $\log_{10}$ and -5.4 $\log_{10}$, respectively. HBeAg seroconversion occurred in 21% and 18% of patients with ETV and LAM, respectively. No genotypic resistance has been reported in treatment-naïve patients [56,57].

Entecavir is partially effective against LAM resistance in HBV but high doses are required. In a phase III trial, 286 LAM-resistant patients were treated with 1 mg ETV daily for 48 weeks. Histological improvement was observed in 55% of patients who received ETV, and in 28% of patients who continued 100 mg LAM. Hepatitis B virus DNA was suppressed to <300 copies/mL in 19% of patients treated with ETV compared with 1% of patients treated with LAM. Alanine aminotransferase normalized in 61% and 15% of patients,

respectively. HBeAg seroconversion was observed in 8% vs. 3%, respectively.

Entecavir is a potent inhibitor of HBV replication. Initial studies suggest that ETV is safe and well tolerated, with a frequency of adverse events similar to that of LAM. The low effective dose of ETV corresponds to the much higher affinity for the wild-type HBV polymerase than the natural 2'-deoxyguanosine 5'triphosphate, with no effect on mitochondrial function. Since the drug is excreted by the kidneys, dose adjustments are required in cases of renal impairment (creatinine clearance <50mL/min). Although ETV is more effective than LAM for viral suppression, after 1 year of treatment HBeAg seroconversion rates are not different between the 2 analogs (21% and 18%, respectively). Due to the lower resistance rates of ETV, HBeAg seroconversion rates could be increased with prolonged treatment, as happens with ADV. This suggests that 1 year of treatment is not an optimal duration. Carcinogenicity after exposure to doses more than 35-fold greater than those administered in humans has been reported in rodents. These lesions include lung and liver adenomas and carcinomas. The cumulative human risk requires post-marketing surveillance.

No genotypic changes in the HBV polymerase associated with phenotypic resistance have been detected in treatment-naïve subjects after 1 year of therapy. Entecavir is known to inhibit LAM-resistant HBV polymerase, although LAM-resistant polymerases show a somewhat reduced susceptibility to ETV – thus, higher doses of ETV (i.e. 1.0 mg) must be used. A complex picture of ETV resistance is emerging, suggesting that new reverse transcriptase changes must be made in combination with those conferring LAM resistance to reduce susceptibility to ETV.

New agents

Tenofovir

Tenofovir and ADV are related molecules with a similar mechanism of action. Tenofovir disoproxil fumarate is the prodrug of TDF. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and by DNA chain termination after incorporation into DNA. The drug is approved at a dose of 300 mg for the treatment of HIV. There is emerging clinical evidence of the efficacy of TDF in chronic hepatitis B, with low nephrotoxicity. The drug is active

against wild-type and precore mutant hepatitis B, as well as LAM-resistant HBV *in vitro* [58-63]. Tenofovir's greater efficacy may be a result of the higher active dose. Small sub-studies in HBV mono-infected and HIV/HBV co-infected patients have demonstrated that TDF is active against HBV. In the ACTG 5127 study, 26 HIV/HBV co-infected patients with LAM resistance were randomized to TDF and 25 were randomized to ADV treatment. Patients receiving 300 mg/day TDF vs. 10 mg/day ADV showed a greater time-weighted average DNA change (DVAG48) and $\log_{10}$ suppression of HBV (4.0 $\log_{10}$ vs. -3.1 $\log_{10}$). A recent trial compared the long-term treatment of TDF (72-130 weeks) and ADV (60-80 weeks) in 53 patients with LAM-resistant HBV infection and high baseline HBV DNA (>1,000,000 copies/mL) [64]. Patients treated with TDF (300 mg/day) had a faster and greater suppression of HBV DNA than those treated with ADV (10 mg/day). The absence of phenotypic HBV resistance to TDF suggests a favorable resistance profile.

Although TDF has not yet been licensed for the treatment of HBV, it may become important for the treatment of highly replicative HBV infection and HIV/HBV-co–infection. The pharmacokinetics of TDF is altered in patients with renal impairment, and the dosing intervals of TDF should be adjusted for creatinine clearance. Lactic acidosis, hepatomegaly, and steatosis have only rarely been reported in patients with HIV infection treated with antiretrovirals and TDF as such treatment may exacerbate hepatitis B. Decreases in bone mineral density have rarely been reported in HIV-positive patients.

Tenofovir may be more effective than ADV in suppressing high levels of HBV replication in treatment-naïve and LAM-resistant chronic hepatitis B. A large-scale, randomized controlled phase III trial comparing the efficacy of ADV and TDF disoproxil in HBeAg-positive and -negative patients is underway. Approval of TDF would expand the choice of HBV treatments.

Emtricitabine

Emtricitabine (2'3'-dideoxy-5'fluoro-3'thiacytidine or FTC) is a 5-fluoro oxathiolane derivative, closely related to LAM. Like LAM, FTC is a cytosine nucleoside analog. Early studies indicate that it is effective in both HBeAg-positive and HBeAg-negative patients [65,66]. Emtricitabine shows a dose-related efficacy with an average 3 $\log_{10}$ decrease in HBV DNA levels after 8 weeks of treatment with the highest doses. In a randomized 48 week study assessing treatment with 25 mg, 100 mg or 200 mg, for the first year, and 200 mg for the second year median decrease in viral load was

2.6 $\log_{10}$, 3.1 $\log_{10}$ and 2.9 $\log_{10}$, respectively. HBeAg seroconversion rates were 23%, 24% and 23%, respectively. Treatment with FTC for a longer period (2 years) resulted in normalization of ALT in 76% of patients and undetectable HBV DNA in 41% of patients. The drug is active against anti-HBe–positive chronic hepatitis B.

Due to its resemblance to LAM, FTC leads to selection of the same mutations that are associated with resistance to HBV polymerase, but less frequently. With the optimal dose of 200 mg/day, genotype resistance occurred in 6% of patients after 1 year and 19% after 2 years of treatment. These resistance rates are relatively high, limiting the role of FTC as a monotherapy, however, similarly to LAM, FTC may prove useful in combination therapy.

HBV-specific L-nucleosides: telbivudine (LdT)

Telbivudine is a thymidine analog and belongs to a new class of β-L-configuration nucleoside analogs with specific activity against hepadnavirus [67-69]. Preliminary studies have shown a pronounced inhibition of HBV replication with a safe profile and no effect on mitochondrial metabolism. Studies in woodchucks showed an impressive 8 $\log_{10}$ copies/mL reduction of woodchuck hepatitis virus DNA after 28 days of treatment. A phase III study randomized 1367 patients to either 600 mg/day LdT (n=680) or 100 mg/day LAM (n=687). In HBeAg-positive patients excellent reductions in HBV DNA were observed with LdT (mean -6.5 $\log_{10}$ copies/mL at week 52, and 60% of patients were negative by PCR). Twenty-two percent of LdT treated patients and 21% of LAM-treated patients seroconverted to anti-HBe after 1 year of treatment. Seventy-seven percent of patients had normal ALT. Interim analysis at week 76 showed that more patients achieved HBeAg loss with continued LdT treatment than with LAM (76% negativity compared to 45%, with an increase in HBeAg seroconversion of 33%). Resistance rates at 1 year were 3% for the LdT arm but they were also low for the LAM arm (8%) which raises a question as to the definition of resistance in this study.

Clevudine

Derived from deoxythymidine, clevudine is a novel L-nucleoside analog with potent anti-HBV activity [27,70-72]. The mechanism of action is mainly inhibition of viral (+) strand DNA synthesis. A marked decrease of 9 $\log_{10}$ copies/mL in viral load was observed in the woodchuck model. In the same model the combination of clevudine with FTC resulted in a marked decrease in viremia levels which was more pronounced than with each drug alone [73]. A unique

characteristic of clevudine is the slow rebound of viremia after cessation of treatment. In patients taking part in an early phase II trial, after 28 days of treatment, the median decrease in HBV DNA levels varied from 2.48 to 2.95 $\log_{10}$ copies/mL with 3 different doses. At the end of the 20-week follow-up period, a slow increase in HBV DNA levels was noted.

Similar results have been reported in HBeAg-positive patients. No serious adverse events have been reported with clevudine. *In vitro* studies suggest that there may be cross-resistance with LAM-resistant HBV mutants. In animal studies, resistance occurred in the B domain of the polymerase gene, after 12 months of treatment.

Resistance to nucleoside analogs

Long-term treatment with a single nucleoside analog often leads to drug resistance due to mutations of the reverse transcription polymerase gene. Hepatitis B virus replicates its genome via reverse (RNA) transcription which results in a reduced proof-reading function. The rate of nucleic acid substitution is estimated at $2x10^{-5}$ per site per annum [74]. The viral half-life is 1-2 days, which implies a *de novo* viral production of 10^{11} virions per day. In patients with high viral load; mutations occur at a rate of 10^3 per day. Hepatitis B virus circulates as a quasispecies, and drug-resistant mutations pre-date antiviral treatment as a minor species [31,75-77]. In virus infection with high rates of replication, selective pressure results in the emergence of drug-resistant mutant strains. This explains the high frequency of LAM-resistance in patients with high rates of replication, or the resistance to ADV in patients with slow declines in HBV DNA after 48 weeks of treatment. An understanding of the patterns of HBV resistance helps determine appropriate therapy following sequential treatment.

CIRRHOSIS

Interferon increases the risk of sepsis and decompensation in patients with advanced cirrhosis as it is a pro-inflammatory cytokine. However, interferon can be used for the treatment of well compensated cirrhosis in selected patients with sufficient hepatic reserve and acceptable levels of liver function. Treatment of cirrhosis should not be based on ALT levels as these may be normal in advanced disease. It is not clear whether patients with lower levels of DNA (<100,000 copies/mL) benefit from treatment, and the threshold

level for treatment has not been determined. Hepatic decompensation may occur due to exacerbation of the disease during treatment with nucleoside analogs, and these patients should be carefully monitored.

It has been established that prolonged suppression of viremia reduces the risk of progression to cirrhosis or the development of decompensated liver disease and possibly HCC. Clinical studies indicate that prolonged and adequate suppression of viremia may stabilize patients and delay or even prevent the need for transplantation. Recent longer-term studies have suggested that HBV DNA suppression with LAM may also be beneficial: based on Kaplan-Meier, in patients with cirrhosis, after 3 years of treatment, disease progression was shown to be reduced from 5% to 21% compared with placebo [78]. However, the development of LAM resistance significantly limits this clinical benefit and although HCC can be reduced with LAM, it remains a persistent risk.

Decompensated cirrhosis and liver transplantation

Interferon is not recommended in patients with decompensated cirrhosis. Such patients should be treated in specialized liver units, because the application of antiviral therapy is complex. Prophylactic therapy is recommended in all patients who will undergo liver transplantation for end-stage hepatitis B, in order to lower levels of HBV DNA to $<10^5$ copies/mL before transplantation. Patients may improve over a period of 3-6 months. However certain patients, such as those with advanced hepatic disease, a high Child-Pugh score, and jaundice, will not benefit from this treatment. Moreover, treatment of decompensated disease with a slow onset of action and suboptimal viral suppression can be detrimental. The aim of treatment is to reduce viremia prior to transplantation. Exacerbation and resistance may occur in patients with cirrhosis. These patients require long-term therapy, with careful monitoring for resistance and flares. Regression of fibrosis has been reported. There are fewer data on the efficacy of newer potent agents such as ETV and TDF in this group.

Recurrent HBV infection in the transplanted liver was a major problem. A retrospective study of liver transplantation in Europe before LAM became available showed that patients with low levels of hepatitis B replication at transplantation and those receiving long-term immunoprophylaxis with hepatitis B immunoglobulin (HBIg) had a reduced risk of recurrent HBV infection and reduced mortality [79]. Lamivudine and ADV have improved these outcomes further. Pre-transplant treatment with LAM resulted in suppression of

HBV DNA levels in 12 of 19 patients [80,81]. Both HBIg and LAM are now used prophylactically and recurrent HBV has become rare [82-84]. However, cases associated with LAM resistance are problematic, because patients with recurrent hepatitis B post-transplant may develop fibrosing cholestatic hepatitis, a manifestation of high levels of viral replication in immunosuppressed patients [85,86].

Adefovir is important for patients with post-transplant LAM resistance. In an open label study, 127 liver transplant patients with LAM-resistant HBV were treated with ADV 10 mg [87]. Treatment resulted in a median 4 $\log_{10}$ copies/mL reduction in HBV DNA concentrations at 48 weeks indicating that ADV should be considered as a second-line therapy in patients who develop LAM resistance in this setting. A pre- and post-transplant regimen of LAM and HBIg reduces the risk of graft infection to <10%, as long as HBV is suppressed before transplantation. Lamivudine, ADV, and the newer, more potent drugs are suitable for treating such patients.

The optimal timing of transplantation has not been established, but selection of resistant strains before surgery should be avoided. Shorter courses of HBIg and other forms of prophylaxis, including ADV in combination with LAM, are being studied. Antiviral therapy to prevent post-transplantation recurrence probably requires lifelong treatment.

PREGANCY

Recent studies suggest that LAM therapy in pregnant women with high levels of viremia during the last trimester of pregnancy, reduces the risk of transmission to newborns who receive HBIg and a vaccine at birth. These studies require confirmation.

EXTRAHEPATIC DISEASE

HBsAg-positive patients with extrahepatic manifestations and active HBV replication may respond to antiviral therapy with either interferon or a nucleoside.

HDV/HBV CO-INFECTION

The mainstay of treatment for HDV co-infection remains long-term interferon with which some patients become HDV RNA-negative, or even HBsAg-negative, with an accompanying improvement in

histology. To date, treatment with nucleoside analogs has proved disappointing but it is hoped that newer agents may prove useful in this indication [88-96].

HIV/HBV CO-INFECTION

The management of hepatitis B is more complex when patients are co-infected with HIV. Hepatitis B virus has little effect on the natural history of HIV infection, or on the treatment for HIV. However, HIV, and HIV treatments profoundly affect the natural history of HBV. This should be considered when choosing treatment for HBV, along with the impact of HBV treatment on HIV. Hepatitis B virus may be reactivated upon HIV infection [97]. Co-infection with HIV and HBV leads to lower rates of HBeAg seroconversion, and higher HBV DNA concentrations. Nucleosides clearly have a role to play in the treatment of HIV/HBV co-infection.

STRATEGIES AND CHOICES FOR TREATMENT

For patients with high levels of viral replication or advanced disease choice of treatment is between a nucleoside analog and pegylated interferon.

In HBeAg-positive patients, anti-HBe–positive patients with high levels of viral replication, or in patients with decompensated cirrhosis, rapid suppression of HBV DNA replication in order to lower the risk of primary non-response or resistance is important, and combination therapies may be indicated. The efficacy of interferon alfa is restricted, but some patients respond and treatment is cost effective.

Which antiviral drug is most appropriate and how can treatment be tailored to optimize response? Who should receive combination therapy *de novo*, and who will respond to monotherapy? Which patients can expect a finite course of treatment, compared to those requiring long-term maintenance suppression? When will the continuation of viral suppression result in HBeAg seroconversion, and hence cessation of therapy?

These questions remain unanswered. Ideally, the drugs used in monotherapy should rapidly reduce levels of viremia, engender few suboptimal responses, and have a low rate of resistance. The potent agents currently in use appear safe if used at appropriate doses. However, development of resistance may necessitate a sequential use of drugs, which could engender multidrug resistant hepatitis B. Different patterns of viral resistance require specific treatment.

In the treatment of chronic HBV combination therapy is critical in order to avoid and overcome the problem of persistence and selection of drug resistant mutants. The arguments for and against combination therapy continue in the absence of data about the most appropriate combinations. Theoretically a synergistic effect would be achieved if agents with dissimilar structures and actions were used. This would enhance rates of viral suppression, and prevent or delay the occurrence of drug resistance. There are no data showing synergy in specific combinations tested to date, although it has been established that resistance to LAM and ADV are reduced when used in combination. This combination, however, has certain drawbacks, because some patients show poor primary responses to ADV. In order to avoid the costs of multidrug-resistant hepatitis B infection it is important that recommendations for combination therapies, such as TDF and ETV, are clarified.

CONCLUSIONS

At present, clinicians, patients and public health authorities must make treatment choices based on data that are incomplete. Although the health outcome measures with new agents have been evaluated with short- and medium-term results in 1- or 2-year registration trials, there is no evidence from longer-term studies, or from combination therapy to optimize treatment of hepatitis B. Studies of combination therapy would be helpful but they are expensive, and for the moment have not received the financial support of the pharmaceutical industry. Policy guidelines should be established, and algorithms for the treatment of hepatitis B should be developed so that monotherapies can be used when appropriate, and combination and concomitant therapies when necessary. The American, European, Asian and Canadian Associations for the Study of the Liver, and the NIH have issued guidelines for the treatment of hepatitis B. Some controversies and areas of disagreement remain and the clinical care of hepatitis B is still evolving. Treatment is influenced by the availability of new interferons, nucleosides and nucleotides, and guidelines must therefore be continually updated.

REFERENCES

1. Ferrari C, Penna A, Bertoletti A Valli A, Antoni AD, Giuberti T, Cavalli A, Petit MA, Fiaccadori F. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. J Immunol 1990;145:3442-3449.
2. Ferrari C, Penna A, Bertoletti A, Cavalli A, Valli A, Missale G, Pilli M, Marchelli S, Giuberti T, Fiaccadori F. Immune pathogenesis of hepatitis B. Arch Virol 1992;4:S11-S18.
3. Webster GJ, Reignat S, Brown D, Ogg GS, Jones L, Seneviratne SL, Williams R, Dusheiko G, Bertoletti A. Longitudinal analysis of CD8+ T cells specific for structural and nonstructural hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. J Virol 2004;78:5707-5719.
4. 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.
5. Lindh M, Hannoun C, Dhillon AP Norkrans G, Horal P. Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers. J Infect Dis 1999;179:775-782.
6. Orito E, Mizokami M, Sakugawa H Michitaka K, Ishikawa K, Ichida T, Okanoue T, Yotsuyanagi H, Iino S. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Hepatology 2001;33:218-223.
7. Tsubota AK, Arase Y, Ren FR, Tanaka H, Ikeda K, Kumada H. Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. J Med Virol 2001;65:257-265.
8. 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.
9. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H, Kawashima T. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797-1801.
10. 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.
11. Kondili LA, Osman H, Mutimer D. The use of lamivudine for patients with acute hepatitis B (a series of cases). J Viral Hepat 2004;11:427-31.
12. Schmilovitz-Weiss H, Ben Ari Z, Sikuler E, Zuckerman E, Sbeit W, Ackerman Z, Safadi R, Lurie Y, Rosner G, Tur-Kaspa R, Reshef R. Lamivudine treatment for acute severe hepatitis B: a pilot study. Liver Int 2004;24:547-551.

13. Petrelli E, Balducci M, Pieretti C, Rocchi MB, Clementi M, Manzin A. Lamivudine treatment failure in preventing fatal outcome of de novo severe acute hepatitis B in patients with haematological diseases. J Hepatol 2001;35:823-826.

14. Reshef R, Sbeit W, Tur-Kaspa R. Lamivudine in the treatment of acute hepatitis B. N Engl J Med 2000;343:1123-1124.

15. 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.

16. Perrillo RP, Gish RG, Peters M, Keeffe EB, Alberti A, Buti M, Cooksley WG, Fried MW, Hadziyannis SJ, Liaw YF, Naoumov NV, Schiff ER, Thio CL, Tsai N, Schalm S. Chronic hepatitis B: a critical appraisal of current approaches to therapy. Clin Gastroenterol Hepatol 2006;4:233-248.

17. McMahon BJ. Selecting appropriate management strategies for chronic hepatitis B: who to treat. Am J Gastroenterol 2006;101:S7-S12.

18. Lok AS. New treatment of chronic hepatitis B. Semin Liver Dis 2004;24:77-82.

19. 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.

20. Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev 2001;14:778-809.

21. Dusheiko GM, Zuckerman AJ. Therapy for hepatitis B. Curr Opin Infect Dis 1991;4:785-794.

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

23. Lau GK, Piratvisuth T, Luo KX Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.

24. Janssen HL, van Zonneveld M, Senturk H Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters HG, Zondervan P, Hansen B, Schalm SW. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.

25. 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.

26. Hoofnagle JH, Di Bisceglie AM, Waggoner JG, Park Y. Interferon alfa for patients with clinically apparent cirrhosis due to chronic hepatitis B. Gastroenterology 1993;104:1116-1121.

27. 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.

28. De Clercq E. Perspectives for the treatment of hepatitis B virus infections. Int J Antimicrob Agents 1999;12:81-95.

29. De Man RA, Heijtink RA, Niesters HGM, Schalm SW. New developments in antiviral therapy for chronic hepatitis B infection. Scand J Gastroenterol 1995;30:S100-S104.

30. Dusheiko GM. Treatment and prevention of chronic viral hepatitis. Pharmacol Ther 1995;65:47-73.

31. Torresi J, Locarnini S. Antiviral chemotherapy for the treatment of hepatitis B virus infections. Gastroenterology 2000;118:S83-S103.

32. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C, Marcellin P, Goodman Z, Delaney WE 4th, Xiong S, Brosgart CL, Chen SS, Gibbs CS, Zoulim F. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004;126:1750-1758.

33. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol 2005;42:302-308.

34. Zoulim F. Antiviral therapy of chronic hepatitis B: can we clear the virus and prevent drug resistance? Antivir Chem Chemother 2004;15:299-305.

35. Boni C, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, Scognamiglio P, Boehme R, Panebianco R, Fiaccadori F, Ferrari C. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. Journal of Clinical Investigation 1998;102:968-975.

36. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. N Engl J Med 1998;339:61-68.

37. Perrillo RP, Lai CL, Liaw YF, Dienstag JL, Schiff ER, Schalm SW, Heathcote EJ, Brown NA, Atkins M, Woessner M, Gardner SD. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36:186-194.

38. Chien RN, Liaw YF, Atkins M. Pretherapy alanine transaminase level as a determinant for hepatitis Be antigen seroconversion during lamivudine therapy in patients with chronic hepatitis B. Asian Hepatitis Lamivudine Trial Group. Hepatology 1999;30:770-774.

39. Zollner B, Petersen J, Puchhammer-Stockl E, Kletzmayr J, Sterneck M, Fischer L, Schroter M, Laufs R, Feucht HH. Viral features of lamivudine resistant hepatitis B genotypes A and D. Hepatology 2004;39:42-50.

40. Lai CL, Leung N, Teo EK Tong M, Wong F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown NA. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis Be antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528-536.

41. Yuen MF, Sablon E, Hui CK Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology 2001;34:785-791.

42. 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.

43. Kramata P, Votruba I, Otova B, Holy A. Different inhibitory potencies of acyclic phosphonomethoxyalkyl nucleotide analogs toward DNA polymerases alfa, delta and epsilon. Mol Pharmacol 1996;49:1005-1011.

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

45. Cundy KC, Barditch-Crovo P, Walker RE, Collier AC, Ebeling D, Toole J, Jaffe HS. Clinical pharmacokinetics of adefovir in human immunodeficiency virus type 1-infected patients. Antimicrob Agents Chemother 1995;39:2401-2405.

46. Shaw JP, Louie MS, Krishnamurthy VV, Arimilli MN, Jones RJ, Bidgood AM, Lee WA, Cundy KC. Pharmacokinetics and metabolism of selected prodrugs of PMEA in rats. Drug Metab Dispos 1997;25:362-366.

47. 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 for the treatment of hepatitis Be antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.

48. 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.

49. Marcellin P, Chang T, Lim F, Sivert W, Tong N, Arterurn S, Xiong F, Brosgart CL, Currie G. Increasing serologic, virologic and biochemical response over time to Adefovir Dipivoxil (ADV) 10 mg in HBeAg+ patients (abstract 73). J Hepatol 2005;42:S31-32.

50. Durantel S, Werle B, Durantel D, Pichoud C, Trépo C, Zoulim F; INSERM U271, Lyon, France; Currie G, Xiong S, Brosgart CL; Gilead Sciences, Foster City, CA. Different profiles of response to adefovir dipivoxil and factors that may influence response in patients with chronic hepatitis B (abstract 1132). Hepatology 2004;40:S654.

51. Colonno RJ, Genovesi EV, Medina I, Lamb L, Durham SK, Huang ML, Corey L, Littlejohn M, Locarnini S, Tennant BC, Rose B, Clark JM. Long-term entecavir treatment results in sustained antiviral efficacy and prolonged life span in the woodchuck model of chronic hepatitis infection. J Infect Dis 2001;184:1236-1245.

52. De Man RA, Wolters LMM, 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 virus infection. Hepatology 2001;34:578-582.

53. Honkoop P, De Man RA. Entecavir: a potent new antiviral drug for hepatitis B. Expert Opin Investig Drugs 2003;12:683-688.

54. Marion PL, Salazar FH, Winters MA, Colonno RJ. Potent efficacy of entecavir (BMS-200475) in a duck model of hepatitis B virus replication. Antimicrob Agents Chemother 2002;46:82-88.

55. 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.

56. Chang TT, Gish RG, De Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-1010.

57. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-1020.

58. Dore GJ, Cooper DA, Pozniak AL, DeJesus E, Zhong L, Miller MD, Lu B, Cheng AK. Efficacy of tenofovir disoproxil fumarate in antiretroviral therapy-naive and -experienced patients coinfected with HIV-1 and hepatitis B virus. J Infect Dis 2004;189:1185-1192.

59. Lacombe K, Gozlan J, Boelle PY, Serfaty L, Zoulim F, Valleron AJ, Girard PM. Long-term hepatitis B virus dynamics in HIV-hepatitis B virus-co-infected patients treated with tenofovir disoproxil fumarate. AIDS 2005;19:907-915.

60. Nelson M, Portsmouth S, Stebbing J, Atkins M, Barr A, Matthews G, Pillay D, Fisher M, Bower M, Gazzard B. An open-label study of tenofovir in HIV-1 and Hepatitis B virus co-infected individuals. AIDS 2003;17:7-10.

61. Benhamou Y, Tubiana R, Thibault V. Tenofovir disoproxil fumarate in patients with HIV and lamivudine-resistant hepatitis B virus. N Engl J Med 2003;348:177-178.

62. Bruno R, Sacchi P, Zocchetti C, Ciappina V, Puoti M, Filice G. Rapid hepatitis B virus-DNA decay in co-infected HIV-hepatitis B virus 'e-minus' patients with YMDD mutations after 4 weeks of tenofovir therapy. AIDS 2003;17:783-784.

63. Kuo A, Dienstag JL, Chung RT. Tenofovir disoproxil fumarate for the treatment of lamivudine-resistant hepatitis B. Clin Gastroenterol Hepatol 2004;2:266-272.

64. Van Bommel F, Wunsche T, Mauss S, Reinke P, Bergk A, Schurmann D, Wiedenmann B, Berg T. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40:1421-1425.

65. Gish RG, Trinh H, Leung N, Chan FK, Fried MW, Wright TL, Wang C, Anderson J, Mondou E, Snow A, Sorbel J, Rousseau F, Corey L. Safety and antiviral activity of emtricitabine (FTC) for the treatment of chronic hepatitis B infection: A two-year study. J Hepatol 2005;43:60-66.

66. Korba BE, Schinazi RF, Cote P Tennant BC, Gerin JL. Effect of oral administration of emtricitabine on woodchuck hepatitis virus replication in chronically infected woodchucks. Antimicrob Agents Chemother 2000;44:1757-1760.

67. 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.

68. 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, Cretton-Scott E, Schinazi RF, Sommadossi JP. Anti-HBV specific beta-L-2'-deoxynucleosides. Nucleosides Nucleotides Nucleic Acids 2001;20:597-607.

69. 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:S119-S129.

70. Kocic I. Clevudine University of Georgia/Abbott/Bukwang/Triangle/Yale University. Curr Opin Investig Drugs 2000;1:308-313.

71. 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. A phase II dose-escalating trial of clevudine in patients with chronic hepatitis B. Hepatology 2004;40:140-148.

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

73. Jacquard AC, Nassal M, Pichoud C, Ren S, Schultz U, Guerret S, Chevallier M, Werle B, Peyrol S, Jamard C, Rimsky LT, Trepo C, Zoulim F. Effect of a combination of clevudine and emtricitabine with adenovirus-mediated delivery of gamma interferon in the woodchuck model of hepatitis B virus infection. Antimicrob Agents Chemother 2004;48:2683-2692.

74. Lewin SR, Ribeiro RM, Walters T, Lau GK, Bowden S, Locarnini S, Perelson AS. Analysis of hepatitis B viral load decline under potent therapy: Complex decay profiles observed. Hepatology 2001;34:1012-1020.

75. Feld J, Locarnini S. Antiviral therapy for hepatitis B virus infections: new targets and technical challenges. J Clin Virol 2002;25:267-283.

76. Locarnini S. Molecular virology of hepatitis B virus. Semin Liver Dis 2004;24:S3-S10

77. Shaw T, Locarnini S. Combination chemotherapy for hepatitis B virus − The path forward? Drugs 2000;60:517-531.

78. Liaw YF, Sung JJ, Chow WC Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-1531.

79. 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. NEJM 1993;329:1842-1847.

80. 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.

81. 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.

82. 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.

83. 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.

84. 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.

85. 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.

86. 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.

87. 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. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post-liver transplantation patients. Hepatology 2003;38:1419-1427.

88. Andreone P, Spertini F, Negro F. Lamivudine-associated remission of chronic hepatitis delta. Ann Intern Med 2000;132:598.

89. Battegay M, Simpson LH, Hoofnagle JH, Sallie R, Di Bisceglie AM. Elimination of hepatitis delta virus infection after loss of hepatitis B surface antigen in patients with chronic delta hepatitis. J Med Virol 1994;44:389-392.

90. Berk L, De Man RA, Housset C Berthelot P, Schalm SW. Alfa lymphoblastoid interferon and acyclovir for chronic hepatitis delta. Prog Clin Biol Res 1991;364:411-420.

91. Bordier BB, Ohkanda J, Liu P, Lee SY, Salazar FH, Marion PL, Ohashi K, Meuse L, Kay MA, Casey JL, Sebti SM, Hamilton AD, Glenn JS. In vivo antiviral efficacy of prenylation inhibitors against hepatitis delta virus. J Clin Invest 2003;112:407-414.

92. Cariani E, Ravaggi A, Puoti M, Mantero G, Albertini A, Primi D. Evaluation of hepatitis delta virus RNA levels during interferon therapy by analysis of polymerase chain reaction products with a nonradioisotopic hybridization assay. Hepatology 1992;15:685-689.

93. Chen TZ, Wu JC, Au LC, Choo KB. Specific inhibition of delta antigen by in vitro system by antisense oligodeoxynucleotide: Implications for translation mechanism and treatment. J Virol Methods 1997;65:183-189.

94. Di Bisceglie AM, Negro F, Smedile A et al. Alfa interferon therapy of chronic delta hepatitis: A pilot study. Prog Clin Biol Res 1991;364:393-397.

95. Farci P, Mandas A, Coiana A, Lai ME, Desmet V, Van Eyken P, Gibo Y, Caruso L, Scaccabarozzi S, Criscuolo D, Ryff JC, Balestrieri A. Treatment of chronic hepatitis D with interferon alfa-2a. N Engl J Med 1994;330:88-94.

96. Farci P, Roskams T, Chessa L, Peddis G, Mazzoleni AP, Scioscia R, Serra G, Lai ME, Loy M, Caruso L, Desmet V, Purcell RH, Balestrieri A. Long-term benefit of interferon alfa therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. Gastroenterology 2004;126:1740-1749.

97. Altfeld M, Rockstroh JK, Addo M, Kupfer B, Pult I, Will H, Spengler U. Reactivation of hepatitis B in a long-term anti-HBs-positive patient with AIDS following lamivudine withdrawal. J Hepatol 1998;29:306-309.

Why Treat Patients with HBeAg-Positive Chronic Hepatitis B with Pegylated Interferon?

C-K. Hui, G. K. Lau

INTRODUCTION

Approximately 2 billion people have been infected with the hepatitis B virus (HBV) worldwide, and 350 million of them have become chronically infected. Individuals with chronic hepatitis B infection are at an increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC); 15 to 40% of these individuals will develop these serious sequelae during their lifetime [1,2]. Two groups of drugs have been approved by the US Federal Drug Administration for the treatment of chronic HBV: immunomodulators such as conventional interferon alfa and pegylated interferon alfa-2a, and nucleos(t)ide analogs such as lamivudine (LAM), adefovir dipivoxil (ADV) and entecavir (ETV). However, not all patients with chronic hepatitis B infection respond to or can tolerate these treatments.

Approved antiviral regimens, especially the nucleos(t)ide analogs, have been shown to improve the outcome of disease while on therapy but do not provide a cure or induce a durable remission in most patients with chronic HBV. Lamivudine which was the first nucleos(t)ide analog to be approved for the treatment of chronic HBV, has a favorable safety profile but long-term therapy with this drug can lead to the selection of drug-resistant mutants. Lamivudine-resistant viruses have a characteristic amino acid substitution in the tyrosine-methionine-aspartate-aspartate (YMDD)-motif of the

RNA-dependent DNA polymerase. The methionine at codon 204 is either replaced by an isoleucine (rtM204I) or by a valine (rtM204V). In addition, the rtM204V mutation is frequently accompanied by a leucine-180-to-methionine (rtL180M) substitution [3,4]. The risk of mutation increases with the duration of treatment. At the end of the first, second, third and fourth years of treatment, the incidences of resistance are: 15 to 32%, 38%, 56% and 67%, respectively [5]. The emergence of these mutants results in phenotypic resistance or viral breakthrough which increased from 43% in year 1 to >80% in year 3, with infrequent hepatic failure [6].

Adefovir dipivoxil is another oral nucleos(t)ide analog that requires long-term therapy. Although ADV-resistant mutants (rtN236T) are rare at week 96 of treatment (only 1.6% in initial reports), ADV-resistant mutants do occur at a cumulative rate of 29% within 5 years [7]. Furthermore, nephrotoxicity requiring switching or termination of treatment has been observed in 4% of those who received ADV for 3 years.

A phase III study on ETV showed that it is better than LAM in hepatitis Be antigen (HBeAg)-positive and -negative nucleos(t)ide-naïve patients [8,9]. With its marked suppression of serum HBV DNA, there is less risk of resistance over time with ETV, with no resistance at 96 weeks in HBeAg-positive LAM-naïve patients [10]. Entecavir resistance in LAM refractory patients is 7% genotypically and 1% phenotypically at 1 year. Entecavir resistance requires pre-existing LAM-resistant substitutions and additional changes at rtT184, rtS202 and rtM250. However, follow-up studies to determine the resistance rate after 2-5 years of therapy are required especially in LAM-resistant patients.

Another major limit of nucleos(t)ide therapy is that the optimal duration of therapy has not been clarified, especially in HBeAg-negative patients. Therapy has been shown to be persistently beneficial in this group of patients with 144 weeks of ADV, but responses were virtually negated when it was withdrawn after 48 weeks of treatment [11,12]. Sustained response after withdrawal of ETV has been shown to be less than optimal in both HBeAg-positive and -negative patients [12,13,14]. In view of the limitations of current therapies for chronic HBV new, more effective agents are needed for this indication.

PEGYLATED INTERFERON ALFA-2a (40 kDa)

Pegylated interferon alfa-2a (40 kDa) (Pegasys) is one of a number of therapeutic agents that are pegylated by incorporating a polyethylene moiety into the active product. Pegylation of the interferon alfa molecule is undertaken to enhance the pharmacokinetic properties of unmodified interferon alfa, allowing once a week dosing. The first pegylated interferon alfa to be developed was 5 kDa in size. However, this drug has limited overall clinical and laboratory benefits. Since then, pegylated interferon alfa-2a (40 kDa) and pegylated interferon alfa-2b (12 kDa) have been developed. As a result of their different sizes and structures, these 2 molecules have different *in vivo* and *in vitro* characteristics.

The pegylation of interferon alfa-2a involves 2 chains of 20 kDa polyethylene glycol conjugated to the lysine residues (position 31, 121, 131 and 134) of the interferon alfa-2a molecule. The peak plasma level is between 72 and 96 hours and the volume of distribution is 8 to 12 L, suggesting that it is highly compartmentalized in the intravascular space. The clearance half-life is between 40 and 80 hours. The serum antiviral activity, as measured by the 2'-5'oligoadenylate synthetase activity, peaks 24-48 hours after administration and remains high for 1 week [15]. Due to its highly intravascular compartmentalization, dose adjustment according to body weight is not necessary.

PEGYLATED INTERFERON ALFA-2a (40 kDa) IN HBeAg-POSITIVE PATIENTS

When pegylated interferon alfa-2a was tested in a phase II study at 90, 180 and 270 μg/week for 24 weeks, compared with conventional interferon alfa-2a, the HBeAg seroconversion was 3%, 35%, 29% and 25%, respectively [16]. The combined response (HBeAg loss, HBV DNA suppression <500,000 copies/mL, alanine aminotransferase [ALT] normalization) was higher in all pegylated interferon alfa-2a doses combined (24% vs. 12%). The response was even higher in difficult-to-treat patients: 27% in patients with <2 times upper limit of normal (ULN) of baseline ALT vs. 11% with interferon alfa-2a; 20% vs. 0% in patients with HBV DNA >11 $\log_{10}$ copies/mL. The side-effects seemed to be dose-dependent and they occurred more often in the 270 μg and 180 μg groups. However, there were no differences in side-effects when the 270 μg group was compared with the 180 μg group.

The beneficial effect of pegylated interferon alfa-2a was further substantiated in 2 multinational phase III studies [17,18]. In the phase III HBeAg-positive study, 814 HBeAg-positive chronic HBV infected patients were randomized to receive either; 1) pegylated interferon alfa-2a180 μg/week; 2) combination therapy with pegylated interferon alfa-2a 180 μg/week plus LAM 100 mg/day; or, 3) LAM 100 mg/day. Treatment lasted for a total of 48 weeks and patients were assessed 24 weeks after the end of therapy [18]. More than 85% of patients in this study were Asians, and the mean HBV DNA was 9.9 to 10.1 $\log_{10}$ copies/mL. About 15 to 18% of patients had severe fibrosis or cirrhosis on liver biopsy at baseline, 9 to 15% had received LAM therapy and 2 to 3% of patients had been previously treated with conventional interferon alfa-2a.

HBeAg seroconversion rates and suppression of HBV DNA to <100,000 copies/mL occurred in significantly more patients with pegylated interferon alfa-2a monotherapy and pegylated interferon alfa-2a plus LAM combination therapy than with LAM monotherapy. More importantly, loss of hepatitis B surface antigen (HBsAg) with the development of hepatitis B surface antibody (anti-HBs) occurred in 8 of the 271 patients (3%) with pegylated interferon alfa-2a monotherapy, 8 of the 271 patients (3%) receiving combination therapy with pegylated interferon alfa-2a plus LAM and none of the 272 patients (0%) receiving LAM monotherapy (p=0.004 for both comparisons) [18].

PEGYLATED INTERFERON ALFA-2a (40 kDa) IN HBeAg-NEGATIVE PATIENTS

In another randomized, partially double-blind, phase III controlled study, 537 HBeAg-negative chronic HBV patients were randomized to receive either: 1) pegylated interferon alfa-2a 180 μg/week; 2) combination therapy with pegylated interferon alfa-2a 180 μg/week plus LAM 100 mg/day; or, 3) LAM 100 mg daily for 48 weeks and followed-up for another 24 weeks after therapy [17]. Patients were included in this trial if they had been HBeAg-negative and anti-HBe–positive for at least 6 months, had an HBV DNA of more than 100,000 copies/mL, serum ALT levels >1 <10 times the ULN and had liver biopsy results within the last 24 months showing significant necro-inflammatory activity. This study had 2 primary end-points assessed 24 weeks after the completion of therapy, normalization of serum ALT and suppression of HBV DNA below 20,000 copies/mL.

After 48 weeks of therapy, suppression of serum HBV DNA from baseline was most significant in patients receiving combination therapy with pegylated interferon alfa-2a plus LAM. However, suppression of HBV DNA from baseline was similar in patients with pegylated interferon alfa-2a monotherapy and LAM monotherapy. Twenty-four weeks after therapy, normalization of serum ALT was more frequent in patients receiving pegylated interferon alfa-2a monotherapy (59%) and combination therapy with pegylated interferon alfa-2a plus LAM (60%) compared to those receiving LAM monotherapy (44%). Virological response was also higher in patients receiving pegylated interferon alfa-2a monotherapy (43%) and combination pegylated interferon alfa-2a plus LAM (44%) than in patients receiving LAM monotherapy (29%). Suppression of HBV DNA to below 400 copies/mL at week 72 was also higher in patients receiving pegylated interferon alfa-2a monotherapy (19%) and combination pegylated interferon alfa-2a plus LAM therapy (20%) than in those on LAM monotherapy alone (7%) [17].

Most importantly, loss of HBsAg occurred in 7 patients receiving pegylated interferon alfa-2a monotherapy (5 Asians and 2 Caucasians) and in 5 patients receiving combination pegylated interferon alfa-2a plus LAM therapy (4 Asians and 1 Caucasian). This was significantly higher than in those receiving LAM monotherapy alone (n=0; p=0.007 and p=0.030, respectively). Clearance of HBsAg with the development of anti-HBs occurred in 8 patients receiving pegylated interferon alfa-2a (5 on pegylated interferon alfa-2a monotherapy and 3 on combination pegylated interferon alfa-2a plus LAM therapy) and in none of the patients receiving LAM monotherapy (p=0.029) [17].

COMBINATION PEGYLATED INTERFERON ALFA-2A (40 kDa) PLUS LAM THERAPY

Unfortunately, data generated from these 2 studies do not support the use of combination therapy with pegylated interferon alfa-2a and LAM for achieving a sustained off-treatment response. In both phase III HBeAg-positive and -negative studies, although the degree of end-of-treatment viral load suppression was higher in those treated with an LAM-containing regimen than with pegylated interferon alfa-2a alone (7.2 $\log_{10}$ vs. 4.5 $\log_{10}$, respectively in the HBeAg-positive study and 5.0 $\log_{10}$ vs. 4.1 $\log_{10}$, respectively in the HBeAg-negative study), the rate of sustained disease remission was higher in the latter [17,18]. This finding suggests that the mechanism of viral load reduction, in addition to the degree of viral suppression,

is an important factor in sustained disease remission. However, a benefit of combination therapy with pegylated interferon alfa-2a plus LAM is fewer YMDD mutations (1 to 4%) compared with LAM monotherapy (18 to 27%) [19].

PREDICTORS OF RESPONSE

Patients infected with HBV genotype A had the highest rate of HBeAg seroconversion 24 weeks after pegylated interferon alfa-2a (±LAM) therapy (52%) compared with patients infected with HBV genotypes B or C (30 to 31%). However, even in these patients the rate of HBeAg seroconversion was still better than in those patients treated with LAM monotherapy [20]. A high baseline ALT level and a low HBV DNA is also predictive of a better response to pegylated interferon alfa-2a. Patients with high baseline ALT levels (>5 times ULN) or low HBV DNA (<9.1 $\log_{10}$ copies/mL) achieved HBeAg seroconversion rates of 41% and 53%, respectively. The authors also found that more significant HBeAg suppression at week 12 of therapy (below 10 IU/mL) was associated with higher HBeAg seroconversion (53%).

Response rates to pegylated interferon alfa-2a were similar in treatment-naïve patients and in patients who had received prior antiviral therapy (LAM or conventional interferon alfa) [21].

DURABILITY OF OFF-THERAPY SUSTAINED RESPONSE

In a longer follow-up study on the durability of off-therapy response with pegylated interferon alfa-2a, 116 HBeAg-negative patients treated with 48 weeks of pegylated interferon alfa-2a and 114 HBeAg-negative patients treated with 48 weeks of combination therapy with pegylated interferon alfa-2a plus LAM were rolled-over into a long-term observational study. The rates of biochemical and virological response 12 and 24 months after the end of treatment were: 50% vs. 45% for ALT normalization, respectively at the end of year-1, and 32% vs. 28%, respectively at the end of year 2. HBV DNA <20,000 copies/mL was 35% vs. 35% in those who had received pegylated interferon alfa-2a and combination therapy with pegylated interferon alfa-2a plus LAM, respectively at the end of year-1, and 29% vs. 25%, respectively at the end of year-2. More importantly, 11 of the 116 patients (9%) who had received pegylated interferon alfa-2a and 14 of the 114 patients (12%) who had received

combination pegylated interferon alfa-2a plus LAM developed sustained loss of HBsAg at the end of 2 years [22].

Similarly, sustained disease remission induced by pegylated interferon alfa-2a in HBeAg-positive patients was also maintained 1 year after the end of treatment [23]. In those who achieved HBeAg seroconversion or HBV DNA <100,000 copies/mL 6 months post-treatment, 86% had a sustained response 1 year post-treatment [23]. In those without HBeAg seroconversion 6 months post-treatment, 14% had HBeAg seroconversion and 10% had HBV DNA <100,000 copies/mL 1 year post-treatment. This suggests that a 48-week course of pegylated interferon alfa-2a can increase the rate of sustained off-treatment disease remission 1 year post-treatment. Furthermore, those with HBeAg seroconversion 6 months after the end of treatment or later, had normalization of ALT 1 year post-treatment [23]. More importantly, those who achieved early HBeAg seroconversion (HBeAg seroconversion before 24 weeks of therapy) achieved HBsAg loss by week 72 [24].

EFFECT ON LIVER HISTOLOGY

The effect of pegylated interferon alfa-2a on liver histology was analyzed by Lau *et al.* and Cooksley *et al.* [25,26]. Both studies found that pegylated interferon alfa-2a therapy can result in histological improvement (defined as a 2-point decrease in the Modified Histologic Activity Index) [27]. Forty-nine percent of HBeAg-positive and 59% of HBeAg-negative patients treated with pegylated interferon alfa-2a had histological improvement on the second liver biopsy 24 weeks after the end of therapy [27].

Histological improvement is more pronounced in patients with a virological response. Thus, HBeAg-positive patients who have achieved ALT normalization, HBV DNA suppression, and HBeAg seroconversion are more likely to have histological improvement. Similarly, HBeAg-negative patients with HBV DNA suppression or ALT normalization also show an improvement in liver histology. Finally, HBeAg-negative patients with normalization of serum ALT and HBV DNA suppression have been shown to have a higher histological response (78% vs. 49%) [25].

SAFETY

The rate of adverse events is similar in patients with pegylated interferon alfa-2a and combination therapy with pegylated interferon

alfa-2a plus LAM but is significantly lower in those receiving LAM monotherapy [17,18]. Pegylated interferon alfa-2a is reasonably well tolerated in HBeAg-negative and -positive patients. The most common adverse events with pegylated interferon alfa-2a are pyrexia, fatigue, myalgia, headache, decreased appetite, arthralgia and alopecia. Most of the patients receiving pegylated interferon alfa-2a experienced at least 1 adverse event with an incidence of 87 to 89% in the pegylated interferon alfa-2a monotherapy and combination groups. However, only 56% of the HBeAg-positive patients in the LAM monotherapy group experienced at least 1 adverse event [18]. The number of HBeAg-negative patients experiencing at least 1 adverse event was also significantly lower in the LAM monotherapy group (48%) [17,18].

Serious adverse events occurred in around 5% of HBeAg-negative patients receiving pegylated interferon alfa-2a monotherapy and in 7% of HBeAg-negative patients receiving combination therapy with pegylated interferon alfa-2a plus LAM. In the group receiving LAM monotherapy, the percentage of serious adverse events was 3% [17]. The most common serious adverse event was infection which occurred in 1 to 2% of all patients. It should be noted that the percentage of serious adverse events in HBeAg-positive patients was 2 to 6% [18] including 2 reported cases of complete liver failure resulting in 1 fatality after the cessation of therapy (both in the LAM monotherapy group). The rate of withdrawal was low in both HBeAg-negative and -positive studies in all 3 treatment groups [17,18].

The safety and tolerance level of pegylated interferon alfa-2a and its effect on the quality of life have been assessed in HBeAg-negative and -positive patients and compared with pooled pegylated interferon alfa-2a monotherapy data from 3 studies in chronic hepatitis C-infected patients [28]. Quality of life was measured with the SF-36 questionnaire in 177 HBeAg-negative and 271 HBeAg-positive patients who received pegylated interferon alfa-2a monotherapy. The questionnaire was completed at baseline, and at weeks 12, 24, 48 and 72 in both the HBV and HCV patients.

The frequency of interferon-related adverse events was generally lower in both Asian and Caucasian HBV patients compared with HCV patients. Both the rate of depression and rate of withdrawal from therapy were lower in HBV patients [28]. The incidence of depression was also lower in Asian compared to Caucasian patients. The incidence of individual events associated with interferon therapy such

as fatigue, myalgia, headache and arthralgia were numerically lower in patients with chronic HBV than in patients with chronic HCV.

OPTIMAL DURATION OF THERAPY WITH PEGYLATED INTERFERON ALFA-2a (40 kDa)

Although these 2 studies demonstrated the efficacy of 48 weeks of pegylated interferon alfa-2a either as monotherapy or in combination with LAM for the treatment of HBeAg-positive and -negative chronic HBV infection [17,18], it is uncertain if a shorter duration of treatment with these drugs will affect the SVR rate. This is because the current licensed duration of therapy with conventional interferon alfa is 16 to 24 weeks. A direct comparison between 24 weeks of pegylated interferon alfa with 48 weeks of pegylated interferon alfa for chronic HBV infection has not been performed.

In a recent review of our experience in treating HBeAg-positive Chinese patients in Hong Kong with either 48 weeks of pegylated interferon alfa-2a or 24 weeks of pegylated interferon alfa-2b, we found that those treated with 48 weeks of pegylated interferon alfa-2a had a higher SVR, defined as HBeAg seroconversion with serum HBV DNA <100,000 $\log_{10}$ copies/mL at week 72 (34% vs. 8%, respectively; p=0.04) [29]. However, due to the small sample size, the use of different pegylated interferon alfas, and the retrospective nature of the study, these results should be interpreted with caution. A large-scale, randomized, prospective study is needed, comparing 24 with 48 weeks of treatment in order to determine the optimal duration of therapy with pegylated interferon alfa.

CONCLUSION

Pegylated interferon alfa-2a can induce sustained off-treatment disease remission in both HBeAg-positive and HBeAg-negative patients. The choice of pegylated interferon alfa-2a as a first-line therapy for chronic HBV is based mostly on its efficacy and acceptable safety profile. Since only a minority of patients will achieve sustained off-therapy responses after treatment with the currently available nucleos(t)ide analogs such as LAM or ADV, pegylated interferon alfa-2a offers a new therapeutic option in the fight against chronic HBV. Pegylated interferon alfa-2a is safe compared to LAM and ADV and can achieve higher rates of off-therapy remission and biochemical normalization than nucleos(t)ide analogs.

REFERENCES

1. Beasley RP. Hepatitis B virus: the major etiology of hepatocellular carcinoma. Cancer 1988;61:1942-1956.
2. McMahon BJ. Hepatocellular carcinoma and viral hepatitis. In: Wilson RA, ed. Viral hepatitis: diagnosis, treatment, prevention. New York, NY: Marcel Deckker 1997;315-330.
3. Wong DK, 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. A meta-analysis. Ann Intern Med 1993;119:312-313.
4. 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.
5. Leung N. Clinical experience with lamivudine. Semin Liver Dis 2002;22:S15-S21.
6. 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.
7. Borroto-Esoda K, Arterburn S, Snow A, Chuck S, Hadziyannis S, Locarnini S, Zoulim F, Pawlotsky JM. Final analysis of virological outcomes and resistance during 5 years of adefovir dipivoxil monotherapy in HBeAg-negative patients. J. Hepatol 2006;44:S179.
8. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-1010.
9. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-1020.
10. Gish RG, Chang TT, De Man RA, Gadano A, Sollano J, Han KH. Entecavir results in substantial virologic and biochemical improvement and HBeAg seroconversion through 96 weeks of treatment in HBeAg+ chronic hepatitis B patients. Hepatology 2005;42:S267.
11. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N Engl J Med 2005;352:2673-2681.
12. Lok AS. The maze of treatments for hepatitis B.N Engl J Med 2005;352:2743-2746.
13. Chang TT, Chao YC, Sollano J, Han KH, Lai CL, Akbar N, et al. Sustained response off-treatment to entecavir and lamivudine after 48 weeks of treatment in

nucleoside-naïve, HBeAg(1) patients: 24-weeks follow-up results of Phase III Study-022 (abstract). Liver Int 2005;25:191.

14. Lai CL, Chang TT, Chao YC, Tanwandee T, Thongsawat S, Lee SD et al. Sustained response off-treatment to entecavir and lamivudine after 48 weeks of treatment in nucleoside-naïve, HBeAg(1) patients: 24-week follow-up results of phase III Study-022 (abstract). Liver Int 2005;25:200.

15. Reddy KR. Development and pharmacokinetics and pharmacodynamics of pegylated interferon alfa-2a (40 kDa). Semin Liver Dis 2004;24 S2:33-38.

16. 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 2002;10:298-305.

17. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N; Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206-1217.

18. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N; Peginterferon Alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.

19. Germanidis G, Marcellin P, Lau G, Bonino F, Farci P, Hadziyannis S, et al. Profound on-treatment viral suppression with peginterferon alfa-2a (40 kDa) (Pegasys) plus lamivudine combination therapy limits the development of YMDD mutations, but does not improve sustained response rates over peginterferon alfa-2a (40kDa) alone. Hepatology 2004;40:S653.

20. Chow WC, Cooksley G, Chang WY, et al. Predicting response in a large, multinational trial of peginterferon a-2a (40 kDa ± lamivudine vs lamivudine alone for HBeAg-positive chronic hepatitis B a-2a (40 kDa) ± lamivudine vs lamivudine alone for HBeAg-positive chronic hepatitis B (abstract). Liver Int 2005;25:138.

21. Lau GK, Luo KX, Paik SW, Chang WY, Piratvisuth T, Chutaputti A, et al. Effect of age, gender, prior anti-HBV therapy and drug exposure on sustained response in Asian patients enrolled in a large multinational study of peginterferon a-2a (40 kDa) ± lamivudine vs lamivudine for chronic hepatitis B (abstract). Liver Int 2005;25:201.

22. Marcellin P, Bonino F, Lau GK, Farci P, Yurdayin C, Piratvisuth et al. Suppression of HBV DNA in patients with HBeAg-negative CHB treated with peginterferon alfa-2a (40 kDa) ± lamivudine: 2-year follow-up results. Hepatology 2006;44:S972.

23. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat G, Cooksley G et al. Durability of response and occurrence of late response to peginterferon alfa-2a one year post-treatment in patients with HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.

24. Piratvisuth T, Lau GK, Marcellin P, Chow WC, Cooksley G et al. Association between HBeAg seroconversion and sustained HBV DNA suppression in patients treated with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.

25. Lau GK, Cooksley G, Marcellin P, Piratvisuth T, Hadziyannis S, Farci P, et al. Predictors of histologic improvement and relationship between sustained response and histology in patients with HBeAg positive (40kDa)(PEGASYS) (abstract). Hepatology 2005;42:587.

26. Cooksley G, Marcellin P, Bonino F, et al. Histologic improvement and sustained response in patients with HBeAg-positive or HBeAg-negative chronic hepatitis B treated with peginterferon a-2a (40 kDa) (abstract). Liver International, 2005;25:134.

27. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN, et al. Histological grading and staging of chronic hepatitis, J Hepatol 1995;22:696-699.

28. Marcellin P, Lau GK, Piratvisuth T, Luo KX, Bonino F, Thongsawat S, et. al. Better safety and quality of life in patients with chronic hepatitis B than in patients with chronic hepatitis C when treated with peginterferon alfa-2a (40 kDa)(PEGASYS) (abstract). Hepatology 2004;40:S668.

29. Lau GK; Hui CK, Lai LS, Lam P, Zhang HY, Fung TT, Lai ST, Wong WM, Lo CM, Fan ST, Leung N, Lau GK. 48 weeks pegylated interferon alpha-2a is superior to 24 weeks of pegylated interferon alpha-2b in achieving hepatitis Be antigen seroconversion in chronic hepatitis B infection. Aliment Pharmacol Ther 2006;23:1171-1178.

Why Treat Patients with HBeAg-Negative Chronic Hepatitis B with Nucleos(t)ide Analogs?

S. J. Hadziyannis

INTRODUCTION

This paper discusses the rationale of nucleos(t)ide analog treatment in patients with hepatitis Be antigen (HBeAg)-negative chronic hepatitis B. Specific indications of this therapeutic approach among the various subsets of HBeAg-negative patients are presented, as well as the selection of the best antiviral compounds in terms of their costs and benefits. Finally, current options for therapeutic schemes, doses, and the optimal duration of nucleos(t)ide analog therapy in HBeAg-negative chronic hepatitis B are evaluated, taking into account realistic and clinically relevant aims and goals.

HISTORICAL BACKGROUND

Nucleos(t)ide analogs were first tried in the treatment of chronic hepatitis B before the era of HIV infection. However, it was only after reverse transcriptase inhibitors that suppressed HIV replication were developed, that their use was extended and approved for the treatment of chronic hepatitis B. The first such drug was lamivudine (LAM) [1-5]. This compound revolutionized the treatment of HBV-induced chronic liver disease, made liver transplantation in patients with end-stage HBV cirrhosis possible, and has saved thousands of lives. Despite the high rate of HBV resistance in long-term LAM monotherapy, this drug is still considered an important antiviral therapy for both HBeAg-negative and

HBeAg-positive chronic hepatitis B [6]. In 2002, the nucleotide analog adefovir dipivoxil (ADV) was approved for the treatment of chronic hepatitis B [7-9]. The main advantage of ADV compared with LAM is that HBV resistance rates are lower. However, although infrequent and delayed, HBV resistance to ADV was found to increase significantly with the duration of ADV therapy [10-14]. There have also been concerns expressed over the low antiviral potency of this compound [15].

Entecavir (ETV), which has high antiviral potency and low HBV resistance rates, is an additional therapeutic option broadening the scope of nucleos(t)ide analogs in hepatitis B. It was adopted for use in the USA in 2005 and in 2006 in Europe [16-18]. Tenofovir (TDF) and telbivudine (LdT) are also promising therapeutic options and are currently in phase III trials [6].

Combinations of several nucleos(t)ide analogs are already used in clinical practice, particularly in patients with chronic hepatitis B that is resistant to one or more compounds and in patients with advanced and decompensated HBV liver disease mostly in the pre- and post-transplantation setting [6,19-23]. Thus the question of why to treat patients with nucleos(t)ide analogs becomes much broader and must include information on who, when, how and with what.

INDICATIONS FOR TREATMENT IN HBeAg-NEGATIVE PATIENTS

To date, treatment strategies have been directed specifically at patients with significant chronic liver damage induced by replicating HBV i.e. to either chronic HBeAg-positive or HBeAg-negative hepatitis B patients rather than to all individuals with chronic HBV infection [2,5,24]. The aim of treatment is to achieve robust and durable suppression of HBV replication. This results in the return of liver enzymes to normal levels, remission of liver necro-inflammation and improvement of hepatic fibrosis [4,8,9].

Chronic hepatitis B is divided into 2 major types: HBeAg-positive and HBeAg-negative (also referred to as anti-HBe–positive and precore HBV mutant chronic hepatitis). In recent years HBeAg-negative chronic hepatitis B has attracted more clinical interest because of: 1) an increased recognition of this type; 2) its greater prevalence worldwide; and, 3) the major difficulties encountered in its management [23-27]. Since currently available therapeutic options rarely, if ever, achieve HBV eradication and a number of HBeAg-negative chronic hepatitis B patients exhibit a very

slow progressive course, it is generally accepted that therapy can be withheld in patients with minimal or mild histological liver disease [2,5]. On the other hand, since the severity of histological liver lesions cannot be predicted by alanine aminotransferase/aspartate aminotransferase (ALT/AST) activity and/or viremia levels, liver biopsy is recommended. Cases with minimal or even mild histological liver disease may be closely followed-up and treatment can be initiated if biochemical and liver disease profiles deteriorate.

Treatment is an unequivocal "must" in: 1) patients with advanced fibrosis/cirrhosis; 2) patients with decompensated chronic liver disease; and, 3) in pre- and post-transplantation setting.

WHY TREAT HBeAg-NEGATIVE CHRONIC HEPATITIS B WITH NUCLEOS(T)IDE ANALOGS?

At present, the decision to begin nucleos(t)ide analog therapy in HBeAg-negative chronic hepatitis B is made in clinical practice when interferon-based therapies fail, if they are refused by the patient or are contra-indicated for any reason [2,3]. The rationale for this therapeutic approach is based on good evidence that in HBeAg-negative chronic hepatitis B patients a 12-month course with interferon alfa, particularly pegylated interferon alfa-2a, is far more effective than a course of nucleoside analogs of the same duration, in relation to the sustained virological response (SVR) after stopping treatment [15,28,29]. It must be noted, however, that the percentage of HBeAg-negative chronic hepatitis B patients who achieve an SVR after stopping interferon-based therapies is generally <30%. Furthermore, this 30% is usually limited to younger patients in relatively early stages of chronic liver disease without complicating factors or co-morbid conditions [15]. At the same time, as already mentioned, it is generally recommended to withhold interferon from patients with advanced and decompensated liver disease [5,8]. Thus most (>70%) HBeAg-negative patients with chronic HBV-induced liver disease, who are in great need of therapy will eventually have to be treated with nucleos(t)ide analogs. The goal of therapy is to achieve strong suppression of HBV replication so that serum HBV DNA declines to levels that are persistently non-detectable by the most sensitive polymerase chain reaction (PCR) assays [30,31] and to maintain this effect by continuous and probably indefinite treatment.

WHAT ARE REALISTIC EXPECTATIONS FOR THIS THERAPY?

Achieving indefinitely durable and potent suppression of HBV replication in chronic hepatitis B is known to be the key to obtaining normal ALT levels and remission of liver necro-inflammation. Improvement of liver fibrosis has also been documented in several studies, particularly with long-term administration of nucleos(t)ide analogs [32-36]. In view of these effects and in order to increase survival and avoid liver transplantation, the long-term clinical goals are a decrease in the rate of both the number of the life-threatening complications of cirrhosis and the development of hepatocellular carcinoma (HCC). These expectations are relative rather than absolute, meaning that the reductions should be compared to untreated patients and complete prevention should not be expected. In fact HCC may develop during chronic HBV infection even without cirrhosis, as well as in certain patients who have achieved HBsAg clearance. This is because HBV DNA becomes integrated into the genome of hepatocytes and may promote hepatocarcinogenesis.

WHICH NUCLEOS(T)IDE ANALOGS SHOULD BE USED AND FOR HOW LONG?

The goal of maintaining a virological response (MVR) with indefinite nucleos(t)ide treatment is clearly inferior to achieving an SVR with a finite course of therapy, because of the potential side effects and high cost of the former. However, MVR is the only option currently available for most HBeAg-negative patients, and it requires that HBV resistance to the administered drugs occurs infrequently and late during therapy; and that when it develops, it is diagnosed early and managed properly [15,21]. In addition a possible SVR after several years of effective nucleos(t)ide analog treatment should always be borne in mind [33, Hadziyannis S unpublished]. For the time being, this possibility should be considered hypothetical, whatever the nucleos(t)ide administered, for periods of 1, 2 and even 3 years. Most HBeAg-negative patients who discontinue nucleos(t)ide therapy for these durations experience virological and biochemical relapses [10,17,37]. Thus, for the moment, nucleos(t)ide analog treatment in patients with HBeAg-negative chronic HBV liver disease should be viewed as indefinite and even lifelong.

Lamivudine

In 1998 LAM became the first oral antiviral agent to be approved for the treatment of chronic hepatitis B. Although it soon became clear that LAM should be given to HBeAg-negative patients for long periods of time [29], the response rates with long-term LAM treatment decreased progressively over time [6,23,25] with increasing rates of viral resistance and virological breakthroughs, resulting also in biochemical breakthroughs [10,25]. Biochemical breakthroughs have adverse effects on liver histology and patient outcome and may result in decompensation, liver failure and death, particularly in patients with histological cirrhosis [21]. However, 30 to 35% of LAM-treated HBeAg-negative patients maintain on-therapy biochemical and virological remission even after 5 years of therapy [25,33,34]. This combined with its low cost and excellent safety profile mean that LAM is still used as an antiviral therapy in HBeAg-negative chronic hepatitis B [6], particularly in Asia, where HBV is endemic. In a Greek cohort study, LAM plus salvage ADV therapy for LAM resistance was associated with a very low risk of major events in noncirrhotic patients, but with a substantial risk of major events including death in cirrhotic patients with HBeAg-negative chronic hepatitis B [39]. In addition, treatment with LAM plus salvage ADV for genotypic LAM resistance has been reported to be safe even in cirrhotic patients with HBeAg-negative chronic hepatitis B who were closely followed-up [21]. After discontinuation of LAM in HBeAg-negative patients with such prolonged on-treatment remission, the optimal duration of therapy and the likely outcome are currently unknown. At the same time, it should be noted that development of HBV-resistance to LAM may adversely affect the efficacy of subsequent ETV and ADV treatment [40,42].

Adefovir dipivoxil

Adefovir dipivoxil, which is a prodrug of an adenosine nucleotide analog, adefovir, is the second available oral anti-HBV agent.

In HBeAg-negative chronic hepatitis B patients, 48 weeks of ADV therapy has been found to achieve: 1) normalization of ALT in 72% of patients vs. 29% in placebo-controls, ($p<0.001$); 2) undetectable serum HBV DNA by a sensitive PCR assay in 51% of patients vs. 0% in placebo-controls, ($p<0.001$); and, 3) a median serum HBV DNA drop of 3.9 $\log_{10}$ vs. 1.35 $\log_{10}$ in the placebo-controls, ($p<0.001$) [7]. However, the response usually disappears after discontinuation of these ADV courses and <10% of patients have undetectable serum

HBV DNA by PCR 48 weeks after stopping a 48-week course of ADV [10]. Long-term ADV therapy has been shown to be safe, well-tolerated and to maintain or even increase satisfactory initial response rates. Indeed, the on-therapy responses have been found to be maintained over the first 3 and even 5 years of ADV therapy in approximately 70% of patients [35,36]. In addition, ADV monotherapy for 5 years was shown to produce significant and increasing improvement in liver fibrosis, and even reversion of histologically established cirrhosis and to result in HBsAg loss in 5% of patients [35,36].

The main advantage of ADV monotherapy compared to LAM is the infrequent development of viral resistance. In naïve patients with HBeAg-negative chronic hepatitis B treated with ADV monotherapy, the cumulative probability of genotypic HBV resistance, regardless of virological and biochemical breakthroughs, has been reported to be 0% at 1, 3% at 2, 11% at 3, 18% at 4, and 29% at 5 years [10,35,36]. The serum HBV DNA levels at 12 months, as assessed by a sensitive PCR assay, seem to be a good predictor of the subsequent emergence of resistance under long-term ADV therapy [42]. Resistance to ADV is mostly associated with the selection of a novel asparagine to threonine mutation at residue rt236 in domain D (rtN236T) and/or with selection of an alanine to valine mutation at residue 181 of the HBV polymerase (rtA181V) [13,14,43]. As in the case of LAM resistance, ADV-resistant chronic hepatitis B may be associated with virological rebounds and liver decompensation. What appears to be the most frequently occurring and clinically most important mutation, rtN236T, has been found to be fully susceptible to LAM both *in vitro* and *in vivo,* and is susceptible to ETV and LdT *in vitro* [43]. The significance of the rtA181V mutation needs further clarification.

Adefovir is the main therapeutic option for patients with LAM resistance and it has a similar antiviral efficacy against all types of LAM-resistant YMDD mutant HBV strains [43]. Whether chronic hepatitis B patients with resistance to LAM should switch to ADV monotherapy immediately or after a period of concurrent LAM therapy or whether they should receive long-term ADV and LAM combination therapy has not yet been answered. It should be noted, however, that the probability of emergence of ADV resistance is higher in patients with LAM resistance than in naïve subjects [41] and in patients with LAM resistance treated with long-term ADV monotherapy than in those treated with long-term ADV plus LAM combination treatment [11,12,20,23]. Thus, in HBeAg-negative chronic hepatitis B patients it seems safer and more efficient to add

ADV with LAM rather than to switch to ADV monotherapy. Patients with cirrhosis, even histological cirrhosis alone, receiving long-term LAM monotherapy should be closely monitored with frequent ALT/AST assays and serum HBV DNA determinations, so that ADV can be added early, ideally upon detection of genotypic resistance to LAM, since a delay in administering ADV may be associated with the development of irreversible decompensation, liver failure and death [20].

Entecavir

Entecavir, which is a carbocyclic analog of guanosine, is the third licensed oral anti-HBV agent. In LAM-naïve patients with either HBeAg-negative or HBeAg-positive chronic hepatitis B, a 48-week course with ETV, at a daily dose of 0.5 mg, has been shown to be significantly more effective than a 48-week course with LAM in suppressing HBV replication, returning ALT to normal and bringing about histological improvement [17,18,45]. HBeAg seroconversion rates, however, were not significantly higher in these patients. Due to its potency and the lack of any reported resistance, treatment has been extended over time and efficacy data for the second and third year of therapy are awaited.

Entecavir has also been used in chronic hepatitis B patients with LAM resistance [16, 17]. In a double-blind, randomized trial including 181 HBeAg-positive or HBeAg-negative chronic hepatitis B patients with LAM resistance, a 48-week course of ETV, at a daily dose of 0.1 mg, 0.5 mg or 1.0 mg, was found to be significantly more effective than continued LAM therapy in inducing virological and biochemical remission [16]. The efficacy of ETV has generally been found to be dose-dependent, and 1.0 mg is more effective (at least for some end-points) than 0.5 mg, and both 1.0 mg and 0.5 mg better than the 0.1 mg dose [16]. Thus, ETV is currently recommended at a daily dose of 0.5 mg in treatment-naïve and 1.0 mg in LAM-resistant chronic hepatitis B patients.

To date, no genotypic resistance to ETV has been detected after 2 years of therapy in any of the many treatment-naïve patients, even in the approximately 10% who develop virological breakthrough or have no response. On the other hand, genotypic resistance during 2 years of ETV therapy has been detected during virological breakthroughs in about 10% of ETV patients with pre-existing LAM resistance [18]. Resistance mutations to ETV usually include rtT184G and/or rtS202I as well as rtI169T and/or rtM250V substitutions of the HBV

polymerase [44]. *In vitro* data suggest that reduced susceptibility to ETV requires the emergence of both ETV and LAM resistance mutations (usually rtM204V and rtL180M) [38,44], which supports clinical data suggesting that resistance to ETV within the first 2 years of therapy only occurs in LAM resistant and not in treatment-naïve chronic hepatitis B patients.

SUMMARY

Sustained post-therapy responses after stopping finite courses of therapy in HBeAg-negative chronic hepatitis B patients are almost exclusively restricted to interferon-based therapies and are limited to <30% of treated patients, mostly young, noncirrhotic individuals. Most HBeAg-negative chronic hepatitis B patients either fail to respond to interferon, are not eligible for, or refuse this therapy. Consequently, effective, long-term/indefinite suppression of HBV replication with nucleos(t)ide analogs remains the main goal of therapeutic management of these patients. When this goal is achieved, there is remission of liver necro-inflammation, improvement (even reversion) of hepatic fibrosis and a decrease in the rate of development of the life threatening complications of cirrhosis and hepatocellular carcinoma. On the other hand, the efficacy of long-term monotherapy is compromised by progressively increasing rates of viral resistance. The long-term resistance profile of ADV is significantly better than that of LAM. Current data for the most potent compound, ETV (limited to 2 years of therapy) are extremely promising in treatment-naïve patients. In approximately 10% of LAM-resistant chronic hepatitis B patients, HBV mutants resistant to ETV are selected during the second year of therapy.

In patients with clinically overt cirrhosis, end-stage liver disease and in the pre-and post-transplantation setting, combination therapy with 2 nucleos(t)ide analogs without cross resistance – as in the case of ADV plus LAM – represents, in the opinion of the author, the treatment of choice.

Combination therapy with ADV and LAM in LAM-resistant patients is also very effective as no cases of ADV-resistance have yet been reported. However, LAM-resistant patients with high baseline viremia levels may not respond completely to the addition of ADV. They may require higher doses or a more potent alternative to ADV, such as TDF, a nucleotide analog with the same resistance profile that is currently commercially available for the treatment of HIV infection.

REFERENCES

1. Maddrey WC. Hepatitis B: an important public health issue. J Med Virol 2000;61:362-366.
2. Papatheodoridis GV, Hadziyannis SJ. Current management of chronic hepatitis B. Aliment Pharmacol Ther 2004;19:25-37.
3. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B. 2000 Summary of a workshop. Gastroenterology 2001;120:1828-1853.
4. Keefe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff E, 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.
5. EASL International consensus conference on hepatitis B. J Hepatol 2003;39:S3-S25.
6. Hadziyannis SJ. New developments in the treatment of chronic hepatitis B. Expert Opin Biol Therapy 2006 (in press).
7. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SL, Goodman Z, Wulfsohn M, Xiong S, Fry J, Brosgart C. Adefovir dipivoxil for the treatment of HBeAg-negative chronic hepatitis B. N Engl J Med 2003;348:800-807.
8. Lok ASF. The maze of treatment of hepatitis B. N Engl J Med 2005;353:2743-2746.
9. Hoofnagle JH. Hepatitis B – preventable and now treatable. N Engl J Med 2006;354:1074-1076.
10. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SL, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart C. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N Engl J Med 2005;352:2673-2681.
11. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, Hussain M, Lok A. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol 2006;44:283-290.
12. Fung SK, Andreone P, Han SH, Rajender Reddy K, Keefe EB, Hussain M, Cursaro C, Richtmyer P, Marrero J, Lok A. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. J Hepatol 2005;43:937-943.
13. 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. J Hepatol 2004;40:S20.
14. Angus P, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C, Brosgart C, Colledge D, Edwards R, Ayres A, Bartholomeusz A, Locarnini S. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 2003;125:292-297.

15. Hadziyannis SJ, Papatheodoridis GV. Hepatitis Be antigen-negative chronic hepatitis B: natural history and treatment. Semin Liver Dis 2006;26:130-141.

16. Chang TT, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, Pastore G, Bacon BR, Poynard T, Joshi S, Klesczeweski K, Thiry A, Rose R, Colonno R, Hindes R. A dose-ranging study of the efficacy and tolerability of entecavir in Lamivudine-refractory chronic hepatitis B patients. Gastroenterology 2005;129:1198-1209.

17. Lai C-L, Shouval D, Lok ASF, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink R, Cross A, Colonno R, Fernandes L. Entecavir versus Lamivudine for Patients with HBeAg-Negative Chronic Hepatitis B. N Engl J Med 2006;354:1011-1020.

18. Chang TT, Gish R, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-Positive Chronic Hepatitis B. N Engl J Med 2006;354:1001-1010.

19. Peters MG, Hann HH, Martin P, Heathcote J, Buggish P, Rubin R, Bourliere M, Kowdley K, Trepo C, Grey Df D, Sullivan M, Kleber K, Ebrhimi R, Xiong S, Brosgart CL. Adefovir dipivoxil alone or in combination with LAM in patients with LAM-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101.

20. Lampertico P, Vigano M, Manenti E, Iavarone M, Lunghi G, Colombo M. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to LAM. Hepatology 2005;421:414-419.

21. Lampertico P, Vigano P, Manenti E, Iavarone M, Lunghi G, Colombo M. Five years of sequential LAM to LAM+ADV therapy suppresses HBV replication in most HBeAg-negative cirrhotics, preventing decompensation but not hepatocellular carcinoma (abstract). Hepatology 2005;42:S582.

22. Fung SK, Chae HB, Fontana R, Conjeevaram H, Marrero J, Oberhelman K, Hussain M, Lok A. HBV genotype D and switch to adefovir (ADV) monotherapy are associated with increased risk of ADV resistance in chronic hepatitis B (CHB) patients (abstract). Hepatology 2005;42:S590.

23. Lampertico P, Marzano A, Battista G, Levrero M, Santantonio T, Andreone P, Brunetto M, DiMarco V, Fagiuoli S, Mazzella G, Raimondo G: A multicenter Italian study of rescue adefovir dipivoxil therapy in LAM resistant patients: a 2-year analysis of 604 patients (abstract). Hepatology 2005;42:S591.

24. Hadziyannis SJ, Vassilopoulos D. Hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.

25. Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, Rizzetto M, Craxi A. Clinical outcome of HBeAg-negative chronic hepatitis B in relation of virological response to LAM. Hepatology 2004;40:883-891.

26. Funk ML, Rosenberg DM, Lok ASF. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat 2002;9:52-61.

27. Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. Hepatology 2004;39:857-861.

28. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Pirtuvisch T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N. Peginterferon alfa-2a alone, LAM alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206-1217.

29. Lau GKK, Piratvisuth T, Luo KX, Marcellin P, Thougsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon alfa-2a, LAM, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.

30. Hochberger S, Althof D, Gallegos de Schrott RG, Nachbaur N, Rock H, Leying H. Fully automated quantitation of Hepatitis B virus (HBV) DNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan System. J Clin Virol 2006;35:373-80.

31. Hadziyannis AS, Mitsoula P, Hadziyannis E, Panopoulou E, Colucci G, Hadziyannis SJ, et al. Monitoring serum HBV-DNA levels with the TaqMan technique during treatment of HBeAg(-) chronic hepatitis B with LAM (abstract). Hepatology 2005;42:S726-S727.

32. Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term LAM monotherapy in patients with HBeAg negative chronic hepatitis B. Hepatology 2000;32:847-851.

33. Fung SK, Wong F, Hussain M, Lok ASF. Sustained response after a 2-year course of LAM treatment of hepatitis Be antigen-negative chronic hepatitis B. J Viral Hepat 2004;11:432-438.

34. Gaia S, Marzano A, Smedile A, Barbon V, Abate ML, Olivero A, Lagget M, Paganin S, Fadda M, Niro G, Rizzetto M. Four years of treatment with LAM: clinical and virological evaluations in HBe antigen-negative chronic hepatitis B. Aliment Pharmacol Ther 2004;20:281-287.

35. Hadziyannis S, Tassopoulos N, Chang T-T, Heathcote J, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart CL. Long-term adefovir dipivoxil treatment induces regression of liver fibrosis in patients with HBeAg-negative chronic hepatitis B: results after 5 years of therapy (abstract). Hepatology 2005;42:S754.

36. Borroto-Esoda K, Anterburn S, Snow A, Chuck S, Hadziyannis SJ, Locarnini S, Pawlotsky J. Final analysis of virological outcomes and resistance during 5 years of adefovir dipivoxil (ADV) monotherapy in HBeAg-negative patients (abstract). J Hepatol 2006;44:S179.

37. Tassopoulos NC, Volpes R, Pastore G , Heathcote J, Buti M, Goldin RP, Hawley S, Barber J, Condreay L, Gray DF. Efficacy of LAM 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.

38. Shaw T, Locarnini S. Entecavir for the treatment of chronic hepatitis B. Expert Rev Anti Infect Ther 2004;2:853-871.

39. Papatheodoridis GV, Dimou E, Dimakopoulos K, Manolakopoulos S, Rapti I, Kitis G, Tzourmakliotis D, Manesis E, Hadzyannis S. Outcome of hepatitis Be

antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with LAM. Hepatology 2005;42:121-129.

40. Tenney DJ, Levine SM, Rose RE, Walsh AW, Wenheimer SP, Discotto L, Plym M, Pokornowski K, Yu C F, Angus P, Ayres A, Bartholomeusz A, Sievert W, Thompson G, Warner N, Locarnini S, Colonno R. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to Lamivudine. Antimicrob Agents Chemother 2004;48:3498-3507.

41. Lee CK, Yeon JE, Hong SP, Kim JH, Brosgart CL, Miler M, et al. More frequent and earlier emergence of adefovir (ADV) resistance mutations in LAM resistant patients treated with ADV compared to previously reported nucleoside-treatment naive patients (abstract). Hepatology 2005;42:S593.

42. Locarnini S, Qi X, Arterburn S, Snow A, Brosgart CL, Currie M. Wulfsohn M, Miller M, Xiong S. Incidence and predictors of emergence of adefovir resistant HBV during four years of adefovir dipivoxil (ADV) therapy for patients with chronic hepatitis B (CHB). J Hepatol 2005;42:S17.

43. 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 LAM-resistant HBV strains in vitro and in liver transplant patients (abstract). Hepatology 2001;34:446.

44. Colonno R, Rose R, Levine S, Baldick J, Pokornowski K, Plym M, Yu C. Entecavir two year resistance update: no resistance observed in nucleoside naive patients and low frequency resistance emergence in LAM refractory patients (abstract). Hepatology 2005;42:S573-S574.

45. Yurdaydin C, Sollano J, Hadziyannis S, et al. Entecavir results in continued virologic and biochemical improvement and HBeAg seroconversion through 96 weeks of treatment in LAM-refractory, HBeAg(+) chronic hepatitis B patients (ETV-026) (abstract). J Hepatol 2006;44:S36

Why Treat Patients with HBeAg-Negative Chronic Hepatitis B with Pegylated Interferon?

R. P. Perrillo

INTRODUCTION

At present, there are 2 different interferon preparations and 4 nucleoside analogs that are specifically licensed for the treatment of chronic hepatitis B virus (HBV). In 2007 this list is likely to grow further. A review of efficacy and safety data from the phase III trials of nucleoside analogs and pegylated interferon is beyond the scope of the current paper. Instead, this paper will focus on how data from these trials can be used with the knowledge of key host-related and viral features in choosing pegylated interferon or nucleoside analogs as first-line therapy.

NUCLEOSIDE ANALOGS: MORE CONVENIENT, BUT MORE EFFECTIVE?

The relative complexity of treating chronic hepatitis B has led to the development of evidence-based treatment guidelines [1]. Current guidelines advocate the use of either interferon or nucleoside analogs and establish alanine aminotransferase (ALT) and HBV DNA thresholds for treatment. For most clinical situations, however, there is no specific advice on whether interferon or nucleoside analogs are preferable as first-line therapy because both treatment options have proven to be effective.

In the United States and elsewhere, many hepatologists and gastroenterologists rarely use pegylated interferon for chronic

hepatitis B because of possible adverse effects and the fact that administration is by injection. In contrast, the decision to treat with nucleoside analogs often is based on an excellent safety profile and the need for less frequent follow-up visits. While it is undeniably easier to manage patients with nucleoside analog therapy, a decision not to use interferon that is primarily or solely based on patient and physician convenience may not be in the best interest of the patient.

In this paper, it is proposed that the treatment of hepatitis B, like most medical disorders, should be based on the specific features of the case so that care is tailored for individuals. Some patients are good candidates for nucleoside analog therapy whilst other patients are better suited for treatment with interferon. The benefits and limitations of current therapies are illustrated in Table 1.

Treatment	Advantage	Disadvantage
Interferon		
	Finite duration of treatment	Given by injection
	Durable treatment response	Frequent side effects
	Loss of HBsAg (5 to 8%*)	Expensive
	Immunomodulatory	Unpredictable immunological effects
	No drug resistance	Lower response rate with high level viremia
		Virological response depends on genotype
Nucleos(t)ide analogs		
	Oral delivery	Drug resistance
	Negligible side effects	Long/indefinite treatment
	Potent inhibition of virus replication	Low rate of HBsAg disappearance
	Less expensive than interferon	Expensive for long-term use
		Potential for multidrug resistant organisms when used sequentially

HBsAg=hepatitis B surface antigen; *based on data with conventional and pegylated interferon

Table 1: Advantages and limitations of currently available antiviral agents

All features listed in this table should be given due consideration prior to making a therapeutic decision. For example, resistance to interferon has not been demonstrated, but resistance to monotherapy with nucleoside analogs often requires switching or adding treatment

using a second nucleoside derivative. This is more expensive and raises some concern that sequential treatment will lead to the emergence of multidrug-resistant HBV. Thus, pegylated interferon may be a sensible choice for first-line therapy in a patient who will probably need long-term treatment with a nucleoside analog (e.g. patients who are hepatitis Be antigen [HBeAg]-negative); if this approach is not successful, nucleoside analog therapy can then be tried. This is particularly important to consider when a nucleoside agent with a low resistance profile is not available. However, pegylated interferon should not only be used if other more preferred therapies are not available. Rather, pegylated interferon may be the preferred therapy when there is a high likelihood of HBeAg and hepatitis B surface antigen (HBsAg) seroconversion as occurs in patients infected with HBV genotype A. Fundamental to the success of such a treatment choice is the identification of patients who are likely to respond best and benefit most from a particular treatment.

FACTORS TO CONSIDER BEFORE MAKING A DECISION

If a therapy that is associated with a significantly higher rate of adverse events is chosen, improved patient outcomes should be more than just marginal compared to less toxic agents. In the present scenario, if interferon is chosen over nucleoside analogs it should lead to a substantially greater chance of short- or long-term patient benefit. I believe that these conditions for the use of pegylated interferon are met in *properly selected patients*. Consider, for example, the impact of interferon on the rates of HBsAg seroconversion. Although clearance of HBsAg is infrequent with all hepatitis B drug therapies, it remains an important event because it signals persistent loss of serum HBV DNA by polymerase chain reaction (PCR) and has been demonstrated to be associated with improved long-term survival, as well as a diminished risk of hepatocellular carcinoma (HCC) [2]. Furthermore, patients who lose HBsAg are much less likely to reactivate when given chemotherapy for cancer [3]. The rates of HBsAg disappearance are said to be too low with pegylated interferon to be clinically meaningful. It is important to understand, however, that the rates of HBsAg loss in the phase III trials of pegylated interferon vary according to ethnicity and geography. The rate in predominantly Asian patients is 3 to 4% [4,5] whilst it is 5 to 7% in Europeans [6]; both figures should be viewed in relation to the annual spontaneous HBsAg seroconversion rate of 1 to 2% in untreated HBV carriers. Even more important, however, is the high rate of HBsAg

disappearance in virological responders to interferon during prolonged post-treatment follow-up. In studies of standard interferon in European and North American patients, the rates of HBsAg loss in sustained virological responders are 50 to 70% during an average follow-up of 5-10 years [7,8]. The highly durable response that typifies interferon therapy is a major factor in this incremental pattern of HBsAg loss. In contrast, most studies using nucleoside analog therapy have shown that the rates of HBsAg seroconversion during the first few years of treatment do not differ from that expected in untreated controls.

The predictors of response to nucleoside analogs and interferon are nearly the same. For example, low baseline ALT identifies patients who are less likely to undergo hepatitis Be antigen (HBeAg) seroconversion whatever the choice of treatment. One way in which the 2 treatments differ, however, is that patients with certain HBV genotypes (A and B) respond more frequently to pegylated interferon than patients with other genotypes (C and D). In a predominantly European population, the rate of HBeAg seroconversion to pegylated interferon alfa-2b was 47%, 44%, 28% and 25% in genotype A, B, C and D patients, respectively [6]. Further analysis revealed that patients with genotype A were significantly more likely to undergo loss of HBsAg; this occurred in 14% of genotype A patients within the first 6 months of follow-up versus 9%, 3% and 2% in genotype B, C and D patients, respectively [9]. Thus, this correlation of incomplete as well as complete virological response by genotype emphasizes the importance of genotyping before deciding to use interferon. In contrast to these findings, the degree to which HBV replication can be suppressed and the rate of HBeAg seroconversion in response to nucleoside analogs does not appear to depend on viral genotype. Another difference between the predictors of response to pegylated interferon and nucleoside analog therapy is that very high levels of serum HBV DNA ($<10^{10}$ copies/mL) predict a higher rate of ultimate drug failure when using interferon-based therapy (Table 2) [10].

Important practical issues

When deciding whether to use pegylated interferon or a nucleoside analog, many practical considerations should also be addressed [11]. One important question for patients is how long will therapy be needed and how much will it cost? For many, the option of taking time-limited therapy with pegylated interferon has many advantages

over prolonged treatment with a nucleoside analog when considering the cost of care, potential changes in third party payers with time, and

	Positive Outcome	**Intermediate Outcome**	**Negative Outcome**
Pegylated interferon			
Baseline ALT	>5 x ULN	>2-5 x ULN	≤2 x ULN
Baseline HBV DNA (copies/mL)	$\leq 10^9$	10^{9-10}	$>10^{10}$
Genotype	A or B	C	D
Nucleoside analogs			
Baseline ALT	>5 x ULN	>2-5 x ULN	≤2 x ULN
Histological activity index	≥10	5-9	0-4

ALT=alanine aminotransferase; ULN=upper limit of normal

Table 2: Predictors of response to pegylated interferon and nucleoside analog therapy in hepatitis Be antigen-positive chronic hepatitis B

the possibility of remote geographic relocation in the future. The incremental cost associated with long-term or indefinite therapy with nucleoside analogs may make this treatment choice impractical for some patients. This is particularly the case in countries where the cost of drugs is either not subsidized or subsidies are limited to certain intervals of treatment, or where private health insurance is inadequate or does not exist.

TREATMENT ADVICE: WHICH WAY TO GO?

At present no single treatment recommendation that applies to all patients with chronic hepatitis B exists, nor is it likely, given the complexity of viral-host interactions and the practical issues involved. Instead, the key to successful treatment of hepatitis B resides in appropriate patient selection and individualized treatment decisions. However, in the author's opinion, certain elements need to be incorporated in sound therapeutic decisions: 1) the aim of treatment should be a durable virological response with the shortest course of therapy, thus minimizing the potential for delayed adverse events and limiting cost; 2) treatment should not increase the chances for the failure of any future therapy; 3) loss of HBsAg which is associated

with a greater certainty of long-term benefit is a valuable additional aim of treatment; and, 4) treatment decisions should not be made solely on projections of virological efficacy. There are many other pragmatic issues that need to be assessed such as the cost of care, the feasibility of intensive monitoring, and the implications that long-term continuous therapy would have on the patient's lifestyle [11]. All of these can be critically important in individual cases.

The patient should understand that treatment with nucleoside analogs is likely to be long-term or even indefinite. Post-withdrawal flares, while infrequent, can be clinically devastating in patients with advanced disease and it is therefore important to consider this factor prior to starting long-term treatment. Long-term monotherapy will ultimately result in some degree of drug resistance. Switchovers in therapy can salvage patients, but this could lead to multidrug-resistant HBV [12,13]. Add-on therapy will often be required.

With these considerations in mind, it is my opinion that a 48-week course of pegylated interferon alfa-2a should be the preferred first-line approach for several patient groups with HBeAg-positive and HBeAg-negative chronic hepatitis B (Table 3). Particular emphasis should be given to selection of individuals with genotypes A or B, low to moderate levels of HBV DNA ($\leq 10^9$ copies/mL), and moderate pre-therapy ALT (>2 to 3 x upper limit of normal [ULN]) because these clearly have been shown to be predictors of response in large phase III trials of pegylated interferon alfa-2a. Such associations are similar to findings with conventional interferon which further confirm their validity. The range of adverse events associated with interferon may be of concern, but in my personal experience if a prescribing physician is enthusiastic about the use of interferon, patients are also likely to be positive about this treatment choice.

It should also be emphasized that there are many patients who are ideal candidates for nucleoside analog treatment (Table 3). Based on data with lamivudine and entecavir, an HBeAg seroconversion rate of >50% may be expected when baseline ALT is above 5 x ULN, and the primary use of these agents in this circumstance will be better tolerated and possibly even more cost-effective than interferon [14]. As the rate of virological response is not as dependent on the pre-therapy level of serum HBV DNA, it is probably better to treat patients with high levels of HBV DNA ($>10^{10}$ copies/mL) with nucleoside analog therapy rather than interferon in most circumstances. Furthermore, only nucleoside analogs are safe for patients with hepatic decompensation. The treating physician should

remember that these agents often stabilize liver disease at an advanced stage and have been shown to be life-saving in some cases [15].

Interferon	Nucleoside analogs
– Age <60, otherwise healthy	– Adult of any age, non-serious comorbid illness
– Baseline HBV DNA $\leq 10^9$ copies/mL	– Baseline HBV DNA $>10^{10}$ copies/mL
– Baseline ALT >2 to 3 x ULN	– Baseline ALT >5 x ULN
– Genotype A or B	– Any genotype
– Noncirrhotic	– Cirrhosis, with or without decompensation
	– Chemotherapy in HBsAg-positive or anti-HBc-positive patients

ALT=alanine aminotransferase; HAI=histological activity index; anti-HBc=antibody to hepatitis B core antigen; HBsAg=hepatitis B surface antigen; ULN=upper limit of normal

Table 3: Preferred initial treatment strategies according to various patient features

Finally, there is reasonable evidence to suggest that long-term nucleoside analog therapy can delay disease progression and reduce the frequency of HCC [16]. Trials in patients with advanced hepatitis C are currently investigating long-term administration of low-dose interferon. This treatment option is not likely to be effective in patients with advanced hepatitis B because it will not adequately control viral replication to the same extent as a nucleoside analog.

CONCLUSIONS

Whatever the drug used to treat chronic hepatitis B, an SVR (however it is defined), is associated with improved biochemical, histological, and clinical outcomes. Choice of the drug used as first-line therapy should not be primarily based on patient (or physician) convenience and acceptance. Instead, the prospect and likelihood of improved long-term outcomes should be the primary consideration. It should be kept in mind that response to interferon tends to be durable, and while there is a small but definite chance of early disappearance of HBsAg, long-term follow-up studies support the frequency of this milestone event increases with time in virological responders. Loss of HBsAg is particularly important in younger individuals with many potential years of HBV infection ahead. Interferon offers the additional advantage of not promoting HBV resistance. The treating clinician should be aware that treatment with a nucleoside analog often involves a long-term commitment. Furthermore, it may be difficult to

stop therapy even if clinical and virologic end-points are achieved, due to a high rate of relapse, particularly in patients with HBeAg-negative chronic hepatitis B. For this reason, any cost advantage to the use of these agents becomes a moot point.

I would like to conclude with a direct answer to the question posed: why do I treat my patients with pegylated interferon? I believe that the most compelling reasons for this choice of treatment are that: 1) it offers time-limited therapy; 2) responses are both durable and often incremental; and, 3) there is a better chance for disappearance of all serum markers of infection. While the mechanisms behind the latter event have not been fully defined, this is most probably related to a greater elimination of the covalently-closed circular DNA template that resides within the hepatocyte nucleus. Thus, a fundamental distinction that can be made between interferon and nucleoside analog therapy is more related to the qualitative rather than to the quantitative nature of the virological response.

REFERENCES

1. Perrillo RP. Overview of treatment of hepatitis B: key approaches and clinical challenges. Semin Liv Dis 2004;24:S23-S29.

2. Fattovich G, Giustina G, Sanchez-Tapias J, Quero C, Mas A, Olivotto PG, Solinas A, Almasio P, Hadziyannis S, Degos F, de Moura MC, Krogsgaard K, Pantalena M, Realdi G, Corrocher R, Schalm SW. European Concerted Action on Viral Hepatitis (EUROHEP). Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. Am J Gastroenterol 1998:93:896-900.

3. Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. Gastroenterology 1991;100:182-188.

4. Marcellin P, Lau GKK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N; Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group. Peginterferon alfa-2a alone, lamivudine alone, and the 2 in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206-1217.

5. Lau GKK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N; PEGinterferon alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group. PEGinterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.

6. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters HG, Zondervan P, Hansen B, Schalm SW; HBV 99-01 Study Group; Rotterdam Foundation for Liver Research. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.

7. van Zonneveld M, Honkoop P, Hansen BE, Niesters HGM, Murad SD, de Man RA, Schalm SW, Janssen HL. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-810.

8. Lau DT, Everhart J, Kleiner DE, Park Y, Vergalla J, Schmid P, Hoofnagle JH. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;113:1660-1667.

9. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HLA. Treatment with Peg-interferon alfa-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol 2006;101:297-303.

10. Cooksley G, Manns M, Lau GKK, Liaw YF, Marcellin P, Chow WC, et al. Effect of genotype and other baseline factors in response to peginterferon alfa-2a (40 kDa) in HBeAg-positive chronic hepatitis B: Results from a large, randomized study (abstract 71). J Hepatology 2006;42:30-31.

11. Perrillo RP. Therapy of hepatitis B-viral suppression or eradication? Hepatology 2006;43:S182-S193.

12. Brunelle MN, Jacquard AC, Pichoud C, Durantel D, Carrouee-Durantel S, Villeneuve JP, Trepo C, Zoulim F. Susceptibility to antivirals of a human HBV strain with mutations conferring resistance to both lamivudine and adefovir. Hepatology 2005;41:1391-1398.

13. Zoulim F. Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic hepatitis B virus infection. Antiviral Res 2004;64:1-15.

14. Perrillo RP, Lai CL, Liaw YF, Dienstag JL, Schiff ER, Schalm SE, Brown NA, Atkins M, Woessner M, Gardner SD. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36:186-194.

15. Perrillo RP, 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; Lamivudine North American Transplant Group. A multicenter United States-Canadian trial to assess lamivudine monotherapy before and after liver transplantation. The Lamivudine North American Transplant Group. Hepatology 2001;33:424-432.

16. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J; Cirrhosis Asian Lamivudine Multicentre Study Group. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-1531.

Management of HBV/HIV Co-Infection

Y. Benhamou

INTRODUCTION

Recommendations for the treatment of chronic hepatitis B in HIV-infected patients are complex due to both a lack of controlled trials and the nature of the activity of therapeutic agents on both viruses. Indications for anti-hepatitis B virus (HBV) therapy should be based on HBV DNA and alanine aminotransferase (ALT) levels and liver lesions. Recommended HBV DNA thresholds for beginning treatment are 20,000 IU/mL and 2000 IU/mL for HBeAg-positive and HBeAg-negative patients, respectively. Using the METAVIR scoring system patients with a fibrosis stage F>2 and activity score A>1 should be considered for anti-HBV therapy. Patients with cirrhosis should receive anti-HBV drugs whatever the HBV DNA level. Agents approved for the treatment of chronic HBV include: 1) interferon alfa; 2) lamivudine (LAM); 3) entecavir (ETV); and, 4) adefovir dipivoxil (ADV). Lamivudine, tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) are approved for the treatment of HIV and are active against HBV. The few existing studies evaluating interferon in HIV/HBV co-infected patients suggest a decreased response in these patients compared to those infected with HBV alone. Lamivudine and FTC are effective against HBV but are associated with a high rate of HBV resistance. Entecavir, ADV and TDF are effective against wild-type and LAM-resistant HBV with a favorable resistance profile for ADV and TDF. Interferon, ADV or ETV are the drugs of choice in HBV-naïve patients who do not require HIV therapy. A combination of TDF plus FTC or LAM should be proposed in patients with a therapeutic indication for both viruses. Tenofovir disoproxil fumarate

should be included in the antiretroviral regimen of patients with HBV that is resistant to LAM.

Since the introduction of combination antiretroviral therapy and the dramatic improvement in the outcome of patients with HIV, liver disease due to chronic hepatitis B and C infection has become an important cause of morbidity and mortality in HIV-infected patients [1-3].

The management of chronic hepatitis B in HIV-infected patients is complex. Proposals for optimal anti-HBV therapy in HIV-infected individuals should be pragmatic and combine knowledge from HBV mono- and HIV/HBV co-infected studies.

The principal goals of HBV treatment are to stop or decrease the progression of liver disease and prevent cirrhosis and hepatocellular carcinoma (HCC). As anti-HBV therapy rarely leads to HBe or HBs seroconversion, long-term sustained suppression of HBV replication may be necessary to reach the therapeutic objective.

The goals of treatment and the treatment plan should reflect the needs of individual patients and will depend on the clinical status of both HIV and HBV, and whether they will be treated concurrently.

SPECIAL CONSIDERATIONS FOR HIV/HBV CO-INFECTED PATIENTS

There are no studies investigating the clinical significance of serum HBV DNA and ALT levels in HIV/HBV co-infected patients. Indications for anti-HBV therapy have recently been recommended by the 1st European Consensus Conference on the Treatment of HBV and HCV in patients co-infected with HIV [4].

The optimal time to start anti-HBV treatment in HIV co-infected patients is not known. There is not enough evidence to conclude that anti-HBV therapy should always be started when antiretroviral therapy is initiated. When it is not started concurrently, HBV treatment should be delayed until HIV replication is controlled or until there is evidence of a progression in liver disease.

When the treatment for HBV also has anti-HIV activity they should be included as components of an antiretroviral regimen. When patients change anti-HIV treatment because of intolerance or lack of efficacy, the anti-HBV component should be continued whenever possible, even if it is not part of the subsequent anti-HIV regimen.

Assessment of treatment efficacy has not been thoroughly studied in HIV/HBV co-infected patients. Although a decline in serum HBV DNA correlates with improvement in liver lesions, no threshold

HBV DNA goal has been established in HIV/HBV co-infected patients.

Highly active antiretroviral therapy (HAART) may be associated with recovery of cell-mediated immunity, leading to immune-mediated HBV-specific liver damage further advocating concurrent HIV-1 and HBV treatment. This immune restoration can either promote clearance of the virus or lead to exacerbation of liver lesions, but, in general, clearance of hepatitis B surface antigen (HBsAg) does not occur. Immune reconstitution has been associated with acute increases in serum aminotransferase levels [5,6]. These flares usually occur soon after beginning HAART in people with high HBV pretreatment viral loads [5,6]. Furthermore, reconstitution flares have been reported to occur despite the inclusion of anti-HBV active agents in the initial HAART regimen [7]. Individuals with high levels of HBV DNA (>4 or 5 $\log_{10}$ copies/mL) or those with a low nadir CD4 count may be particularly at risk.

ANTI-HBV THERAPY

There are 5 therapeutic agents licensed for the treatment of chronic HBV: 1) interferon alfa; 2) pegylated interferon alfa-2a; 3) LAM (100 mg daily); 4) ADV; and, 5) ETV. Three agents with anti-HBV activity are licensed for the treatment of HIV: 1) LAM (300 mg daily); 2) TDF; and, 3) FTC (Table 1). All these drugs have been tested in HIV/HBV co-infected patients [8-14]. A number of newer agents are also under development.

TREATMENT ALGORITHM

Since treatment of HIV/HBV co-infection has been poorly investigated the treatment algorithm should be pragmatic. The choice of HBV therapies will depend on the clinical status of the patient, and whether HIV is being treated at the same time. Single agents have been recommended for HBV infection alone. However, the high rate of resistance to LAM monotherapy in co-infected patients and the need for an indefinite duration of anti-HBV therapy supports the use of combination antiviral therapy. In addition, agents with dual activity against HBV and HIV should not be used as a monotherapy in patients not receiving antiretrovirals. This practice could compromise future anti-HIV treatments (Figure 1).

	IFN	LAM	ETV	FTC	TDF	ADV*
Duration (weeks)	12-24	48	48	48	24-48	48-192
Anti-HBV activity tested in HIV patients	wt	wt	LAM-R	wt	wt, LAM-R	LAM-R
HBV DNA decline (log_{10} copies/mL)	26%†	2.7	4.2	-	4.4	4.7-6*
(%) HBe seroconversion	9	11	-	-	4	7
(%) ALT normalization	12-20	30-50	49	-	-	35-66*
Histological improvement	-	-	-	-	-	33-50*

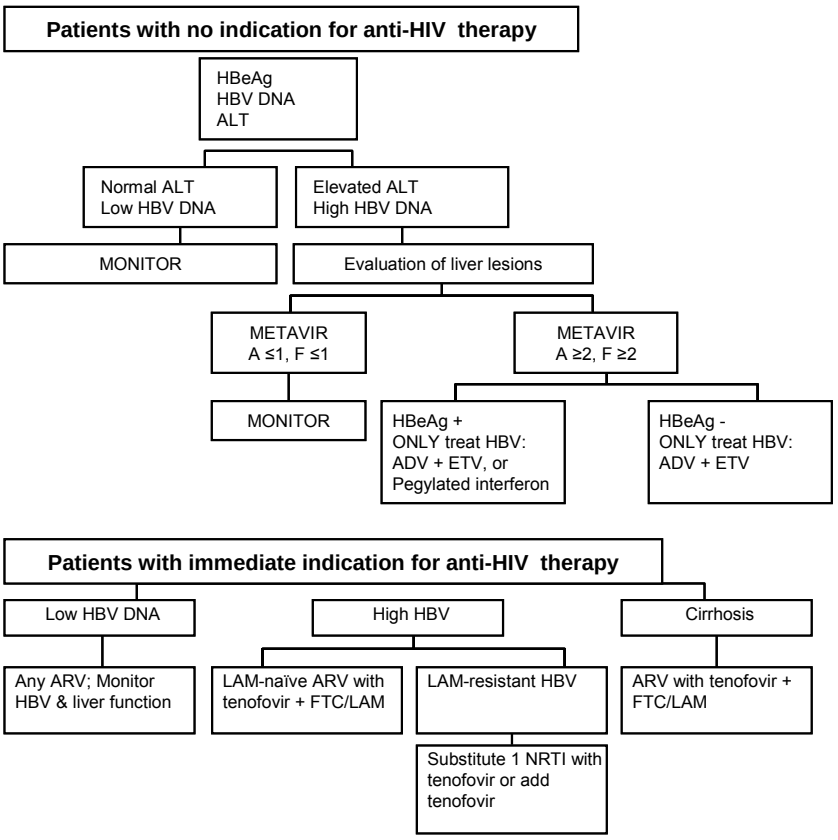
ADV=adefovir dipivoxil; ETV=entecavir; FTC=emtricitabine; IFN=standard interferon; LAM=lamivudine; LAM-R=lamivudine HBV-resistant strain; TDF=tenofovir disoproxil fumarate; wt=wild type; *results at 48-192 weeks of ADV; †proportion of patients with serum HBV DNA <6 log_{10} copies/mL

Table 1: Responses to anti-hepatitis B virus agents tested in HIV/HBV co-infected patients

HBV-naïve patients

Patients who need anti-HBV therapy and have no anti-HIV indication

Patients who do not require HIV therapy should not receive therapy for HBV infection that also has activity against HIV (LAM, TDF, FTC). This may lead to early HIV resistance, limiting later HIV therapeutic options. Under these circumstances HBeAg-positive patients can be offered pegylated interferon alfa-2a (optimal dose and duration of treatment are unknown), ADV or ETV. Newer drugs that have no anti-HIV activity (telbivudine, clevudine) may also be useful. There are not enough data to support the use of pegylated interferon in HBeAg-negative patients.



ADV=adefovir dipivoxil; ALT=alanine aminotransferase; ARV=antiretroviral; ETV=entecavir; FTC=emtricitabine; LAM=lamivudine; NRTI=nucleoside reverse transcriptase inhibitor; PEG IFN=pegylated interferon
*high HBV DNA: ≥20,000 IU/mL in HBeAg-positive patients, ≥20,000 IU/mL in HBeAg-negative patients

Figure 1: Summarized treatment algorithm of chronic hepatitis B in HIV co-infected patients [adapted from reference 4]

Patients with indications for both HBV and HIV therapy

Agents with dual activity (TDF, FTC, LAM) should be included in the antiretroviral regimen. The combination of a nucleoside and a nucleotide analog is the preferred association to prevent long-term

resistance (TDF + LAM or TDF + FTC). Adefovir may be an alternative if TDF cannot be used, and ETV is an alternative to FTC or LAM. Although not the first choice, monotherapy remains an option. In this case, nucleotides (TDF) are preferable to nucleosides (FTC, LAM) because of a more favorable resistance profile.

Patients who need anti-HIV therapy and no anti-HBV therapy

Patients with persistent controlled HBV replication (serum HBV DNA <4 $\log_{10}$ copies/mL) may not need drugs with dual activity. These patients should be monitored for ALT and serum HBV DNA every 3 or 4 months. Some HIV/HBV co-infected patients may have a high serum HBV DNA (>4 to 5 $\log_{10}$ copies/mL) and no or mild liver disease. The strategy should be the same as in patients with indications for both viruses, to prevent immune reconstitution hepatitis.

Patients with HBV resistance to lamivudine

Tenofovir disoproxil fumarate should be included in the antiretroviral regimen and LAM maintained. Entecavir or ADV are alternative options.

Patients with cirrhosis

The sustained control of HBV replication in patients with cirrhosis is critical to prevent liver decompensation, HCC and death. Compliance to therapy and prevention of resistance are essential. Therefore, patients with cirrhosis should be treated with combination therapy.

CONCLUSION

In HIV/HBV co-infected patients HIV and HBV can be treated individually or concurrently. Combination therapy is generally preferable in patients who need anti-HBV and anti-HIV therapy. Anti-HBV monotherapy is the option for HIV-naïve patients who need anti-HBV therapy. Studies are needed to identify correlation between disease progression and treatment responses. Clinical trials are needed to address the value of combination therapies. Finally, the role of new anti-HBV drugs in HIV/HBV co-infected patients should also be evaluated.

REFERENCES

1. Thio C, Seaberg E, Skolasky R, Phair J, Visscher B, Munoz A, Thomas DL. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter AIDS Cohort Study (MACS). Lancet 2002;360:1921-1926.
2. Colin JF, Cazals-Hatem D, Loriot M, Martinot-Peignoux M, Pham BN, Auperin A, Degott C, Benhamou JP, Erlinger S, Valla D, Marcellin P. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. Hepatology 1999:29;1306-1310.
3. Benhamou Y. Antiretroviral therapy and HIV/Hepatitis B co-infection. Clin Infect Dis 2004;38:S98-S103.
4. Alberti A, Clumeck N, Collins S, Gerlich W, Lundgren J, Palu G, Reiss P, Thiebaut R, Weiland O, Yazdanpanah Y, Zeuzem S; ECC Jury. Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. J Hepatol 2005;42:615-624.
5. Carr A, Cooper D. Restoration of immunity to chronic hepatitis B infection in HIV-infected patient on protease inhibitor. Lancet 1997;346:995-996.
6. Proia L, Ngui S, Kaur S, Kessler H, Trenholme G. Reactivation of hepatitis B in patients with human immunodeficiency virus infection treated with combination antiretroviral therapy. Am J Med 2000;108:249-251.
7. Drake A, Mijch A, Sasadeusz J. Immune reconstitution hepatitis in HIV and hepatitis B coinfection, despite lamivudine therapy as part of HAART. Clin Inf Dis 2004;39:129-143.
8. Wong DK, Yim C, Naylor CD, Chen E, Sherman M, Vas S Wanless IR, Read S, Li H, Heathcote EJ. Interferon alfa treatment of chronic hepatitis B: Randomized trial in a predominantly homosexual male population. Gastroenterology 1995;108:165-171.
9. Benhamou Y, Katlama C, Lunel F, Coutellier A, Dohin E, Hamm N, Tubiana R, Herson S, Poynard T, Opolon P. The effects of lamivudine on replication of hepatitis B virus in HIV-infected men. Ann Intern Med 1996;125:705-712.
10. 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. Lancet 2001;358:718-723.
11. Benhamou Y, Thibault V, Vig P, Calvez V, Marcelin AG, Fievet MH, Currie G, Chang CG, Biao L, Xiong S, Brosgart C, Poynard T. Safety and efficacy of adefovir dipivoxil in patients infected with lamivudine-resistant hepatitis B and HIV-1. J Hepatol 2006;44:62-67.
12. Benhamou Y, Fleury H, Trimoulet P, Pellegrin I, Urbinelli R, Katlama C. Anti-hepatitis B virus efficacy of tenofovir disoproxil fumarate in HIV-infected patients. Hepatology 2006;43:548-555.

13. Raffi F, Snow A, Borroto-Esoda K, et al. Anti-HBV activity of emtricitabine in patients co-infected with HIV and hepatitis B virus. Abstracts of the 2nd International AIDS Society Conference on HIV Pathogenesis and Treatment. Paris (abstract). 2003:215.

14. Pessoa W, Gazzard B, Huang A, Brandao-Mello C, Cassetti L, Correa M et al. Entecavir in HIV/HBV-co-infected Patients: Safety and Efficacy in a Phase II Study (ETV-038). Conference on Retroviruses and Opportunistic Infections, Boston, MA (abstract). 2005:123.

Treatment of Patients with Chronic Hepatitis Delta

P. Farci

Chronic hepatitis D is a severe and rapidly progressive liver disease caused by persistent infection with hepatitis D virus (HDV), one of the most interesting and unusual human pathogens [1]. Hepatitis D virus is the smallest animal virus and the only one to possess a circular RNA genome (1700 nucleotides) and a single structural protein, hepatitis delta antigen (HDAg), encapsidated by the hepatitis B surface antigen (HBsAg) [2]. Hepatitis D virus does not resemble any known transmissible agent of animals and is unique in several aspects. The most peculiar feature of HDV is the fact that it is a defective virus that requires the obligatory helper function of hepatitis B virus (HBV) for its assembly and transmission [3]. It does not encode a polymerase of its own, but exploits a host cellular enzyme for its replication [4]. The only enzymatic activity that is inherent to HDV is mediated by RNA elements termed ribozymes, which cleave the circular genome producing a linear RNA molecule [5,6]. The HDV genome replicates via a double rolling-circle model similar to that proposed for some plant viroids [7]. Due to the obligatory link with HBV, infection with HDV occurs only in persons who simultaneously harbor HBV. There are 2 principal modes of HDV infection: simultaneous coinfection with HBV and superinfection of an HBsAg carrier. Whereas co-infection evolves to chronicity in only 2% of cases, superinfection results in chronic infection in over 90%. Being immune to HBV, persons carrying anti-HBs are also protected from HDV infection.

It has been estimated that 5% of HBsAg carriers are also co-infected with HDV worldwide, leading to a total of 15 million people infected with HDV. Over the past decade, there has been a dramatic decline in the prevalence of HDV in Southern Europe [8,9],

which is probably due to the implementation of universal HBV vaccination and other precautions. As a consequence, new, florid forms of hepatitis D have become rare. Nevertheless, residual disease is still present in patients who were infected when HDV infection was endemic. These patients pose a major therapeutic challenge because most of them present with advanced liver disease. Chronic hepatitis D is the least common but the most severe form of viral hepatitis, leading to cirrhosis in about 80% of cases [10] within 5-10 years from the onset of acute hepatitis. This figure is almost 3-times higher than for hepatitis B or C. Once established, HDV-associated cirrhosis may be a stable disease for many years, although the risks of mortality and HCC are 2 and 3 times those observed in patients with compensated HBV-associated cirrhosis alone [11].

The unique replication process of HDV and its high pathogenic potential make chronic hepatitis D a particularly challenging target for antiviral therapy. No specific inhibitor of HDV has been developed so far. Strikingly, in spite of the vital relationship between HDV and HBV, drugs that specifically block HBV have little or no effect on HDV replication. This is most likely due to the fact that the contribution of HBV replication to HDV pathogenesis is negligible, as suggested by the fact that HBV replication levels are usually low during chronic hepatitis D [12]. The only helper function that HBV provides to HDV is the envelope, HBsAg, which is efficiently expressed in most HBV carriers regardless of the level of HBV replication. Thus effective therapy would probably require marked suppression of HBsAg expression, which current therapies for HBV do not achieve. As a consequence, drugs that potently inhibit HBV replication such as lamivudine (LAM), a second-generation nucleoside analog that does not typically reduce HBsAg levels, fail to show any efficacy on HDV viremia or liver disease activity in patients with chronic hepatitis D [13,14]. Similarly, neither viremia nor HDV-related liver disease were reduced after treatment with another nucleoside analog, famciclovir [15]. In addition, immunomodulators such as steroids, thymosin, levamisole, thymic humoral factor-gamma 2, and other antiviral agents, such as acyclovir and ribavirin (RBV), have been proven to be ineffective against HDV [14]. Recently, clevudine, a nucleoside analog that suppresses HBV replication [16], was shown to reduce hepatitis D viremia levels in chronically infected woodchucks [17], but data in humans are lacking. Prenylation inhibitors that block a post-translational modification of the large HDV antigen, a critical determinant of viral assembly, represent a new class of antiviral agents [18].

The only option currently available for the treatment of chronic hepatitis D is interferon alfa, which is the most extensively studied and the only licensed drug for the treatment of this disease [19]. The earliest observations on the efficacy of interferon alfa in chronic hepatitis D date back to the mid 1980s when it was reported that a short course of interferon alfa was associated with an improvement, although transient, of chronic hepatitis D [20]. Subsequently, several clinical trials, most uncontrolled, have been carried out in order to evaluate the effects of long-term treatment with interferon alfa [14]. The results of these clinical trials, however, were difficult to compare because of large variations in patient characteristics, dose and duration of treatment, study end-points and, most importantly, the variable sensitivity and lack of standardization of HDV RNA assays for treatment monitoring and assessment. Despite these limitations, the results showed that interferon alfa is effective in chronic hepatitis D, but the rate of relapse is high and its efficacy is related to the dose and duration of treatment. The highest rate of response was achieved using high doses of interferon alfa (9 MU 3-times per week) for 12 months, with alanine aminotransferase (ALT) normalization in 71% of patients [21]. Although HDV viremia remained detectable in all patients by qualitative polymerase chain reaction (PCR) regardless of ALT response, based on a semiquantitative assay, patients with a biochemical response showed a significant reduction (up to 4 $\log_{10}$) in the levels of HDV viremia at the end of treatment (22). Strikingly, the effects of treatment with high doses of interferon alfa were long-lasting. In patients with ALT normalization, the virological response persisted for up to 14 years, leading to the clearance of serum HDV RNA and eventually of HBV DNA in some patients. These beneficial effects were associated with a marked improvement in liver histology (complete disappearance of advanced fibrosis), normalization of liver-enzyme values and significant improvement in hepatic synthesis. High doses of interferon alfa were found to significantly improve the long-term clinical outcome and survival of patients with chronic hepatitis D, even in the presence of initial cirrhosis [22].

Although interferon alfa remains the only therapy of proven benefit for chronic hepatitis D, treatment is still not satisfactory [14,19]. Efficacy is limited, relapses are common, treatment is poorly tolerated at the doses needed and it is not indicated in patients with decompensated cirrhosis. To increase the efficacy of interferon alfa therapy, several strategies have been investigated, including longer duration of treatment or even continuous therapy for up to

12 years [23], but most patients still do not respond and the relapse rate remains high. Following the wave of success obtained with combination therapy in chronic hepatitis B and especially C, a few small studies have investigated the efficacy of standard interferon alfa in combination with LAM or RBV. A slightly higher rate of sustained complete response was seen in patients treated with interferon alfa plus LAM (28%) compared with 18% with interferon alfa alone [24] whereas the addition of RBV failed to show any beneficial effects, while relatively important side effects occurred [25,26]. Thus, in contrast to the significantly higher response rates achieved in chronic hepatitis C, combination of interferon alfa plus RBV does not increase the response rates in chronic hepatitis D. The molecular mechanisms for the difference in effectiveness of RBV in chronic hepatitis C and D are unknown.

More recently, 2 studies evaluated the efficacy and safety of pegylated interferon alone or in combination with RBV for the treatment of chronic hepatitis D [27]. Pegylated interferon, the product of conjugation of the original recombinant interferon alfa with an inert molecule of polyethelene glycol results in prolongation of the half-life so that only 1 dose is required to maintain effective levels in the blood. Given the increased response rates observed with pegylated interferon compared to conventional interferon alfa in chronic hepatitis B and C, its effectiveness was assessed in chronic hepatitis D. Castelnau and colleagues [28] evaluated the efficacy of pegylated interferon alfa-2b monotherapy, 1.5 μg/kg per week for 12 months, in 14 patients with chronic hepatitis D. A virological response at the end of treatment, defined as undetectable HDV RNA by qualitative polymerase chain reaction PCR, was seen in 57% of the patients and 43% were still virological responders 6-49 months after cessation of therapy, even though most of the patients (85%) were previous non-responders to high doses of standard interferon alfa. The rate of biochemical response (normalization of ALT levels) was higher at the end of follow-up (57%) than at the end of therapy (36%), indicating that in some patients, ALT normalization was achieved during post-treatment follow-up. Until now, virological response has been monitored primarily by qualitative assays. Castelnau and colleagues used real-time PCR to quantify the levels of viremia, and they found that HDV RNA levels significantly decreased during the first 3-6 months of treatment in patients with a virological response at the end of treatment, whereas no significant changes were observed in the non-responder group. Hepatitis D virus RNA became undetectable in 6 out of 8 responders at month 6 of treatment, but in none of the

non-responders. However, HDV RNA also significantly decreased in 2 patients who relapsed after the end of treatment, indicating that early reduction of viremia does not differentiate responders from relapsers. It should be noted that there was a delayed decrease in HDV RNA at the end of the treatment period in 2 non-responders. These patients may represent "slow responders" who might benefit from a longer course of interferon alfa therapy. In the second study Niro and colleagues reported the results of a randomized controlled trial designed to compare the efficacy and safety of pegylated interferon alfa alone (1.5 μg/kg/week) for 72 weeks (16 patients) or in combination with RBV (800 μg/day) for 48 weeks followed by pegylated interferon monotherapy for 24 additional weeks (22 patients) [29]. Most of the patients had cirrhosis (74%) and were unresponsive (79%) to standard interferon alfa alone or in combination with LAM. Clearance of HDV occurred in 25% of these patients, as measured by a sensitive qualitative PCR assay 6 months after the end of treatment, whereas the addition of RBV, as seen with standard interferon alfa, did not result in a higher rate of sustained virological response (18%). Interestingly, as previously documented with standard interferon alfa [22], the rate of virological response increased in both groups during the post-treatment follow-up; the response was seen in 3 out of the 8 patients who had HDV RNA levels below 1000 copies/mL at the end of treatment. This delayed viral response is probably due to the long-term immunomodulatory effects of interferon alfa, as seen in chronic hepatitis B [30]. The rates of biochemical response were similar in patients treated with monotherapy and combination therapy, both at the end of treatment (37% vs. 41%) and at the end of follow-up (25% vs. 27%), although the overall rate of relapse was high, even with pegylated interferon alfa. Tolerance of therapy was poor with treatment discontinuation in 25% of patients and dose modification in 58% mainly due to exacerbation of neutropenia and thrombocytopenia in patients with cirrhosis.

Although these 2 studies were small and most patients were previous non-responders to standard interferon alfa, the results provide new information for the treatment and monitoring of patients with chronic hepatitis D. Considering the excellent results of pegylated interferon alfa in chronic hepatitis B and C, these preliminary results suggest that this treatment should become the standard treatment choice in chronic hepatitis D. In contrast, the addition of RBV to pegylated interferon alfa does not improve the response rate. The use of long-acting interferon alfa once a week

would probably also result in better compliance during the long duration of treatment necessary for chronic hepatitis D patients. Although these studies also confirm and reinforce the importance of using quantitative assays of viremia in treatment monitoring, there is no standardized assay available and the detection of HDV RNA still relies on home-made assays. Once quantitative HDV RNA testing is standardized and widely adopted, enabling the identification of a decline in serum HDV RNA during treatment, patients with significant decreases in viremia who might benefit from a more prolonged course of therapy even though they test positive on a qualitative assay, can be treated accordingly. Even using quantitative methods for viremia, there is no way to predict who will have a sustained viral loss or who will relapse after therapy. Although more recently acquired disease may respond better to therapy, clear predictors of response have not been confirmed and the timing of response is unpredictable both with standard and pegylated interferon. Thus, treatment with high doses of interferon alfa or pegylated interferon alfa for at least 1 year should be offered to all patients with compensated chronic hepatitis D at diagnosis, before a patient becomes a non-responder. One of the major challenges is to decide when to stop treatment in a patient with a good response as a loss of serum HDV RNA may not reflect viral clearance and a significant decline in viremia may lead to a delayed virological response. As the major goal is eradication of both HDV and HBV, therapy should be continued as long as possible in responders until HDV RNA and HBsAg disappear, adjusting the interferon alfa dose according to tolerance and to the patient's ALT and possibly HDV RNA levels. Side effects are common during interferon alfa treatment and therefore continuing medical monitoring is essential for the early detection and management of medical and psychiatric complications [31]. Although the first trials with pegylated interferon alfa in chronic hepatitis D have provided some encouraging results, the low response rate and the high rate of relapse emphasize the need to determine predictors of response and to use innovative molecular approaches to identify new antiviral agents that may benefit patients with chronic hepatitis D.

REFERENCES

1. Rizzetto M, Canese MG, Arico S, Crivelli O, Bonino F, Trepo CG, Bonino F, Verme G. Immunofluorescence detection of a new antigen/antibody system (delta/anti-delta) associated with hepatitis B virus in liver and serum of HBsAg carriers. Gut 1977;18:997-1003.
2. Wang KS, Choo QL, Weiner AJ, Ou JH, Najarian C, Thayer G, Mullenbach GT, Denniston KJ, Gerin JL, Houghton M. Structure, sequence and expression of the hepatitis delta viral genome. Nature 1986;323:508-514.
3. Taylor JM. Hepatitis delta virus. Virology 2006;344:71-76.
4. Lai MM. RNA replication without RNA-dependent RNA polymerase: surprises from hepatitis delta virus. J Virol 2005;79:7951-7958.
5. Sharmeen L, Kuo MY, Dinter-Gottlieb G, Taylor J. Antigenomic RNA of human hepatitis delta virus can undergo self-cleavage. J Virol 1988;62:2674-2679.
6. Ferre-D'Amare AR, Zhou K, Doudna JA. Crystal structure of a hepatitis delta virus ribozyme. Nature 1998;395:567-574.
7. Branch AD, Robertson HD. A replication cycle for viroids and small infectious RNAs. Science 1984;223:450-455.
8. Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Stornaiuolo G, Lobello S, Sagnelli E, Brunetto MR, Rizzetto M. Chronic hepatitis D: a vanishing disease? An Italian multicenter study. Hepatology 2000;32:824-827.
9. Navascues CA, Rodriguez M, Sotorrio NG, Sala P, Linares A, Suarez A, Rodrigo L. Epidemiology of hepatitis D virus infection: changes in the last 14 years. Am J Gastroenterol 1995;90:1981-1984.
10. Rizzetto M, Verme G, Recchia S, Bonino F, Farci P, Arico S, Calzia R, Picciotto A, Colombo M, Popper H. Chronic hepatitis in carriers of hepatitis B surface antigen, with intrahepatic expression of the delta antigen. An active and progressive disease unresponsive to immunosuppressive treatment. Ann Intern Med 1983;98:437-441.
11. 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.
12. Sakugawa H, Nakasone H, Nakayoshi T, Kawakami Y, Yamashiro T, Maeshiro T, Kinjo F, Saito A, Zukeran H. Hepatitis B virus concentrations in serum determined by sensitive quantitative assays in patients with established chronic hepatitis delta virus infection. J Med Virol 2001;65:478-484.
13. Lau DT, Doo E, Park Y, Kleiner E, Schmid P, Kuhns MC, Hoofnagle JH. Lamivudine for chronic delta hepatitis. Hepatology 1999;30:546-549.
14. Niro GA, Ciancio A, Tillman HL, Lagget M, Olivero A, Perri F, Fontana R, Little N, Campbell F, Smedile A, Manns MP, Andriulli A, Rizzetto M. Lamivudine therapy in chronic delta hepatitis: a multicentre randomized-controlled pilot study. Aliment Pharmacol Ther 2005;22:227-232.

15. Yurdaydin C, Bozkaya H, Gurel S, Tillmann HL, Aslan N, Okcu-Heper A, Erden E, Yalcin K, Iliman N, Uzunalimoglu O, Manns MP, Bozdayi AM. Famciclovir treatment of chronic delta hepatitis. J Hepatol 2002;37:266-271.

16. 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. A phase II dose-escalating trial of clevudine in patients with chronic hepatitis B. Hepatology 2004;40:140-148.

17. Casey J, Cote PJ, Toshkov IA, Chu CK, Gerin JL, Hornbuckle WE, Tennant BC, Korba BE. Clevudine inhibits hepatitis delta virus viremia: a pilot study of chronically infected woodchucks. Antimicrob Agents Chemother 2005;49:4396-4399.

18. Glenn JS. Prenylation of HDAg and antiviral drug development. Curr Top Microbiol Immunol 2006;307:133-149.

19. Hoofnagle JH, Di Bisceglie AM. The treatment of chronic viral hepatitis. N Engl J Med 1997;336:347-356.

20. Farci P. Delta hepatitis: an update. J Hepatol 2003;39:S212-S219.

21. Farci P, Mandas A, Coiana A, Lai ME, Desmet V, Van Eyken P, Gibo Y, Caruso L, Scaccabarozzi S, Criscuolo D, Ryff JC, Balestrieri A. Treatment of chronic hepatitis D with interferon alfa-2a. N Engl J Med 1994;330:88-94.

22. Farci P, Roskams T, Chessa L, Peddis G, Mazzoleni AP, Scioscia R, Serra G, Lai ME, Loy M, Caruso L, Desmet V, Purcell RH, Balestrieri A. Long-term benefit of interferon alpha therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. Gastroenterology 2004;126:1740-1749.

23. Lau DT, Kleiner DE, Park Y, Di Bisceglie AM, Hoofnagle JH. Resolution of chronic delta hepatitis after 12 years of interferon alfa therapy. Gastroenterology 1999;117:1229-1233.

24. Canbakan B, Senturk H, Tabak F, Akdogan M, Tahan V, Mert A, Sut N, Ozaras R, Midilli K, Ozbay G. Efficacy of interferon alpha-2b and lamivudine combination treatment in comparison to interferon alpha-2b alone in chronic delta hepatitis: a randomized trial. J Gastroenterol Hepatol 2006;21:657-663.

25. Gunsar F, Akarca US, Ersoz G, Kobak AC, Karasu Z, Yuce G, Ilter T, Batur Y. Two-year interferon therapy with or without ribavirin in chronic delta hepatitis. Antivir Ther 2005;10:721-726.

26. Kaymakoglu S, Karaca C, Demir K, Poturoglu S, Danalioglu A, Badur S, Bozaci M, Besisik F, Cakaloglu Y, Okten A. Alpha interferon and ribavirin combination therapy of chronic hepatitis D. Antimicrob Agents Chemother 2005;49:1135-1138.

27. Farci P. Treatment of chronic hepatitis D: New advances, old challenges. Hepatology 2006;44:536-539.

28. Castelnau C, Le Gal F, Ripault MP, Gordien E, Martinot-Peignoux M, Boyer N, Pham BN, Maylin S, Bedossa P, Deny P, Marcellin P, Gault E. Efficacy of peginterferon alpha-2b in chronic hepatitis delta: Relevance of quantitative RT-PCR for follow-up. Hepatology 2006;44:728-735.

29. Niro GA, Ciancio A, Gaeta GB, Smedile A, Marrone A, Olivero A, Stanzione M, David E, Brancaccio G, Fontana R, Perri F, Andriulli A, Rizzetto M. Pegylated interferon alpha-2b as monotherapy or in combination with ribavirin in chronic hepatitis delta. Hepatology 2006;44:713-720.

30. Lok AS. The maze of treatments for hepatitis B. N Engl J Med 2005;352:2743-2746.

31. Gaudin JL, Faure P, Godinot H, Gerard F, Trepo C. The French experience of treatment of chronic type D hepatitis with a 12-month course of interferon alpha-2B. Results of a randomized controlled trial. Liver 1995;15:45-52.

Clinical Case: Management of Antiviral-Resistant Hepatitis B

A. S. F. Lok

INTRODUCTION

Substantial advances have been made in the treatment of chronic hepatitis B in the past decade. Currently, there are 5 approved therapies: standard and pegylated interferon, lamivudine (LAM), adefovir (ADV) and entecavir (ETV). Although nucleos(t)ide analogs are more convenient than interferon and have fewer side effects, sustained viral suppression is achieved in a very small percentage of patients after withdrawal of nucleos(t)ide analogs, necessitating long and in many cases, indefinite treatment. Unfortunately, long-term nucleos(t)ide analog treatment is associated with an increasing risk of the development of drug resistance. Emergence of antiviral-resistant hepatitis B virus (HBV) mutants leads to loss of initial response, hepatitis flares, and in rare instances, hepatic decompensation and death [1]. Furthermore, mutations to 1 nucleos(t)ide analog may be cross-resistant to another nucleos(t)ide analog, thus limiting future treatment options. Finally, there have been reports of multidrug-resistant HBV due to sequential monotherapy [2-5]. Therefore, the decision to initiate hepatitis B treatment and the choice of initial therapy must balance long-term benefits against long-term risks. The following case discussion highlights the complexities of hepatitis B treatment and the need for careful monitoring.

CASE REPORT OF AN ASYMPTOMATIC HEPATITIS B SURFACE ANTIGEN-POSITIVE PATIENT

Patient history and first examination – March 2002

A 25 year-old Chinese man was found to have mildly elevated aminotransferases during an incidental blood test in March 2002. Subsequent testing showed that he tested positive for hepatitis B surface antigen (HBsAg), hepatitis Be antigen (HBeAg), and had high serum HBV DNA levels (Table 1). He could not recall any past history of acute hepatitis or jaundice, and denied risk factors for hepatitis B. He was born in Taiwan and had been in the US for 3 years. He was single and attending graduate school. An uncle in the family was diagnosed with liver cancer at the age of 55. His mother and 1 brother also tested positive for hepatitis B. He had no symptoms and did not have any other medical problems. He had no stigmata of chronic liver disease, and no hepatosplenomegaly.

Hemoglobin	15 g/dL	Albumin	4.3 g/dL
WBC	5.4 x 10^9/L	AST	34 IU/L (ULN 35)
Platelet	225 x 10^9/L	ALT	46 IU/L (ULN 40)
INR	0.9 mg/dL	Bilirubin	0.6 mg/dL
HBsAg	Positive	Alkaline phosphatase	91 IU/L
HBeAg	Positive	AFP	5.9 ng/mL
HBV DNA	8.7 $\log_{10}$ copies/mL		
Ultrasound	Normal liver size and texture, spleen not enlarged		

ULN=upper limit of normal; AST=aspartate aminotransferase; ALT=alanine aminotransferase; AFP=alfa-fetoprotein; HBsAg=hepatitis B surface antigen; HBeAg=hepatitis Be antigen; INR=international normalized ratio

Table 1: Initial test results of an HBeAg-positive patient with high serum HBV DNA and minimially elevated ALT

Initial management with lamivudine 100 mg – June 2002

Repeat tests 1 month later showed that alanine aminotransferase (ALT) was 42 IU/L and HBV DNA 9.4 $\log_{10}$ copies/mL. The gastroenterologist managing this patient was very concerned that the serum HBV DNA level had more than doubled and that the ALT remained elevated. A liver biopsy was discussed but the patient

refused the procedure. Treatment was recommended in view of the high and rising serum HBV DNA levels and the family history of liver cancer. At that time, the only approved therapies included standard interferon and LAM. Neither the patient nor the physician wanted to try interferon because of the need for parenteral administration, the concern being that side effects, such as fatigue, would hinder the patient's studies, and the low likelihood of response due to the minimally elevated ALT and high serum HBV DNA levels. Lamivudine 100 mg daily was prescribed in June 2002.

Initial response to lamivudine: 5 $\log_{10}$ decrease in serum HBV DNA – December 2002

Serum HBV DNA decreased by >3 $\log_{10}$ to 5.8 $\log_{10}$ copies/mL by September 2002. In December 2002, ALT fell to 29 IU/mL, and HBV DNA to 4.5 $\log_{10}$ copies/mL while HBeAg remained positive. The patient was reassured by this excellent response. In July 2003 he remained well and laboratory tests showed that his ALT was still normal at 31 IU/mL, alfa-fetoprotein (AFP) was 7.1 ng/mL, and ultrasound was normal. Hepatitis B markers were not tested.

Treatment switch to adefovir monotherapy due to lamivuline resistance – March 2004

The patient graduated and found a job in a different city. He continued to take LAM. In February 2004, he experienced severe fatigue but was not jaundiced. Laboratory tests revealed ALT 237 IU/mL, bilirubin 0.9 mg/dL, international normalized ratio (INR) 1.0, HBeAg-positive, HBV DNA 8.7 $\log_{10}$ copies/mL.

Lamivudine resistance was suspected. In March 2004, his new physician advised him to stop LAM and to start ADV 10 mg daily. His symptoms slowly improved and ALT normalized after 4 months but HBV DNA decreased very slowly. After 6 months on ADV, HBV DNA remained at 6.4 $\log_{10}$ copies/mL. The patient was reassured that at the time, no resistance was reported to be associated with ADV.

Adefovir resistance diagnosed – February 2006

Over the next few months, serum HBV DNA decreased to a trough of 5.2 $\log_{10}$ copies/mL by April 2005 and remained at that level until November 2005 when HBV DNA increased to 5.9 $\log_{10}$ copies/mL.

The patient was asymptomatic and ALT remained normal. In February 2006, HBV DNA increased to 6.5 million copies/mL and the patient was referred to a hepatologist. The patient felt well but ALT had increased from 27 IU/L to 39 IU/L by April 2005. Antiviral resistance testing showed a N236T mutation (asparagine to threonine substitution) but no change in the YMDD motif.

The question is what would be the optimal further management of this patient: 1) stop ADV and observe; 2) stop ADV and treat with pegylated interferon; 3) stop ADV and switch to ETV; 4) stop adevovir and switch to tenofovir (TDF); 5) continue ADV alone; 6) continue ADV and reintroduce LAM; 7) continue ADV and add ETV; or, 8) stop ADV and switch to Truvada (combination of emtricitabine [FTC] and TDF)?

ANTIVIRAL-RESISTANT HBV

Incidence and risk factors of antiviral-resistant HBV

Before discussing how this patient should be managed, it is worth discussing the incidence and risk factors of antiviral-resistant HBV. One reason that the knowledge about antiviral-resistant HBV is confusing is the lack of standardization in nomenclature. At the National Institutes of Health Meeting on HBV in 2006, standardized nomenclature was proposed [6].

Although antiviral-resistant HBV mutations can occur spontaneously, these mutants are present as a minor virus species (<0.1%) in a large pool of viruses in most HBV carriers who have not been exposed to nucleos(t)ide analogs. Thus, unless special techniques are used that selectively amplify the mutants, antiviral-resistant HBV cannot be detected in patients who have not received nucleos(t)ide analog treatment.

The incidence of genotypic resistance is related to viral, host, and treatment factors. Among patients receiving a specific nucleos(t)ide analog, the frequency of detection of antiviral-resistant HBV mutants correlates with pretreatment serum HBV DNA levels, rapidity of viral suppression, and duration of treatment. Several studies have shown that patients receiving LAM or telbivudine (LdT) treatment whose serum HBV DNA remained above $3 \log_{10}$ copies/mL (1,000 copies/mL) after 6 months of treatment had significantly higher rates of antiviral resistance [7,8]. Similarly, it has been reported that patients receiving ADV treatment whose serum HBV DNA remained

above 3 $\log_{10}$ copies/mL after 12 months of treatment had significantly higher rates of ADV resistance [9]. The incidence of genotypic resistance also varies with the sensitivity of the methods used to detect resistant mutations and the patient population being studied.

Direct sequencing is necessary to identify mutations resistant to new treatments and to detect all changes in the HBV polymerase gene. However, direct sequencing is insensitive and can consistently detect resistant mutants only if they constitute at least 40% of the virus population. Other assays such as restriction fragment length polymorphism (RFLP) and reverse hybridization (line probe) are more sensitive and can detect resistant mutants when they comprise 10% of the virus population [10].

The first manifestation of antiviral resistance is virological breakthrough – an increase in serum HBV DNA levels by >1 $\log_{10}$ copies/mL from nadir. However, emergence of antiviral resistance may occur prior to virological breakthrough in patients who have incomplete viral suppression – persistently detectable serum HBV DNA during continued treatment. Thus, studies testing all patients with detectable serum HBV DNA using sensitive polymerase chain reaction (PCR) assays tend to report higher rates of genotypic resistance than those in which only patients with virological breakthrough or viral rebound are tested for antiviral-resistant mutations.

Incidence and risk factors

The key mutations associated with resistance to ADV involve substitutions of the amino acid asparagine for threonine (N236T) and of alanine for valine or threonine (A181V/T) [2,3]. *In vitro* studies showed that these mutations decrease susceptibility to ADV by 3- to 15-fold but viral rebound (>100-fold increase in serum HBV DNA levels), hepatitis flares and hepatic decompensation have been reported to be associated with the selection of these mutations [11].

Compared to LAM, ADV treatment is associated with a lower rate of drug resistance. Direct sequencing of all samples with detectable HBV DNA by PCR after 48 weeks of ADV treatment in 2 phase III trials showed no evidence of genotypic resistance [12]. A follow-up report of 70 HBeAg-negative patients who received 5 years of ADV found that the rate of genotypic resistance as determined by direct sequencing increased from 0% in year 1 to 3%, 11%, 18%, and 29% in years 2, 3, 4 and 5, respectively [13]. A retrospective analysis of

467 patients who received ADV for LAM-resistant HBV found that genotypic resistance to ADV was detected in only 4 patients, all of whom had stopped LAM [14]. None of the patients who received combination treatment with LAM and ADV were found to have ADV-resistant mutations. Several recent studies using more sensitive techniques have reported that ADV-resistant mutations can be detected in approximately 5% of patients after 1 year and in up to 20% after 2 years of treatment [4,15,16]. The inclusion of patients with prior LAM resistance, who were switched to ADV monotherapy may have contributed in part to the higher rate of ADV resistance seen in these studies.

Increasing data indicate that while resistance to ADV is less common than to LAM, it is more common than previously reported. Adefovir resistance is particularly more frequent among patients who have slow or inadequate viral suppression, and who switched to ADV monotherapy because of prior LAM resistance.

Treatment

In vitro studies showed that ADV-resistant HBV mutants are susceptible to LAM and ETV, but are partially resistant to TDF [3]. Based on these *in vitro* data, it has been suggested that patients with ADV-resistant HBV be treated with addition of LAM. Case reports have confirmed *in vivo* efficacy of LAM in suppressing ADV-resistant HBV [2,3,11]. However, the duration of follow-up in these reports is short. Thus, the durability of the antiviral efficacy of LAM in suppressing ADV-resistant HBV is unknown. This is of particular concern in patients with prior LAM resistance as multiple studies have shown that LAM-resistant mutations can be detected more than 12 months after LAM is withdrawn. In addition, there have been reports that LAM-resistant mutations are rapidly selected upon reintroduction of LAM. We recently reported the re-emergence of LAM-resistant mutations within 4 months of reintroducing LAM in a patient with ADV-resistant HBV, even though LAM had been stopped 34 months earlier and LAM-resistant mutations were not detected by direct sequencing or a more sensitive line probe assay at the time LAM was reintroduced [11]. Thus, while LAM may be an appropriate treatment for LAM-naïve patients with ADV-resistant HBV, its long-term efficacy in LAM-experienced patients with ADV-resistant HBV remains to be established.

Theoretically, ETV would be an appropriate option for the treatment of ADV-resistant hepatitis B. However, there are very few

data on its *in vivo* efficacy. We recently reported our experience in 3 patients who were switched to ETV after the detection of ADV resistance [2]. All patients had undetectable serum HBV DNA levels within 5 months of the start of ETV. Although serum HBV DNA remained undetectable at the last follow-up, 11-16 months after the change in treatment, longer follow-up is needed to determine the durability of response since all 3 patients had prior LAM resistance.

Clinical studies found that while resistance to ETV has not yet been detected in LAM-naïve patients [17], ETV-resistant mutations have been detected in 4% of LAM-refractory patients prior to the start of ETV therapy, increasing to 7% after 1 year, and 12% after 2 years of ETV treatment [18]. These data indicate that pre-existing LAM-resistant mutations increase the risk of ETV resistance. This finding is supported by *in vitro* data demonstrating that the presence of ETV-resistant mutations alone (changes at positions 169, 184, 202 or 250 in the reverse transcriptase region of the HBV polymerase gene) decrease susceptibility to ETV by 1- to 9-fold, but the co-existent presence of LAM- and ETV-resistant mutations decrease susceptibility to ETV by 7- to >740-fold [19].

Tenofovir, a nucleotide analog is structurally similar to ADV. *In vitro* studies showed that TDF and ADV are equipotent in suppressing wild-type HBV [20]. However, TDF has a better safety profile and a higher dose is approved for clinical use. Clinical studies, predominantly in patients with LAM-resistant HBV many of whom were co-infected with HIV, reported that TDF was more potent in suppressing serum HBV DNA levels than ADV [21,22]. In addition, there have been 2 reports documenting viral rebound when 4 patients who had suppression of serum HBV DNA while receiving TDF for LAM-resistant HBV were switched to ADV 10 mg [23,24]. More recently, van Bommel *et al.* reported that 19 of 20 patients with chronic HBV infection who were switched to TDF monotherapy after a poor virological response to ADV monotherapy (serum HBV DNA >4 $\log_{10}$ copies/mL after 4-28 months) had undetectable serum HBV DNA levels after a median of 12 months on TDF treatment [25]. These data indicate that TDF at the approved dose of 300 mg is more potent than ADV 10 mg in suppressing wild-type and LAM-resistant HBV, and may overcome ADV resistance.

Our own experience with patients who had suboptimal response to ADV therapy was similar to that of van Bommel *et al.* in that all 9 patients had >3 $\log_{10}$ decrease in serum HBV DNA levels after being switched to TDF, but only 4 had undetectable serum HBV DNA levels after a median of 18 months of TDF treatment. However, 2 patients

with confirmed ADV resistance had <3 $\log_{10}$ copies/mL decrease in serum HBV DNA levels and a second ADV-resistant mutation was detected in 1 of these patients after treatment was switched to TDF. Thus, limited data suggest that TDF may not be an optimal treatment for patients with ADV-resistant HBV. It is possible that TDF in combination with FTC, coformulated as Truvada, will be more effective since FTC has antiviral activity similar to LAM (3TC).

In theory, interferon should be as effective in suppressing nucleos(t)ide analog-resistant HBV as in suppressing wild-type HBV. However, clinical data confirming its efficacy in patients with antiviral-resistant HBV are scanty. Interferon may be tried in patients who have no underlying cirrhosis or severe hepatitis flares.

How should the case be managed?

Based on the foregoing discussions, there is no simple solution for the patient described in the case, and none of the available options have been properly studied. We elected to stop ADV and switched the patient to Truvada. Serum HBV DNA decreased by 2.5 $\log_{10}$ copies/mL within 3 months after the start of Truvada. However, longer follow-up is needed to determine the durability of response. The option of stopping ADV and observing the patient is reasonable as long as the patient is closely monitored and treatment initiated when ALT levels become persistently elevated.

What are the lessons learned from this case?

The most important lesson is that "Prevention is better than Cure". All efforts should be made to prevent antiviral resistance. Once antiviral-resistant mutations have developed, treatment is problematic. The problem is worse in patients who have acquired resistance to more than 1 nucleos(t)ide analog. The first step in preventing antiviral-resistant HBV is to avoid unnecessary treatment. In this patient, ample data from clinical trials of interferon and nucleos(t)ide analogs indicate that the likelihood of HBeAg seroconversion will be low regardless of treatment [26], necessitating very long durations of treatment if nucleos(t)ide analog therapy is chosen. Although this patient is predicted to have a high risk of cirrhosis and hepatocellular carcinoma (HCC) if he continues to have high serum HBV DNA levels for the next 2 decades [27,28], the risk of cirrhosis and HCC in the near future (next 10 years) is probably low. Furthermore, the risk in the distant future (20-40 years later) may be substantially reduced if

he undergoes spontaneous HBeAg seroconversion within the next 5-10 years and remains in the inactive carrier state. Initiation of antiviral therapy at an early stage would be beneficial for this patient if HBV can be permanently suppressed with a finite course of treatment or with long-term therapies that are safe and affordable. In this instance, while serum HBV DNA was intermittently suppressed to lower levels, it is not clear that the benefits outweigh the transient flare associated with the emergence of LAM resistance and the depletion of many treatment options in such a young patient.

Would this patient present now, LAM would not be the treatment of choice even if a decision is made to treat this patient. Nucleos(t)ide analogs with lower rates of resistance such as ETV would be preferred and combination therapy should be considered. Although none of the combination therapies evaluated to date have been shown to have additive or synergistic antiviral effects, resistance rates have decreased.

Had this patient been more closely monitored in 2003, LAM resistance might have been detected earlier. Recent studies found that ADV is more effective in suppressing LAM-resistant HBV when it is initiated at the time genotypic resistance or virological breakthrough is recognized than when treatment is implemented after biochemical breakthrough or hepatitis flare has occurred [29]. Furthermore, increasing data support that the combination of LAM and ADV is associated with a lower risk of ADV resistance than switching to ADV monotherapy [4,14].

This case highlights the complexities of hepatitis B therapies. Despite increased treatment options and the availability of more potent therapies with fewer side effects, finding an exit through the maze of hepatitis B treatments continues to be a challenge and physicians should not send patients into this maze, lightly, without a plan for monitoring and redirecting the patient to ensure a safe exit.

REFERENCES

1. Fung SK, Lok AS. Management of hepatitis B patients with antiviral resistance. Antivir Ther 2004;9:1013-1026.
2. Angus P, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C, Brosgart C, Colledge D, Edwards R, Ayres A, Bartholomeusz A, Locarnini S. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 2003;125:292-297.
3. 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.
4. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, Hussain M, Lok AS. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol 2006;44:283-290.
5. Yim HJ, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology 2006(in press).
6. Proceedings of NIH Meeting: Management of Chronic Hepatitis B 2006.
7. Lai CL, Leung N, Teo EK, Tong M, Wong F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown NA. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528-536.
8. Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors preceding hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology 2001;34:785-791.
9. Locarnini S, Qi X, Arterburn S, Snow A, Brosgart C, Currie G, Wulfsohn M. Incidence and predictors of emergence of adefovir resistant HBV during 4 years of adefovir dipivoxil (ADV) therapy for patients with chronic hepatitis B (CHB) (abstract). J Hepatol 2005;42:17.
10. Hussain M, Fung S, Libbrecht E, Sablon E, Cursaro C, Andreone P, Lok AS. Sensitive line probe assay that simultaneously detects mutations conveying resistance to lamivudine and adefovir. J Clin Microbiol 2006;44:1094-1097.
11. Fung SK, Andreone P, Han SH, Rajender Reddy K, Regev A, Keeffe EB, Hussain M, Cursaro C, Richtmyer P, Marrero JA, Lok AS. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. J Hepatol 2005;43:937-943.
12. Westland CE, Yang H, Delaney WE 4th, Gibbs CS, Miller MD, Wulfsohn M, Fry J, Brosgart CL, Xiong S. Week 48 resistance surveillance in two phase 3 clinical studies of adefovir dipivoxil for chronic hepatitis B. Hepatology 2003;38:96-103.

13. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart CL. Long-Term Adefovir Dipivoxil Treatment induces regression of Liver Fibrosis in Patients with HBeAg-Negative Chronic Hepatitis B: Results after 5 years of Therapy (abstract). Hepatology 2005;42:754.

14. Snow A, Thibault V, Qi X, Zhu Y, Westland C, Arterburn S, Benhamou Y. Combination of Adefovir Dipivoxil (ADV) and Lamivudine (LAM) prevented emergence of ADV resistance Mutations in chronic Hepatitis B (CHB) patients with LAM-Resistant HBV (abstract). Gastroenterology 2005;128:945.

15. Yeon JE, Yoo W, Hong SP, Chang YJ, Yu SK, Kim JH, Seo YS, Chung HJ, Moon MS, Kim SO, Byun KS, Lee CH. Resistance to adefovir dipivoxil (ADV) in lamivudine-resistant chronic hepatitis B patients treated with ADV Gut 2006; epub Feb. 4, 2006: accessed May 31, 2006 at http://gut.bmjjournals.com.proxy.lib.umich.edu/cgi/content/abstract/gut.2005.077099v077091.

16. Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, Chung YH, Lee YS, Yoo W, Kim SO. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology 2006;43:1385-1391.

17. Colonno R, Rose F, CJ B. High barrier to resistance results in no emergence of entecavir resistance in nucloside-naïve subjects during the first two years of therapy (abstract 490). J Hepatol 2006;44:S182.

18. Colonno R, Rose R, Levine S, Baldick J, Pokornowski K, Plym M, Yu C. Entecavir two year resistance update: no resistance observed in nucleoside naïve patients and low frequency resistance emergence in lamivudine refreactory patients (abstract). Hepatology 2005;42:573.

19. Tenney D, Levine S, Rose R, Walsh A, Weinheimer S, Discotto L. Clinical emergence of entecavir-reisitant hepatitis B virus requires additonal subsititutions in virus already resistant to Lamivudine. Antimicrob Agents Chemother 2004;48:3498-3507.

20. Brunelle MN, Jacquard AC, Pichoud C, Durantel D, Carrouee-Durantel S, Villeneuve JP, Trepo C, Zoulim F. Susceptibility to antivirals of a human HBV strain with mutations conferring resistance to both lamivudine and adefovir. Hepatology 2005;41:1391-1398.

21. van Bommel F, Wunsche T, Mauss S, Reinke P, Bergk A, Schurmann D, Wiedenmann B, Berg T. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40:1421-1425.

22. Peters MG, Anderson J, Lynch P, Jacobs J, Sherman K, Alton Smith B, Swindells S. Tenofovir disoproxil fumarate is not inferior to adefovir dipivoxil for the treatment of hepatitis B virus in subjects who are co-infected with HIV: results of ACTG A5127 (abstract). 12th Conference on retroviruses and opportunistic infections. Boston, MA. 2005:A124.

23. van Bommel F, Berg T. Reactivation of viral replication after replacement of tenofovir by adefovir. Hepatology 2005;42:239-240.

24. Schildgen O, Hartmann H, Gerlich WH. Replacement of tenofovir with adefovir may result in reactivation of hepatitis B virus replication. Scand J Gastroenterol 2006;41:245-246.

25. van Bommel F, Zollner B, Sarrazin C, Spengler U, Huppe D, Moller B, Feucht HH, Wiedenmann B, Berg T. Tenofovir rescue therapy for patients with lamivudine-resistant hepatitis B virus (HBV) infection showing persistent high level replication during adefovir therapy. Hepatology 2006;44:309-313.

26. Perrillo RP, Lai CL, Liaw YF, Dienstag JL, Schiff ER, Schalm SW, Heathcote EJ, Brown NA, Atkins M, Woessner M, Gardner SD. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36:186-194.

27. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.

28. Iloeje U, Yang H, Su J, Jen C, You S, Chen C. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-686.

29. Lampertico P, Vigano M, Manenti E, Iavarone M, Lunghi G, Colombo M. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. Hepatology 2005;42:1414-1419.