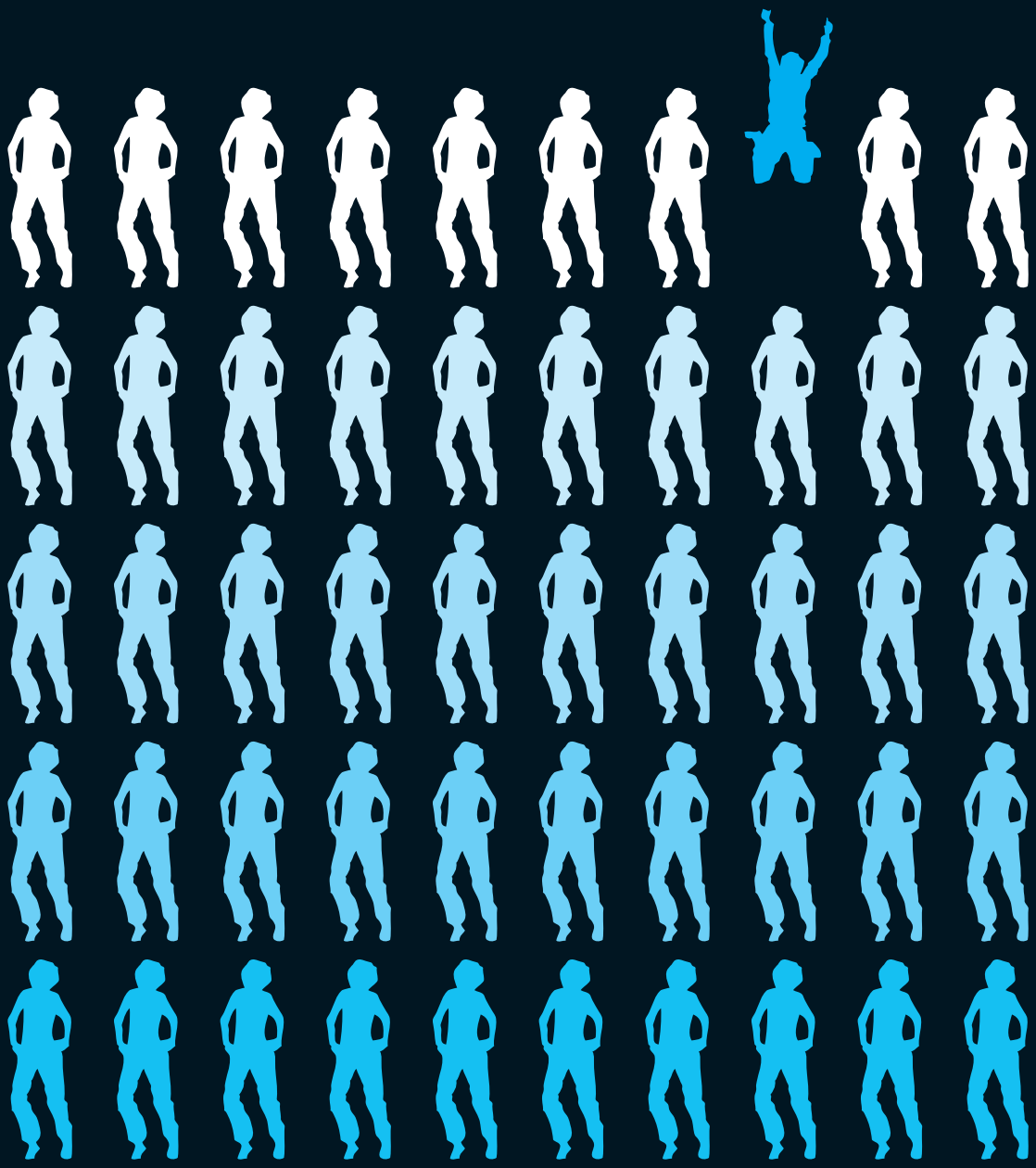

Chronic Hepatitis B:
Individualized Antiviral Therapy

E.H.C.J. Buster

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Colofon

ISBN: 978-90-8559-569-4

Printed by: Optima Grafische Communicatie

The work presented in this thesis was conducted at the Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands.

Financial support for printing this thesis was kindly given by the Department of Gastroenterology and Hepatology of the Erasmus MC University Medical Center Rotterdam, de Nederlandse Vereniging voor Hepatologie, Abbott Diagnostics, Ferring Pharmaceuticals, Gilead Sciences, GlaxoSmithKline, Janssen Cilag, Novartis, Roche, Schering-Plough and Tramedico.

Chronic Hepatitis B: Individualized Antiviral Therapy

Chronische hepatitis B: Geïndividualiseerde antivirale behandeling

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

vrijdag 23 oktober 2009 om 11:30

door

Erik Henricus Cornelius Johannes Buster

geboren te Goirle

PROMOTIECOMMISSIE

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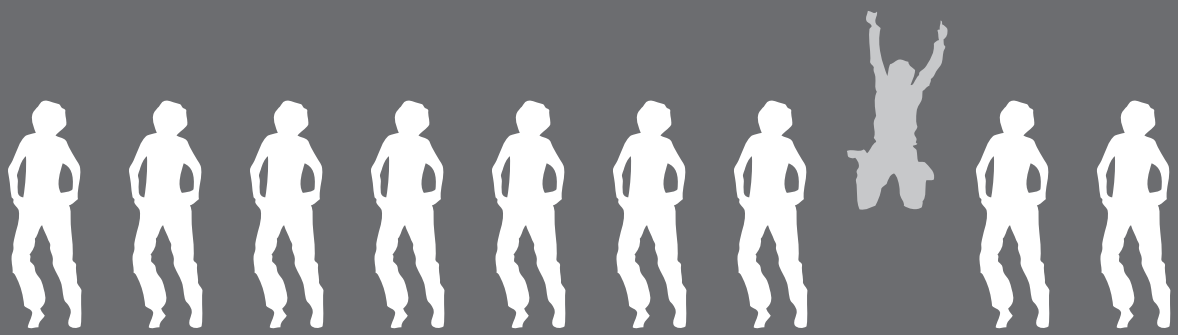


General Introduction

The hepatitis B virus (HBV) was discovered in 1966 with the identification of the Australia antigen in Aboriginals by Dr. Baruch Blumberg, who received the 1976 Nobel Prize in Medicine for his work. We now know the Australia antigen as hepatitis B surface antigen (HBsAg).

The hepatitis B virus

HBV belongs to a family of closely related DNA viruses called the hepadnaviruses. The viral genome of HBV is a partially double- stranded circular DNA of approximately 3200 base pairs that encodes four overlapping open reading frames: the surface or envelope gene, the core gene, the polymerase gene and the X gene. The core gene can also produce a soluble small molecular weight protein called hepatitis B e antigen (HBeAg) by an alternate start codon and post-translational modification. After entry in the hepatocyte, the HBV DNA is transported to the nucleus and converted to covalently closed circular DNA (cccDNA), which serves as the stable template for transcription of both messenger RNA (for translation of viral proteins) and pre-genomic RNA (for reverse transcription into genomic DNA). Because the cccDNA is highly resistant to antiviral therapy and the host's immunological response, complete eradication of HBV from the liver is probably not feasible.[1] HBV is non-cytopathic, cellular injury in HBV infected persons appears immune-mediated.[2]

Acute hepatitis B

Acute hepatitis B virus (HBV) infection is usually asymptomatic, less than 30% of adults present with icteric hepatitis and symptomatic disease is even less frequent in younger patients. The risk of chronic infection is highly dependent on the age of infection. Perinatal infection from the infected mother evolves to chronicity in 90% of cases.[3] Infection in early childhood (1-5 years) is also associated with a high risk of chronic infection of about 30%. In adults, in contrast, infection resolves with development of anti-HBs in 95% of cases.[4] Chronic infection is characterized by the persistence of serum HBsAg and anti-HBc.

Chronic hepatitis B

Chronic hepatitis is always associated with active HBV replication. There are two types of chronic hepatitis B, which differ by the HBeAg and anti-HBe status. Seroconversion from HBeAg to anti-HBe is the key event in the natural course of chronic hepatitis B since HBeAg seroconversion is followed by resolution of biochemical and histologic signs of inflammatory activity in the 67-80% of patients.[5, 6] Some patients may have persistence or recurrence of disease activity with elevated serum ALT and high serum HBV DNA levels despite HBeAg clearance. HBeAg seroconversion, whether spontaneous or after antiviral therapy, reduces the risk of hepatic decompensation and improves survival.[6-8]

Epidemiology

Chronic HBV infection is an important health problem. Current estimates are that about one-third of

world's population has evidence of past or present infection with HBV and that 400 million people are chronically infected.[9, 10] Primary liver cancer is ranked the 6th most common cancer globally and over 50% of cases are caused by chronic HBV infection.[11] The prevalence of HBV infection and patterns of transmission vary greatly throughout the world. Approximately 45% of the global population live in areas of high HBV endemicity (HBV prevalence >8%).[12] These areas include many African and Asian countries. In these countries the source of infection is mainly through perinatal transmission from the chronically infected mother or through infection during early childhood. The source of infection in areas with low prevalence of HBV infection is mainly through unsafe sexual contacts and needle sharing among injecting drug users. Eight genotypes of HBV have been identified (A-H), each with a specific geographic distribution. HBV genotype A is most common in the United States and Northern Europe, B and C in Asia, and D in Mediterranean counties and the Middle East.[13, 14] Recent data suggest that HBV genotypes may play an important role in the progression of HBV-related liver disease as well as response to interferon (IFN) therapy.[13, 15]

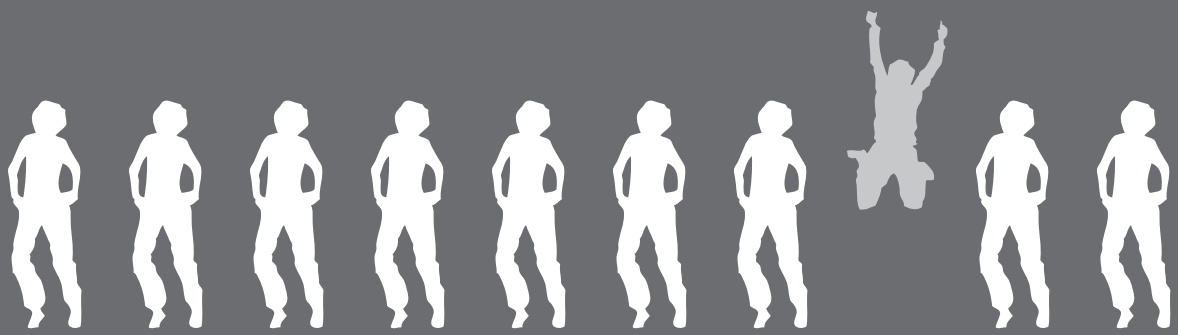
Antiviral therapy for chronic hepatitis B

The goal of therapy for hepatitis B is to improve quality of life and survival by preventing progression to cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC) and death. This goal can be achieved if HBV replication can be suppressed in a sustained manner. Reduction in serum HBV DNA levels is generally accompanied by a reduction in necroinflammatory activity, thereby limiting the risk of cirrhosis and decreasing the risk of HCC.[8, 16] Seven drugs are now licensed for the treatment of chronic hepatitis B (conventional interferon alpha, pegylated interferon alpha, lamivudine, adefovir, entecavir, telbivudine and tenofovir). The practicing clinician has to choose between two therapeutical approaches: a strategy aimed at inducing sustained off-treatment response (mainly IFN-based therapy) versus a strategy aimed at maintained viral suppression during prolonged antiviral therapy (nucleoside and nucleotide analogues).

REFERENCES

1. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. Hepatology 2007;45(4):1056-75.
2. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol. 1995;13:29-60.
3. McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, et al. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. Journal Infect Dis. 1985;151(4):599-603.
4. Tassopoulos NC, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL, Purcell RH. Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. Gastroenterology 1987;92(6):1844-50.
5. Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002 ;35(6):1522-7.
6. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology (Baltimore, Md. 2007 Jan 26;45(2):507-39.
7. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B.

The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Hepatology 1995;21(1):77-82.
8. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39(3):804-10.
9. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11(2):97-107.
10. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337(24):1733-45.
11. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94(2):153-6.
12. Weinbaum CM, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, et al. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. MMWR Recomm Rep. 2008;57(RR-8):1-20.
13. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology 2004;40(4):790-2.
14. Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004;47(6):289-309.
15. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol. 2006;101(2):297-303.
16. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351(15):1521-31.



Treatment of chronic hepatitis B virus infection - Dutch National Guidelines.

Erik H.C.J. Buster, Karel J. van Erpecum, Solko W. Schalm, C. Minke Bakker, Johannes T. Brouwer, Huub C. Gelderblom, Robert J. de Knegt, Hans L. Zaaijer, Henk W. Reesink, Harry L.A. Janssen for the Netherlands Association of Gastroenterolists and Hepatologists.

The Netherlands Journal of Medicine 2008; 66(7): 292-306.

SUMMARY

The development of this guideline was initiated and coordinated by the Dutch Society of Gastroenterologists and Hepatologists (Nederlands Genootschap van Maag-Darm-Leverartsen). The aim is the establishment of National standards in the evaluation and antiviral treatment of patients with chronic hepatitis B virus (HBV) infection. This includes recommendations on the initial evaluation of patients, choice and duration of antiviral therapy, follow-up after antiviral therapy and monitoring of patients not currently requiring antiviral therapy.

The initial evaluation of chronic HBV infected patients should include testing of liver biochemistry, virus serology and abdominal imaging. In patients without cirrhosis, antiviral treatment is recommended for those with serum HBV DNA of at least 1.0 x 10^5 copies/mL (>=2.0 x 10^4 IU/mL) in combination with: a) elevation of serum alanine aminotransferase (ALT) level above twice the upper limit of normal during at least three months, and/or b) histological evidence of porto-portal septa or interface hepatitis on liver histology. In patients with cirrhosis, antiviral treatment is recommended if serum HBV DNA is 1.0 x 10^4 copies/mL (>=2.0 x 10^3 IU/mL) or higher, independent of ALT levels or histological findings. If the patient has decompensated cirrhosis, antiviral treatment is recommended if serum HBV DNA is 1000 copies/mL (>=200 IU/mL) or higher.

Patients who do not have an indication for antiviral treatment should be monitored because there is a risk of (re)activation of disease activity. Monitoring every three to six months is recommended for HBeAg positive and HBeAg negative patients with high viremia (HBV DNA >=1.0 x 10^5 copies/mL or >=2.0 x 10^4 IU/mL) and normal ALT levels. For patients with serum HBV DNA below 1.0 x 10^5 copies/mL (<2.0 x 10^4 IU/mL) the recommended frequency of monitoring is every three to six months for HBeAg positive patients and every six to 12 months for HBeAg negative patients.

Peginterferon (PEG-IFN) therapy should be considered as initial therapy in both HBeAg positive and HBeAg negative patients without contraindications for treatment with this drug because of the higher chance of achieving sustained response compared to nucleos(t)ide analogue therapy. In patients starting nucleos(t)ide analogue therapy, the use of lamivudine is not preferred if long-term antiviral treatment is expected due to the high risk of antiviral resistance against this drug. Of currently licensed nucleos(t)ide analogues, entecavir has the lowest risk of antiviral resistance (compared to lamivudine, adefovir and telbivudine), while suppression of viral replication seems most profound with either entecavir or telbivudine.

The recommended duration of treatment with PEG-IFN is one year for both HBeAg positive and HBeAg negative patients. In HBeAg positive patents, nucleos(t)ide analogue therapy should be at least continued until HBeAg seroconversion and a decline in HBV DNA to below 400 copies/mL (80 IU/mL) has been achieved and maintained for six months during therapy. Whether nucleos(t)ide analogue therapy can be safely discontinued in HBeAg negative patients is unknown, usually prolonged or indefinite antiviral treatment is necessary.

Patients receiving PEG-IFN should be monitored once monthly, while three monthly monitoring suffices for those receiving nucleos(t)ide analogues. Genotypic analysis of the HBV polymerase is

indicated if an increase in serum HBV DNA of at least 1 log10 copies/mL (IU/mL) compared to the nadir value is observed during nucleos(t)ide analogue therapy. Antiviral therapy should be changed as soon as possible in case of confirmed genotypic resistance. Adding a second antiviral agent seems beneficial over switching to another agent.

With the availability of multiple new antiviral drugs for the treatment of chronic hepatitis B, effective treatment is now possible for more patients and for longer periods. However, the complexity of HBV therapy has also increased. Nowadays, virtually all chronic HBV infected patients can be effectively managed, either by inducing sustained off-treatment response or by maintaining an on-treatment response.

INTRODUCTION

About one-third of the world's population has evidence of HBV infection and chronic hepatitis B affects about 400 million people worldwide.[1,2] More than 500,000 people yearly die of HBV related liver disease, largely due to complication of cirrhosis or hepatocellular carcinoma.[3] The Netherlands is a low endemic country for HBV infection, the estimated seroprevalence of HBsAg and anti-HBc is about 0.2% and 2.1%, respectively.[4] Risk groups with a higher prevalence of HBV infection include immigrants from areas with intermediate or high prevalence of HBV infection, males who have sex with males and people with multiple sexual contacts. Despite the availability of a safe and effective vaccine for over 20 years now, HBV infection remains an important health problem. Antiviral treatment of chronic hepatitis B has dramatically changed over the last decade; with the availability of multiple new antiviral agents the treatment of chronic HBV infection has become more effective, but more complex as well.

Multiple consensus guidelines for the treatment of chronic hepatitis B have been published in the last few years.[3,5,6] However, there currently is no standard of care for the management and antiviral treatment of chronic HBV infected patients in the Netherlands. Therefore, a committee was convened by the Dutch Society of Gastroenterologists and Hepatologists (Nederlands Genootschap van Maag-Darm-Leverartsen) to formulate consensus based guidelines for the management and treatment of chronic HBV infected adults.

The guideline provides recommendations on the initial evaluation of chronic HBV infected patients, choice of (initial) antiviral therapy, follow-up during and after antiviral therapy and monitoring of patients currently not requiring antiviral therapy. Management of patients with coinfections of HBV and hepatitis C virus (HCV), hepatitis delta virus (HDV) or human immunodeficiency virus (HIV) is not discussed in this guideline. The recommendation in this guideline have been defined in accordance with recent international literature, data presented at international symposia and guidelines of the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL) en the Asian-Pacific Association for the Study of the Liver (APASL).[3,5,6]

The level of recommendation was performed according to the Dutch Institute for Healthcare Im-

provement (CBO) (http://www.cbo.nl/product/ richtlijnen/handleiding_ebro/article20060207153532) (table 1a and 1b).

Table 1a. Quality of studies on which a recommendation is based

Grade	Definition
A1	Systematic review of at least two independent studies of A2 level.
A2	Randomised double-blind controlled study of adequate quality and size.
B	Comparative study not fulfilling the characteristics of A2 level studies (including case-control studies and cohort studies).
C	Non-comparative studies.
D	Expert opinion

Table 1b. Quality of evidence on which a recommendation is based

Grade	Definition
I	Study of level A1 or at least two independent studies of level A2.
II	Single level A2 study or at least two independent level B studies.
III	Single level B or C study.
IV	Expert opinion.

NATURAL HISTORY

Infection with HBV at adulthood is usually not associated with symptomatic disease and results in chronic infection in less than 5% of cases.[3] However, infection during childhood is associated with a much higher risk of chronicity, up to 90% in case of perinatal transmission.[3] Chronic HBV infection is defined as detectable hepatitis B surface antigen (HBsAg) in serum for at least six months.

Phases of infection

Chronic HBV infected patients typically present in one of four phases of infection (figure 1).[7] In the immunotolerant phase, hepatitis B e antigen (HBeAg) is detectable and serum HBV DNA is high (>1.0 x 10^5 copies/mL or >2.0 x 10^4 IU/mL), while serum alanine aminotransferase (ALT) is normal. Patients infected in early childhood usually remain in this phase of infection for 10-30 years.[8] In the immuno-active phase, an active host's immune response against the virus results in a rise in ALT accompanied by a decline in HBV DNA; loss of HBeAg with seroconversion to anti-HBe can occur. The immune control phase follows HBeAg seroconversion and is characterised by low viremia (<1.0 x 10^4 copies/mL or <2.0 x 10^3 IU/mL) and normalization of ALT. Although HBV replication persists, it is profoundly suppressed by an active immune response. In a significant proportion of HBeAg negative patients, viral replication and hepatic inflammation persist or recur, usually due to

the selection of HBV variants with mutation in the HBV genome (precore or core promoter mutants) which hamper the production of HBeAg. These patients develop HBeAg negative chronic hepatitis. Patients with chronic hepatitis B who acquire infection in adulthood often skip the immunotolerant phase and enter the immuno-active phase shortly after the infection.
Reactivation of HBV infection can occur in case of immunosuppression, it is therefore recommened to determine HBV status in all patients prior to the start of chemotherapy or treatment with selective antibodies.

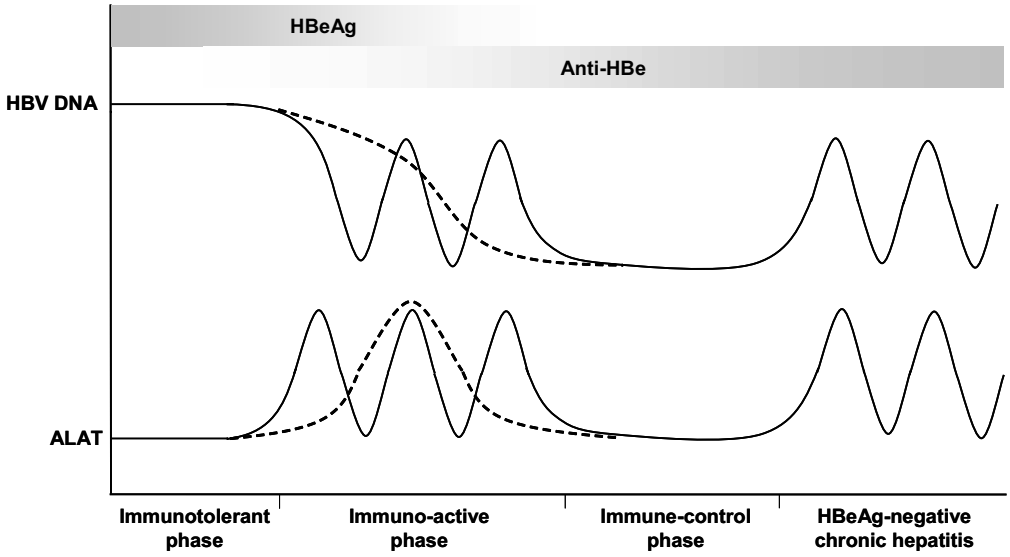


Figure 1. Phases of chronic hepatitis B virus infection
This figures shows the phases of chronic HBV infection. Patients can be categorised based on HBeAg-status, serum HBV DNA and ALT levels.

Cirrhosis

The annual incidence of cirrhosis in patients with chronic hepatitis B is about 6%, with a 5-year cumulative incidence of 20%.[9] The course of disease strongly varies among individual patients, progression of liver damage particularly occurs in those with persistent hepatic inflammation.[10] Factors associated with an increased risk of developing liver cirrhosis include high serum HBV DNA, coinfection with HCV, HDV or HIV, repeated episodes of acute exacerbation and severe necroinflammation at diagnosis.[3,11,12] The annual risk of hepatic decompensation is about 3% in patients with pre-existent cirrhosis.[3] Presence of liver cirrhosis is associated with a diminished 5-year survival rate of about 84%, for patients with decompensated cirrhosis this is only 14-30%.[13-15]

Hepatocellular carcinoma

Cirrhosis is a major risk factor for the development of hepatocellular carcinoma (HCC), the majority of patients with HCC has underlying cirrhosis (80-90%).[16] The annual incidence of HCC in

European chronic hepatitis B patients is about 2%. In patients from Asia, the risk of developing HCC is higher, with an annual incidence of about 3%.[16] Factors associated with an increased risk of developing HCC in patients with cirrhosis include high age, male sex, persistent hepatic inflammation, HBV DNA >1.0 x 10^4 copies/mL (>2.0 x 10^3 IU/mL), HBeAg positivity, coinfection with HCV or HIV, and alcohol abuse.[16-19]

INITIAL EVALUATION

The initial evaluation of patients with chronic HBV infection should include a detailed history, with special emphasis on risk factors for infection with blood-borne viruses and sexual transmitted diseases, alcohol use, and family history of HBV infection and liver cancer. Physical examination should emphasize signs of chronic liver disease and cirrhosis (palmar erythema, spider nevi, gynaecomasty, flapping tremor and testicular atrophy), portal hypertension (ascites, splenomegaly and abdominal wall collaterals) and liver failure (jaundice and hepatic encephalopathy).

Laboratory tests should include assessment of liver enzymes (aminotransferases), liver function tests (albumin, bilirubin and prothrombin time), full blood count and kidney function tests. Virus serology should include markers of HBV replication (quantification of HBV DNA, HBeAg and anti-HBe) and in patients at increased risk, also tests for coinfection with HCV, HDV, or HIV. Determination of HBV genotype may be of use in patients starting antiviral therapy, as this may guide the choice of therapy.

Abdominal ultrasound should be performed in all patients, with special emphasis on signs of cirrhosis (irregular liver surface, blunt liver edge and narrowed hepatic veins), portal hypertension (diminished portal flow speed, splenomegaly, venous collaterals and ascites) and focal liver lesions.

Performing a liver biopsy is often indicated, but does not have to be routinely performed in all chronic HBV infected patients. A liver biopsy should particularly be considered in patients with an indication for antiviral therapy in order to assess baseline necroinflammatory activity and fibrosis stage. In case there is doubt about the need of starting antiviral therapy, liver biopsy is probably of even greater value as it may give additional information as to whether antiviral therapy or a conservative approach is justified. For patients in the immune-control phase (inactive HBsAg carrier state) a liver biopsy should be considered in case presence of cirrhosis is suspected. Patients in the immune-control and HBeAg negative hepatitis phase are usually older, are infected with HBV longer and more often have developed advanced fibrosis or cirrhosis as compared to patients in other phases of infection.[20] If liver histology shows presence of cirrhosis it is not recommended to discharge the patient because of the risk of developing HCC in these patients, even in patients with inactive disease. Patients in the immunotolerant phase on the other hand rarely have significant fibrosis and progression of disease should only be suspected in case of transition to the immunoactive phase.[21] Performing a liver biopsy can therefore usually be postponed in such cases. Surveillance for HCC, by abdominal ultrasound every 6 to 12 months, is recommended for all

chronic HBV infected patients with cirrhosis, in particular in those at increased risk of developing.[6,22] Patients at increased risk of developing HCC include Asian males over 40 years, Asian females over 50 years, patients with a family history of HCC, Africans over 20 years, patients with high HBV DNA levels and those with persistent hepatic inflammation.[23] Also in patients without cirrhosis but with an increased risk of developing HCC, surveillance for HCC should be considered. Surveillance for HCC results in detection of HCC at an earlier stage and thereby improved survival.[24] Routine measurement of alfa-fetoprotein is in general not useful as this does not improve the efficacy of screening and leads to increase in false-positive findings.[23] In patients with cirrhosis, upper gastrointestinal endoscopy should be considered to confirm or exclude the presence of esophageal varices.[25]

Hepatitis A virus (HAV) immunity should be established in all patients with chronic hepatitis B, since the risk of a fulminant course of acute HAV infection is increased compared to healthy controls.[26,27] Despite the fact that the actual risk of fulminant HAV is low, HAV vaccine is recommended for all chronic HBV infected patients not immune to HAV.

RECOMMENDATIONS

Grade Ⅳ	The initial evaluation of chronic HBV infected patients should include a detailed history and physical examination. Blood chemistry, full blood count, virus serology, including quantification of serum HBV DNA, and abdominal ultrasound should be performed. Performing a liver biopsy should particularly be considered in case of active hepatitis and in case there is doubt about the need for starting antiviral therapy.
Grade Ⅲ	Surveillance for hepatocellular carcinoma by abdominal ultrasound every 6 to 12 months is recommended in patients with cirrhosis..
Grade Ⅲ	Surveillance for hepatocellular carcinoma by abdominal ultrasound every 6 to 12 months is recommended in patients with cirrhosis..

INDICATIONS FOR ANTIVIRAL THERAPY

In a considerable proportion of chronic HBV infected patients there is no need for antiviral treatment.[28] Whether or not antiviral treatment should be started depends on multiple factors (table 2). First, active viral replication should be present, as shown by serum HBV DNA of at least 1.0 x 10^5 copies/mL (2.0 x 10^4 IU/mL). In HBeAg negative patients, the risk of (re)activation of disease activity and progression of disease seems already increased in those with serum HBV DNA above 1.0 x 10^4 copies/mL (2.0 x 10^3 IU/mL) compared to patients with lower HBV DNA levels.[29] In addition to HBV DNA, the degree of hepatic fibrosis and inflammation plays an important role in assessing the need for antiviral therapy. This is represented by serum ALT levels and necroinflammatory activity on liver histology. Serum ALT of at least two times the upper limit of normal (ULN) during three to six

months is usually considered an indication for antiviral therapy. In patients with serum HBV DNA above 1.0 x 10^5 copies/mL (2.0 x 10^4 IU/mL) and persistent mild hepatic inflammation (ALT 1-2 x ULN), but with significant liver fibrosis (porto-portal septa) or interface hepatitis antiviral therapy should also be considered. If serum ALT is elevated but serum HBV DNA is low, other causes of hepatitis should be considered. If no other underlying aetiology can be found and liver biopsy shows hepatitis B virus associated inflammation, antiviral treatment should be considered.

In patients with compensated cirrhosis, antiviral treatment should be considered if HBV DNA is 1.0 x 10^4 copies/mL (2.0 x 10^3 IU/mL) or higher. HBV DNA above this level is associated with an increased risk of progression to decompensated cirrhosis or HCC.[17] Patients with decompensated cirrhosis should be offered antiviral therapy if HBV DNA is 1,000 copies/mL (200 IU/mL) or higher, as suppression of viral replication can significantly improve liver function and survival in these patients.[30,31]

Over 90% of babies born to HBsAg positive mothers is effectively protected by passive-active immunization. However, in pregnant patients with very high viremia (HBV DNA >=1.0 x 10^9 copies/mL or >=2.0 x 10^8 IU/mL), the risk of vaccination failure in the newborn is about 30%.[32,33] In these women, nucleos(t)ide analogue therapy from week 32 of pregnancy can significantly lower this risk.[33,34] Lamivudine is the antiviral agent of choice because of extensive clinical experience in pregnancy, particularly in HIV infection.[33-35] Switching to another antiviral agent after delivery can be considered if prolongation of antiviral therapy is indicated. In patients becoming pregnant during nucleos(t)ide analogue therapy, the risks of stopping antiviral therapy (in particular acute exacerbation) should be balanced against the risk for the unborn child when continuing the drug. Recommendations on what to do in such cases are not possible as scientific evidence is lacking, consulting a centre with expertise on treatment of chronic HBV infection is recommended.

In HBsAg positive patients starting chemotherapy or treatment with selective antibodies, prophylactic antiviral treatment with a nucleos(t)ide analogue is recommended until six months after the completion of the immunosuppressive therapy. Prophylactic antiviral therapy has been shown to significantly reduce the risk of reactivation and hepatitis B related death.[6,36] In patients requiring prophylactic antiviral treatment, who have baseline HBV DNA of above 1.0x 10^4 copies/mL (>2.0 x 10^3 IU/mL), the regular endpoints of antiviral therapy should be applied (see also Choice and duration of antiviral therapy).[6] Prophylactic antiviral therapy can also be considered in anti-HBc positive patients, since these patients are also at risk for reactivation in case of severe immunosuppression.[36]

RECOMMENDATIONS

Grade I	In patients without cirrhosis, antiviral therapy is recommended in those with serum HBV DNA of at least 1.0 x 10^5 copies/mL (2.0 x 10^4 IU/mL) in combination with serum ALT above twice the upper limit of normal during at least 3 months, and/or presence of interface hepatitis or significant fibrosis on liver histology.

Grade II	In patients with cirrhosis, antiviral therapy is recommended if serum HBV DNA is 1.0 x 10^4 copies/mL (2.0 x 10^3 IU/mL) or higher, irrespective of serum ALT or HBeAg status.
Grade III	In patients with decompensated cirrhosis, antiviral therapy should be considered in those with serum HBV DNA of 1,000 copies/mL (200 IU/mL) or higher, irrespective of serum ALT or HBeAg status.
Grade II	Antiviral therapy from week 32 of pregnancy until delivery can be considered in pregnant women with serum HBV DNA of 1.0 x 10^9 copies/mL (2.0 x 10^8 IU/mL) of higher in order to lower the risk of failure of passive-active immunization in the newborn.

MONITORING OF PATIENTS NOT REQUIRING ANTIVIRAL THERAPY

Patients who do not have an indication for antiviral therapy should be monitored since disease activity may fluctuate over time (table 2). Three to six monthly monitoring of serum ALT is recommended for HBeAg positive patients with high viremia (HBV DNA >=1.0 x 10^5 copies/mL or >=2.0 x 10^4 IU/mL) and normal ALT, with more frequent monitoring when ALT becomes elevated. For HBeAg negative patients with high serum HBV DNA (>=1.0 x 10^5 copies/mL or >=2.0 x 10^4 IU/mL) and normal ALT, monitoring is also recommended every three to six months. In those with low viremia, six to 12 monthly monitoring suffices.

RECOMMENDATION

Grade I	Patients who are currently not candidates for antiviral therapy should be monitored since disease activity may fluctuate over time. For both HBeAg positive and HBeAg negative patients with high serum HBV DNA (>=1.0x 10^5 copies/mL or >=2.0x 10^4 IU/mL) and normal ALT, three to six monthly monitoring is recommended. For patients with low serum HBV DNA (<1.0x 10^5 copies/mL or <2.0x 10^4 IU/mL) the recommended frequency of follow-up is once per three to six months for HBeAg positive patients and once per six to 12 months for HBeAg negative patients.

GOALS OF ANTIVIRAL THERAPY

The ultimate goal of antiviral therapy for chronic HBV infected patients is clearance of HBsAg and appearance of anti-HBs. However, since HBsAg-seroconversion can only be achieved in a small proportion of patients, other surrogate endpoints of antiviral therapy have been chosen. These endpoints can generally be assessed after one year of treatment and are associated with favourable long-term outcome. The most important endpoints of antiviral therapy include HBeAg seroconversion (loss of HBeAg with appearance of anti-HBe) in previously positive patients, decline in serum HBV

Table 2. Recommendations of management of chronic hepatitis B based on HBeAg status, ALT en HBV DNA.

Severity of disease	HBeAg status	ALT	HBV DNA copies/mL (IU/mL)	Recommended management
Chronic hepatitis	HBeAg positive	>=2x ULN	>=1.0 x 10^5 (>=2.0 x 10^4)	Antiviral therapy
		<2x ULN	>=1.0 x 10^5 (>=2.0 x 10^4)	3-monthly monitoring, consider liver biopsy in case of persistently elevated ALT (and antiviral therapy in case of active necroinflammation)
		<2x ULN	<1.0 x 10^5 (<2.0 x 10^4)	3-monthly monitoring
		>=2x ULN	<1.0 x 10^5 (<2.0 x 10^4)	Exclude other cause of hepatitis, consider liver biopsy
	HBeAg negative	>=2x ULN	>=1.0 x 10^5 (>=2.0 x 10^4)	Antiviral therapy
		<2x ULN	>=1.0 x 10^5 (>=2.0 x 10^4)	3-6 monthly monitoring, consider liver biopsy in case of persistently elevated ALT (and antiviral therapy in case of active necroinflammation)
		<2x ULN	<1.0 x 10^5 (<2.0 x 10^4)	6-12 monthly monitoring
		>=2x ULN	>=1.0 x 10^4 - <1.0 x 10^5 (>=2.0 x 10^3 - <2.0 x 10^4)	Antiviral therapy if no other causes of hepatitis are present.
			<1.0 x 10^4 (<2.0 x 10^3)	Exclude other cause of hepatitis, consider liver biopsy.
Compensated cirrhosis	-	-	>=1.0 x 10^4 (>=2.0 x 10^3)	Antiviral therapy
Decompensated cirrhosis	-	-	>300 (>60)	Antiviral therapy

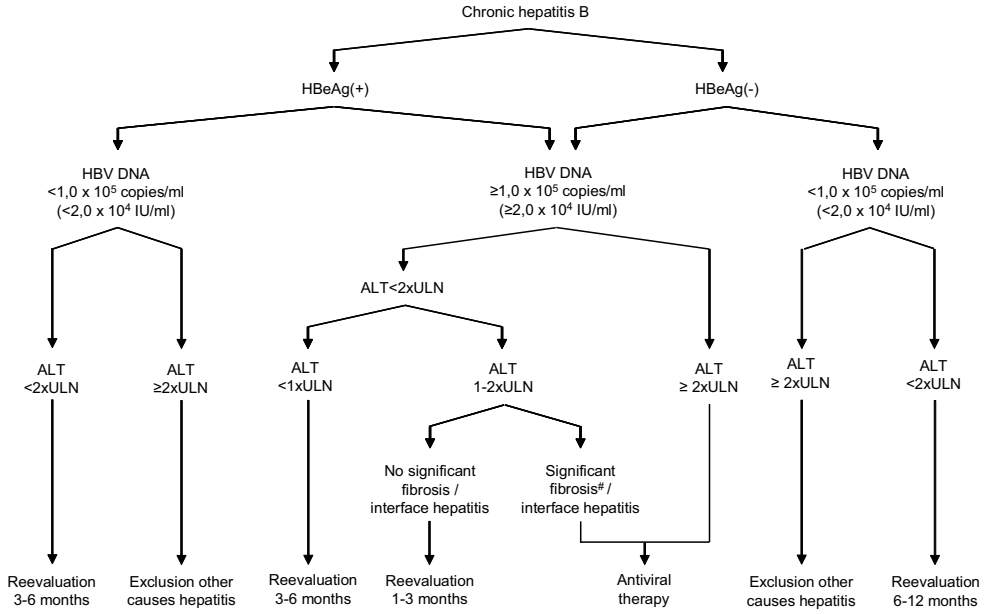


Figure 2: Management of chronic hepatitis B virus infected patients.
This flowchart shows the recommendations on the management of chronic HBV infected patients based on HBeAg status, serum HBV DNA and ALT levels. #Significant fibrosis: at least porto-portal septa on liver histology.

DNA below the lower limit of detection of a sensitive polymerase chain reaction (PCR) assay (or comparable test), biochemical response (normalisation of ALT) and improvement of liver histology (decrease in necroinflammatory activity and no increase in fibrosis). These endpoints indicate the presence of inactive disease in patients with previous active hepatitis. Furthermore, responses can be distinguished in those sustained after discontinuation of therapy versus those that need to be maintained by antiviral therapy. In case of sustained response there is an active immune-response against the virus, as shown by HBeAg or HBsAg seroconversion. In case of treatment-maintained response there is persistent suppression of viral replication by the antiviral drug, but no active immune-response. Sustained response is particularly achieved with (peg)interferon therapy, while treatment-maintained response can be achieved with long-term nucleos(t)ide analogue therapy in the majority of patients. Higher rates of sustained HBeAg seroconversion have been achieved with interferon compared to lamivudine.[37] It is not clear whether with the new potent nucleos(t)ide analogues, substantial rates of sustained response can be reached after HBeAg seroconversion and subsequent withdrawal of therapy.

ANTIVIRAL DRUGS FOR THE TREATMENT OF CHRONIC HBV INFECTION

Peginterferon

Interferon-alfa (IFN-alfa) has been used for the treatment of chronic HBV infection since the 1980's.

Interferons are natural occurring cytokines with immunomodulatory, antiproliferative and antiviral activity.[38] IFN-alfa has been a mainstay in the treatment of chronic HBV infection since it was licensed for this indication in the early 1990's, both in HBeAg positive and HBeAg negative chronic hepatitis B. In the majority of HBeAg positive patients IFN-induced HBeAg seroconversion is durable (87%) and eventually leads to HBsAg loss in about 50% of these responders.[39] The risk of developing and HCC is significantly lower for responders to IFN therapy compared to non-responders.[39]

The addition of a polyethylene glycol molecule (PEG) to the IFN has resulted in a significant increase of half-life, thereby allowing administration once weekly. The last few years clinical research has focussed on the use of peginterferon (PEG-IFN) for the treatment of chronic hepatitis B. Two types of peginterferons have been developed (peginterferon alfa-2a and peginterferon alfa-2b), of which peginterferon-alfa-2a has been licensed for the treatment of chronic HBV infection in the Netherlands in a weekly dose of 180 microgram (subcutaneous) for 48 weeks in both HBeAg positive and HBeAg negative patients.

In HBeAg positive patients, PEG-IFN appears at least as effective as conventional IFN with loss of HBeAg in 35% and seroconversion to anti-HBe in 29-32% of patients.[40-43] Addition of lamivudine did not lead to an increase in sustained response rates compared to PEG-IFN monotherapy. PEG-IFN induced HBeAg loss is sustained in 80-86% of HBeAg-positive patients.[44,45] HBsAg-seroconversion occurs in 3-7% of PEG-IFN treated patient within six months after the end of therapy (10-20% of those with HBeAg loss). The likelihood of HBeAg loss after PEG-IFN therapy is associated with the HBV genotype; patients with genotype A or B have a higher chance of achieving HBeAg loss than those with genotype C or D.[42,46] Genotype A infected patients significantly more often show loss of HBeAg and HBsAg than those infected with genotype D.[42,47]

Only one large randomised trial of PEG-IFN therapy has been performed in HBeAg negative chronic hepatitis B.[48] Combined response of HBV DNA below 2.0 x 10^4 copies/mL and normalization of ALT occurred in 36% of patients. At 24 weeks post-treatment, serum HBV DNA below 400 copies/mL was observed in 19%. As in HBeAg positive chronic hepatitis B, the addition of lamivudine did also not increase response rates in these patients. HBsAg seroconversion occurred in 4% of PEG-IFN treated HBeAg negative patients (over 10% of those with combined response).[48]

Major disadvantages of PEG-IFN therapy are the subcutaneous administration and frequent side effects (table 3). Particularly flu-like symptoms, cytopenia and psychiatric adverse events are frequently observed,[49] but rarely require discontinuation of therapy.[49,50]

PEG-IFN is contraindicated in patients with advanced cirrhosis (albumin <35g/l, bilirubin >34 µmol/l or prolongation of prothrombin time by more than 4 seconds) because of the increased risk of decompensation in case of acute exacerbation.[51,52] Other important contraindications of treatment with PEG-IFN are severe psychiatric comorbidity (depression and suicidal ideation), severe cardiac disease and autoimmune hepatitis (or other autoimmune disorders). A major advantage of PEG-IFN therapy is the high rate of sustained of response (over 80% in HBeAg positive patients and about 40% of HBeAg negative patients who initially responded to the treatment).[44,45,53]

Table 3. Undesirable effects during treatment with peginterferon alfa[49,100-102]

Frequency	Undesirable effects
>30% (very frequent)	Flu-like symptoms
	Headache
	Fatigue
	Pyrexia
	Chills
	Myalgia
	Thrombocytopenia
	Induction of auto-antibodies
1-30% (frequent)	Anorexia
	Erythema at injection site
	Insomnia
	Alopecia
	Lack of motivation
	Lack of concentration
	Irritability, agitation
	Emotional instability
	Depression
	Diarrhoea
	Auto-immune disease (thyreoiditis, Sjögren's disease)
	Neutropenia
	Change of taste
<1% (rare)	Polyneuropathy
	Paranoia of suicidal ideation
	Diabetes mellitus
	Retinopathy
	Optic neuritis
	Hearing loss
	Seizures
	Loss of libido
	Cardiotoxicity

Nucleos(t)ide analogues

In the last decade there has been a major advance in the treatment of chronic hepatitis B with nucleos(t)ide analogues. These antiviral agents inhibit the viral polymerase and thereby viral replication. Advantages of nucleos(t)ide analogues are the oral administration, rapid decline in HBV DNA and minimal side effects. A major disadvantage is that the majority of patients needs prolonged or even indefinite therapy, as sustainability of response after discontinuation of therapy is limited. Furthermore, the risk of antiviral resistance increases with the duration of antiviral therapy. Antiviral resistance is caused by the selective selection of naturally occurring mutations in the HBV polymerase. Rapid and profound viral suppression reduce the risk of antiviral resistance.[54]

Lamivudine

Lamivudine was the nucleoside analogue licensed for the treatment of chronic HBV infection in 1999. Lamivudine should be given is a dosage of 100mg daily and has excellent safety and tolerability. In HBeAg positive patients, treatment with lamivudine for one year results in HBeAg seroconversion plus serum HBV DNA below 1.0 x 10^5 copies/mL (2.0 x 10^4 IU/mL) in 16-22% of patients.[55-60] The rate of HBeAg seroconversion increases with increasing duration of therapy to 29%, 40% and 47% after two, three and four years of therapy, respectively.[57,58,61] Decline in HBV DNA below 7,0 x 10^5 copies/mL (1,4 x 10^5 IU/mL) was observed in 65% of patients.[62]

In HBeAg negative patients, serum HBV DNA below 400 copies/mL (<80 IU/mL) was observed in 68-73% of patients after one year of lamivudine. Sixty-eight to 96% of these patients also had biochemical response.[48,63,64] In HBeAg negative patients the rate of virological response declined with increasing duration of therapy, largely due to the increasing risk of antiviral resistance. Response rates at year two, three and four were 67%, 60% and 39%, respectively.[63,65,66] Treatment with lamivudine has been shown to result in a decrease of disease progression and development of HCC in patients with advanced fibrosis or cirrhosis compared to untreated controls.[67]

The major disadvantage of lamivudine is the high incidence of antiviral resistance. The majority of patients with viral breakthrough has mutations in the tyrosine-methionine-aspartate-aspartate motif (YMDD) of the HBV polymerase.[66] The most frequently observed mutation is a substitution of methionine for valine or isoleucine at position 204 of the HBV polymerase.[68] Lamivudine resistance occurred in 24% of patients after one year, which increased to 71% after 5 years.[69] The selection of resistance mutations is often followed by an increase in ALT.[66] Another disadvantage of lamivudine is the high risk of relapse after discontinuation of therapy; half of patients with lamivudine induced HBeAg seroconversion had relapse at 2-3 years after therapy.[37,70]

Adefovir

Adefovir is a nucleotide analogue with activity against wild-type and lamivudine-resistant HBV. Adefovir was licensed for the treatment of chronic hepatitis B in the Netherlands in 2003 in a daily dosage of 10mg. Higher dosages may be more effective, but are associated with nephrotoxicity.[71]

In HBeAg positive patients, a one-year course of adefovir resulted in HBeAg seroconversion in 12%, serum HBV DNA below 1.0 x 10^3 copies/mL (200 IU/mL) in 21% and normalisation of ALT in 48% of patients.[71] The rate of HBeAg-seroconversion increased with increasing duration of therapy to 29% after 2 years and 43% after three years of treatment. The proportion of patients with HBV DNA below 1.0 x 10^3 copies/mL (200 IU/mL) increased to 45% and 56% after two and three years, respectively.[72]

Serum HBV DNA below 1.0x 10^3 copies/mL (200 IU/mL) and normalisation of ALT were observed in 51% and 72% of HBeAg-negative patients after one year of adefovir.[73] After five years of therapy, the proportion of patients with HBV DNA below 1.0 x 10^3 copies/mL (200 IU/mL) increased to 67% and to 69% for ALT normalization.[74,75] Histologic response was observed in 75-80% of adefovir treated patients at year five.[74] However, more recent studies suggested lower response rates dur-

ing adefovir therapy, with serum HBV DNA above 1.0 x 10^4 copies/mL (2.0 x 10^3 IU/mL) in 50% of patients after six months of therapy.[76]

HBeAg loss occurred in 20% of lamivudine resistant patients treated with adefovir.[77] The proportion of lamivudine resistant patients with HBV DNA below 400 copies/mL (80 IU/mL) was 19% after one year of adefovir therapy.[77]

Antiviral resistance to adefovir occurs less frequent and later during the course of therapy compared to lamivudine. The most important mutations in the HBV polymerase associated with adefovir resistance include a substitution of asparagine for threonine at position 236 and a substitution of alanine for valine or threonine at position 181.[75,78] The reported incidence of adefovir resistance is 0% at year one, 22% at year two and 28% at year five of antiviral therapy.[74,76] In lamivudine resistant patients treated with adefovir monotherapy the rate of antiviral resistance was 6-18% after one year and 21-38% after two years.[77,79,80]

Entecavir

Entecavir is a guanine analogue, which was licensed for the treatment of chronic hepatitis B in the Netherlands in 2006. In nucleoside naïve patients, entecavir is given in a daily dosage of 0.5 mg. A daily dose of 1 mg should be used in patients with pre-existent lamivudine resistance. Three large randomised trials have compared entecavir to lamivudine for the treatment chronic hepatitis B.[62,81,82] Decline in HBV DNA was significantly greater with entecavir than lamivudine in both HBeAg positive and HBeAg negative patients.[62,81]

HBeAg seroconversion occurred in 21% of entecavir treated HBeAg positive patients after one year, while serum HBV DNA below 300 copies/mL (60 IU/mL) or below 7,0x 10^5 copies/mL (1,4x 10^5 IU/mL) was observed in 67% and 91% of patients.[62] The cumulative proportion of patients with undetectable HBV DNA (300 copies/mL (60 IU/mL) increased to 82% after three years of therapy.[83] After one year of entecavir treatment, serum ALT normalised in 68% of patients, which increased to 90% of patients after three years.[83] The proportion of patients with HBeAg loss also increased, to 39% after three years of therapy.[83]

In HBeAg negative patients, treatment with entecavir resulted in normalization of ALT and HBV DNA below 300 copies/mL (200 IU/mL) in 78% and 89%, and 90% and 94% of patients after one and two years of therapy, respectively.[81,84] Histologic response was observed in 70% of entecavir treated patients after one year.[81]

Response to entecavir was lower in lamivudine resistant patients, with undetectable HBV DNA in 19% and ALT normalization in 61% of patients after 48 weeks of entecavir therapy.[82] HBeAg loss was observed in 8% of these patients.[82]

The rate of entecavir resistance was extremely low in nucleoside naive patients with antiviral resistance observed in less than 1% of patients after 4 years of entecavir therapy.[85-87] However, in lamivudine resistant patients the risk of antiviral resistance is much higher with entecavir resistance in 12%, 20%, 25% and 40% after 1-4 years of therapy, respectively.[86-88] These findings implicate cross-resistance of lamivudine and entecavir, entecavir resistance requires pre-existent

lamivudine resistance mutations.

Telbivudine

Telbivudine is a nucleoside analogue belonging to the same group of antiviral agents as lamivudine. Telbivudine is orally administrated in a daily dosage of 600 mg. Telbivudine has been licensed for the treatment of chronic hepatitis B in the Netherlands since 2007. In both HBeAg positive and HBeAg negative patients, telbivudine resulted in more profound viral suppression than lamivudine.[89] After one year of treatment with telbivudine HBeAg seroconversion occurred in 22% of HBeAg positive patients, increasing to 29% after two years of therapy.[89,90] The proportion of patients with serum HBV DNA below 300 copies/mL (60 IU/mL) was 60% after one year and 54% after two years of telbivudine therapy. Eighty percent of patients who stopped telbivudine treatment after achieving HBeAg seroconversion had sustained response after a mean period of 35 weeks post-treatment, which was comparable to lamivudine therapy.[91]

Treatment with telbivudine resulted in HBV DNA below 300 copies/mL (60 IU/mL) in 88% of HBeAg negative patients after one year and 79% after two years. Combined response of HBV DNA below 1.0×10^5 copies/mL ($<2.0 \times 10^4$ IU/mL) and normalisation of ALT was observed in 75% and 74% of patients after one and two years of therapy, respectively.

Since telbivudine and lamivudine belong to the same group of nucleoside analogues, there is cross-resistance between the two drugs. A substitution of methionine for isoleucine at position 204 is associated with telbivudine resistance. Telbivudine resistance was observed in 2-3% of patients after one year and in 7-17% after two years of telbivudine therapy.[89,90] The risk of antiviral resistance was strongly associated with viral load at week 24 of treatment. The two-year rate of telbivudine resistance was 4% in HBeAg positive patients and 2% in HBeAg negative patients if serum HBV DNA was below 300 copies/mL (60 IU/mL) after 24 weeks of therapy. At week 24, HBV DNA below this level was observed in 45% and 80% of HBeAg positive and HBeAg negative patients, respectively.[89]

Choice and duration of (initial) therapy

When deciding on the antiviral drug to be given, several factors have to be taken into account (table 4). The major advantage of PEG-IFN is the higher chance of achieving sustained response compared to nucleos(t)ide analogues with a finite duration of therapy. Disadvantages are the subcutaneous administration and the frequent occurrence of side effects. The major advantage of nucleos(t)ide analogues are the favourable tolerability and the oral administration. Disadvantages are the long duration of therapy and the subsequent risk of antiviral resistance. The costs of a one-year course of nucleos(t)ide analogue therapy are lower than of PEG-IFN, but will easily be higher when long-term therapy is needed.

PEG-IFN should be always be considered as first-line therapy in eligible patients because of the higher chance of achieving sustained off-treatment response compared to nucleos(t)ide analogues (table 5), particularly in HBeAg positive patients. Sustained transition to the immune-control phase

(inactive HBsAg carrier state) can be achieved in 30-35% of HBeAg positive patients and 25% of HBeAg negative patients treated with PEG-IFN, implicating that treatment induced response is sustained in about 85% and 40% of HBeAg positive and HBeAg negative patients, respectively.[45,53]

Table 4. Choice of initial therapy based on patient characteristics.

Patient characteristics	Peginterferon	Nucleos(t)ide analogue
HBeAg status	HBeAg positive	HBeAg negative
HBV genotype	A or B	C or D
HBV DNA	>=1.0 x10^9 copies/mL (2.0 x 10^8 IU/mL)	>1.0 x 10^9 copies/mL (2.0 x 10^8 IU/mL)
ALAT	>2-10 ULN	1-2 or >10 ULN
Severity of liver disease	Compensated	Compensated or decompensated

ULN = upper limit of normal. The above mentioned characteristics may be of help in choosing an antiviral agent, but do not provide strict recommendations.

Relapse occurs in at least 40% and 90% of HBeAg positive and HBeAg negative patients after discontinuation of nucleos(t)ide analogue therapy, respectively.[64,73,75,92] The latter applies especially to the older nucleos(t)ide analogs. Sufficient data for the newer, more potent, nucleos(t)ide analogues is not available.

Patients with a high chance of response to PEG-IFN therapy are those with genotype A or B, with serum HBV DNA below 1.0 x 10^9 copies/mL (2.0 x 10^8 IU/mL) and serum ALT above twice the upper limit of normal.[42,93,94] The licensed duration of peginterferon therapy is one year for both HBeAg positive and HBeAg negative chronic hepatitis B. However, the optimal duration of PEG-IFN therapy has not been established. In HBeAg positive patients, response rates after 24-32 weeks of treatment course seem comparable to those observed after one year, but head-to-head comparison is not available.[40-43] Since early prediction of response to PEG-IFN is not possible in chronic HBV infected patients, the recommended the duration of therapy is one year for all patients.

Nucleos(t)ide analogue therapy should be considered in patients not responding to or not eligible for PEG-IFN therapy. This includes patients with autoimmune disease, pre-existent psychiatric disorders or advanced cirrhosis (signs of diminished liver function or portal hypertension). When choosing a nucleos(t)ide analogue, potency and risk of resistance play an important role (table 5). Because of the high risk of antiviral resistance, lamivudine should no longer be considered as initial therapy in patients who require long-term therapy. However, because of extensive clinical experience, lamivudine can be given to pregnant patients with very high viremia during the last trimester of pregnancy.[33]

Of currently available nucleos(t)ide analogues, entecavir has the most favourable resistance profile (in comparison with lamivudine, adefovir and telbivudine) (table 5),[87] while entecavir and telbivudine

seem most potent.[62,89,95] However, fewest is known about the long-term safety of entecavir and telbivudine. In patients with lamivudine resistance, treatment with entecavir is not recommended because of the high risk of antiviral resistance. Adefovir add-on therapy is recommended for these patients. The role of telbivudine in the treatment is not yet established, as is the role of de-novo combination therapy of nucleos(t)ide analogues. A combination of antiviral drugs could potentially prevent the selection of resistance mutations, but supporting scientific evidence is not available.
In HBeAg positive patients, nucleos(t)ide analogue therapy should be continued at least until HBeAg seroconversion and HBV DNA below 400 copies/mL (80 IU/mL) have been achieved and maintained for six months. It is unclear when nucleos(t)ide analogue therapy can be safely discontinued in HBeAg negative patients, this may be possible in case of HBsAg seroconversion and HBV DNA below 400 copies/mL (80 IU/mL). Nucleos(t)ide analogue therapy therefore needs to be continued for long periods in virtually all HBeAg negative patients.

RECOMMENDATIONS

Grade I	PEG-IFN should be considered as first-line therapy in patients without contra-indications because of the higher chance of achieving sustained response compared to nucleos(t)ide analogues.
Grade I	Nucleos(t)ide analogue therapy should be considered in patients not eligible for, not tolerating or not responding to PEG-IFN therapy.
Grade I	Lamivudine is not recommended in patients in whom long-term nucleos(t)ide analogue is expected because of the high risk of antiviral resistance.
Grade II	Of currently available nucleos(t)ide analogues, entecavir has the lowest risk of antiviral resistance (compared to lamivudine, adefovir and telbivudine). Entecavir and telbivudine seem to provide most potent viral suppression.
Grade III	The recommended duration of PEG-IFN therapy is one year for both HBeAg positive and HBeAg negative patients.
Grade II	In HBeAg positive patents, nucleos(t)ide analogue therapy should be at least continued until HBeAg seroconversion and a decline in HBV DNA below 400 copies/mL (80 IU/mL) have been achieved and maintained for six months during therapy.
Grade II	In HBeAg negative patients, it is unknown whether nucleos(t)ide analogues can be safely discontinued. Long-term or indefinite antiviral treatment is usually necessary.

MONITORING OF ANTIVIRAL THERAPY

PEG-IFN treated patients should be monitored monthly; an additional visit at week two can be considered. Frequently occurring side effects such as depression, irritability, neutropenia and throm-

Table 5. Response after one year of antiviral therapy and antiviral resistance after 1-5 years of therapy.

	HBeAg-positive		HBeAg-negative						
	HBeAg-seroconversion		Undetectable HBV DNA		Antiviral resistance				
Antiviral therapy	**End of therapy**	**Post-treatment**	**End of therapy**	**Post-treatment**	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Alpha-interferon	35%[38,103-107]	30% [39,41,103-108]	60%[109-115]	35%[109-112,115]	-	-	-	-	-
Peginterferon	40%[40-43]	35%[40-44]	63%[48]	19%[48]	-	-	-	-	-
Lamivudine	19%[55, 56, 58-60]	12%[44,70,92]	65%[48,63,65]	10%[48,64]	24%[116]	42%[116]	53%[116]	70%[116]	74%[117]
Adefovir	12%[71]	NA	51%[73]	NA	0%[74]	3%[74]	11%[74]	18%[74]	28%[74]
Adefovir in lamivudine resistance	20%[77]	NA	19%[#,77]	NA	6-18% [77,79,80]	21-38% [77,79,80]	NA	NA	NA
Entecavir	21%[62]	NA	90%[81]	NA	0,1%[85]	0,3%[85]	0,4%[87]	0,8%[86]	NA
Entecavir in lamivudine resistance	8%[82]	NA	26%[#,118]	NA	12%[85]	20%[85]	25%[87]	40%[86]	NA
Telbivudine	22%[89]	NA	88%[89]	NA	2-3%[89]	7-17%[90]	NA	NA	NA

[#]HBV DNA <400 copies/mL in a mixed group of HBeAg positive and HBeAg negative patients. NA: not available.

bocytopenia require monthly monitoring and blood count. If necessary, PEG-IFN dosage should be reduced or treatment temporarily discontinued. PEG-IFN dosage should be reduced if the neutrophil count is below 0.75 x 10^9/l or platelet count below 50 x 10^9/l. The dose can reduced by 25% of the original dose until the respective cell fractions have normalised. Temporary discontinuation of PEG-IFN therapy is indicated if the neutrophil count is below 0.50 x 10^9/l or platelet count below 25 x 10^9/l. Severe side-effects such as depression or severe flu-like may also require dose-reduction or even (temporary) discontinuation of therapy.

Recommendations on laboratory testing during PEG-IFN and nucleos(t)ide analogue therapy are shown in table 6. The recommended frequency of HBV DNA quantification during antiviral therapy is every three to six months, dependent on the risk of antiviral resistance. Testing of serum ALT is recommended every 3 months. Prior to the start of nucleos(t)ide analogue therapy a quantitative HBV DNA test and HBV serology should be performed in order to evaluate response to therapy. The recommended frequency of monitoring during nucleos(t)ide analogue therapy is every 3 months, particularly for the early detection of antiviral resistance. Monitoring of serum creatinin is indicated every 3 months and nucleos(t)ide analogue dosage or frequency of administration should be reduced in case of severely decreased creatinin clearance (<50 ml/min) (table 7). Although nucleos(t)ide analogue therapy generally has an excellent safety and tolerability profile, severe side-effects such as lactic acidosis have been described.[96]

RECOMMENDATION

Grade II	The recommended frequency of monitoring is monthly during PEG-IFN therapy and every three months during nucleos(t)ide analogue therapy.

ANTIVIRAL RESISTANCE

Primary non-response is defined as a less than 2log10 copies/mL (or IU/mL) decline in HBV DNA after 24 weeks of nucleos(t)ide analogue therapy [6]. Potential causes of primary non-response include antiviral resistance, but also non-compliance, decreased absorption or rapid breakdown [97]. The risk of antiviral resistance is minimal if serum HBV DNA is 400 copies/mL or lower after 24 weeks of therapy. Addition of adefovir or switching to entecavir is recommended in telbivudine-treated patients with serum HBV DNA above 400 copies/mL (80 IU/mL) after 24 weeks of therapy. This should also be considered in patients treated with lamivudine. Antiviral resistance is rare in adefovir treated patients during the first year of antiviral treatment. However, addition of telbivudine or switching to entecavir should be considered in patient with serum HBV DNA above1000 copies/mL (200 IU/mL) after 12 months of therapy because of the increasing risk of antiviral resistance in these patients.

Antiviral resistance should be suspected if serum HBV DNA increases during nucleos(t)ide analogue therapy. If virological breakthrough, defined as a 1log10 copies/mL (or IU/mL) increase in

Table 6. Recommendations on minimal laboratory testing during antiviral therapy.

	Start of therapy	PEG-IFN 4-weekly*	PEG-IFN 3-monthly	Nucleos(t)ide analogues 3-monthly
Aminotransferases (AST, ALT)	x	x		x
Lever function (bilirubin, albumin, prothrombin time)	x		x	x
Kidney function (creatinin)[§]	x		x	x
Blood count (platelets, neutrophil count)	x	x		
Endocrinology (TSH)	x[#]		x	
Virus serology (HBsAg,[¥] anti-HBs,[¥] HBeAg, anti-HBe)	x		x	x
Quantitative HBV DNA	x		x[£]	x[£]

*Also after 2 weeks of therapy.

[§]Assessment of 24-hour creatinin clearance is recommended in patients with elevated creatinin.

[#]Only for PEG-IFN treated patients.

[¥]HBsAg and anti-HBs only after HBeAg-seroconversie or repeatedly undetectable HBV DNA (HBV DNA <400 copies/mL or <80 IU/mL).

[£]Quantitative HBV DNA every 3-6 months

Table 7. Adjustment of nucleos(t)ide analogue dosing in accordance with creatinin clearance.

	Lamivudine	Adefovir	Entecavir in naïve patients	Entecavir in lamivudine resistance	Telbivudine
Creatinin clearance:					
<5 ml/min / haemodialysis / CAPD	10 mg/day (starting dose 35 mg)	10 mg/7 days¥	0.05 mg/day	0.1 mg/day	600 mg/4 days‡
5-9 ml/min / haemodialysis / CAPD	15 mg/day (starting dose 35 mg)	10 mg/7 days¥	0.05 mg/day	0.1 mg/day	600 mg/4 days‡
10-14 ml/min	15 mg/day (starting dose 35 mg)	10 mg/3 days	0.15 mg/day	0.3 mg/day	600 mg/3 days
15-19 ml/min	25 mg/day (starting dose 100 mg)	10 mg/3 days	0.15 mg/day	0.3 mg/day	600 mg/3 days
20-29 ml/min	25 mg/day (starting dose 100 mg)	10 mg/2 days	0.15 mg/day	0.3 mg/day	600 mg/3 days
30-49 ml/min	50 mg/day (starting dose 100 mg)	10 mg/2 days	0.25 mg/day	0.5 mg/day	600 mg/2 days

CAPD = continuous ambulant peritoneal dialysis

¥No recommendations can be made for adefovir treated patients with creatinin clearance <10 ml/min.

‡Telbivudine should be administered after haemodialysis

Source: Farmacotherapeutisch Kompas (http://www.fk.cvz.nl/)

HBV DNA, is observed in a compliant patients, genotypic analysis of the HBV polymerase is indicated.[97] A rise in HBV DNA is the first sign of antiviral resistance and is often followed by a rise in ALT without intervention.[97]

In case of antiviral resistance it is recommended to change antiviral therapy as soon as possible since response to the second drug is better when started at the time of virological breakthrough than at the time of biochemical breakthrough.[98] Adding a second drug seems favourable over switching to another drug, since this significantly reduces the risk of antiviral resistance to the second drug.[99] However, adding a second nucleos(t)ide analogue does not lead to more profound viral suppression compared to monotherapy of the new drug.[99] Adding adefovir is preferred over switching to entecavir in lamivudine resistance patients because of the lower risk of antiviral resistance. If entecavir is started, lamivudine should be discontinued. In adefovir-resistant patients, treatment with entecavir is preferred.[6] Table 8 shows the recommendations for the management of antiviral resistance. In case of resistance to other antiviral agents or multiple drugs, consulting a centre with expertise on this topic is recommended.

Table 8. Treatment options for patients with antiviral resistance

Type of antiviral resistance	Treatment options‡
Lamivudine resistance	Adefovir add-on (Switch to entecavir)
Adefovir resistance	Entecavir add-on (Telbivudine add-on) (Lamivudine add-on) (Switch to entecavir)
Entecavir resistance	Adefovir add-on
Telbivudine resistance	Adefovir add-on (Switch to entecavir)

‡Controlled studies are often not available

Treatment options between brackets are not preferred.

FOLLOW-UP AFTER ANTIVIRAL THERAPY

Liver biochemistry, quantification of HBV DNA and HBV serology should be repeated three to six months post-treatment in PEG-IFN treated patients to assess the presence of sustained response. This is also recommended for responders to nucleos(t)ide analogue treatment who stopped antiviral therapy, although more frequent monitoring after discontinuation can be considered because of the higher risk of relapse compared to PEG-IFN.[37]

In HBeAg negative patient with serum HBV DNA below 1.0 x 10^5 copies/mL (2.0 x 10^4 IU/mL) and normal ALT, yearly monitoring of ALT during three years suffices. These patients could also be monitored by their general practitioner. The Dutch Society of General Practitioners (NHG) also

recommends this frequency of follow-up in their guideline "Viral hepatitis and other liver diseases". If a rise in serum ALT is observed, the patient should be referred to the treating specialist. In patients with HBsAg seroconversion (loss of HBsAg and anti-HBs >10 IU/l), monitoring of disease activity and prophylactic treatment should only be considered in case of severe immunosuppression, e.g. chemotherapy or treatment with selective antibodies.

RECOMMENDATIONS

Grade II	In telbivudine treated patients, changing antiviral therapy is recommended if serum HBV DNA is higher than 400 copies/mL (80 IU/mL) after 24 weeks of therapy because of the risk of antiviral resistance.
Grade IV	In adefovir treated patients, changing antiviral therapy is recommended if serum HBV DNA is higher than 1,000 copies/mL (200 IU/mL) after 12 months of therapy because of the risk of antiviral resistance.
Grade I	Confirmation of the HBV DNA measurement and genotypic analysis of the HBV polymerase is indicated in compliant patients with virological breakthrough, as defined by a more than 1log10 copies/mL (or IU/mL) increase in serum HBV DNA, as this often is associated with antiviral resistance.
Grade II	Antiviral treatment should be changed as soon as possible in case of antiviral resistance.
Grade III	Adding a second nucleos(t)ide analogue is preferable over switching to another drug because of the lower risk of antiviral resistance to the second drug.
Grade II	In lamivudine resistant patients, addition of adefovir is preferred over switching to entecavir because of the high risk of entecavir resistance in these patients.

REFERENCES

1. Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13:S47-9.
2. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-45.
3. de Franchis R, Hadengue A, Lau G, et al. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39:S3-25.
4. Veldhuijzen IK, Conyn-van Spaendonck MAE, Dorigo-Zetsma JW. Seroprevalentie van hepatitis B en C in de Nederlandse bevolking. Infectieziekten Bulletin 1999;10:182-184.
5. Liaw YF, Leung N, Guan R, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update. Liver Int 2005;25:472-89.
6. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
7. Wong SN, Lok AS. Treatment of hepatitis B: who, when, and how? Arch Intern Med 2006;166:9-12.
8. Chang MH. Natural history of hepatitis B virus infection in children. J Gastroenterol Hepatol 2000;15 Suppl:E16-9.
9. Fattovich G, Brollo L, Giustina G, et al. Natural history and prognostic factors for chronic hepatitis type

B. Gut 1991;32:294-8.
10. Mathurin P, Moussalli J, Cadranel JF, et al. Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. Hepatology 1998;27:868-72.
11. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-86.
12. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349:825-32.
13. de Jongh FE, Janssen HL, de Man RA, et al. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. Gastroenterology 1992;103:1630-5.
14. Fattovich G, Giustina G, Schalm SW, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Hepatology 1995;21:77-82.
15. Realdi G, Fattovich G, Hadziyannis S, et al. Survival and prognostic factors in 366 patients with compensated cirrhosis type B: a multicenter study. The Investigators of the European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol 1994;21:656-66.
16. Fattovich G, Stroffolini T, Zagni I, et al. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127:S35-50.
17. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
18. Iloeje UH, Yang HI, Su J, et al. Viral load is a strong predictor of hepatocellular carcinoma risk in people chronically infected with hepatitis B virus and with normal serum alanine aminotransferase level. J Viral Hepat 2005;42:179.
19. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347:168-74.
20. Cadranel JF, Lahmek P, Causse X, et al. Epidemiology of chronic hepatitis B infection in France: risk factors for significant fibrosis--results of a nationwide survey. Aliment Pharmacol Ther 2007;26:565-76.
21. Hui CK, Leung N, Yuen ST, et al. Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. Hepatology 2007;46:395-401.
22. Trevisani F, De NS, Rapaccini G, et al. Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: effects on cancer stage and patient survival (Italian experience). Am J Gastroenterol 2002;97:734-44.
23. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208-36.
24. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol 2004;130:417-22.
25. Garcia-Tsao G, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. Hepatology 2007;46:922-38.
26. Chu CM, Liaw YF. Increased incidence of fulminant hepatic failure in previously unrecognized HBsAg carriers with acute hepatitis independent of etiology. Infection 2005;33:136-9.
27. Reiss G, Keeffe EB. Review article: hepatitis vaccination in patients with chronic liver disease. Aliment Pharmacol Ther 2004;19:715-27.
28. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis 2005;25 Suppl 1:3-8.
29. Manesis EK, Papatheodoridis GV, Sevastianos V, et al. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis B e antigen-negative chronic hepatitis B virus infection. Am J Gastroenterol 2003;98:2261-7.
30. Fontana RJ, Hann HW, Perrillo RP, et al. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. Gastroenterology 2002;123:719-27.
31. Villeneuve JP, Condreay LD, Willems B, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000;31:207-10.

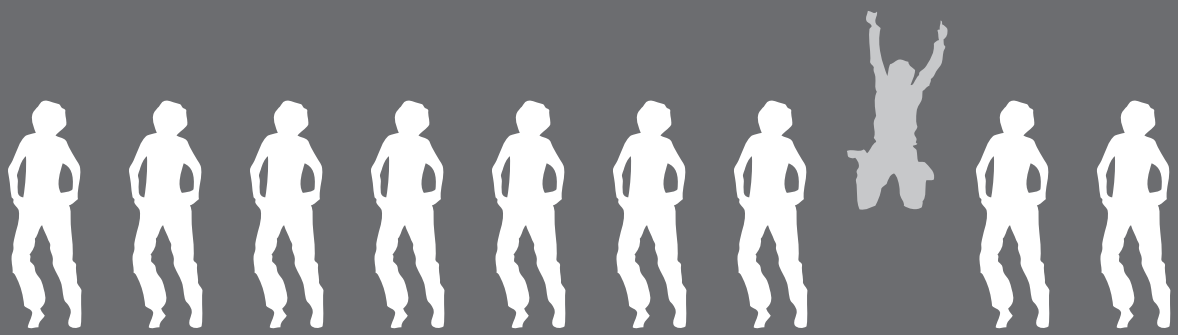
32. del Canho R, Grosheide PM, Mazel JA, et al. Ten-year neonatal hepatitis B vaccination program, The Netherlands, 1982-1992: protective efficacy and long-term immunogenicity. Vaccine 1997;15:1624-30.
33. van Zonneveld M, van Nunen AB, Niesters HG, et al. Lamivudine treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. J Viral Hepat 2003;10:294-7.
34. Xu WM, Cui YT, Wang L, et al. Efficacy and safety of lamivudine in late pregnancy for the prevention of mother-child transmission of hepatitis B: a multicenter, randomized, double-blind, placebo-controlled study. Hepatology 2004;40:272A-273A.
35. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. Clin Gastroenterol Hepatol 2006;4:936-62.
36. Kohrt HE, Ouyang DL, Keeffe EB. Systematic review: lamivudine prophylaxis for chemotherapy-induced reactivation of chronic hepatitis B virus infection. Aliment Pharmacol Ther 2006;24:1003-16.
37. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-4.
38. Haria M, Benfield P. Interferon-alpha-2a. A review of its pharmacological properties and therapeutic use in the management of viral hepatitis. Drugs 1995;50:873-96.
39. van Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-10.
40. Chan HL, Leung NW, Hui AY, et al. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alpha2b and lamivudine with lamivudine alone. Ann Intern Med 2005;142:240-50.
41. Cooksley WG, Piratvisuth T, Lee SD, et al. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.
42. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-9.
43. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-95.
44. Chan HL, Hui AY, Wong VW, et al. Long-term follow-up of peginterferon and lamivudine combination treatment in HBeAg-positive chronic hepatitis B. Hepatology 2005;41:1357-64.
45. Lau GK, Piratvisuth T, Luo KX, et al. Durability of response and occurrence of late response to peginterferon alpha-2a (40KD) one year post-treatment in patients with HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.
46. Zhao H, Kurbanov F, Wan MB, et al. Genotype B and younger patient age associated with better response to low-dose therapy: a trial with pegylated/nonpegylated interferon-alpha-2b for hepatitis B e antigen-positive patients with chronic hepatitis B in China. Clin Infect Dis 2007;44:541-8.
47. Flink HJ, van Zonneveld M, Hansen BE, et al. Treatment with peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol 2006;101:297-303.
48. Marcellin P, Lau GK, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206-17.
49. van Zonneveld M, Flink HJ, Verhey E, et al. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. Aliment Pharmacol Ther 2005;21:1163-71.
50. Janssen HL, Berk L, Vermeulen M, et al. Seizures associated with low-dose alpha-interferon. Lancet 1990;336:1580.
51. Janssen HL, Brouwer JT, Nevens F, et al. Fatal hepatic decompensation associated with interferon alfa. European concerted action on viral hepatitis (Eurohep). BMJ 1993;306:107-8.
52. Pugh RN, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal

varices. Br J Surg 1973;60:646-9.
53. Marcellin P, Bonino F, Lau GKK, et al. The majority of patients with HBeAg-negative chronic hepatitis B treated with peginterferon alfa-2a (40KD) [Pegasys®] sustain responses 2 years post-treatment. J Hepatol 2006;44:S275.
54. di Bisceglie A, Lai CL, Gane E, et al. Telbivudine GLOBE trial: Maximal early HBV suppression is predictive of optimal two-year efficacy in nucleoside-treated hepatitis B patients. Hepatology 2006;44:230A-231A.
55. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256-63.
56. Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998;339:61-8.
57. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527-32.
58. Liaw YF, Leung NW, Chang TT, et al. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. Gastroenterology 2000;119:172-80.
59. Schalm SW, Heathcote J, Cianciara J, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. Gut 2000;46:562-8.
60. Schiff ER, Dienstag JL, Karayalcin S, et al. Lamivudine and 24 weeks of lamivudine/interferon combination therapy for hepatitis B e antigen-positive chronic hepatitis B in interferon nonresponders. J Hepatol 2003;38:818-26.
61. Chang TT, Lai CL, Chien RN, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol 2004;19:1276-82.
62. Chang TT, Gish RG, de Man R, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-10.
63. Hadziyannis SJ, Papatheodoridis GV, Dimou E, et al. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2000;32:847-51.
64. Santantonio T, Mazzola M, Iacovazzi T, et al. Long-term follow-up of patients with anti-HBe/HBV DNA-positive chronic hepatitis B treated for 12 months with lamivudine. J Hepatol 2000;32:300-6.
65. Buti M, Cotrina M, Jardi R, et al. Two years of lamivudine therapy in anti-HBe-positive patients with chronic hepatitis B. J Viral Hepat 2001;8:270-5.
66. Gaia S, Marzano A, Smedile A, et al. Four years of treatment with lamivudine: clinical and virological evaluations in HBe antigen-negative chronic hepatitis B. Aliment Pharmacol Ther 2004;20:281-7.
67. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-31.
68. Tipples GA, Ma MM, Fischer KP, et al. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. Hepatology 1996;24:714-7.
69. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology. 2003 Dec;125(6):1714-22.
70. Song BC, Suh DJ, Lee HC, et al. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology. 2000 Oct;32(4 Pt 1):803-6.
71. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003 Feb 27;348(9):808-16.
72. Marcellin P, Chang TT, Lim SG, et al. Long term efficacy and safety of adefovir dipivoxil (ADV) 10mg in HBeAg+ chronic hepatitis B patients: increasing serologic, virologic and biochemical response over time. Hepatology 2004;40:655A.
73. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800-7.

74. Hadziyannis S, Tassopoulos NC, Chang TT, et al. 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;42:754A.
75. 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;352:2673-81.
76. Fung SK, Chae HB, Fontana RJ, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol 2006;44:283-90.
77. Lee YS, Suh DJ, Lim YS, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology 2006;43:1385-91.
78. Bartholomeusz A, Locarnini SA, Ayres A, et al. Molecular modelling of hepatitis B virus polymerase and adefovir resistance identifies three clusters of mutations. Hepatology 2004;40:A165.
79. Chen CH, Wang JH, Lee CM, et al. Virological response and incidence of adefovir resistance in lamivudine-resistant patients treated with adefovir dipivoxil. Antivir Ther 2006;11:771-8.
80. Yeon JE, Yoo W, Hong SP, et al. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. Gut 2006;55:1488-95.
81. Lai CL, Shouval D, Lok AS, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-20.
82. Sherman M, Yurdaydin C, Sollano J, et al. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. Gastroenterology 2006;130:2039-49.
83. Chang TT, Chao YC, Kaymakoglu S, et al. Entecavir maintained virological suppression through 3 years of treatment in antiviral-naïve HBeAg(+) patients (ETV 022/901). Hepatology 2006;44:229A.
84. Shouval D, Akarca US, Hatzis G, et al. Continued virologic and biochemical improvement through 96 weeks of entecavir treatment in HBeAg(-) chronic hepatitis B patients (study ETV-027). J Hepatol 2006;44:S32-22.
85. Colonno RJ, Rose R, Baldick CJ, et al. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology 2006;44:1656-65.
86. Colonno RJ, Rose RE, Pokornowski K, et al. Four year assessment of ETV resistance in nucleoside-naive and lamivudine refractory patients. J Hepatol 2007;46:S294.
87. Colonno RJ, Rose RE, Pokornowski K, et al. Assessment at three years shows high barrier to resistance is maintained in entecavir-treated nucleoside naïve patients while resistance emergence increases over time in lamivudine refractory patients. Hepatology 2006;44:229A-230A.
88. Tenney DJ, Rose RE, Baldick CJ, et al. Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. Antimicrob Agents Chemother 2007;51:902-11.
89. Lai CL, Gane E, Liaw YF, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576-88.
90. Lai CL, Gane E, Hsu CW, et al. Two-year results from the GLOBE trial in patients with hepatitis B: greater clinical and antiviral efficacy for telbivudine (LdT) vs. lamivudine. Hepatology 2006;44:222A.
91. Poynard T, Chutaputti A, Hwang SG, et al. Sustained off-treatment HBeAg response in telbivudine and lamivudine treated HBeAg-positive patients from the GLOBE study. J Hepatol 2007;46:S27.
92. Dienstag JL, Cianciara J, Karayalcin S, et al. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology 2003;37:748-55.
93. Bonino F, Lau GKK, Marcellin P, et al. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized, partially double-blind study of peginterferon-alfa-2a (4-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone. Hepatology 2004;40:A1142.
94. Cooksley G, Lau GKK, Liaw YF, et al. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large,

randomised study. J Hepatol 2005;42:S30.
95. Hadziyannis SJ, Papatheodoridis GV. Adefovir dipivoxil in the treatment of chronic hepatitis B virus infection. Expert Rev Anti Infect Ther 2004;2:475-83.
96. Carr A, Miller J, Law M, et al. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. Aids 2000;14:F25-32.
97. Locarnini S, Hatzakis A, Heathcote J, et al. Management of antiviral resistance in patients with chronic hepatitis B. Antivir Ther 2004;9:679-93.
98. Lampertico P, Vigano M, Manenti E, et al. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. Hepatology 2005;42:1414-9.
99. Lampertico P, Marzano A, Levrero M, et al. Adefovir and lamivudine combination therapy is superior to adefovir monotherapy for lamivudine-resistant patients with HBeAg-negative chronic hepatitis B. Hepatology 2006;44:693A-694A.
100. Fattovich G, Giustina G, Favarato S, et al. A survey of adverse events in 11,241 patients with chronic viral hepatitis treated with alfa interferon. J Hepatol 1996;24:38-47.
101. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2002;36:S237-44.
102. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. Gut 2006;55:1350-9.
103. Hoofnagle JH, Peters M, Mullen KD, et al. Randomized, controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. Gastroenterology 1988;95:1318-25.
104. Janssen HL, Gerken G, Carreno V, et al. Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1999;30:238-43.
105. Korenman J, Baker B, Waggoner J, et al. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.
106. Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. N Engl J Med 1990;323:295-301.
107. Wong DK, Cheung AM, O'Rourke K, et al. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312-23.
108. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-97.
109. Fattovich G, Farci P, Rugge M, et al. A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HBeAg. Hepatology 1992;15:584-9.
110. Hadziyannis S, Bramou T, Makris A, et al. Interferon alfa-2b treatment of HBeAg negative/serum HBV DNA positive chronic active hepatitis type B. J Hepatol 1990;11 Suppl 1:S133-6.
111. Lampertico P, Del Ninno E, Manzin A, et al. A randomized, controlled trial of a 24-month course of interferon alfa 2b in patients with chronic hepatitis B who had hepatitis B virus DNA without hepatitis B e antigen in serum. Hepatology 1997;26:1621-5.
112. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. Gastroenterology 2001;120:1828-53.
113. Lok AS, Wu PC, Lai CL, et al. A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. Gastroenterology 1992;102:2091-7.
114. Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. Gastroenterology 2001;121:101-9.
115. Pastore G, Santantonio T, Milella M, et al. Anti-HBe-positive chronic hepatitis B with HBV-DNA in the serum response to a 6-month course of lymphoblastoid interferon. J Hepatol 1992;14:221-5.

116. Lai CL, Dienstag J, Schiff E, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003;36:687-96.
117. Moskovitz DN, Osiowy C, Giles E, et al. Response to long-term lamivudine treatment (up to 5 years) in patients with severe chronic hepatitis B, role of genotype and drug resistance. J Viral Hepat 2005;12:398-404.
118. Chang TT, Gish RG, Hadziyannis SJ, et al. A dose-ranging study of the efficacy and tolerability of entecavir in Lamivudine-refractory chronic hepatitis B patients. Gastroenterology 2005;129:1198-209.



Predicting response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa.

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Gastroenterology, in press.

ABSTRACT

Background

Therapy with pegylated interferon alpha (PEG-IFN) results in sustained response in a minority of chronic hepatitis B virus (HBV) infected patients and has considerable side-effects. We combined data from individual patients from the 2 largest global trials of hepatitis B e Antigen (HBeAg)-positive chronic hepatitis B to determine which patients are most likely to respond to PEG-IFN therapy.

Methods

Five-hundred-forty-two patients treated with PEG-IFN alpha-2a (180 µg/week, 48 weeks) and 266 patients treated with PEG-IFN alpha-2b (100 µg/week, 52 weeks) were included. Eighty-seven patients were excluded, leaving 721 patients for analysis. A sustained response was defined as HBeAg loss and HBV DNA $<2.0 \times 10^4$ IU/ml at 6 months post treatment. Logistic regression analysis was used to identify predictors of sustained response and a multivariable model was constructed.

Results

HBV genotype, high ALT (>=2 x ULN) , low HBV DNA ($<2.0 \times 10^8$ IU/ml), female sex, older age, and absence of previous IFN therapy predicted sustained response. Genotype A patients with either high ALT and/or low HBV DNA had a high (>30%) predicted probability of sustained response. High ALT was the strongest predictor in genotype B and low HBV DNA level in genotype C. Genotype D patients had a low chance of sustained response, irrespective of ALT or HBV DNA.

Conclusion

The best candidates are genotype A patients with either high levels of ALT or low HBV DNA, and genotype B and C patients with high levels of ALT and low HBV DNA. Genotype D patients have a low chance of sustained response.

INTRODUCTION

Hepatitis B is a major global health problem. The World Health Organization reported that there are more than 400 million carriers in the world, approximately 75% of whom reside in Asia and the Western Pacific.[1] In this part of the world, HBV infection is usually acquired perinatally or in early childhood. Most patients from these areas typically have HBeAg-positive chronic hepatitis B with high HBV DNA levels and they develop moderate to severe hepatic inflammation with elevated alanine aminotransferase (ALT) levels after 10–30 years of infection.[2] In contrast, patients infected in late childhood, adolescence or adulthood present with elevated aminotransferases after a shorter duration of infection. Although spontaneous HBeAg seroconversion occurs in the majority of HBeAg-positive patients, the duration of hepatic inflammation can be prolonged and severe, and may result in liver cirrhosis. Therefore, antiviral treatment is indicated in patients who remain HBeAg positive with high HBV DNA levels after a 3-6 month period of elevated ALT levels.[3]

Successful treatment of chronic hepatitis B virus (HBV) infection with loss of hepatitis B e antigen (HBeAg), decline in serum HBV DNA and normalization of ALT is associated with favorable long-term outcome, independent of the antiviral drug used.[4, 5] In HBeAg-positive patients, sustained clearance of HBeAg from serum is associated with a higher likelihood of losing hepatitis B surface antigen (HBsAg), reduced incidence of cirrhosis and hepatocellular carcinoma (HCC), and improved survival.[5-8] Of currently available drugs for the treatment of chronic hepatitis B, pegylated interferon (PEG-IFN) still results in the highest rate of off-treatment sustained response after a one-year course of therapy.[9-12] Furthermore, responders to IFN-based therapy have a considerable chance of losing HBsAg, which has been observed in 12-65% of patients within 5 years after HBeAg loss.[7, 8, 13-18] Treatment with PEG-IFN is however often complicated by the occurrence of side-effects such as flu-like symptoms, cytopenia and depression.[19] Nucleos(t)ide analogues such as lamivudine, adefovir, entecavir and tenofovir on the other hand are well tolerated, but because of the modest seroconversion rate and the high risk of post-treatment relapse, prolonged therapy is usually required.[20] Nowadays, maintenance of virological response over prolonged periods is feasible,[21] but may still pose a considerable risk for resistance in the long-term.[22-24]

Since both treatment with PEG-IFN and nucleos(t)ide analogues has proven effective, but also have their advantages and limitations, the question arises what treatment regimen should be used as first-line therapy in which patients. Both the chance of achieving response and specific patient characteristics play a role in the decision on what type of antiviral therapy should be started. Recently performed studies with one year of PEG-IFN in HBeAg-positive patients identified high baseline alanine aminotransferase (ALT), low baseline HBV DNA, absence of previous IFN therapy, low baseline HBeAg and HBV genotype A or B as predictors of response.[10, 25, 26] Current guidelines do not provide specific recommendations as to which patients should be treated with peginterferon,[27] the above mentioned studies were considered to provide insufficient evidence for such recommendations. The aim of this study therefore was to develop a solid model for the prediction of sustained response to PEG-IFN in individual HBeAg-positive patients, which will allow physicians

throughout the globe to choose the optimal candidates for treatment with this drug.

METHODS

Patients and study design

Individual data of 542 patients treated with PEG-IFN alpha2a 180 µg per week for 48 weeks (271 patients with added lamivudine 100 mg daily) and 266 patients treated with PEG-IFN alpha2b 100 µg per week for 52 weeks (130 patients with added lamivudine 100 mg daily) were analyzed.[10, 11] Post-treatment follow-up lasted six months. Addition of lamivudine did not influence response rates at the end of follow-up (6 months post-treatment) in any way. For the current study, sustained virological response (SVR) was defined as clearance of HBeAg from serum and HBV DNA <10,000 copies/ml at six months post-treatment.

The inclusion and exclusion criteria were reported in detail previously and were similar for the two studies.[10, 11] In short, patients were eligible if they had been HBsAg positive for at least 6 months, were HBeAg positive, had elevated serum ALT between one and 10 times the upper limit of normal (ULN), had serum HBV DNA >1.0 x 10^5 copies/ml (2.0 x 10^4 IU/ml) and had findings on a liver biopsy within the preceding 12 months that were consistent with the presence of chronic hepatitis B. Exclusion criteria included decompensated liver disease, antiviral therapy within 6 months prior to randomization, viral coinfections (hepatitis C virus, hepatitis delta virus or human immunodeficiency virus), or pre-existent neutropenia or thrombocytopenia.

Laboratory testing

During therapy and post-treatment follow-up, all patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally and therefore expressed as times upper limits of normal (ULN). HBV DNA was assessed monthly using an in-house developed Taqman PCR assay based on the Eurohep standard (lower limit of detection 373 copies/ml) or the Cobas Amplicor HBV Monitor Test (Roche Diagnostics).[28] HBeAg, anti-HBe, HBsAg and anti-HBs were measured with the use of the AxSYM test (Abbott, Abbott Park, IL, USA). HBV genotype analysis was performed by INNO-LiPA Assay (Innogenetics, Gent, Belgium).

Statistical analysis

Statistical analysis was performed using the SPSS 15.0 program (SPSS Inc. Chicago, IL) and the R 2.3.1 Project for Statistical Computing (Harrell's Design, HMisc and Foreign libraries). A p-value <0.05 was considered statistically significant (all two-tailed). We performed univariate logistic regression analysis to identify predictors of SVR among the variables age, sex, HBV genotype (A-D), serum HBV DNA ($\log_{10}$ copies/ml), ALT (ln ALT x ULN), treatment allocation (PEG-IFN monotherapy or combination therapy of PEG-IFN and lamivudine), and previous treatment with interferon or lamivudine. We used multivariable logistic regression analysis with backward stepwise

selection, using a *p*-value greater than 0.05 for removal of variables, to construct a multivariable linear model that provides a natural logarithm transformed prediction of SVR. We used restricted cubic spline functions to assess the linearity of the effect of continuous variables. Interactions between variables were explored. Odds ratios (ORs) were calculated with 95% confidence intervals (95%-CI). Since there were interactions between HBV genotype and other factors, ORs for HBV genotype were calculated for 33-year old (mean age), IFN-naïve males with ALT and HBV DNA fixed at 2 x ULN and 1.0 x 10^9 copies/ml (2.0 x 10^8 IU/ml), respectively.

Discrimination, which is the ability to distinguish patients who will achieve SVR from those who will not, was quantified by the area under the receiver-operating characteristic curve (AUC). An AUC of 0.5 indicates no discriminative ability at all, whereas an AUC of 1.0 indicates perfect discrimination. Internal validity was assessed with bootstrap sampling.[29, 30] Two-hundred bootstrap samples were drawn with replacement and with the same size as the original sample. The final prediction model was constructed by applying the penalized maximum likelihood estimation acquired from the bootstrap samples.[31] Nomograms for IFN-naïve patients were constructed based on the logistic regression formulas. In order to develop a simple rule for each of the genotypes independent of sex and age, the predicted probability of SVR in different patient subgroups was calculated with the logistic regression formulas. Since sex and age also predicted SVR, but were not included in the flowchart, age was fixed at the respective mean value of each subgroup and the mean predicted probability of SVR for males and females was calculated. For serum ALT, a low level (<2 x ULN) was considered to be between 1 x ULN and 2 x ULN, and a high level (>=2 x ULN) was considered to be between 2 x ULN and 10 x ULN. For serum HBV DNA, a low level (<copies/ml [<2.0 x 10^8 IU/ml]) was considered to be between 1.0 x 10^7 copies/ml (2.0 x 10^6 IU/ml) and 1.0 x 10^9 copies/ml (2.0 x 10^8 IU/ml), and a high level (>=1.0 x 10^9 copies/ml [>=2.0 x 10^8 IU/ml]) was considered to be between 1.0 x 10^9 copies/ml (2.0 x 10^8 IU/ml) and 1.0 x 10^{11} copies/ml (2.0 x 10^{10} IU/ml). These cut-offs were chosen because the majority of patients had ALT (95%) and HBV DNA (80%) levels between these values. In addition, these cut-off levels are generally used in clinical practice and recommended by international guidelines for the treatment of chronic hepatitis B.[3, 27, 32, 33]

RESULTS

Of 808 patients eligible for participation in this study, 87 were excluded because of missing values (n=76) or infection with HBV genotype other than A to D (n=11), leaving 721 patients for analysis. There were no differences in baseline characteristics between patients enrolled and those excluded from participation in this study, except for a lower rate of previous IFN therapy among the participants than the excluded patients (14% vs. 24%, p=0.01). HBeAg loss, HBeAg seroconversion and HBV DNA <10,000 copies/ml (<2.0 x 10^3 IU/ml) were observed in 254 (35.2%), 232 (32.2%) and 174 (24.1%) of 721 patients, respectively. Sustained virological response (SVR), defined as HBeAg loss and HBV DNA <10,000 copies/ml (<2.0 x 10^3 IU/ml) at six month post-treatment, was observed in 158 of 721 patients (21.9%). SVR was observed in 22.4% of patients treated with PEG-IFN

alone and in 21.4% of those treated with PEG-IFN and lamivudine combination therapy (p=0.73). Baseline characteristics of patients with and without SVR are given in table 1. Patients with SVR were older, more often were female, had higher baseline ALT and lower HBV DNA levels, and were more likely to have genotype A but less likely to have genotype D infection compared to those without SVR. The proportion of patients that was previously treated with IFN or lamivudine therapy did not differ between patients with SVR and those without.

Table 1: Baseline characteristics and univariate logistic regression analysis

Characteristic	Sustained virological response† (n = 158)	No sustained virological response (n = 563)	Odds Ratio	95% Confidence Interval Lower	Upper	p
Age	34.8 ± 11.4	32.4 ± 10.6	1.02	1.00	1.04	0.01
Female sex	47 (29.7%)	120 (21.3%)	1.56	1.05	2.32	0.03
Serum ALT (x ULN)	4.3 ± 3.0	3.9 ± 3.5	1.31	1.02	1.69	0.03
HBV DNA (log_{10} copies/ml)	9.4 ± 1.7	9.8 ± 1.8	0.85	0.77	0.95	0.003
HBV genotype						<0.001
A	42 (26.6%)	73 (13.0%)	1.00			
B	41 (25.9%)	125 (22.2%)	0.57	0.34	0.96	
C	67 (42.4%)	266 (47.2%)	0.44	0.28	0.70	
D	8 (5.1%)	99 (17.6%)	0.14	0.06	0.32	
Previous interferon therapy	22 (13.9%)	79 (14.0%)	0.99	0.60	1.65	0.97
Previous lamivudine therapy	16 (10.1%)	64 (11.4%)	0.88	0.49	1.57	0.66

†Sustained virological response: HBeAg loss and HBV DNA <10,000 copies/ml at 6 months post-treatment; ULN: upper limit of normal For age, ALT, GGT and HBV DNA the mean ± SD is given.

Predictors of sustained virological response

Factors associated with an increased likelihood of SVR included HBV genotype A infection, high baseline ALT, low baseline HBV DNA, female sex, older age, (table 1). There was no association between SVR and previous treatment with interferon or lamivudine on univariate analysis. Using multivariate analysis, high baseline ALT was found to be an independent predictor of SVR (OR 1.57 per $1log_{10}$ x ULN increase [95%-CI, 1.19 – 2.09], p=0.002). In addition, HBV genotype was associated with SVR, with higher rates of SVR in patients with genotype A (OR 1, reference) than B ($OR_{B\ vs.\ A}$ 0.46 [95%-CI, 0.21 – 0.99], p=0.05), C ($OR_{C\ vs.\ A}$ 0.30 [95%-CI, 0.16 – 0.59], p<0.001) or D ($OR_{D\ vs.\ A}$ 0.08 [95%-CI, 0.02 – 0.31], p<0.001). The influence of sex, age, HBV DNA, and previous IFN therapy was significantly different across HBV genotypes (p<0.02 for the interaction between HBV genotype and each of these factors). These variables were therefore also included

in the model. We here describe the most important predictive factors. Genotype C and D infected females had a significantly higher chance of sustained response compared to males (OR 2.78 [1.51 - 5.11] and OR 7.69 [1.48 - 39.90], p<0.02). Older age was associated with a significantly higher chance of sustained response in genotype A infected patients (OR 1.04 per year increase in age [1.01 - 1.08], p=0.01). High baseline HBV DNA was associated with a lower likelihood of sustained response in patients with genotype A (OR 0.57 [0.40 - 0.82], p=0.003) and C (OR 0.77 [0.65 - 0.91], p=0.002). Previous IFN therapy resulted in a significantly lower chance of sustained response in patients with genotype A or D (OR 0.21 [0.07 - 0.58], p=0.003). We found no differences in the predictors of response for the two treatment groups.

Performance of the model

The distribution of the predicted probabilities of SVR in genotypes A – D is shown in figure 1. The agreement between the predicted probabilities and the observed frequency of SVR was good (p=0.27 by the Hosmer–Lemeshow goodness-of-fit test). A multivariable model including the variables age, sex, ALT, HBV DNA, HBV genotype and previous interferon therapy had adequatediscriminative ability as shown by an area under the receiver-operating characteristic curve (AUC) of 0.72 (95%-CI, 0.67 – 0.77). The AUC was 0.75 (95%-CI, 0.65 – 0.85), 0.65 (95%-CI, 0.55 – 0.75), 0.68 (95%-CI, 0.61 – 0.75) and 0.78 (95%-CI, 0.65 – 0.92) for genotypes A to D, respectively. After bootstrap validation, the area under the ROC curve was 0.69 (95%-CI, 0.60 – 0.77). Since the influence of the predictors was significantly different across genotypes, a validated formula for the prediction of SVR was generated for each HBV genotype separately. The PEG-IFN HBV Treatment Index is based on these formulas (figure 2). An automated calculator can be found on www.liver-gi.nl/peg-ifn.

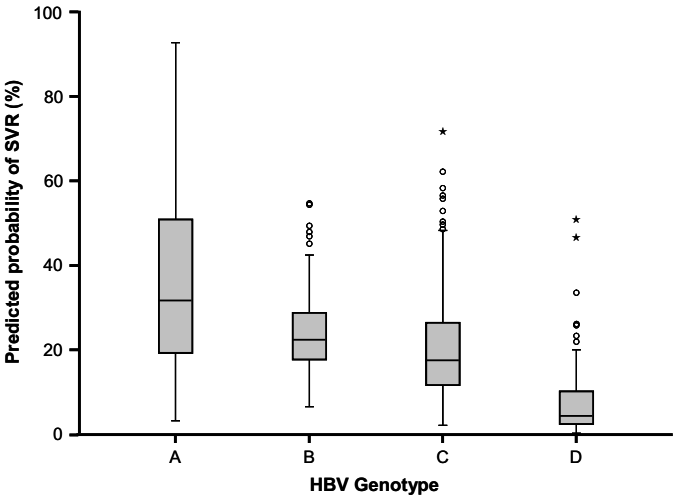
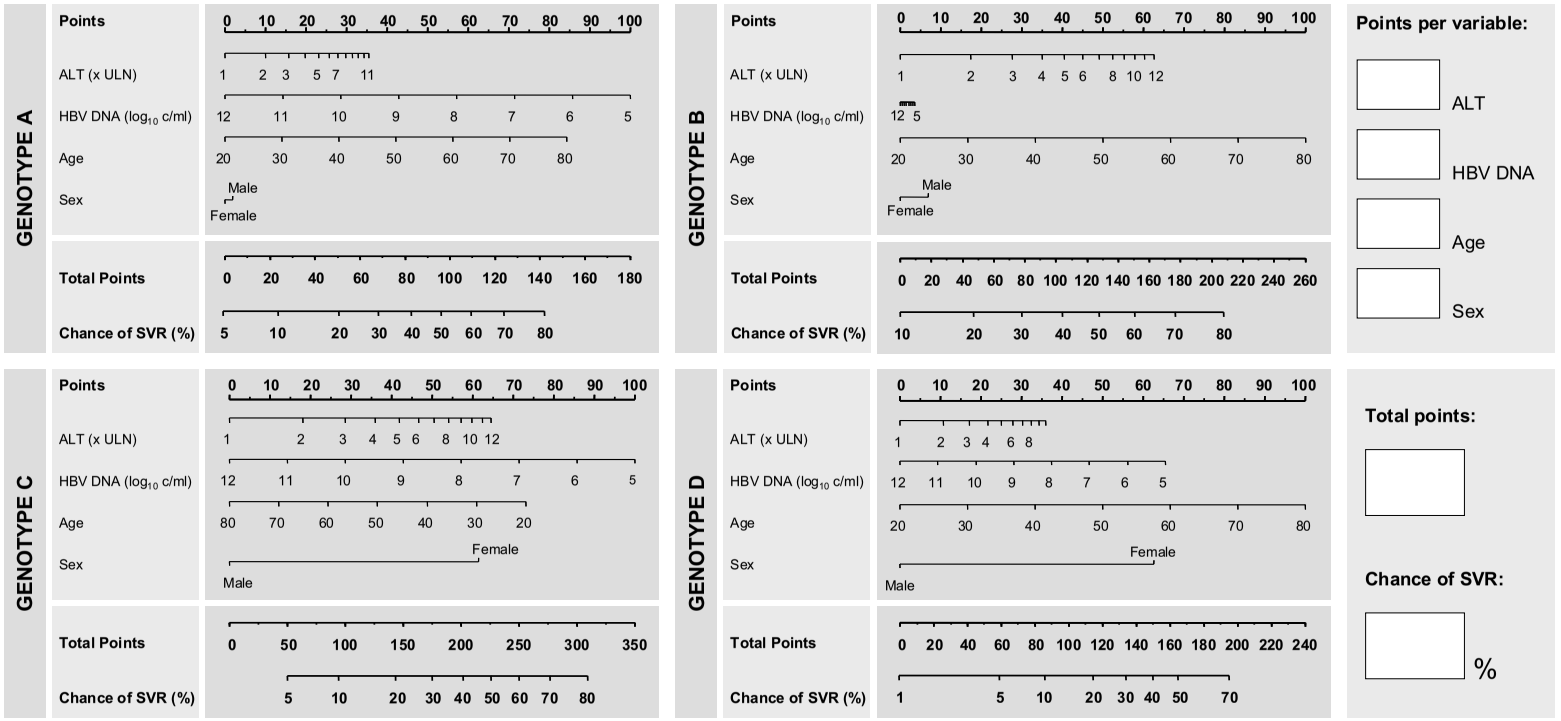


Figure 1: Distribution of predicted probabilities of sustained virological response in patients with HBV genotype A-D.

Boxplots show the distribution of the predicted probabilities of sustained virological response (SVR), defined as HBeAg loss and HBV DNA <10,000 copies/ml at six months post treatment, in patients with genotype A (n=115), B (n=166), C (n=333) or D (n=107).



Logistic regression formulas for HBV genotypes A-D:
A: Logit(p) = 1.48 - 0.05 (sex = female) + 0.04 (age) + 0.43 (ln ALT) -0.43 (log HBV DNA) – 1.46 (previous IFN = yes)
B: Logit(p) = -2.70 - 0.12 (sex = female) + 0.03 (age) + 0.43 (ln ALT) + 0.01 (log HBV DNA) + 0.67 (previous IFN = yes)
C: Logit(p) = 0.72 + 1.03 (sex = female) - 0.02 (age) + 0.43 (ln ALT) - 0.24 (log HBV DNA) + 0.67 (previous IFN = yes)
D: Logit(p) = -2.38 + 1.75 (sex = female) + 0.05 (age) + 0.43 (ln ALT) - 0.26 (log HBV DNA) - 1.46 (previous IFN = yes)

Figure 2: PEG-IFN HBV treatment index

These nomograms can be used to obtain a patient-tailored predicted probability of sustained virological response in IFN-naïve patients with genotype A-D based on ALT, HBV DNA, sex and age. The probability of SVR is calculated by drawing a vertical line to the top Points axis for each of the four variables ALT, HBV DNA, age and sex. The points for each variable are then summed and located on the Total points axis, and a vertical line is projected from the Total points axis to the bottom scale to get the predicted probability of SVR. For example: a genotype C infected female (62 points), 25 years old (67 points), with serum ALT of 2.7 x ULN (25 points) and serum HBV DNA of 9.2 log_{10} copies/ml (40 points) has a total score of 194 points which converts to a probability of SVR of 37%.

Application of the model in clinical practice

In order to allow for application of the model in clinical practice, a nomogram for IFN-naïve patients was generated from the validated formula for each of the HBV genotypes separately (figure 2). These nomograms can be used for calculating the probability of SVR in individual HBeAg-positive patients based on their sex, age, and ALT and HBV DNA level. Average response rates based on the presence of low (<2 x ULN) or high ALT levels (e"2 x ULN), and low (<9log$_{10}$ copies/ml [<2.0 x 10^8 IU/ml]) or high HBV DNA levels (>=9log$_{10}$ copies/ml [>=2.0 x 10^8 IU/ml]) are shown in figure 3.

HBV genotype	**A**			**B**		
ALT	≥2 x ULN	1-2 x ULN	1-2 x ULN	≥2 x ULN	1-2 x ULN	1-2 x ULN
	and	*or*	*and*	*and*	*or*	*and*
HBV DNA (copies/ml)	<9log	≥9log	≥9log	<9log	≥9log	≥9log
Average chance of SVR	57%	31%	23%	30%	28%	21%

HBV genotype	**C**			**D**		
ALT	≥2 x ULN	1-2 x ULN	1-2 x ULN	≥2 x ULN	1-2 x ULN	1-2 x ULN
	and	*or*	*and*	*and*	*or*	*and*
HBV DNA (copies/ml)	<9log	≥9log	≥9log	<9log	≥9log	≥9log
Average chance of SVR	36%	22%	16%	17%	9%	6%

Figure 3: Flowcharts to easily obtain average predicted probabilities of sustained virological response in patients infected with HBV genotype A-D.

These flowcharts show the average predicted probability of SVR depending on HBV genotype, ALT (above or below 2 x ULN) and HBV DNA (above or below 9log$_{10}$ copies/ml). For a precise estimate of the probability of SVR in an individual patient, the nomograms in figure 2 can be used.

DISCUSSION

We combined the data of the two largest studies investigating PEG-IFN in HBeAg-positive chronic hepatitis B in order to develop a model for the prediction of response to PEG-IFN in all HBV genotypes. Although the model is based on the data of patients enrolled in randomized clinical trials with predefined inclusion and exclusion criteria, generalizability of our results is probably good because of the large sample size and wide geographic distribution of the patients. We provided nomograms which can be used to calculate the predicted probability of response in individual patients. A rapid estimate can be obtained from the provided flowcharts.

We recommend to start PEG-IFN therapy in patients with the highest chance of achieving sustained response (table 2). We arbitrarily chose those with a predicted probability of sustained response

Table 2: Recommendations for the use of peginterferon (PEG-IFN) as initial antiviral therapy

HBV genotype	General recommendations for HBeAg positive chronic hepatitis B patients
A	Either high ALT (>=2 x ULN) or low HBV DNA levels (<9log10 copies/ml)
B and C	Both high ALT (>=2 x ULN) and low HBV DNA levels (<9log10 copies/ml)
D	PEG-IFN therapy is not recommended

The recommendation to consider PEG-IFN therapy is based on an average predicted probability of SVR of at least 30%. Predicted SVR rates may be higher or lower in selected subgroups of patients. In patients with a predicted probability of SVR <30%, co-factors such as age and co-morbidity can be taken into account when deciding whether or not to start PEG-IFN therapy.

of at least 30% to be good candidates for PEG-IFN therapy. About 25% of patients included in this study had a predicted probability of sustained response above this level. This includes all HBV genotype A infected patients except for those with low ALT and high HBV DNA levels. In addition, genotype C infected patients with high ALT and low HBV DNA levels have a high likelihood of response to PEG-IFN. All remaining patients are moderate candidates for PEG-IFN except for those with genotype D, who have a rather low chance of achieving sustained response and are in our view generally not candidates for treatment with PEG-IFN. It should be noted that table 2 provides recommendations for patient groups, not individual patients. In selected patients, the given response rate in the table may thus slightly differ from the more accurate predicted probability of response obtained from the nomograms.

With the licensing of pegylated interferon and an additional five nucleos(t)ide analogues for the treatment of chronic hepatitis B in the last years, choice of antiviral therapy has become more important and more complex at the same time. Since both treatment with IFN-based therapy and nucleos(t)ide analogue therapy have proven effective and can improve long-term outcome, the pros and cons of these drugs as well as patient-specific characteristics should be taken into consideration. All of the major practice guidelines have advocated IFN-based therapy as potential first-line therapy for both HBeAg-positive and HBeAg-negative patients,[3, 27, 32, 33] particularly because sustained response and HBsAg loss seem to occur more often with IFN and PEG-IFN than with the direct antiviral agents.[20] In order to reduce the risk of relapse, nucleos(t)ide analogue therapy can be extended for several months after HBeAg seroconversion as this reduces relapse rates.[34, 35] HBeAg seroconversion was sustained in 86% of patients treated with telbivudine or lamivudine who discontinued therapy after at least six months of maintenance therapy.[36, 37] Whether these responses can also be sustained in the long-term is however still unknown.

However, the use of PEG-IFN currently accounts for no more than 10% of all prescriptions for the treatment of chronic hepatitis B.[38] The relatively low usage of PEG-IFN may be explained by the it's significant side effects and need for administration by injection. Furthermore, recommendations on the use of PEG-IFN in specific subsets of patients who are most likely to have a sustained

response and HBsAg seroconversion were lacking. When we are able to identify patients with a high likelihood of response to PEG-IFN, the proportion of patients achieving sustained response after treatment with this drug can probably be increased.

Most studies investigating IFN-based therapy in HBeAg-positive chronic hepatitis B found that high baseline ALT, low baseline HBV DNA and HBV genotype A or B were associated with of response.[10, 25, 26, 39] In addition to these factors, we identified sex and age as predictors of response to PEG-IFN. It should be mentioned that in both studies more men than women were included. We found that the influence of sex, age, HBV DNA and previous IFN therapy was significantly different across HBV genotypes. HBV genotype thus has great influence on the outcome of PEG-IFN therapy. Therefore, contrary to a statement on this topic in the newest guidelines from the European Association for the Study of the Liver (EASL),[27] we believe that determination of HBV genotype is essential in all patients in whom sustained off-treatment response is pursued. Other potential approaches to tailor PEG-IFN therapy in chronic hepatitis B include quantification of serum HBeAg and HBsAg.[40] These approaches are still being validated and not routinely available to most physicians. Because of limited availability in clinical practice, we also chose not to include liver histology.

Previously we presented a model based on 266 HBeAg-positive patients participating in a single randomised trial.[41] However, the vast majority of these patients were infected with HBV genotype A or D, only a small proportion of patients harboured HBV genotype B or C. To gain a good prediction model for all HBeAg positive patients, we now combined the data of the two largest randomised trials investigating PEG-IFN in HBeAg-positive chronic hepatitis B. We showed that a model based on readily available baseline factors can provide an adequate prediction of sustained response. Ideally, a large confirmatory group would have been used for external validation. Such a group is unfortunately not available. Clinical trials that are currently still ongoing may allow for further validation of the model in the near future.

Since substantial viral replication may persist despite HBeAg loss in some patients, a combined endpoint of HBeAg clearance from serum and low HBV DNA is crucial in HBeAg positive chronic hepatitis B. Particularly patients with HBV genotype non-A infection can develop mutations in the precore or core promoter region and may still be at risk for progressive liver disease despite HBeAg loss.[6, 42] Both clearance of HBeAg and suppression of HBV replication are key events in the natural course and during antiviral therapy in HBeAg positive chronic hepatitis B. HBeAg loss after IFN-based therapy was associated with reduced progression to cirrhosis and HCC, and improved survival.[5, 43] In addition, large population studies have established a clear link between HBV viremia and the risk for HBV-related complications.[44, 45] Serum HBV DNA was the strongest predictor of progression to cirrhosis and HCC, with a significantly higher risk for patients with HBV DNA above 10,000 copies/ml (2,000 IU/ml) as compared to those with serum HBV DNA <300 copies/ml (relative risk 2.5 [95%-CI 1.6-3.8] and 2.3 [95%-CI 1.1-4.9] for developing cirrhosis and HCC, respectively). Although the proportion of patients with undetectable HBV DNA was relatively low shortly after PEG-IFN therapy,[10] it further increased with prolonged duration of follow-up.[6]

Because presence of anti-HBe at 6 months post-treatment was not associated with long-term sustainability of response to PEG-IFN,[6] the combined endpoint of HBeAg loss and low HBV DNA seems optimal.

The parties involved in this study agreed not to perform a direct comparison between the two formulations of PEG-IFN. However, the previously reported results of two studies included in this retrospective analysis were very similar.[10, 11] Unfortunately, we cannot provide recommendations for HBeAg-negative patients, since we only had data of HBeAg-positive patients treated with PEG-IFN. Due to the low sustained response rate in HBeAg-negative patients,[46] PEG-IFN is relatively less often given to HBeAg-negative as compared to HBeAg positive patients. Prediction of response to PEG-IFN therefore seems of greater importance in HBeAg-positive than in HBeAg-negative chronic hepatitis B.

In conclusion, we provide a practical tool to calculate the probability of sustained response to PEG-IFN in individual HBeAg-positive patients, which can be easily used in clinical practice and can thus allow for the selection of the optimal candidates for PEG-IFN therapy. We were unfortunately not able to perform external validation because such a database is not available. Clearly, this should be done when an appropriate patient group is available. We recommend to consider PEG-IFN therapy in all genotype A patients with either high ALT or low HBV DNA levels, in genotype B and C infected patients with both high ALT and low HBV DNA. HBeAg-positive genotype D infected patients are generally not good candidates for treatment with PEG-IFN.

ACKNOWLEDGMENT

We thank Dr. Richard Batrla (Roche, Basel, Switzerland), Dr. Philip McCloud (Roche, Dee Why, Australia), Peter Button (Roche, Dee Why, Australia) and Schering-Plough International (Kenilworth, NJ, USA) for supporting this study. In addition, we thank all investigators of the Peginterferon Alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group and the HBV99-01 Study Group.[10, 11]

REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
2. Lok AS, Lai CL, Wu PC, Leung EK, Lam TS. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology 1987;92:1839-43.
3. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
4. 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;351:1521-31.
5. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, Schalm SW, Janssen HL. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology

2004;39:804-10.

6 Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, Feinman SV, Mach T, Akarca US, Schutten M, Tielemans W, van Vuuren AJ, Hansen BE, Janssen HL. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459-467.

7. 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;113:1660-7.

8. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-5.

9. 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;354:1001-10.

10. 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;365:123-9.

11. 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;352:2682-95.

12. 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 B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-16.

13. Carreno V, Castillo I, Molina J, Porres JC, Bartolome J. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. J Hepatol 1992;15:102-6.

14. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1997;26:1338-42.

15. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.

16. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-97.

17. Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. Gastroenterology 1993;105:1833-8.

18. 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;334:1422-7.

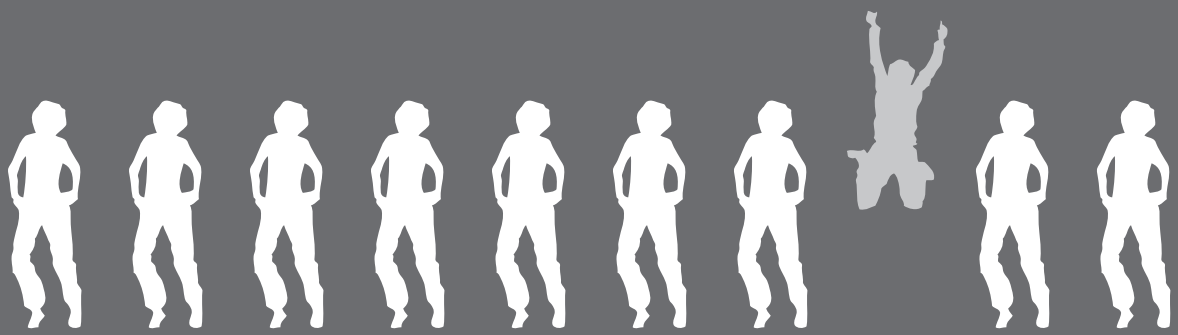
19. van Zonneveld M, Flink HJ, Verhey E, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Hansen BE, Schalm SW, Janssen HL. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. Aliment Pharmacol Ther 2005;21:1163-71.

20. van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, Heathcote J, Song BC, Janssen HL, de Man RA, Schalm SW. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-4.

21. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, Lim SG, Wu PC, Dent JC, Edmundson S, Condreay LD, Chien RN. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527-

32.
22. Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, Ng KY, Nicholls GJ, Dent JC, Leung NW. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol 2004;19:1276-82.
23. Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, Walsh A, Fang J, Hsu M, Mazzucco C, Eggers B, Zhang S, Plym M, Klesczewski K, Tenney DJ. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology 2006;44:1656-65.
24. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Brosgart CL, Borroto-Esoda K, Arterburn S, Chuck SL. Long-term Therapy With Adefovir Dipivoxil for HBeAg-Negative Chronic Hepatitis B for up to 5 Years. Gastroenterology 2006;131:1743-51.
25. Cooksley G, Lau GKK, Liaw YF, Marcellin P, Chow WC, Thongsawat S, Gane E, Fried MW, Zahm FE. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol 2005;42:S30.
26. Bonino F, Lau GKK, Marcellin P, Hadziyannis S, Kitis G, Jin R, Yao GB, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, McCloud P, Brunetto MR, Farci P. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized, partially double-blind study of peginterferon-alfa-2a (4-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone. Hepatology 2004;40:A1142.
27. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol 2009;50:227-42.
28. Pas SD, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-901.
29. Steyerberg EW, Eijkemans MJ, Harrell FE, Jr., Habbema JD. Prognostic modelling with logistic regression analysis: a comparison of selection and estimation methods in small data sets. Stat Med 2000;19:1059-79.
30. Steyerberg EW, Harrell FE, Jr., Borsboom GJ, Eijkemans MJ, Vergouwe Y, Habbema JD. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol 2001;54:774-81.
31. Harrell FE. Regression Modeling Strategies with Applications to Linear Models, Logistic Regression, and Survival Analysis. Springer-Verlag New York, LLC, 2006.
32. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, Mele A, Paumgartner G, Pietrangelo A, Rodes J, Rosenberg W, Valla D. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39:S3-25.
33. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008:DOI 10.1007/s12072-008-9080-3.
34. Chien RN, Yeh CT, Tsai SL, Chu CM, Liaw YF. Determinants for sustained HBeAg response to lamivudine therapy. Hepatology 2003;38:1267-73.
35. Ryu SH, Chung YH, Choi MH, Kim JA, Shin JW, Jang MK, Park NH, Lee HC, Lee YS, Suh DJ. Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospective study. J Hepatol 2003;39:614-9.
36. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, B zowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576-88.

37. Poynard T, Hou JL, Chutaputti A, Manns M, Naoumov N. Sustained durability of HBeAg seroconversion in chronic hepatitis B patients after treatment with telbivudine. J Hepatol 2008;48:S263-S264.
38. Zoulim F, Perrillo R. Hepatitis B: reflections on the current approach to antiviral therapy. J Hepatol 2008;48 Suppl 1:S2-19.
39. Craxi A, Di Bona D, Camma C. Interferon-alpha for HBeAg-positive chronic hepatitis B. J Hepatol 2003;39 Suppl 1:S99-105.
40. Fried MW, Piratvisuth T, Lau GK, Marcellin P, Chow WC, Cooksley G, Luo KX, Paik SW, Liaw YF, Button P, Popescu M. HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. Hepatology 2008;47:428-34.
41. Buster EH, Hansen BE, Zeuzem S, Schalm SW, Steyerberg EW, Janssen HL. Predicting sustained HBeAg loss after treatment with peginterferon alpha-2b: development and validation of a practical model. Hepatology 2007;46:684A-685A.
42. 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 pre-core promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity. J Hepatol 2000;33:430-9.
43. Lin SM, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, Liaw YF. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol 2007;46:45-52.
44. 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.
45. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-86.
46. Marcellin P, Piratvisuth T, Brunetto MR, Bonino F, Lau GK, Farci P, Yurdaydin C, Wu J, Popescu M. Virological and biochemical response in patients with HBeAg-negative chronic hepatitis B treated with peginterferon alfa-2a (40kD) with or without lamivudine: results of 4-year follow-up. J Hepatol 2008;48:S46.



Peginterferon alpha-2b is safe and effective in HBeAg positive chronic hepatitis B patients with advanced fibrosis.

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Hepatology 2007; 46(2): 388-394.

3

ABSTRACT

Background

Chronic hepatitis B (CHB) patients with advanced fibrosis are often not considered for treatment with peginterferon (PEG-IFN) since IFN therapy may precipitate immunological flares, potentially inducing hepatic decompensation.

Methods

We investigated in HBeAg-positive CHB efficacy and safety of 52 weeks of PEG-IFN alpha-2b (100µg weekly) alone or in combination with lamivudine (100mg daily) in 70 patients with advanced fibrosis (Ishak fibrosis score 4-6) and 169 patients without advanced fibrosis, all with compensated liver disease.

Results

Virologic response, defined as HBeAg-seroconversion and HBV DNA <10,000 copies/ml at week 78, occurred significantly more often in patients with advanced fibrosis compared to those without (25% vs. 12%, respectively; p=0.02). Also patients with cirrhosis (n=24) exhibited a virologic response more frequently than non-cirrhotics (30% vs. 14%, respectively; p=0.02). Improvement of liver fibrosis occurred more frequently in patients with advanced fibrosis (66% vs. 26%, p<0.001). HBV genotype A was more prevalent among patients with advanced fibrosis than those without (57% vs. 24%, p<0.001). Most adverse events, including serious adverse events, were observed equally in patients with and without advanced fibrosis. Fatigue, anorexia and thrombocytopenia occurred more often in patients with advanced fibrosis than those without (p<0.01). Necessity for dose reduction or discontinuation of therapy was comparable for both patient groups (p=0.92 and p=0.47, respectively).

Conclusion

PEG-IFN is effective and safe in HBeAg-positive patients with advanced fibrosis. Since PEG-IFN therapy results in high rates of sustained off-therapy response, patients with advanced fibrosis or cirrhosis but compensated liver disease should not be excluded from PEG-IFN treatment.

INTRODUCTION

Hepatitis B virus (HBV) is one of the most prevalent viral pathogens of man with almost a third of the world's population having evidence of infection with HBV and about 400 million people being chronically infected.[1] Chronic HBV is a leading cause of cirrhosis and hepatocellular carcinoma (HCC). The most important objective in treatment of chronic HBV is to halt progression of liver injury by persistent suppression of the virus. Complete response, with loss of hepatitis B surface antigen (HBsAg) and appearance of anti-HBs, is difficult to achieve with currently available antiviral drugs and most likely does not imply complete eradication but rather control of residual virus by a persistent host's immune response.[2] Therefore, surrogate markers of short-term treatment response, such as loss of HBeAg and decrease of HBV DNA, are most often used to evaluate the effectiveness of antiviral therapy.

Treatment with interferon (IFN) results in loss of HBeAg in about one-third of patients.[3] Somewhat higher response rates have been reported for peginterferon (PEG-IFN) therapy in hepatitis B e antigen (HBeAg) positive chronic HBV.[4-6] HBeAg loss after IFN-based therapy is durable in the majority of patients,[7] and has been shown to result in decreased progression of fibrosis, reduced risk of developing hepatocellular carcinoma and improved survival .[8,9] Persistence of HBeAg on the other hand results in unfavorable long-term outcome.[10,11] IFN induces immune-mediated hepatitis flares in 20-40% of patients, which may precipitate hepatic decompensation in patients with advanced liver disease.[12]

Studies on treatment of chronic HBV with conventional IFN or PEG-IFN have identified similar predictors of response. These factors included high pretreatment alanine aminotransferase (ALT), low pretreatment HBV DNA, high degree of necroinflammatory activity, infection at adult age and HBV genotype A or B.[4,5,13-16] PEG-IFN is often not given to HBV infected patients with advanced fibrosis or cirrhosis due to the presumed lack of efficacy and fear for hepatic flares and other toxicity.

In this study we investigated response to and safety of PEG-IFN alpha-2b with or without lamivudine in patients with HBeAg-positive chronic hepatitis B and advanced fibrosis.

PATIENTS AND METHODS

We investigated the efficacy and safety of 52 weeks of PEG-IFN alpha-2b alone or in combination with lamivudine in patients with HBeAg-positive chronic HBV and advanced fibrosis. The patients and results of the multicenter randomized controlled study investigating PEG-IFN alpha-2b monotherapy and PEG-IFN alpha-2b in combination with lamivudine in HBeAg-positive chronic HBV have been described previously (5). All patients were positive for hepatitis B s antigen (HBsAg) for at least 6 months prior to randomization. Patients were eligible if they were HBeAg positive on two occasions within 8 weeks prior to randomization, had elevated serum ALT of >2 - 10 x the upper limit of normal (ULN), and had serum HBV DNA above 1.0×10^5 copies per milliliter (copies/

ml). Major exclusion criteria were: antiviral therapy within 6 months prior to randomization, serum antibodies against hepatitis C virus, hepatitis D virus or human immunodeficiency virus, pre-existent leucopenia (white blood cell count <3,000/mm3, neutrophil count <1,800/mm3) or thrombocytopenia (platelets <100,000/mm3), or decompensated liver disease. Patients were randomized in a 1:1 ratio to receive PEG-IFN alpha-2b (100µg weekly) with placebo or with lamivudine (100mg daily) for 52 weeks. After 32 weeks, PEG-IFN alpha-2b dosage was lowered to 50µg per week to prevent side effects and early treatment discontinuation. Follow-up after discontinuation of therapy lasted 26 weeks. The primary outcome measure was loss of HBeAg at week 78.

During therapy and post treatment follow-up, patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally in accordance with standardized procedures and therefore expressed as times upper limits of normal (ULN). Hepatitis flares were defined as a threefold increase in serum ALT compared to baseline levels.[17] Serum HBV DNA levels were measured monthly using an in-house developed Taqman PCR assay (lower limit of detection 373 copies/ml) based on the Eurohep standard.[18] HBeAg and HBsAg (AxSYM, Abbott, Abbott Park, IL, USA) were assessed at week 0, 32, 52 and week 78 (end of follow-up). HBV genotype and YMDD mutation analysis were performed by INNO-LiPA assay (Innogenetics, Gent, Belgium). Since both HBeAg-positivity and HBV DNA above 10,000 copies/ml have been described to be detrimental,[8,19] particularly in patients with advanced fibrosis, we defined virologic response in this study as HBeAg seroconversion (loss of HBeAg and appearance of anti-HBe) in combination with serum HBV DNA <10,000 copies/ml. A baseline biopsy sample was available for 239 patients, biopsy at the end of treatment was optional. Paired biopsies were taken in 110 patients. Histological scoring was performed according to the modified histological activity index (HAI) by one experienced pathologist,[20] who was unaware of the chronological order of biopsies, treatment allocation and outcome measures. Advanced fibrosis was defined as fibrosis score 4 - 6 (HAI). Improvement of liver histology was defined as a reduction of at least two points in necroinflammatory score (range 0-18) or one point in fibrosis score (range 0-6).

Statistical analysis was performed using the SPSS 14.0 program (SPSS Inc. Chicago, IL). Chi-square, Fisher's exact test and Mann-Whitney U test were used where appropriate. The Kaplan-Meier method was used to compare incidence of dose reduction and discontinuation of therapy. The relation between characteristics at baseline and during therapy, and relapse and post treatment response was examined by logistic regression analyses. Univariate analysis was used to assess the importance of prognostic factors. To investigate the independence of these factors, multivariate logistic regression analyses was performed with all characteristics with a p-value <0.2 in univariate analysis. A p-value <=0.05 was considered statistically significant.

RESULTS

Of 239 patients included in this study, 70 had advanced fibrosis (29%) and 24 had cirrhosis (10%).

Table 1: Baseline characteristics

Characteristic	Advanced fibrosis* (n = 70)	No advanced fibrosis† (n = 169)	p
Age	41.3 ± 13.9	31.8 ± 10.5	<0.001
Male sex	60 (86%)	126 (75%)	0.06
Weight	75.4 ± 13.2	71.9 ± 14.5	0.09
Race			0.72
- Caucasian	54 (77%)	121 (72%)	
- Asian	13 (19%)	35 (21%)	
- Other	3 (4%)	13 (7.7%)	
Geographic region			0.49
- North-Western Europe	28 (40%)	60 (36%)	
- Eastern Europe	11 (16%)	16 (10%)	
- Mediterranean	19 (27%)	62 (37%)	
- East Asia	8 (11%)	19 (11%)	
- North-American	4 (6%)	12 (7%)	
Route of transmission			0.003
- Vertical	20%	24%	
- Sexual or parenteral	39%	18%	
- Unknown	41%	58%	
Serum ALT	4.2 ± 2.4	4.5 ± 3.9	0.48
HBV DNA (log)	9.1 ± 0.9	9.1 ± 1.0	0.90
Platelet count (x 10^9/l)	175 ± 43	215 ± 60	<0.001
HBV genotype			<0.001
- A	38 (57%)	39 (24%)	
- B	4 (6%)	17 (10%)	
- C	10 (15%)	26 (16%)	
- D	15 (22%)	81 (50%)	
Necroinflammation	6.6 ± 2.0	4.9 ± 2.1	<0.001
Previous interferon	19%	20%	0.78
Previous lamivudine	7%	15%	0.11

Mean ± SD is given for continuous variables, *Ishak fibrosis score 4-6, †Ishak fibrosis score 1-3, ULN = upper limit of normal

Baseline demographics are shown in table 1. Five of 27 patients without a baseline liver biopsy sample had thrombocytopenia (platelet count range: 133-144 x 10^9/l), which could indicate presence of cirrhosis. None of the 27 patients had signs of decompensated liver disease; bilirubin was slightly elevated in 2 patients (17.1 and 19.5 µmol/l). Patients with advanced fibrosis were older than those without and more often acquired their infection via sexual or parenteral transmission. Baseline platelet count was significantly lower and necroinflammatory score higher in patients with advanced fibrosis. Genotype A (32%) and D (40%) were the most prevalent genotypes in our mainly Caucasian patient population. Distribution of genotypes was significantly different between the patient groups. Patients with genotype A more often had advanced fibrosis (49%) than patients with genotype B (19%), C (28%) or D (17%) (p=0.01, p=0.03 and p<0.001, respectively).

Response to treatment

At the end of follow-up, all treatment outcomes were observed equally in patients treated with PEG-IFN alpha-2b alone or combination therapy with lamivudine, both in patients with and without advanced fibrosis. Different response rates in patients with and without advanced fibrosis are given in table 2. Virologic response at week 78, defined as HBeAg-seroconversion and HBV DNA <10,000 copies/ml, occurred significantly more often in patients with advanced fibrosis compared to those without (p=0.02), as well as a decrease in fibrosis score (p<0.001). Although not statistically significant, the rate of HBsAg-seroconversion was twice as high in patients with advanced fibrosis compared to those without. Similar trends were observed for improvement of necroinflammation and HBV DNA <400 copies/ml. Response rates were comparable between patients who had no liver biopsy taken at baseline and those with available baseline liver histology (18% vs. 16% for virologic response at week 78; p=0.76).

Response rates in the subgroup of patients with cirrhosis were similar to those in the total group of patients with advanced fibrosis. Thirty-five percent of patients with cirrhosis showed virologic response at week 78 compared to 14% in patients without cirrhosis (p=0.02). For HBsAg-seroconversion, these rates were 13% and 5%, respectively (p=0.13).

HBV genotype was found to influence outcome at the end of follow-up. Rates of virologic response were higher in patients with genotype A (30%) and B (26%) than genotype C (3%) or D (6%) infection (A vs. C, p=0.002; A vs. D, p<0.001; B vs. C, p=0.02; B vs. D, p=0.02). Among patients with advanced fibrosis a similar trend was observed for genotype A and D infection (33% vs. 8%, p=0.14). Among patients with genotype A infection, virologic response occurred equally in those with or without advanced fibrosis (33% vs. 27%; p=0.54).

Logistic regression analysis of factors predicting response at week 78

For the prediction of virologic response at the end of follow-up, the following variables were included in univariate logistic regression analysis: age, sex, weight, race, route of transmission, baseline serum ALT, baseline HBV DNA level, HBV genotype, liver histology, previous interferon or lamivudine therapy, and treatment allocation. Increasing age, sexual or parenteral transmission,

Table 2: Response to PEG-IFNalpha-2b with or without lamivudine at week 78

	Advanced fibrosis (n = 70)	No advanced fibrosis (n = 169)	p
HBeAg seroconversion	36%	29%	0.29
HBsAg seroconversion	9%	4%	0.21
HBV DNA <10,000 c/ml	30%	17%	0.04
HBV DNA <400 c/ml	13%	7%	0.19
Mean decline HBV DNA	$2.58\log_{10}$	$2.27\log_{10}$	0.45
Virologic response¥	25%	12%	0.02
Normalization of ALT	44%	50%	0.45
Improvement fibrosis‡	66%	26%	<0.001
Improvement necroinflammation‡	53%	51%	0.84

*Second biopsy taken at week 52 (n=47 for patients with advanced fibrosis; n=90 for patients without advanced fibrosis), ¥HBeAg seroconversion and HBV DNA <10,000 copies/ml, ‡1 point for fibrosis, 2 points for necroinflammation.

presence of advanced fibrosis or cirrhosis, and high HAI scores were found to be associated with increased likelihood of virologic response (table 3). In multivariate analysis, HBV genotype and high necroinflammatory score (OR 1.31, CI95% 1.05-1.65, p=0.02) independently predicted virologic response at week 78. HBV genotype was found to be the strongest predictor of virologic response. Genotype A infection was associated with higher response rates compared to genotype C (OR 11.30, CI95% 1.38-92.57, p=0.02) or D (OR 4.28, CI95% 1.39-13.21, p=0.01). Genotype B infection also resulted in a higher likelihood of response compared to genotype C (OR 12.13, CI95% 1.24-118.30, p=0.03) or D (OR 4.59, CI95% 1.14-18.43, p=0.03). In multivariate analysis, presence of advanced fibrosis did not alter the likelihood of achieving virologic response (OR 0.98, CI95% 0.17-5.23, p=0.98).

Safety

Most adverse events occurred equally in patients with or without advanced fibrosis (table 4). Fatigue, anorexia and thrombocytopenia were observed more frequently in patients with advanced fibrosis than in those without (p<0.01). These adverse events also occurred more frequently in the subgroup of patients with cirrhosis compared to those without, as well as hepatitis flares (33% vs. 12%, p=0.008) and dizziness (21% vs. 7%, p=0.05). The higher rate of thrombocytopenia in patients with advanced fibrosis did not result in an increase of significant bleeding problems. Nevertheless, epistaxis tended to occur more often in patients with advanced fibrosis (p=0.08). Necessity for PEG-IFN dose reduction was comparable for patients with (33%) and without advanced fibrosis (34%), as well as premature discontinuation of therapy (11% and 8%, respectively) (figure

Table 3: Univariate analysis for the prediction of virologic response at week 78

Variable	Odds Ratio	95%-CI Lower	95%-CI Upper	p value
Age	1.05	1.02	1.08	0.003
Sex - male	0.86	0.36	2.06	0.74
Weight	1.01	0.99	1.04	0.40
Race				
- Caucasian	1.00			
- Asian	0.67	0.24	1.87	0.44
- Other	1.89	0.56	6.45	0.31
Route of transmission				
- Vertical	1.00			
- Sexual or parenteral	13.36	2.91	61.48	0.001
- Other	3.39	0.74	15.62	0.12
Advanced fibrosis	2.43	1.13	5.21	0.02
Cirrhosis	3.38	1.23	9.25	0.02
Baseline serum ALT	1.04	0.93	1.17	0.48
Baseline viral load	0.84	0.57	1.23	0.37
HBV genotype				
- A	1.00			
- B	0.84	0.27	2.64	0.77
- C	0.07	0.01	0.58	0.01
- D	0.15	0.05	0.43	<0.001
Baseline HAI score				
- Necroinflammation	1.37	1.11	1.67	0.003
- Fibrosis	1.40	1.08	1.82	0.01
Previous interferon	0.58	0.19	1.75	0.33
Previous lamivudine	1.01	0.32	3.16	0.98
Treatment allocation				
- PEG-IFN monotherapy	1.00			
- Combination therapy	1.36	0.64	2.88	0.43

1). The reason for dose reduction of PEG-IFN in patients with advanced fibrosis was more often thrombocytopenia than in patients without advanced fibrosis (32% vs. 5%, p=0.01). For both pa-

tients with and without advanced fibrosis, the majority of dose reductions took place during the first 16 weeks of therapy (79% and 66%, p=0.25). Serious adverse events occurred equally in patients with or without advanced fibrosis (4% and 5%, respectively, p=1.00). In patients with cirrhosis, dose reduction was necessary more often than in patients without cirrhosis (63% vs. 30%, p=0.002), as well as premature discontinuation of therapy (21% vs. 8%, p=0.05). Timing of dose adjustments or discontinuation of therapy did not differ between the patient groups.

Table 4: Safety of PEG-IFN alpha-2b alone or in combination with lamivudine in patients with or without advanced fibrosis.

	Advanced fibrosis (n = 70)	No advanced fibrosis (n = 169)	p
Flu like syndrome	46 (66%)	114 (68%)	0.80
Fatigue	39 (56%)	57 (34%)	0.002
Leucopenia	33 (47%)	70 (41%)	0.42
Local reaction injection site	24 (34%)	42 (25%)	0.14
Myalgia	22 (31%)	55 (33%)	0.87
Neutropenia	18 (26%)	42 (25%)	0.89
Anorexia	18 (26%)	21 (12%)	0.01
Thrombocytopenia	18 (26%)	11 (7%)	<0.001
Hepatitis flare	14 (20%)	19 (11%)	0.07
Arthralgia	13 (19%)	26 (15%)	0.54
Insomnia	9 (13%)	19 (11%)	0.72
Irritability	7 (10%)	19 (11%)	0.78
Depression	6 (9%)	17 (10%)	0.72
Epistaxis	3 (4%)	1 (1%)	0.08
Ophthalmologic problems	1 (1%)	2 (1%)	0.88
Serious adverse event	3 (4%)	9 (5%)	1.00
Dose reduction	24 (34%)	55 (33%)	0.80

DISCUSSION

In this study, using PEG-IFN therapy, virologic response, as defined by HBeAg seroconversion and HBV DNA 10,000 copies/ml at the end of follow-up, occurred significantly more often in patients with advanced fibrosis than in those without. The rate of HBsAg-seroconversion was twice as high in patients with advanced fibrosis, although this difference was not statistically significant.

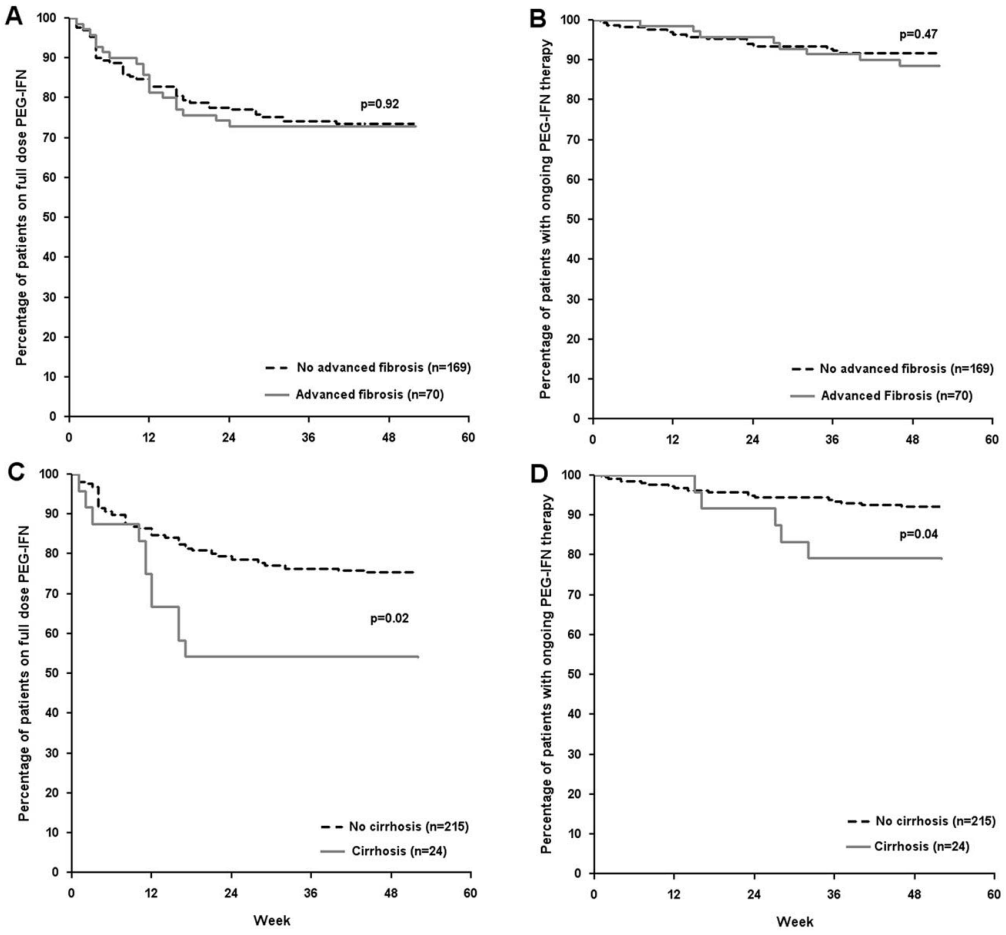


Figure 1: Dose reduction and premature discontinuation of PEG-IFN ?-2b therapy in patients with or without advanced fibrosis.

Frequency and timing of dose reduction (A) and premature discontinuation (B) of PEG-IFN alpha-2b therapy was comparable for patients with or without advanced fibrosis. However, dose reduction (C) and premature discontinuation of therapy (D) were observed more frequently in the subgroup of patients with cirrhosis than in those without.

Because IFN-based therapy is often presumed to be less effective in patients with advanced fibrosis, PEG-IFN is frequently withheld from these patients. In a retrospective study of standard IFN in 200 HBeAg-positive patients, response rates were reported to be significantly lower in patients with advanced fibrosis (Metavir fibrosis score 3-4; 17.5%) compared to patients with minimal or no fibrosis (Metavir 0-1; 35.5%, p=0.02).[21] Lower stage of liver fibrosis and higher grade of necroinflammation were identified as independent predictors of response to standard IFN in this study.[21] Our results clearly contradict these findings. The higher response rates in patients with advanced fibrosis in our study might be due to the antiviral drug used (PEG-IFN vs. standard IFN),

the longer duration of therapy (1 year vs. 4 months) or the predominant HBV genotypes.

Multivariate analysis showed no relation between presence of advanced fibrosis and response to PEG-IFN alpha-2b. HBV genotype was identified as strongest independent predictor of virologic response at the end of follow-up. Previous studies also have shown a higher likelihood of response to (PEG-)IFN therapy for patients harboring genotype A .[5,14,22] The higher response rates in patients with advanced fibrosis compared to those without appears thus also related to the higher prevalence of genotype A in these patients. Since multivariate analysis showed no influence of advanced fibrosis on response to PEG-IFN therapy, other factors appear to be of greater influence on response rates in these patients. Nevertheless, our finding that the presence of advanced fibrosis does in fact not compromise efficacy of PEG-IFN is important. Furthermore, our findings emphasize the importance of testing for HBV genotype prior to the initiation of antiviral therapy.

Nucleos(t)ide analogues are often considered preferential over PEG-IFN in patients with advanced fibrosis since prolonged treatment with these drugs may reduce progression to decompensated liver disease and development of hepatocellular carcinoma in patients with advanced fibrosis.[23] However, improved survival and decreased incidence of HCC have also been observed in patients with or without preexisting cirrhosis who responded to IFN therapy.[8,9,24]

Concerns are often raised about the safety of PEG-IFN in patients with advanced fibrosis. We found PEG-IFN alpha-2b to be safe in these patients; most adverse events were observed equally among patients with and without advanced fibrosis. As expected with lower baseline platelet levels, thrombocytopenia occurred more frequently in patients with advanced fibrosis. Thrombocytopenia also more often led to dose reduction in these patients, while the frequency and timing of both dose reduction and premature discontinuation of therapy was comparable between patient groups. In the subgroup of patients with cirrhosis, dose adjustment and premature treatment discontinuation were more often necessary, but despite less drug exposure lower response rates were not observed. Only one patient with advanced fibrosis presented with temporarily elevated bilirubin but no other signs of compromised liver function were encountered.

Another reason why PEG-IFN is often not given to patients with advanced fibrosis has been the concern of the potential precipitation of hepatitis flares and subsequent hepatic decompensation. We found a trend towards higher incidence of hepatitis flares in patients with advanced fibrosis and flares occurred more frequently in patients with cirrhosis than those without. However, the flares in our patients, who had well compensated liver disease at the start of therapy, did not lead to any clinical problems. Previous studies on IFN confirm the absence of complications in patients with compensated cirrhosis. Less than 1% of HBeAg-positive patients developed hepatic decompensation, while up to 60% of patients included in these trials had cirrhosis.[25,26] In contrast, IFN therapy leads to significant side effects due to bacterial infection and exacerbation of liver disease in patients with Child's class B or C cirrhosis.[27,28] In a large European study, 9 cases of fatal hepatic decompensation were identified among 2490 IFN treated patients. All of these patients had documented cirrhosis and 5 of them had signs of hepatic decompensation prior to the start of IFN therapy.[29] These observations clearly show that IFN-based therapy should not be given to hepatitis

B patients with signs of hepatic decompensation. The setting of a clinical trial and exclusion of patients with signs of advanced liver disease or portal hypertension could have led to our finding that serious adverse events were observed equally in patients with and without cirrhosis. In our experience, routine monthly follow-up and anticipation for the occurrence of adverse events is sufficient in patients with advanced fibrosis and compensated liver disease.

In conclusion, our findings show that PEG-IFN alpha-2b is at least as effective in patients with advanced fibrosis as in those without. Although patients with cirrhosis more often require dose reduction or early discontinuation of therapy, treatment is safe and outcome is similar to patients without cirrhosis. Therefore, when aiming for sustained off-treatment response, patients with advanced fibrosis and well compensated liver disease, in particular those with genotype A and B, should be offered the possibility of a finite course of PEG-IFN, rather than initiation of possibly indefinite nucleos(t)ide analogue therapy.

ACKNOWLEDGEMENTS

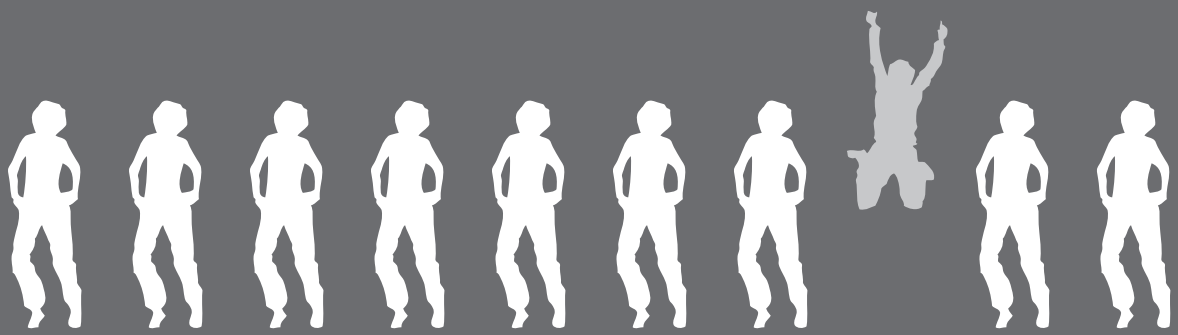
Other members of the HBV 99-01 Study Group include: Netherlands - BCM Vroom (University Medical Center, Utrecht); CMJ van Nieuwkerk (VU University Medical Center, Amsterdam); J Jansen, J Drenth, SJ van den Hazel (University Medical Centre Radboud, Nijmegen); JW den Ouden-Muller (St Franciscus Hospital, Rotterdam); AC Tan (Canisius Wilhelmina Hospital, Nijmegen); KJ van Erpecum (University Medical Center Utrecht, Utrecht); RA de Vries (Rijnstate Hospital, Arnhem). Belgium - F Nevens (University Hospital Gasthuisberg, Leuven); H van Vlierberghe (University Hospital, Gent); J Henrion (Hôpital de Jolimont, Haine-St-Paul). Germany - S Bein, U Treichel (University Hospital, Essen); MP Manns, J Hadem (Medizinische Hochschule, Hannover). Denmark - MR Buhl, IM Hansen (Skejby Hospital, Arhus); K Krogsgaard (Copenhagen University Hospital, Hvidovre). Poland - J Cianciara, J Jablonska, J Kozlowska (Medical Academy of Warsaw, Warsaw); D Prokopowicz (Medical Academy of Bialystok, Bialystok). Spain - A Valdes, R Esteban (Hospital Valle Hebron, Barcelona); M Rodriguez, M Garcia Espiga (Hospital Central de Asturias, Oviedo). Italy - A Andriulli, G Stornaiulo, GB Gaeta (Ospedale Gesù e Maria, Napoli); G Montalto, F D'Antona (Università di Palermo, Palermo). Greece - G Kitis, P Xiarchos Panagiotis (George Papanikolaou General Regional Hospital,Thessaloniki); NC Tassopoulos (West Attica Hospital, Athens). Turkey - G Ersöz (Ege University Faculty of Medicine, Izmir); C Yurdayin, H Bozkaya (Medical School Cebeci Kampusu, Ankara); Y Balaban (Hacettepe University Faculty of Medicine, Ankara), F Tabak (Istanbul University Cerrahpasa Medical School, Istanbul). Israel-Y Lurie (Sauraski Medical Center, Tel-Aviv). Canada - S Greenbloom (General Hospital, Etobicoke). Singapore - R Guan (Mount Elizabeth Medical Center Singapore). Malaysia - I Merican (Hospital Selayang, Institute for Medical Research, Kuala Lumpur).

REFERENCES

1. Lai CL, Ratziu V, Yuen MF, et al. Viral hepatitis B. Lancet 2003;362:2089-2094.
2. Ferrari C, Missale G, Boni C, et al. Immunopathogenesis of hepatitis B. J Hepatol 2003;39 Suppl 1:S36-42.
3. Wong DK, Cheung AM, O'Rourke K, et al. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312-323.
4. Cooksley WG, Piratvisuth T, Lee SD, et al. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.

5. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.
6. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.
7. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and
8. alanine aminotransferase. Gut 2003;52:420-424.
van Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-interferon treatment of
9. patients with chronic hepatitis B. Hepatology 2004;39:804-810.
Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with
10. interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422-1427.
de Jongh FE, Janssen HL, de Man RA, et al. Survival and prognostic indicators in hepatitis B surface
11. antigen-positive cirrhosis of the liver. Gastroenterology 1992;103:1630-1635.
Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in
12. patients with chronic hepatitis B. Hepatology 2002;35:1522-1527.
Peters M, Davis GL, Dooley JS, et al. The interferon system in acute and chronic viral hepatitis. Prog
13. Liver Dis 1986;8:453-467.
de Franchis R, Hadengue A, Lau G, et al. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39
14. Suppl 1:S3-25.
Erhardt A, Blondin D, Hauck K, et al. Response to interferon alfa is hepatitis B virus genotype depen-
15. dent: genotype A is more sensitive to interferon than genotype D. Gut 2005;54:1009-1013.
Wai CT, Chu CJ, Hussain M, et al. HBV genotype B is associated with better response to interferon
16. therapy in HBeAg(+) chronic hepatitis than genotype C. Hepatology 2002;36:1425-1430.
Bonino F, Lau GKK, Marcellin P, et al. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized, partially double-blind study of peginterferon-alfa-2a (4-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone.
17. Hepatology 2004;40:A1142.
Flink HJ, Sprengers D, Hansen BE, et al. Flares in chronic hepatitis B patients induced by the host or the virus? Relation to treatment response during Peg-interferon {alpha}-2b therapy. Gut 2005;54:1604-
18. 1609.
Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepa-
19. titis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-2901.
Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral
20. load. Gastroenterology 2006;130:678-686.
Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol
21. 1995;22:696-699.
22. Shindo M, Hamada K, Nishioji K, et al. The predictive value of liver fibrosis in determining the effectiveness of interferon and lamivudine therapies for chronic hepatitis B. J Gastroenterol 2004;39:260-267.
Flink HJ, van Zonneveld M, Hansen BE, et al. Treatment with peg-interferon alpha-2b for HBeAg-positive
23. chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol 2006;101:297-303.
24. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-1531.
25. Lin SM, Yu ML, Lee CM, et al. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol 2007;46:45-52.
26. Lok AS, Wu PC, Lai CL, et al. A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. Gastroenterology 1992;102:2091-2097.

27. Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. N Engl J Med 1990;323:295-301.
28. Hoofnagle JH, Di Bisceglie AM, Waggoner JG, et al. Interferon alfa for patients with clinically apparent cirrhosis due to chronic hepatitis B. Gastroenterology 1993;104:1116-1121.
29. Perrillo R, Tamburro C, Regenstein F, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. Gastroenterology 1995;109:908-916.
Janssen HL, Brouwer JT, Nevens F, et al. Fatal hepatic decompensation associated with interferon alfa. European concerted action on viral hepatitis (Eurohep). BMJ 1993;306:107-108.



Low incidence of retinopathy during peginterferon alpha-2b and lamivudine therapy for chronic hepatitis B.

Erik H.C.J. Buster, Martijn J. ter Borg, Johannes R. Vingerling, Harry L.A. Janssen.

Journal of Hepatology 2006; 45(1): 160-161.

4

INTRODUCTION

With interest we read the paper by d'Alteroche et al. about ophthalmologic side-effects during interferon (IFN) therapy for viral hepatitis.[1] In this study retinopathy was found on fundoscopic examination in 24% of patients after IFN therapy, none of whom experienced ophthalmologic symptoms. Factors associated with an increased risk of developing retinopathy included a history of arterial hypertension, age above 45 years and treatment with pegylated alpha-interferon. Based on their findings, d'Alteroche et al. recommended regular fundoscopic examination for all IFN treated patients, particularly during the first months of treatment. We here present our experience on retinopathy in chronic hepatitis B infected patients treated with peginterferon-alpha-2b (PEG-IFN).

PATIENTS AND METHODS

As part of a global randomized controlled trial we performed routine ophthalmological examination in PEG-IFN treated patients of our own center.[2] Twenty-eight HBeAg-positive chronic hepatitis B infected patients were included in this study and randomized to PEG-IFN alone in a dosage of 100µg per week or its combination with lamivudine (table 1). Before and during treatment, corrected visual acuity testing and routine examination of the retina by indirect ophthalmoscopy and slit-lamp biomicroscopy was performed.

RESULTS

None of the patients had ophthalmological symptoms or abnormalities on pre-treatment analysis. Ophthalmological examination during treatment was performed after a median treatment period of 14 weeks (range 5-33 weeks). During treatment, 3 patients complained of blurred vision, without clear underlying ophthalmological etiology. Overall, visual acuity did not decrease during PEG-IFN treatment compared to baseline (mean visual acuity 0.05 and 0.02 logMAR - logarithm of the minimum angle of resolution, respectively, p=0.18). One of three patients with complaints of blurred vision showed retinal hemorrhage on fundoscopic examination, which resolved spontaneously within 5 weeks on continued therapy. No abnormalities were observed on fundoscopic examination in any of the other patients during treatment. In all three patients with ophthalmologic symptoms, spontaneous recovery was observed during therapy without a need for dose adjustment.

DISCUSSION

IFN associated abnormalities that can be found on fundoscopic examination include retinal hemorrhage, cotton wool spots, micro aneurysms, optic disc hyperemia and macular edema.[3-5] The reported incidence of retinopathy varies between studies, rates between 18% and 86% have been reported.[4,5] The observed incidence of retinopathy in our study was significantly lower compared

Table 1: Clinical characterstics of peginterferon-alfa (and lamivudine) treated chronic hepatitis B patients.

Clinical characteristics at baseline (n=28)		
Age		
Mean age	34 ± 4.3 years (range 16-80 years)	
18-44 years	24/28 (83%)	
45 years or above	4/28 (17%)	
Sex		
Male	20/28 (71%)	
Biochemistry and virology		
Mean ALT	150 ± 33.5 U/l (range 47-394 U/l)	
Median HBV DNA	1.6 x 10^9 copies/ml (range 2.0 x 10^7 - 1.4 x 10^{10})	
Risk factors for retinopathy		
Arterial hypertension	0/28 (0%)	
Diabetes mellitus	0/28 (0%)	
Antiviral treatment		
PEG-IFN + lamivudine	14/28 (50%)	
PEG-IFN + placebo	14/28 (50%)	
Ophthalmologic examination	*Baseline*	*During treatment*
Blurred vision	0/28 (0%)	3/28 (11%)
Mean corrected visual acuity	0.05 logMAR	0.02 logMAR*
Retinopathy	0/28 (0%)	1/28 (4%)

*p=0.18 (compared to baseline by paired t test)

to that observed by D'Alteroche et al. (4% vs. 24%, p=0.01 by Chi-Square test), and may be explained by multiple factors. First, we performed fundoscopic examination relative early during treatment (median 14 weeks). However, in other studies retinopathy was particularly observed within the first three months of treatment.[1,4,5] Second, in our study mean age was profoundly lower (34 ± 4.3 years), with only 4 patients (17%) aged 45 or above, which was found to be associated with an increased risk of retinopathy by d'Alteroche et al. Diabetes mellitus and arterial hypertension are other risk factors for developing retinopathy. In contrast to the study by d'Alteroche et al. none of our patients had a history of either risk factor. When excluding patients with hypertension and diabetes mellitus form analysis, our rate of retinopathy was still lower than found by D'Alteroche et al. (4% vs. 20%, p=0.052 by Fisher's Exact test). Correction for patient age was not possible with available data. Third, hepatitis C patients may be, irrespective of age and other risk factors, more at risk to develop retinopathy than hepatitis B patients. Retinopathy was found in 32% of

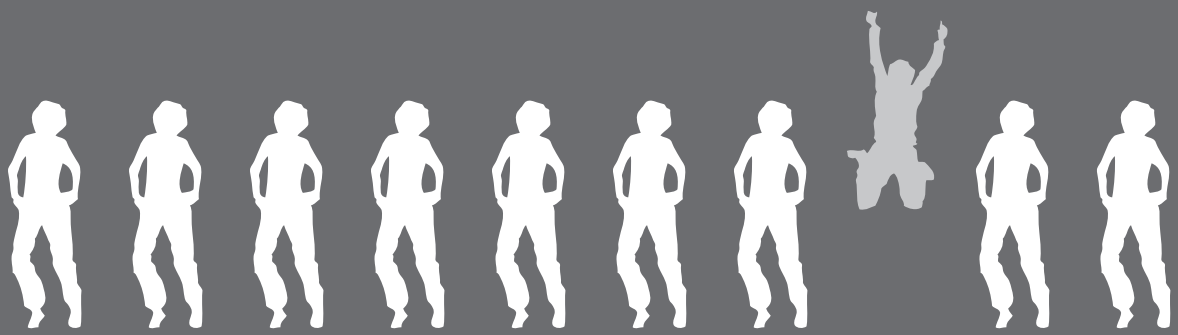
untreated HCV patients, compared to 6% in non-HCV-infected controls.[6] A relation between retinopathy and type II cryoglobulinemia in HCV infection has been suggested.[7,8] The rate of retinopathy in our study was comparable to that in the subgroup of HBV patients in the study of D'Alteroche et al. HCV infection in combination with diabetes mellitus, arterial hypertension and higher age may thus predispose patients to develop retinopathy on IFN therapy.

Despite the high frequency of retinopathy in many studies, symptomatic ocular adverse events are infrequently reported during IFN therapy (0.4% of patients).[1] Although isolated cases of severe ophthalmologic complications have been reported, the observed abnormalities in IFN associated retinopathy are usually transient.

Based on the transient character of PEG-IFN-related retinopathy, it's association with other risk factors and the significantly lower incidence in hepatitis B infected patients without these risk factors in our study, we question whether routine fundoscopic examination should be performed in this patient group. In our opinion, further studies should prove whether in hepatitis B infected patients to be treated with PEG-IFN, fundoscopic examination should be performed in all patients. So far, we recommend to examine only those with an increased risk for developing retinopathy or in patients with known pre-existing retinopathy.

REFERENCES

1. d'Alteroche L, Majzoub S, Lecuyer AI, et al. Ophthalmologic side effects during alpha-interferon therapy for viral hepatitis. J Hepatol 2006;44(1):56-61.
2. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365(9454): 123-9.
3. Tu KL, Bowyer J, Schofield K, et al. Severe interferon associated retinopathy. Br J Ophthalmol 2003;87(2):247-8.
4. Hayasaka S, Nagaki Y, Matsumoto M, et al. Interferon associated retinopathy. Br J Ophthalmol 1998;82(3):323-5.
5. Schulman JA, Liang C, Kooragayala LM, et al. Posterior segment complications in patients with hepatitis C treated with interferon and ribavirin. Ophthalmology 2003;110(2):437-42.
6. Abe T, Nakajima A, Satoh N, et al. Clinical characteristics of hepatitis C virus-associated retinopathy. Jpn J Ophthalmol 1995;39(4):411-9.
7. Abe T, Sakuragi S, Kuramitsu OT. Retinopathy associated with hepatitis C virus. Jpn J Clin Ophthalmol (Rinsho Ganka) 1993;47:297-300.
8. Zegans ME, Anninger W, Chapman C, et al. Ocular manifestations of hepatitis C virus infection. Curr Opin Ophthalmol 2002;13(6):423-7.



Relapse after treatment with peginterferon apha-2b alone or in combination with lamivudine in HBeAg positive chronic hepatitis B.

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Adapted from: Gut 2007; 56(10): 1485-1486.

5

ABSTRACT

Background

Interferon-induced loss of HBeAg is sustained in a majority of chronic hepatitis B infected patients, we investigated the frequency of relapse after peginterferon (PEG-IFN) alpha-2b therapy alone or in combination with lamivudine in HBeAg positive patients.

Methods

266 HBeAg positive patients were treated with PEG-IFN alpha-2b (100µg / week) in combination with placebo (n=136) or lamivudine (100mg / day) (n=130) for 52 weeks. Post-treatment follow-up lasted 26 weeks. Relapse was defined as HBeAg negativity at week 52 and recurrence of HBeAg at week 78.

Results

HBeAg loss was observed in 44% of patients on combination therapy and 29% on monotherapy at the end of treatment (p=0.01). Relapse occurred in 39% and 13% of patients receiving combination therapy or PEG-IFN alone, respectively (p=0.005). In the combination therapy group, relapse occurred significantly less frequently if anti-HBe was detectable at week 52 (21% vs. 63%, p=0.002); for PEG-IFN monotherapy a similar trend was found (7% vs. 30%; p=0.09). In multivariate analysis, PEG-IFN and lamivudine combination therapy (OR 3.9, CI95% 1.1-13.2, p=0.03), absence of anti-HBe at week 52 (OR 9.8, 95% CI 3.2-30.3, p<0.001) and absence of HBsAg-loss at week 52 (OR 10.3, 95% CI 1.09-97.89, p=0.04) independently predicted HBeAg relapse.

Conclusion

HBeAg relapse occurs more frequently after PEG-IFN alpha-2b and lamivudine combination therapy than after PEG-IFN alpha-2b monotherapy. Full HBeAg seroconversion with appearance of anti-HBe, rather than HBeAg loss, seems the best endpoint of PEG-IFN based therapy in HBeAg positive chronic HBV.

INTRODUCTION

Sustained loss of hepatitis B e antigen (HBeAg) from serum is associated with loss of hepatitis B surface antigen (HBsAg), reduced incidence of hepatocellular carcinoma (HCC) and improved survival.[1] Treatment with interferon (IFN) for chronic hepatitis B virus (HBV) infection results in HBeAg loss in about one-third of patients.[2] Interferon-induced HBeAg loss is generally sustained in up to 90% of patients.[1,3,4] A study by Song et al. in a Korean population demonstrated older age and presumed vertical transmission of HBV to be independent predictors for relapse after conventional IFN treatment.[5]

Nucleo(t)side analogues are well capable of inhibiting HBV replication, and have been shown to induce HBeAg loss in 15-30% of patients after 1 to 2 years of treatment.[6,7] However, prolonged treatment with lamivudine or adefovir is associated with the emergence of therapy resistant HBV strains and relapse occurs frequently after cessation of treatment with these drugs.[8,9] Predictors for sustained HBeAg seroconversion after lamivudine therapy include prolonged duration of therapy after HBeAg seroconversion and HBV genotype A.[10] Shorter interval of undetectable HBV DNA (<0.7 log10 IU/ml) was found to predict relapse following lamivudine therapy.[11] A prolonged period of undetectable HBV DNA by an additional month reduced the risk of relapse by 50%.

Recently performed studies with one year of pegylated interferon (PEG-IFN) alone or in combination with lamivudine in HBeAg positive patients showed high baseline alanine aminotransferase (ALT), low baseline HBV DNA, absence of previous IFN therapy, low baseline HBeAg and HBV genotype as independent predictors of response.[12-14] Predictors for relapse after PEG-IFN therapy are still unknown. In this study we investigated the frequency and possible predictors of relapse after treatment with PEG-IFN alpha-2b alone or in combination with lamivudine.

PATIENTS AND METHODS

Data for this study were extracted from a multicenter randomised controlled trial which compared PEG-IFN alpha-2b monotherapy to its combination with lamivudine in patients with HBeAg positive chronic hepatitis B. The inclusion and exclusion criteria were reported previously.[12] In short, patients were eligible if they had been HBsAg positive for more than 6 months, were HBeAg positive on two occasions within 8 weeks prior to randomisation, had elevated serum ALT of at least twice the upper limit of normal (ULN), and had serum HBV DNA >1.0 x 10^5 copies/ml. Major exclusion criteria were: antiviral therapy within 6 months prior to randomisation, serum antibodies against hepatitis C virus, hepatitis D virus or human immunodeficiency virus (HIV), pre-existent leucopenia or thrombocytopenia (white blood cell count [WBC] <3,000/mm^3, neutrophils <1,800/mm^3, platelets <100,000/mm^3), or decompensated liver disease.

Patients were randomised in a 1:1 ratio to receive PEG-IFN alpha-2b (100µg weekly) with placebo or with lamivudine (100mg daily) for 52 weeks. After 32 weeks, PEG-IFN alpha-2b dosage was

lowered to 50µg to prevent side effects and early treatment discontinuation. Follow-up after discontinuation of therapy lasted 26 weeks.

During therapy and post-treatment follow-up, patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally and therefore expressed as times upper limits of normal (ULN). HBV DNA was assessed monthly using an in-house developed Taqman PCR assay (lower limit of detection 373 copies/ml) based on the Eurohep standard.[15] HBeAg, anti-HBe, HBsAg and anti-HBs (AxSYM, Abbott, Abbott Park, IL, USA) were assessed at week 0, 32, 52 and week 78 (end of follow-up). HBV genotype and YMDD mutation analysis were performed by INNO-LiPA Assay (Innogenetics, Gent, Belgium).

Liver histology was assessed at baseline in all patients. Biopsy at the end of treatment was optional. Paired biopsies were available for 110 patients. Histological scoring was performed by one experienced pathologist according to the histological activity index, modified by Ishak.[16] The pa-

Table 1. Baseline characteristics of responders at the end of treatment (n = 97), divided by the occurrence of relapse (n=27) or sustained response (n=70) after therapy.

Variable	Relapse (n = 27)	Sustained response (n = 70)	p
Age*	36.3 ± 11.1	38.1 ± 12.4	0.50
Weight*	76.1 ± 14.1	75.6 ± 16.7	0.91
Race			0.64
Caucasian	22 (82%)	52 (74%)	
Asian	4 (15%)	12 (17%)	
Other	1 (3%)	6 (9%)	
Serum ALT (x ULN)*	5.5 ± 3.7	4.6 ± 3.0	0.22
HBV DNA (log_{10} copies/ml)*	9.0 ± 1.0	8.9 ± 0.9	0.66
HBV genotype			0.22
A	11 (41%)	35 (50%)	
B	2 (7%)	6 (9%)	
C	2 (7%)	8 (11%)	
D	12 (44%)	16 (23%)	
Other	0 (0%)	5 (7%)	
Histology†			
Necroinflammation	5 (2 - 10)	6 (2 - 10)	0.36
Fibrosis	3 (0 - 6)	3 (0 - 6)	0.69

ULN = upper limit of normal

*Mean ± SD

†Median (range)

thologist was blinded for information about the chronological order of biopsies, treatment allocation and outcome measures. Improvement of histology was defined as a reduction of at least two points in necroinflammatory score (range 0-18) or one point in fibrosis score (range 0-6).

RESULTS

At the end of treatment, 97 of 266 patients (36%) lost HBeAg, 57 (44%) in the combination therapy group and 40 (29%) in the monotherapy group (p=0.01). Twenty-seven of these 97 responders (28%) had recurrence of HBeAg at the end of follow-up (relapse), and 70 (72%) remained HBeAg negative throughout post-treatment follow-up (sustained response). Virological response (HBV DNA <200,000 copies/ml) occurred in 96 (74%) patients receiving combination therapy and 40 (29%) receiving monotherapy at the end of treatment (p<0.001); ALT normalisation was observed in 66 (51%) and 46 (33%) patients at the end of therapy, respectively (p=0.005). Baseline characteristics of patients with HBeAg relapse and sustained HBeAg response are shown in table 1. No differences in baseline variables were found between these groups.

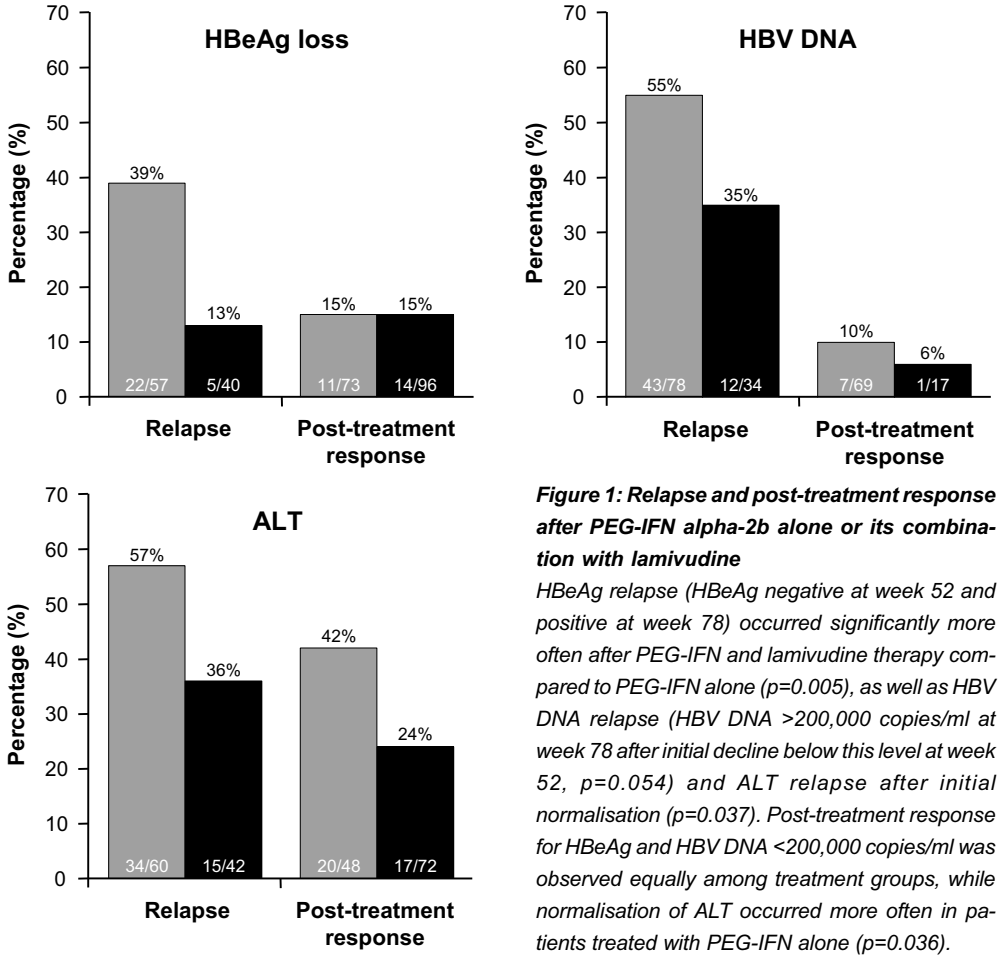


Figure 1: Relapse and post-treatment response after PEG-IFN alpha-2b alone or its combination with lamivudine

HBeAg relapse (HBeAg negative at week 52 and positive at week 78) occurred significantly more often after PEG-IFN and lamivudine therapy compared to PEG-IFN alone (p=0.005), as well as HBV DNA relapse (HBV DNA >200,000 copies/ml at week 78 after initial decline below this level at week 52, p=0.054) and ALT relapse after initial normalisation (p=0.037). Post-treatment response for HBeAg and HBV DNA <200,000 copies/ml was observed equally among treatment groups, while normalisation of ALT occurred more often in patients treated with PEG-IFN alone (p=0.036).

Relapse and post-treatment response for HBeAg, HBV DNA and ALT

HBeAg relapse occurred more often in patients treated with PEG-IFN alpha-2b and lamivudine combination therapy compared to PEG-IFN alpha-2b alone (figure 1A): 22 patients (39%) in the combination therapy group and 5 (13%) in the monotherapy group relapsed (p=0.005). Post-treatment response occurred in 15% of patients from both treatment groups. Relapse and post-treatment response rates for biochemical and virological response are shown in figure 1B and 1C. For both endpoints, patients in the combination therapy group more often relapsed than those in the monotherapy group (p=0.054 and p=0.037 for virological response and biochemical response, respectively). Patients with HBeAg relapse were more likely to have relapse of HBV DNA >200,000 copies/ml than sustained HBeAg responders (76% vs. 20%, p<0.001), as well as relapse of ALT (69% vs. 24%, p=0.007).

Prediction of HBeAg relapse

Among patients treated with combination therapy, 7 of 33 patients (21%) with detectable anti-HBe at the end of therapy relapsed compared to 15 of 24 patients (63%) without detectable anti-HBe (p=0.002). A similar trend was observed in patients treated with PEG-IFN alpha-2b alone, 2 of 30 patients (7%) with detectable anti-HBe and 3 of 7 patients without (30%) relapsed (p=0.09). Seven of 10 patients (70%) in the monotherapy group did not relapse despite the absence of anti-HBe compared to 9 of 24 patients (38%) treated with combination therapy (p=0.13). Three patients treated with PEG-IFN alpha-2b alone (30%) and 4 treated with combination therapy (17%), who had no detectable anti-HBe at the end of treatment, developed anti-HBe at the end of follow-up (p=0.39). HBV genotype tended to influence HBeAg relapse rates, 29% of patients harbouring genotype A relapsed compared to 56% of patients with genotype D (p=0.09). Although not statistically significant, possibly due to the limited number of lamivudine resistant patients among initial responders (with HBeAg loss at week 52), emergence of lamivudine resistance seemed to result in an increased risk of HBeAg relapse. Three of 4 patients (75%) with lamivudine resistance showed HBeAg relapse compared to 19 of 53 patients (36%) without lamivudine resistance (p=0.29).

Serum HBV DNA in relation to HBeAg relapse

Mean HBV DNA levels in patients with HBeAg relapse and sustained responders are shown in figure 2 for patients treated with PEG-IFN alpha-2b alone or its combination with lamivudine. In patients treated with PEG-IFN alone, decline in HBV DNA was 4.8 $\log_{10}$ copies/ml for patients with relapse compared to 3.8 $\log_{10}$ copies/ml for sustained responders at the end of treatment (p=0.30). Decline in HBV DNA was 5.8 $\log_{10}$ copies/ml for all patients in the combination therapy group at the end of treatment. In both treatment groups mean HBV DNA was significantly higher in relapsers than sustained responders at week 78 (9.2$\log_{10}$ vs. 4.6 log10 for monotherapy and 7.7 $\log_{10}$ vs. 3.7 $\log_{10}$ for combination therapy, p<0.001).

In patients treated with PEG-IFN alpha-2b alone, combination of HBeAg loss and HBV DNA <10,000 copies/ml at week 52 was associated with a significantly lower rate of relapse compared to partial

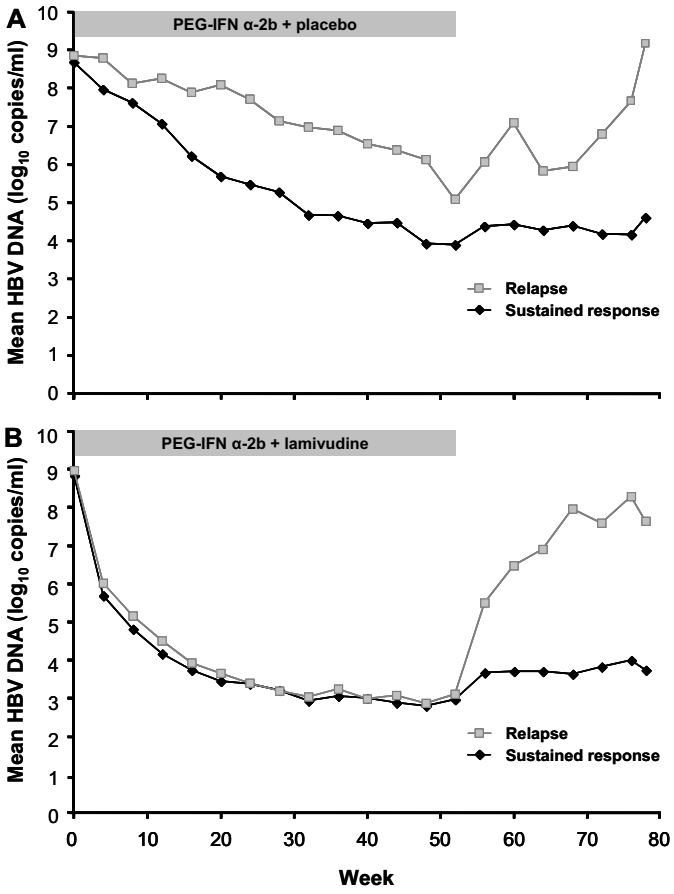


Figure 2: Mean HBV DNA in patients with HBeAg relapse and sustained response after PEG-IFN alpha-2b alone or its combination with lamivudine.

Among patients treated with PEG-IFN monotherapy, decline in HBV DNA was more pronounced in sustained responders than relapsers (A). This difference was however not observed in patients treated with PEG-IFN and lamivudine combination therapy (B).

response of HBeAg loss but HBV DNA of 10,000 copies/ml or higher (table 2, p=0.01), while HBeAg negativity in combination with other HBV DNA cut-off levels did not correlate with HBeAg relapse. In the combination therapy group, HBeAg relapse could not be predicted by serum HBV DNA at the end of treatment. Furthermore, in the monotherapy group, duration of low viremia also influenced relapse rates. In none of 19 patients treated with PEG-IFN alpha-2b alone, who had HBV DNA <10,000 copies/ml for at least 4 weeks prior to discontinuation of therapy HBeAg relapse occurred, while this occurred in 5 of 20 patients (25%) with HBV DNA <10,000 copies/ml at week 52 only (p=0.04). HBV DNA below 1.0 x 10^5 or 1.0 x 10^6 copies/ml on at least 2 consecutive tests by the end of treatment was not related with HBeAg relapse (p=0.58 and p=0.14 for HBV DNA <10^5 or <10^6 copies/ml, respectively). At the end of follow-up, 56% of patients with sustained HBeAg response had HBV DNA <10,000 copies/ml compared to 4% of patients with relapse (p<0.001).

Table 2: HBeAg relapse in patients with negative HBeAg in combination with various HBV DNA levels at week 52 at the end of treatment

	HBeAg relapse	p
HBV DNA <1,000 vs. >=1,000 copies/ml	0% vs. 22%	0.14
HBV DNA <10,000 vs. >=10,000 copies/ml	0% vs. 29%	0.01
HBV DNA <100,000 vs. >=100,000 copies/ml	12% vs. 14%	1.00
HBV DNA <1,000,000 vs. >=1,000,000 copies/ml	12% vs. 20%	0.53

Logistic regression analysis of factors predicting HBeAg relapse and post-treatment HBeAg response

For the prediction of HBeAg relapse and post-treatment HBeAg response, the following baseline variables were included in univariate logistic regression analysis: age, sex, weight, race, mode of transmission, serum ALT, HBV DNA level, HBV genotype, liver histology and treatment allocation. In addition to these baseline variables, HBeAg and anti-HBe status at week 32; serum ALT, HBV DNA, anti-HBe, HBsAg (HBeAg relapse only) and liver histology at week 52; and time points of ALT normalisation and HBV DNA response were included (table 3). In multivariate time dependent analysis, combination therapy of PEG-IFN alpha-2b with lamivudine (OR 3.9, 95% CI 1.1 - 13.2,

Table 3: Factors significantly associated with HBeAg relapse in multivariate analysis

	Odds	95%-CI		p
Variable	**Ratio**	**Lower**	**Upper**	**value**
Relapse				
Combination therapy	4.4	1.15	12.93	0.007
Anti-HBe positive week 32	0.29	0.10	0.82	0.02
Anti-HBe positive week 52	0.15	0.06	0.39	<0.001
Post-treatment response				
Sex - male	0.25	0.11	0.61	0.002
Weight	0.95	0.92	0.99	0.01
Body mass index (BMI)	0.90	0.82	0.99	0.03
Baseline necroinflammation	1.25	1.00	1.55	0.05
HBeAg negative week 32	13.16	3.03	57.09	0.001
Anti-HBe positive week 32	8.03	1.98	32.58	0.004
Time point of ALT normalisation	0.98	0.96	0.99	0.007
Time point of HBV DNA response	0.98	0.97	1.00	0.009

CI, confidence interval

p=0.03), absence of HBsAg loss at week 52 (OR 10.3, 95% CI 1.09-97.89, p=0.04) and absence of anti-HBe at week 52 (OR 9.8, 95% CI 3.2 - 30.3, p<0.001) independently predicted HBeAg relapse. Absence of anti-HBe at the end of treatment was found to be the strongest predictor of HBeAg relapse.

DISCUSSION

In HBeAg-positive chronic hepatitis B, it is assumed that relapse occurs frequently after discontinuation of nucleos(t)ide analogue therapy, while response appears more durable after interferon-based treatment because of its immunomodulatory effects. In the current study we found 39% relapse after discontinuation of PEG-IFN alpha-2b and lamivudine combination therapy and 13% after PEG-IFN alpha-2b alone. These relapse rates are consistent with findings of previous studies, which showed HBeAg relapse in 22 - 40% of lamivudine treated patients[3,17] and 10% of IFN treated patients.[3,18] Most likely, the higher relapse rates (preceded by higher response rates) after cessation of combination therapy compared to PEG-IFN alpha-2b monotherapy can be explained by lamivudine induced HBeAg loss.

In multivariate analysis, treatment allocation, absence of anti-HBe and absence of HBsAg loss at the end of treatment independently predicted HBeAg recurrence. Factors predicting relapse after conventional IFN have previously been described and include older age and presumable vertical transmission.[5] We did not find a relation between relapse and these factors in both univariate and multivariate analysis. Possibly our findings differ from this Korean study because of differences in patient population and type or duration of the antiviral therapy.

Among PEG-IFN alpha-2b and lamivudine treated patients in our study, 21% of those positive for anti-HBe at the end of therapy relapsed compared to 63% of patients who lacked anti-HBe. In HBeAg positive patients treated with PEG-IFN alpha-2a, HBeAg seroreversion (after full HBeAg-seroconversion) occurred in 18% of patients treated with PEG-IFN alpha-2a monotherapy and 22% of patients on combination therapy with lamivudine.[19] Response was sustained in 86% of responders at 1 year post-treatment in this study.[20] Although relapse rates in patients without detectable anti-HBe in our study are much higher compared to PEG-IFN alpha-2a treated patients with full HBeAg-seroconversion, relapse rates for patients with loss of HBeAg and detectable anti-HBe at the end of treatment seem comparable between the two studies.

Presence of anti-HBe may be less important for PEG-IFN alpha-2b monotherapy than combination therapy, since the majority of patients treated with PEG-IFN alpha-2b monotherapy (7 of 10 patients) did not relapse despite the absence of anti-HBe at the end of therapy. In patients with HBeAg relapse, HBV DNA levels declined during treatment, but less profoundly compared to sustained responders (figure 2). HBV DNA <10,000 copies/ml prior to discontinuation of treatment was found to significantly decrease the risk of relapse in PEG-IFN alpha-2b treated patients, while other HBV DNA threshold levels did not correlate with HBeAg relapse. This is consistent with findings on relapse after cessation of lamivudine in patients with prolonged suppression of HBV

DNA.[11] In addition to HBeAg response, decline in HBV DNA (below 10,000 copies/ml) seems thus important for maintaining PEG-IFN induced response and may be associated with immune control of the virus. Patients with a partial response, as defined by HBeAg loss but insufficient decline of HBV DNA (not below 10,000 copies/ml) at the end of therapy, might benefit from prolonged therapy, as this has previously been shown to increase response to standard IFN.[21] Maintaining HBV DNA below 10,000 copies/ml after discontinuation of therapy also appears important since recent studies showed that serum HBV DNA below this level reduces the risk of progression to cirrhosis, decompensated liver disease and hepatocellular carcinoma.[22,23] Fifty-six percent of sustained responders had HBV DNA <10,000 copies/ml at the end of follow-up, while virtually all patients with relapse had HBV DNA above this level. In HBeAg positive patients treated with monotherapy of PEG-IFN alpha-2a, a comparable rate of HBV DNA below 10,000 copies/ml was observed in responders (66%) at the end of follow-up.[24]

HBV genotype independently predicted loss of both HBeAg and HBsAg in HBeAg-positive patients treated with PEG-IFN alpha-2b, with higher response rates in patients with genotype A compared to those with genotype D.[12,25] A similar trend was observed in a population of mainly Asian patients treated with PEG-IFN alpha-2a.[19,26] HBV genotype also tended to influence HBeAg relapse after discontinuation of PEG-IFN alpha-2b with or without lamivudine, with genotype D infected patients having a higher risk of relapse than those with genotype A.

In conclusion, HBeAg relapse occurs more often after treatment of PEG-IFN alpha-2b in combination with lamivudine than after PEG-IFN alpha-2b monotherapy. Absence of anti-HBe at the end of treatment was found to be the strongest predictor of relapse, particularly in patients treated with combination therapy. HBeAg loss thus is less suitable as sole endpoint of PEG-IFN based therapy in HBeAg positive chronic HBV. More profound suppression of HBV DNA below 10,000 copies/ml further decreases the risk of relapse. Patients with a partial response might benefit from prolonged PEG-IFN treatment, further reducing HBV DNA and thereby possibly increasing the development of anti-HBe and reducing the risk of relapse after discontinuation of therapy.

ACKNOWLEDGEMENTS

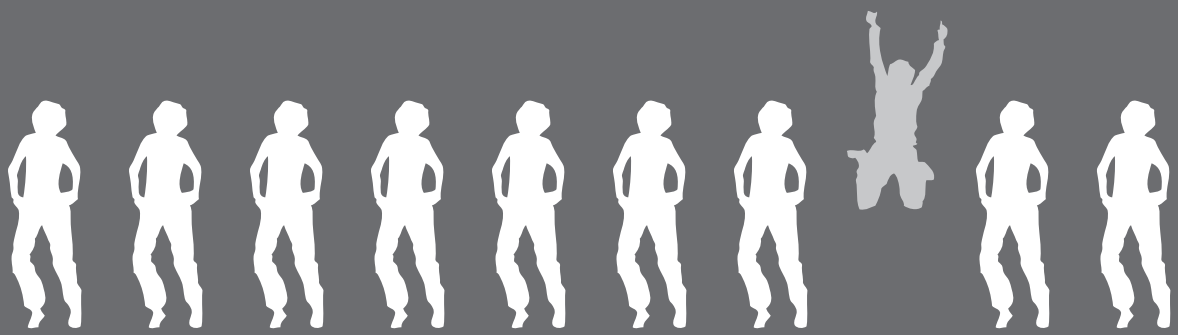
Other members of the HBV 99-01 Study Group: Netherlands - KJ van Erpecum, BCM Vroom (University Medical Center, Utrecht); CMJ van Nieuwkerk (VU University Medical Center, Amsterdam); J Jansen, J Drenth, SJ van den Hazel (University Medical Centre Radboud, Nijmegen); JW den Ouden-Muller (St Franciscus Hospital, Rotterdam); AC Tan (Canisius Wilhelmina Hospital, Nijmegen). Belgium - DM Adler (Hopital Erasme, Brussels); P Michielsen (University Hospital, Antwerp); H van Vlierberghe (University Hospital, Gent); J Delwaide (Centre Hospitalier Universitaire, Liège); J Henrion (Hopital de Jolimont, Hainet Paul). Germany - S Bein, U Treichel (University Hospital, Essen); MP Manns, J Hadem (Medizinische Hochschule, Hannover); C Niederau (St Jozef Hospital, Oberhausen). Denmark - MR Buhl, IM Hansen (Skejby Hospital, Arhus); K Krogsgaard (Copenhagen University Hospital, Hvidovre). Poland - J Jablonska, J Kozlowska (Medical Academy of Warsaw, Warsaw); D Prokopowicz, R Flisiak (Medical Academy of Bialystok, Bialystok). Spain - M Buti, A Valdes, R Esteban (Hospital Valle Hebron, Barcelona); M Rodriguez, M Garcia Espiga (Hospital Central de Asturias, Oviedo). Italy - A Andriulli, G Stornaiulo, GB Gaeta (Ospedale Gesù e Maria, Napoli); G Montalto, F D'Antona (Università di Palermo, Palermo). Greece - P Xiarchos Panagiotis (George Papanikolaou General

Regional Hospital,Thessaloniki); NC Tassopoulos (West Attica Hospital, Athens). Turkey - G Ersöz (Ege University Faculty of Medicine, Izmir); C Yurdayin, H Bozkaya (Medical School Cebeci Kampusu, Ankara); Y Balaban (Hacettepe University Faculty of Medicine, Ankara), F Tabak (Istanbul University Cerrahpasa Medical School, Istanbul). Israel-Y Lurie (Sauraski Medical Center, Tel-Aviv). Canada - S Greenbloom (General Hospital, Etobicoke). Indonesia - DA Sulaiman (Ciptomangunkusomo Hospital, Jakarta). Singapore - R Guan (Mount Elizabeth Medical Center Singapore).

REFERENCES

1. van Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-10.
2. Wong DK, Cheung AM, O'Rourke K, et al. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312-23.
3. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-4.
4. Korenman J, Baker B, Waggoner J, et al. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.
5. Song BC, Suh DJ, Lee HC, et al. Which patients with chronic hepatitis B are more likely to relapse after interferon alpha-induced hepatitis B e antigen loss in Korea? J Clin Gastroenterol 2004;38:124-9.
6. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-16.
7. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256-63.
8. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527-32.
9. Sung JJ, Wong ML, Bowden S, et al. Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained response to therapy. Gastroenterology 2005;128:1890-7.
10. Chien RN, Yeh CT, Tsai SL, et al. Determinants for sustained HBeAg response to lamivudine therapy. Hepatology 2003;38:1267-73.
11. Ito K, Tanaka Y, Orito E, et al. Predicting relapse after cessation of Lamivudine monotherapy for chronic hepatitis B virus infection. Clin Infect Dis 2004;38:490-5.
12. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-9.
13. Cooksley G, Lau GKK, Liaw YF, et al. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol 2005;42:S30.
14. Bonino F, Lau GKK, Marcellin P, et al. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized, partially double-blind study of peginterferon-alfa-2a (4-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone. Hepatology 2004;40:A1142.
15. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-901.
16. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-9.
17. Yoon SK, Jang JW, Kim CW, et al. Long-term results of lamivudine monotherapy in Korean patients with HBeAg-positive chronic hepatitis B: response and relapse rates, and factors related to durability of

HBeAg seroconversion. Intervirology 2005;48:341-9.
18. van Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-10.
19. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-95.
20. Lau GK, Piratvisuth T, Luo KX, et al. Durability of response and occurrence of late response to peginterferon alpha-2a (40KD) one year post-treatment in patients with HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.
21. Janssen HL, Berk L, Schalm SW, et al. Antiviral effect of prolonged intermittent lymphoblastoid alpha interferon treatment in chronic hepatitis B. Gut 1992;33:1094-8.
22. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
23. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-86.
24. Piratvisuth T, Lau GKK, Marcellin P, et al. Association between HBeAg seroconversion and sustained HBV DNA suppression in patients treated with peginterferon alfa-2a (40KD) for HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.
25. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with peginterferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol 2006;101:297-303.
26. Hadziyannis S, Lau GKK, Marcellin P, et al. Sustained HBsAg seroconversion in patients with chronic hepatitis B treated with peginterferon alpha-2a (40kDa) (Pegasys®). J Hepatol 2005;42:S178.



Withdrawal flares occur frequently and are non-beneficial in HBeAg-positive chronic hepatitis B patients treated with peginterferon alpha-2b.

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Submitted.

9

ABSTRACT

Background and aim

Hepatitis flares are a well-known and potentially dangerous phenomenon during interferon-based and after nucleos(t)ide analogue therapy. We investigated the frequency of severe flares after peginterferon (PEG-IFN) therapy in HBeAg positive chronic hepatitis B.

Methods

One-hundred-thirty-six patients were treated with PEG-IFN alpha -2b (100µg/week for 52 weeks) and followed for 26 weeks after therapy. Hepatitis flares were defined as an increase in ALT levels to >=10x the upper limit of normal (ULN) and >=2x the nadir value during (on-treatment flare) or after therapy (withdrawal flare). Combined response was defined as HBeAg loss and HBV DNA <10,000 copies/ml at 6 months post-treatment.

Results

Withdrawal flares were observed in 19 patients (14%) and on-treatment flares in 22 patients (16%). Mean maximum ALT of withdrawal flares was 14.8 ± 6.5 x ULN compared to 15.3 ± 4.8 x ULN of on-treatment flares (p=0.79). Withdrawal flares were particularly observed in non-genotype-A infected patients (odds ratio [OR] 5.1, 95%-CI 1.1-23.2), those with previous lamivudine therapy (OR 3.5, 95%-CI: 1.1-10.8) or with high ALT at the end of treatment (OR 1.4, 95%-CI 1.1-1.8). No association between baseline factors and on-treatment flares was observed. Combined response was observed in 12% of patients without a flare, 5% of patients with a withdrawal flare and 18% of patients with an on-treatment flare.

Conclusion

Withdrawal flares occur frequently after PEG-IFN therapy and are non-beneficial. Therefore, close monitoring of patients at increased risk, and rapid re-initiation of nucleos(t)ide analogue therapy in those developing a withdrawal flare is recommended.

INTRODUCTION

Chronic hepatitis B virus (HBV) infected patients can suffer from acute exacerbations under various conditions. These exacerbations of inflammatory activity (hepatitis flares) in general represent an increased host immunity against HBV infected hepatocytes due to spontaneous or treatment-associated increases in viral replication, or as a result of therapeutic intervention with immunomodulatory dugs such as interferon, corticosteroids, or chemotherapy.[1] It has been reported that viremia markedly increased before an increase in serum alanine aminotransferase (ALT) level is observed.[2] Flares have been observed in 16-19% of patients after stopping lamivudine therapy.[5, 9, 10] Hepatitis flares can be severe or even life threatening, particularly in patients with underlying cirrhosis.[3] Acute exacerbations of chronic hepatitis B are associated with hepatocellular damage and repeated exacerbations or reactivations can lead to progressive fibrosis.[1]

Hepatitis flares are a well known phenomenon during treatment with (peg)interferon (PEG-IFN), as well as in patients developing antiviral resistance or stopping nucleos(t)ide analogue therapy .[1, 4, 5] During treatment with standard IFN or PEG-IFN, flares occurred in 25-40% of patients and have been associated with an increased likelihood of virological response.[4, 6-8] Flares during IFN-based therapy are potentially associated with the increase in T cell cytolytic activity and natural killer cell function during IFN therapy and typically occur during the second to third month of treatment.[7] Two types of flares have been distinguished during PEG-IFN therapy. Virus induced flares, which occur after an increase in HBV DNA level, and most probably are indicative for increased expression of viral antigens, did generally not lead to treatment response. In contrast, host induced flares which were followed by a decrease in serum HBV DNA were highly associated with treatment response.[4] A uniform definition of flares was recently proposed in the newest edition of the AASLD consensus guideline for the treatment of chronic hepatitis B.[11] The incidence of and outcome after such flares associated with PEG-IFN monotherapy is still unknown.

PATIENTS AND METHODS

We investigated the occurrence of flares after (withdrawal flares) and during (on-treatment flares) treatment with PEG-IFN alpha-2b in HBeAg-positive chronic hepatitis B patients.[12] One-hundred-thirty-six patients treated with PEG-IFN alpha-2b for 52 weeks were included in this study. After 32 weeks, PEG-IFN alpha-2b dosage was lowered from 100µg to 50µg per week to prevent side effects and early treatment discontinuation. Follow-up after discontinuation of therapy lasted 26 weeks.

Patients were eligible if they were positive for hepatitis B surface antigen (HBsAg) for at least 6 months, hepatitis B e antigen (HBeAg) positive on two occasions within 8 weeks prior to randomization, had elevated serum ALT of >2 to >=10 x the upper limit of normal (ULN), and had serum HBV DNA above 1.0 x 10^5 copies per milliliter (copies/ml). Major exclusion criteria were: antiviral therapy within 6 months prior to randomization, serum antibodies against hepatitis C virus (HCV),

hepatitis delta virus (HDV) or human immunodeficiency virus (HIV), pre-existent leucopenia (white blood cell count <=3,000/mm^3, neutrophil count <=1,800/mm^3) or thrombocytopenia (platelets >=100,000/mm^3), or decompensated liver disease.

During therapy and post treatment follow-up, patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally in accordance with standardized procedures and therefore expressed as times the ULN. Serum HBV DNA levels were measured monthly using an in-house developed Taqman PCR assay (lower limit of detection 373 copies/ml) based on the Eurohep standard.[13] HBeAg and HBsAg (AxSYM, Abbott, Abbott Park, IL, USA) were assessed at week 0, 32, 52 (end of treatment) and week 78 (end of follow-up). HBV genotype and YMDD mutation analysis were performed by INNO-LiPA assay (Innogenetics, Gent, Belgium).

Hepatitis flares were defined as intermittent elevations of ALT levels to at least 10 times the upper limit of normal and at least twice the nadir value, as defined in the AASLD consensus guideline for the treatment of chronic hepatitis B.[11] A flare was defined as "virus induced" when preceded by an increase in HBV DNA (>=1log$_{10}$ copies/ml) within four months. A flare was defined as "host induced" when the flare was followed by a decline in HBV DNA (>=1log$_{10}$ copies/ml) within four months. Flares that did not meet one of these criteria were classified as indeterminate.[4]

Statistical analysis was performed using the SPSS 15.0 program (SPSS Inc. Chicago, IL). Chi-square, Fisher's exact test and Mann-Whitney U test were used where appropriate. The relation between characteristics at baseline and at the end of therapy, and the occurrence of flares was examined by logistic regression analyses. A p-value <= 0.05 (all 2-sided) was considered statistically significant.

RESULTS

Hepatitis flares were observed in 38 of 136 patients (28%); 16 patients (12%) developed a flare within 6 months after therapy (withdrawal flare), 19 patients (14%) developed a flare during PEG-IFN therapy (on-treatment flare), and 3 patients (2%) had a flare both during and after therapy. Patient characteristics are shown in table 1. The mean maximum ALT of withdrawal flares was 14.8 ± 6.5 x ULN compared to 15.3 ± 4.8 x ULN of on-treatment flares (p=0.79). Withdrawal flares occurred after a mean period of 12.2 ± 8.9 weeks after therapy, with 79% occurring within 12 weeks after stopping PEG-IFN therapy. On-treatment flares occurred after a mean treatment duration of 16.6 ± 11.9 weeks, with 50% of flares occurring in the first 12 weeks of therapy.

Patterns of flares

Different patterns of flares could be distinguished (figure 1). The observed frequency of the three patterns of acute exacerbation (virus-induced, host-induced and indeterminate flares) was significantly different between withdrawal flares and on-treatment flares (p=0.006). Virus-induced flares were the most frequently observed type of withdrawal flares, while more than half of the flares that

Table 1: Patient characteristics

Characteristic	No flare (n = 98)	Withdrawal flare (n = 16)	On-treatment flare (n=19)	Both types of flares (n=3)	p
Baseline:					
Age	35.7 ± 14.3	32.0 ± 10.2	39.3 ± 12.9	28.7 ± 3.8	>0.08
Male sex	76 (78%)	13 (81%)	15 (79%)	2 (67%)	0.95
ALT (x ULN)	4.1 ± 3.1	4.9 ± 3.5	4.4 ± 2.3	4.1 ± 1.8	>0.39
HBV DNA (log_{10} copies/ml)	9.0 ± 1.1	9.2 ± 0.6	9.1 ± 0.6	9.4 ± 0.9	>0.38
HBV genotype					0.18
A	38 (39%)	2 (13%)	7 (37%)	0 (0%)	
B	8 (8%)	2 (13%)	2 (11%)	0 (0%)	
C	14 (14%)	2 (13%)	3 (16%)	2 (67%)	
D	35 (36%)	10 (63%)	5 (26%)	1 (33%)	
Other	3 (3%)	0 (0%)	2 (11%)	0 (0%)	
End of treatment (week 52):					
HBeAg negative	28 (29%)	2 (13%)	9 (47%)	1 (33%)	0.16
HBeAg seroconversion	22 (22%)	1 (6%)	6 (32%)	1 (33%)	0.28
ALT (x ULN)	1.5 ± 1.0	3.2 ± 2.5	2.6 ± 2.9	2.6 ± 0.3	<0.006†
HBV DNA (log_{10} copies/ml)	6.8 ± 2.6	7.6 ± 1.9	5.8 ± 2.7	8.0 ± 0.6	0.04‡
End of follow-up (week 78):					
HBeAg negative	35 (36%)	6 (38%)	8 (42%)	0 (0%)	0.57
HBeAg seroconversion	27 (28%)	6 (38%)	6 (32%)	0 (0%)	0.58
ALT (x ULN)	1.9 ± 1.8	4.1 ± 4.0	2.0 ± 1.9	5.3 ± 2.7	<0.003¥

†Patients without a flare vs. those with an on-treatment flare or withdrawal fare.

‡Patients with an on-treatment flare vs. those with a withdrawal flare.

¥Patients without a flare vs. those with a withdrawal flare or with both an on-treatment flare and a withdrawal flare.

occurred during PEG-IFN therapy were of the host-induced type.

Factors influencing the occurrence of flares after or during PEG-IFN therapy

The distribution of the HBV genotypes among patients with and without a flare is shown in table 1. All withdrawal flares occurred in patients who were still HBeAg positive at the end of treatment, no withdrawal flares were observed in those with end of treatment response (p=0.13). Withdrawal flares occurred significantly less often in genotype A infected patients compared to those with other genotypes (4% vs. 19%, p=0.02). Factors associated with the occurrence of withdrawal flares include genotype non-A infection (odds ratio [OR] 5.1, 95%-CI 1.1-23.2), previous lamivudine therapy (OR 3.5, 95%-CI 1.1-10.8) and high ALT at week 52 (OR 1.4, 95%-CI 1.1-1.8). On-treatment

flares occurred equally across genotypes (15%, 17%, 24% and 12% in patients with genotype A-D, respectively; p=0.44). None of the baseline factors age (p=0.39), sex (p=0.93), weight (p=0.71), ALT (p=0.82), HBV DNA (p=0.59), genotype (p=0.48), BMI (p=0.74), previous IFN (p=0.35) or previous lamivudine therapy (p=0.48) was associated with the occurrence of on-treatment flares on logistic regression analysis.

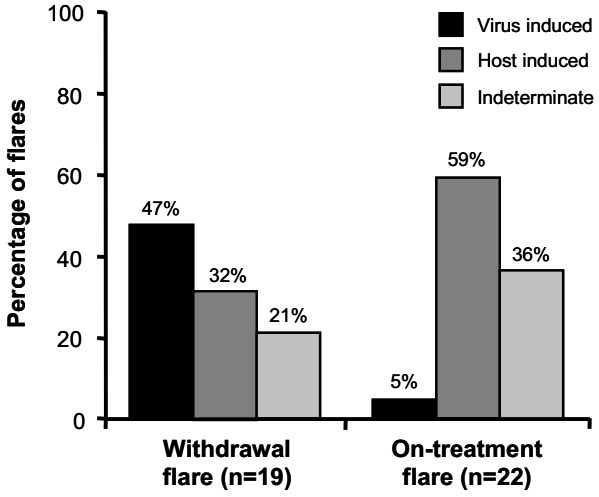


Figure 1: Patterns of hepatitis flares occurring during or after PEG-IFN therapy.
Three pattern of acute exacerbation can be distin-guished: virus induced flares, host induced flares and indeterminate flares. The frequency of the different patterns of acute exacerbation was significantly different between withdrawal flares and on-treatment flares (p=0.006).

Flares and response to therapy

At the end of treatment, combined response was observed in 16 of 136 patients (12%) and was not influenced by the occurrence of on-treatment flares (9% vs. 12% in patients with and without an on-treatment flare, p=0.67). Combined response at 6 months post-treatment was observed in 13% of patients. There was no difference in the rate of combined response at 6 months post-treatment between patients with and those without a flare (13% vs. 12%, p=0.89). Four of 19 patients (21%) with an on-treatment flare achieved combined response at 6 months post-treatment, all of whom had a virus-induced flare. Combined response at 6 months post-treatment was observed in 1 of 16 patients (6%) with a withdrawal flare. None of the three patients with both an on-treatment and a withdrawal flare showed combined response at 6 months post-treatment.

Influence of flares on liver function

Significant hepatic decompensation was not observed in patients with flares, neither in those without. A rise in bilirubin to >=1.5 x ULN during PEG-IFN therapy was observed in 14% of patients with an on-treatment flare compared to 11% of those without (p=0.77). Clinically overt jaundice was not observed in any of the patients. Bilirubin elevations were also observed equally in patients with and without a withdrawal flare (16% vs. 12%, p=0.61). A prolongation of prothrombin time was observed in two patients. The first patient had a persistently prolonged prothrombin time throughout therapy and post-treatment follow-up (2.1 – 2.5 x ULN) of unclear origin, no underlying etiology or

use of anticoagulants was reported. The other had a prolongation of prothrombin time to 1.6 x ULN after developing a withdrawal flare with a maximum ALT level of 15.6 x ULN. Bilirubin levels remained normal and the patient did not have underlying cirrhosis.

DISCUSSION

Hepatitis flares are a well known and potentially dangerous phenomenon after discontinuation or development of antiviral resistance during nucleos(t)ide analogue, particularly in patients with advanced liver disease. Acute exacerbations during PEG-IFN therapy on the other hand have been reported to be beneficial, resulting in higher response rates.[4, 8] In the current study we defined flares as an ALT level of >=10 times the upper limit of normal and >=2 times the nadir value as recently proposed in the AASLD practice guidelines. Such severe acute exacerbations occurred in 16% of patients during treatment with PEG-IFN alpha-2b and in 14% of patients after discontinuation of therapy. The maximum ALT level during the flare was comparable for the two types of flares and no signs of hepatic decompensation were observed.

The observed frequency of withdrawal flares in our study seems comparable to the rates observed in previous studies of lamivudine and/or interferon therapy (2-19%).[5, 9, 10, 14] Recent studies with nucleos(t)ide analogues provide little data on post-treatment follow-up, since nucleos(t)ide analogue therapy is now generally given for longer periods.[15-17] A higher frequency of withdrawal flares might be expected in nucleos(t)ide analogue treated patients, since post-treatment virological relapse may occur more frequently after stopping nucleos(t)ide analogue than PEG-IFN therapy.[12, 18-20] In particular a rapid increase in serum HBV DNA has been shown to precipitate hepatitis flares.[2] However, in entecavir treated patients, withdrawal flares (ALT level >2x reference [lesser of the baseline and the end-of-dosing ALT values] and >10x ULN) were observed in only 2% of the patients who discontinued antiviral therapy.[21] This low rate of withdrawal flares may be due to the high rate of sustained HBeAg seroconversion of about 75% at six months post-treatment. Schiff et al. found elevations of at least twice baseline levels and at least 500U/l in 11% of patients treated with lamivudine and only 2% of patients treated with IFN and lamivudine combination therapy.[14] The differences in the observed frequency of flares may be explained by differences in the definition of a flare and the duration of follow-up. We chose the lowest ALT level during or after treatment as the reference value for on-treatment and withdrawal flares, respectively. If baseline and end of treatment ALT would have been chosen as the reference value, patients with high baseline ALT levels would by definition have had a lower risk of developing a flare.

The majority of on-treatment flares were of the host-induced type. Among patients with an on-treatment flare, all of those who achieved combined response at six months post-treatment had a host-induced flare. On the other hand, we frequently observed a rise in HBV DNA prior to the rise in ALT levels among patients developing a withdrawal flare (virus-induced flares). None of the patients with a withdrawal flare had HBeAg loss at the end of treatment. Loss of PEG-IFN induced HBV DNA decline seems thus responsible for the observed withdrawal flares.

We did not observe signs of hepatic decompensation in our patients, who all had fully compensated liver disease at inclusion (no prolongation of prothrombin time by >3 seconds, albumin concentration >=35 g/L, bilirubin <35 µmol/L and no history of ascites, variceal bleeding, or hepatic encephalopathy). In IFN-treated patients with signs of hepatic decompensation prior to the start of IFN therapy or with Child's class B or C cirrhosis, severe adverse events including fatal hepatic decompensation have been previously reported and IFN-based therapy is therefore contra-indicated in these patients.[3, 22, 23]

We conclude that flares occur frequently in HBeAg positive patients during and after PEG-IFN therapy. Withdrawal flares particularly occurred in those who are still HBeAg positive at the end of therapy and are non-beneficial. Therefore, close monitoring of patients at increased risk for developing withdrawal flares is recommended during the first months after discontinuation of therapy. In addition, rapid re-initiation of nucleos(t)ide analogue therapy should be considered in patients developing a withdrawal flare.

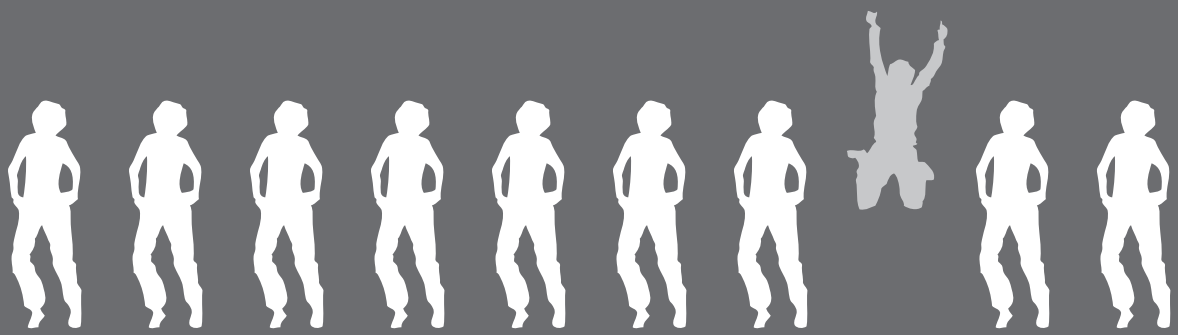
ACKNOWLEDGEMENTS

Members of the HBV99-01 Study Group includes the following investigators: The Netherlands: R.A. de Man, H.G.M. Niesters, P.E. Zondervan (University Medical Center Rotterdam), B.C.M. Vroom (University Medical Center Utrecht) C.M.J. van Nieuwkerk (VU University Medical Center Amsterdam), R.A. de Vries (Rijnstate Hospital Arnhem), J. Jansen, J. Drenth, S.J. van den Hazel (University Medical Centre Radboud Nijmegen), J.W. den Ouden-Muller (St. Franciscus Hospital Rotterdam), A.C. Tan (Canisius Wilhelmina Hospital Nijmegen); Belgium: D.M. Adler (Hopital Erasme Brussels), P. Michielsen (University Hospital Antwerp), H. van Vlierberghe (University Hospital Gent), F. Nevens (University Hospital Leuven), J. Delwaide (Centre Hospitalier Universitaire Liège), J. Henrion (Hopital de Jolimont, Haine St. Paul); Germany: S. Zeuzem (Saarland University Hospital, Homburg/Saar), G.Gerken, S Bein, U. Treichel (University Hospital Essen), J. Trojan (J.W. Goethe Universität Frankfurt), M.P. Manns, J. Hadem (Medizinische Hochschule Hannover), C. Niederau (St. Jozef Hospital Oberhausen); Denmark: M.R. Buhl, I.M. Hansen (Skejby Hospital, Arhus), K. Krogsgaard (Copenhagen University Hospital Hvidovre); Poland: C. Simon (Medical University, Wroclaw), J. Cianciara, J. Jablonska, J Kozlowska (Medical Academy of Warsaw), D. Prokopowicz, R. Flisiak (Medical Academy of Bialystok), T. Mach (Collegium Medicum UJ Kraków); Spain: M. Buti, A. Valdes, R Esteban (Hospital Valle Hebron, Barcelona), M. Rodriguez, M. Garcia Espiga (Hospital Central de Asturias, Oviedo); Italy: A. Andriulli, G. Stornaiulo, G.B. Gaeta (Ospe. Gesù e Maria, Napoli), G. Montalto, F. D'Antona (Università di Palermo); Greece: G.E. Kitis, P. Xiarchos Panagiotis (George Papanikolaou General Regional Hospital,Thessaloniki), N.C. Tassopoulos (West Attica Hospital Athens); Turkey: U.S. Akarca, G. Ersöz (Ege University Faculty of Medicine Izmir), S. Karayalcin, C. Yurdayin, H. Bozkaya (Medical School Cebeci Kampusu Ankara), H. Simsek, Y. Balaban (Hacettepe University Faculty of Medicine Ankara), H. Senturk, F. Tabak (Istanbul University Cerrahpasa Medical School, Istanbul), Y. Cakaloglu (Medical Faculty, University of Istanbul, Istanbul) ; Israel: Y. Lurie (Sauraski Medical Center Tel-Aviv); Canada: J. Heathcote (Toronto Western Hospital, Toronto); S.V. Feinman (Mount Sinai Hospital Toronto); S. Greenbloom (General Hospital Etobicoke); Indonesia: D.A. Sulaiman (Ciptomangunkusomo Hospital Jakarta); Singapore: R. Guan (Mount Elizabeth Medical Center Singapore); Malaysia: I. Merican (Institute for Medical Research Kuala Lumpur); China: T.M.K. So (Princess Margaret Hospital, Hong Kong).

REFERENCES

1. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology 2001;120:1009-1022.
2. Mels GC, Bellati G, Leandro G, Brunetto MR, Vicari O, Borzio M, Piantino P, et al. Fluctuations in viremia, aminotransferases and IgM antibody to hepatitis B core antigen in chronic hepatitis B patients with disease exacerbations. Liver 1994;14:175-181.
3. Janssen HL, Brouwer JT, Nevens F, Sanchez-Tapias JM, Craxi A, Hadziyannis S. Fatal hepatic decompensation associated with interferon alfa. European concerted action on viral hepatitis (Eurohep). BMJ 1993;306:107-108.
4. Flink HJ, Sprengers D, Hansen BE, van Zonneveld M, de Man RA, Schalm SW, Janssen HL. Flares in chronic hepatitis B patients induced by the host or the virus? Relation to treatment response during Peg-interferon {alpha}-2b therapy. Gut 2005;54:1604-1609.
5. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. Hepatology 2000;32:635-639.
6. Alexander GJ, Brahm J, Fagan EA, Smith HM, Daniels HM, Eddleston AL, Williams R. Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. Lancet 1987;2:66-69.
7. Peters M, Davis GL, Dooley JS, Hoofnagle JH. The interferon system in acute and chronic viral hepatitis. Prog Liver Dis 1986;8:453-467.
8. Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? Hepatology 2001;34:1021-1026.
9. Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med 1995;333:1657-1661.
10. Nevens F, Main J, Honkoop P, Tyrrell DL, Barber J, Sullivan MT, Fevery J, et al. Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. Gastroenterology 1997;113:1258-1263.
11. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
12. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.
13. Pas SD, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-2901.
14. Schiff ER, Dienstag JL, Karayalcin S, Grimm IS, Perrillo RP, Husa P, de Man RA, et al. Lamivudine and 24 weeks of lamivudine/interferon combination therapy for hepatitis B e antigen-positive chronic hepatitis B in interferon nonresponders. J Hepatol 2003;38:818-826.
15. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-1010.
16. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576-2588.
17. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-1020.
18. Flink HJ, Buster EH, Merican I, Nevens F, Kitis G, Cianciara J, de Vries RA, et al. Relapse after treatment with peginterferon alpha-2b alone or in combination with lamivudine in HBeAg positive chronic hepatitis B. Gut 2007;56:1485-1486.
19. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med

2005;352:2682-2695.
20 van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, Heathcote J, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-424.
21. Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, Han KH, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. Gastroenterology 2007;133:1437-1444.
22. 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.
23.. Perrillo R, Tamburro C, Regenstein F, Balart L, Bodenheimer H, Silva M, Schiff E, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. Gastroenterology 1995;109:908-916.



Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b.

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Gastroenterology 2008; 135(2): 459-467.

7

ABSTRACT

Background and aims

The aim of this study was to evaluate the long-term sustainability of response in HBeAg positive chronic hepatitis B patients treated with peginterferon alpha-2b (PEG-IFN) alone or in combination with lamivudine.

Methods

All 266 patients enrolled in the HBV99-01 study were offered participation in a long-term follow-up (LTFU) study. Patients were treated with PEG-IFN alpha-2b (100µg/week) alone or in combination with lamivudine (100mg/day) for 52 weeks. Initial response was defined as HBeAg negativity at 26 weeks post-treatment. For the LTFU study, patients had one additional visit after the initial study (mean interval 3.0 ± 0.8 years).

Results

Of 266 patients enrolled in the initial study, 172 (65%) participated in the LTFU study. At LTFU, HBeAg and HBsAg negativity were observed in 37% and 11% of 172 patients, respectively. Sixty-four patients were classified as initial responders and 108 as non-responders. Among the initial responders, sustained HBeAg negativity and HBsAg loss were observed in 81% and 30%, respectively. HBV genotype was associated with long-term sustainability of response, with significantly higher rates of HBeAg negativity in genotype A infected initial responders compared to those with genotype-non-A (96% vs. 71%, p=0.02), as well as HBsAg loss (58% vs. 11%, p<0.001).

Conclusions

HBeAg loss after treatment with PEG-IFN alone or in combination with lamivudine is sustained in the majority of patients and is associated with a high likelihood of HBsAg loss, particularly in genotype A infected patients. Therefore, PEG-IFN remains an important treatment option in this era of nucleos(t)ide analogue therapy.

INTRODUCTION

It is estimated that 350 million persons worldwide are chronically infected with hepatitis B virus (HBV).[1] Chronic HBV infection increases the risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC).[2] During their lifetime, about 15% to 40% of (untreated) chronically HBV infected individuals will develop serious complications from liver disease.[3] Despite the availability of a safe and effective vaccine for more than two decades, new infections with HBV remain common and chronic HBV infection still is a major health problem.[4]

Hepatitis B e antigen (HBeAg) loss from serum is usually associated with a marked decrease in serum HBV deoxyribonucleic acid (DNA) concentration and normalization of alanine aminotransferase (ALT) levels.[5] Although reactivation of disease activity may occur in patients with HBeAg negative disease, the occurrence of liver-related complications is rare.[6] Since loss of hepatitis B surface antigen (HBsAg), which is referred to as clearance of HBV infection, can be observed only in a small proportion of patients after antiviral therapy, loss of HBeAg is often used as a surrogate end point.

In recent studies, about 35% of patients lost HBeAg after an one-year course of pegylated interferon (PEG-IFN) therapy.[7-9] Adding lamivudine to PEG-IFN therapy did not result in higher rates of HBeAg loss.[7, 8] It is unknown whether PEG-IFN induced HBeAg loss is sustained over prolonged periods and is associated with favorable long-term outcome. The aim of this study therefore was to evaluate the long-term virological response and clinical outcome in patients treated with PEG-IFN alpha-2b alone or in combination with lamivudine.

METHODS

Participants

All 266 patients enrolled in the HBV99-01 study (42 centers in 15 countries) were eligible for inclusion in this long-term follow-up (LTFU) study.[7] Patients were treated with peginterferon alpha-2b 100µg weekly (PegIntron, Schering-Plough, Kenilworth, NJ, USA) in combination with placebo or lamivudine 100mg daily (Zeffix, GlaxoSmithKline, Greenford, UK) for 52 weeks. After 32 weeks, PEG-IFN alpha-2b dosage was lowered to 50µg per week to prevent early discontinuation of therapy due to side-effects.[7] The primary outcome measure was loss of HBeAg at 26 weeks post-treatment (week 78). For the current LTFU study, patients with loss of HBeAg at week 78 were classified as initial responders. HBeAg relapse was defined as negative serum HBeAg at 26 weeks post-treatment and positive HBeAg at LTFU.

The inclusion and exclusion criteria were reported previously.[7] In short, patients were eligible if they had been HBsAg positive for more than 6 months, were HBeAg positive on two occasions within 8 weeks prior to randomisation, had elevated serum ALT of at least twice the upper limit of normal (ULN), and had serum HBV DNA >1.0 x 10^5 copies/ml. Major exclusion criteria were: antiviral therapy within 6 months prior to randomisation, viral coinfections, pre-existent cytopenia or

decompensated liver disease.

For the LTFU study, patients were re-evaluated by one additional visit at the local participating center. The local investigator assessed clinical signs and symptoms of liver disease, complications of liver disease (hepatocellular carcinoma, ascites, variceal bleeding, encephalopathy or jaundice), liver transplantation, mortality and administration of (other) antiviral therapy after the initial study according to predefined criteria on standardized questionnaires. If the patient had been retreated, local data prior to retreatment were also collected. Patients were enrolled in the LTFU study after they had given informed consent according to standards of the local ethics committees. Follow-up time was calculated from the end of the initial study (week 78) until the visit for the LTFU study. The primary outcome for the LTFU study was sustainability of HBeAg negativity. Secondary outcome measures were HBV DNA <10,000 and <400 copies/ml, ALT normalization, HBsAg negativity, HBeAg relapse and need for retreatment. Patients who were retreated after the initial study were considered non-responders for all categorical outcome measures, except for HBeAg response for which the local test result at the time of restarting antiviral therapy was taken into account.

Laboratory testing

During therapy and post treatment follow-up of the initial study, patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally in accordance with standardized procedures and therefore expressed as times upper limits of normal (ULN). Serology and quantification of serum HBV DNA was performed at the coordinating center in Rotterdam by staff unaware of treatment allocation and outcome at the end of treatment and end of the initial study. For the initial study, serum HBV DNA levels were measured monthly using an in-house developed TaqMan polymerase chain reaction (PCR) assay (lower limit of detection 373 copies/ml) based on the EuroHep standard.[10] Testing for markers of HBV infection (HBeAg, anti-HBe, HBsAg and anti-HBs) was performed with a commercial radioimmunoassay (AxSYM, Abbott, Abbott Park, IL, USA) at week 0, 32, 52 and 78. HBV genotype analysis was performed by INNO-LiPA assay (Innogenetics, Gent, Belgium). Quantification of serum HBV DNA levels for the LTFU study was performed with the Cobas TaqMan HBV assay (Roche Molecular Systems, Branchburg, NJ, USA), with a dynamic range of quantification of 174 - 6.4 x 108 copies/ml (30 - 1.1 x 10^8 IU/ml). Since serum HBV DNA was expressed in copies/ml in the initial study, all HBV DNA measurements for the LTFU study were recalculated to copies/ml (1 IU/ml = 5.8 copies/ml). To assure comparability of the two HBV DNA quantification assays, 40 samples were tested with both assays and the results were compared. There was an excellent correlation between the two assays ($r=0.930$, $p<0.001$) and plotting the difference against the average of the assays showed no significant correlation (Bland-Altman test; $r=0.12$, $p=0.49$), strengthening the conclusion that both assays are comparable in the dynamic range.[11] All initial responders who had serum HBV DNA >5.0 x 10^3 copies/ml after HBeAg loss were tested for the presence of core promoter (A1762T/G1764A) and precore mutations (G1896A) by sequence analy-

sis. Testing for HBeAg, anti-HBe, HBsAg and anti-HBs for the LTFU study was performed with the commercially available Enzyme-Linked Immuno Sorbent Assays (ELISA) of DiaSorin (DiaSorin S.p.A., Saluggia, Italy).

Statistical analysis

For the comparison of frequencies between or within groups, Chi-square test, Fisher's Exact test and McNemar test were used where appropriate. Student's t-test and Mann-Whitney test were used to compare means between groups. The Kaplan-Meier method was used to estimate cumulative response rates, differences between groups were compared by Log Rank testing. Four-year cumulative response rates were used, since an adequate proportion of patients was still at risk at this time point. This method was used in addition to a cross-sectional analysis because the timing of the LTFU visit was different for the patients enrolled. A cross-sectional analysis alone could overestimate or underestimate response rates due to the differences in duration of follow-up after the initial study. Cox regression analysis was used for the identification of factors influencing HBeAg relapse in responders. The hazard ratio (HR) and 95% confidence interval (CI-95%) is given for factors associated with HBeAg relapse. Patients with missing data and patients who were retreated were considered non-responders. A p-value of 0.05 was considered to be statistically significant (all 2-sided). Statistical analysis was performed with the SPSS14.0 program (SPSS Inc., Chicago, IL).

RESULTS

Patients

Of 266 patients from 41 centers participating in the HBV99-01 study, 172 patients (65%) from 28 centers (68%) were enrolled in the LTFU study. The LTFU study overview is shown in figure 1. Patients were followed for a mean period of 3.0 ± 0.8 years (range 1.6 - 5.0) after the end of the initial study. The majority of the 94 patients who were not enrolled in the LTFU study did not participate because the local study site did, for variable reasons, not take part in this study (n=52, 55%) or because the patient was lost to follow-up (n=23, 24%). Three patients died after the initial study, all three were non-responder. Baseline characteristics and outcome at the end of the initial study of patients who did not participate in the LTFU study (n=94) and those who were included in the LTFU study (n=172) were comparable, except for mean baseline ALT (3.7 ± 2.1 vs. 4.7 ± 4.0; p=0.03).

Baseline characteristics and outcome in the initial study

Baseline characteristics and outcome at the end of the initial study for patients enrolled in the LTFU study (n=172) and all patients from the initial study (n=266) are shown in table 1. The patient groups were similar in terms of baseline demographics and disease characteristics. Ninety-one patients treated with PEG-IFN alone and 81 patients treated with PEG-IFN in combination with

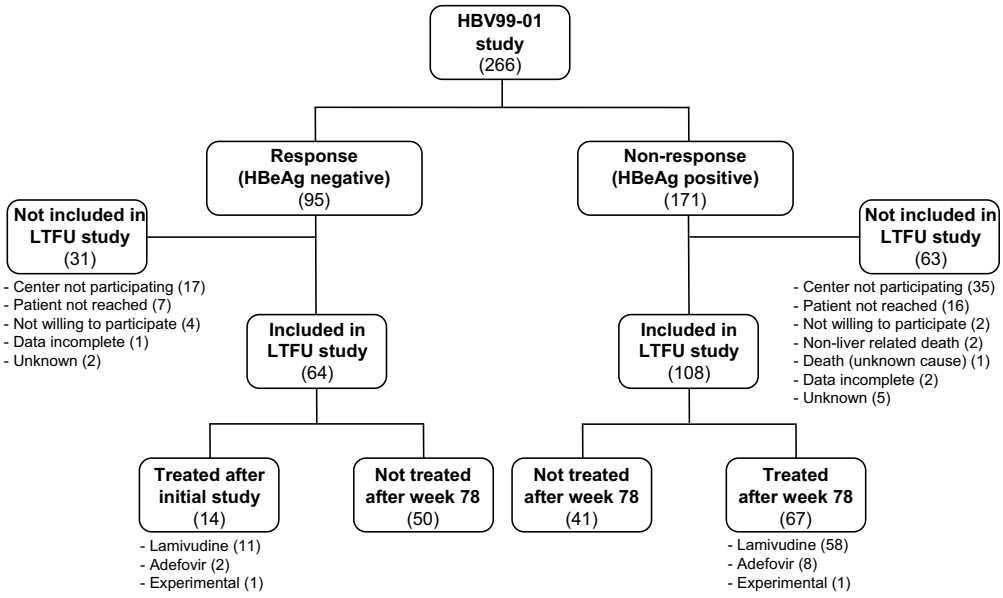


Figure 1: Study overview

This figure shows inclusion of patients from the initial study (HBV99-01 study) in the long-term follow-up (LTFU) study and reasons for not participating for those not enrolled in the LTFU study.

lamivudine were included in the LTFU study. Outcome at the end of the initial study was comparable between the two patient groups.

Sixty-four of 172 patients (37%) were HBeAg negative at the end of the initial study and were classified as initial responders (figure 1). Fourteen of these 64 initial responders (21%) received additional nucleos(t)ide analogue therapy compared to 67 of 108 initial non-responders (62%) ($p<0.001$). Of 14 responders who were retreated, 7 had HBeAg reversion prior to the initiation of nucleos(t)ide analogue therapy, while the remaining 7 remained HBeAg negative but had active disease (high HBV DNA and/or elevated ALT).

HBeAg

Rates of HBeAg negativity at the end of the initial study and at LTFU are shown in figure 2 for patients treated with PEG-IFN and lamivudine combination therapy or PEG-IFN alone. When combining the two groups at LTFU, HBeAg was negative in 63 of 172 patients (37%). In 11 of 41 initial non-responders (27%) who were not retreated, loss of HBeAg occurred after the end of the initial study. HBeAg loss was sustained in 52 of 64 (81%) initial responders (figure 3). By Kaplan-Meier analysis, the 4-year cumulative probability of sustained HBeAg negativity in these patients was 69% (figure 3).

At the end of the initial study, HBeAg seroconversion was observed in 53 of 172 patients (31%). At LTFU, HBeAg seroconversion was observed in 25% and 35% of patients receiving combination

therapy and PEG-IFN monotherapy, respectively (p=0.14). HBeAg seroconversion was sustained in 37 of 53 patients (70%) with HBeAg seroconversion at the end of the initial study.

Table 1. Baseline characteristics and outcome at the 26 weeks post-treatment

	Initial Study (n=266)	LTFU Study (n=172)	p
Baseline (start of treatment):			
Peginterferon monotherapy	136 (51%)	91 (53%)	0.72
Age (mean ± SD)	35.0 ± 12.9	35.5 ± 13.3	0.66
Male	207 (78%)	137 (80%)	0.65
Ethnicity			0.88
- Caucasian	196 (74%)	124 (72%)	
- Asian	53 (20%)	35 (20%)	
- Other	17 (6%)	13 (8%)	
ALT (mean ± SD)	4.3 ± 3.5	4.7 ± 4.0	0.36
Log HBV DNA (mean ± SD)	9.1 ± 0.9	9.0 ± 0.9	0.72
HBV genotype			0.83
- A	90 (34%)	53 (31%)	
- B	23 (9%)	13 (8%)	
- C	39 (15%)	32 (19%)	
- D	103 (39%)	66 (38%)	
- Other	11 (4%)	8 (5%)	
Necroinflammation (median)	5 (1-10)	5 (1-10)	0.91
Fibrosis (median)	3 (0-6)	3 (0-6)	0.95
Previous IFN	55 (21%)	40 (23%)	0.52
Previous lamivudine	33 (12%)	22 (13%)	0.92
26 weeks post-treatment (end of initial study):			
HBeAg loss	95 (36%)	64 (37%)	0.75
HBeAg seroconversion	77 (29%)	53 (31%)	0.68
Log HBV DNA (mean ± SD)	6.8 ± 2.6	6.7 ± 2.6	0.95
HBV DNA <400 copies/ml	21 (8%)	14 (8%)	0.93
ALT (mean ± SD)	2.4 ± 3.6	2.2 ± 2.0	0.47
ALT normalization	92 (37%)	61 (36%)	0.78
HBsAg loss	18 (7%)	12 (7%)	0.93
HBsAg seroconversion	16 (6%)	11 (6%)	0.87

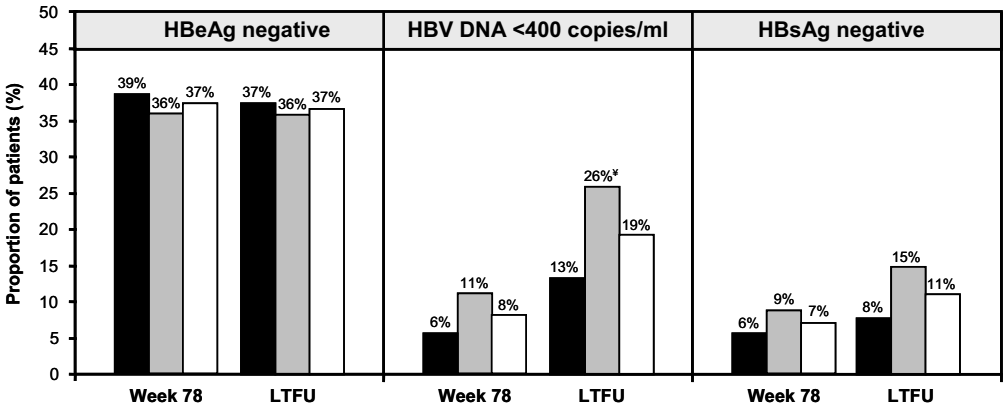


Figure 2: Response rates at the end of the initial study (week 78) and at long-term follow-up (LTFU) in 172 patients included in the LTFU study.

This figure shows the rate of HBeAg negativity, HBV DNA <400 copies/ml, and HBsAg negativity in patients treated with PEG-IFN alone (■, n=91), its combination with lamivudine (▒, n=81) and overall response rates (□, n=172) at the end of the initial study (week 78) and at long-term follow-up (LTFU). Data for the LTFU study were collected after a mean period of 3.0 ± 0.8 years (range 1.6 - 5.0 years) after the end of the initial study (¥p=0.03 for the difference between the two treatment groups).

HBV DNA

At LTFU, 48 (28%) and 33 (19%) of 172 patients had HBV DNA <10,000 and <400 copies/ml, respectively. Patients treated with PEG-IFN and lamivudine significantly more often had HBV DNA <400 copies/ml than those treated with PEG-IFN alone (figure 2) at LTFU, while HBV DNA <10,000 copies/ml was observed equally in both treatment groups (31% vs. 25%, p=0.42). At LTFU, the proportion of patients with HBV DNA <400 copies/ml increased in both treatment groups as compared to the respective proportions at the end of the initial study (p<0.02).

HBV DNA below 10,000 copies/ml was observed significantly more often in patients with sustained HBeAg negativity than in patients who remained HBeAg positive throughout follow-up (67% vs. 6%; p<0.001). Fifty-eight percent of initial responders had HBV DNA <10,000 copies/ml at LTFU and 45% of them had HBV DNA <400 copies/ml (figure 3). By Kaplan-Meier analysis, the 4-year cumulative probability of HBV DNA <10,000 and <400 copies/ml in initial responders was 78% and 52%, respectively (figure 3).

ALT

At LTFU, 52 of 172 patients (30%) had normal ALT, which was similar to the proportion of patients with normal ALT at the end of the initial study (36%; p=0.65). Normal ALT was observed equally in patients treated with combination therapy and those receiving PEG-IFN alone at LTFU (33% vs. 28%, p=0.40). Among the 64 initial responders, 77% percent had normal ALT at LTFU, with a 4-year cumulative rate of 85% by Kaplan-Meier analysis (figure 3).

HBsAg

Although not statistically significant, HBsAg loss was observed almost twice as often in patients treated with PEG-IFN and lamivudine compared to those treated with PEG-IFN alone at LTFU (figure 2; p=0.14). Overall, HBsAg was negative in 19 of 172 patients (11%) participating in the LTFU study (figure 2), including 11 patients who had lost HBsAg by the end of the initial study and 8 patients with HBsAg loss after the end of the initial study. HBsAg seroreversion (HBsAg positive and anti-HBs negative) was not observed among patients with HBsAg seroconversion at the end of the initial study, although one patient with HBsAg loss (but negative anti-HBs) had relapse of HBsAg positivity. Among initial responders, HBsAg was negative in 19 of 64 patients (30%) at LTFU, with a 4-year cumulative probability of 29% by Kaplan-Meier analysis (figure 3).

HBV genotype

Response rates at LTFU for initial responders with genotypes A-D are given in figure 4, five patients with other genotypes (two with genotype E, two with genotype F and two with genotype G) were excluded. HBeAg negativity was sustained in 96% of genotype A infected patients compared to 76% those with non-A genotype (p=0.06). Initial responders with genotype A were more likely to maintain serum HBV DNA <10,000 copies/ml compared to those with genotype-non-A (81% vs. 42%, p=0.002). In addition, HBV DNA <400 copies/ml and ALT normalization were observed significantly more often in patients with genotype A than in those with other genotypes (65% vs. 27% and 96% vs. 64%, respectively; p<0.003). Finally, HBV genotype strongly influenced the likelihood of HBsAg negativity at LTFU. Among the 172 patients enrolled, the highest rate of HBsAg negativity of 28% was observed in genotype A infected patients as compared to 3% in those with genotype non-A infection (p<0.001 for genotype A vs. non-A). In 64 initial responders, HBsAg negativity occurred also significantly more often in genotype A infected patients than in those with genotype-non-A infection (58% vs. 6%, p<0.001).

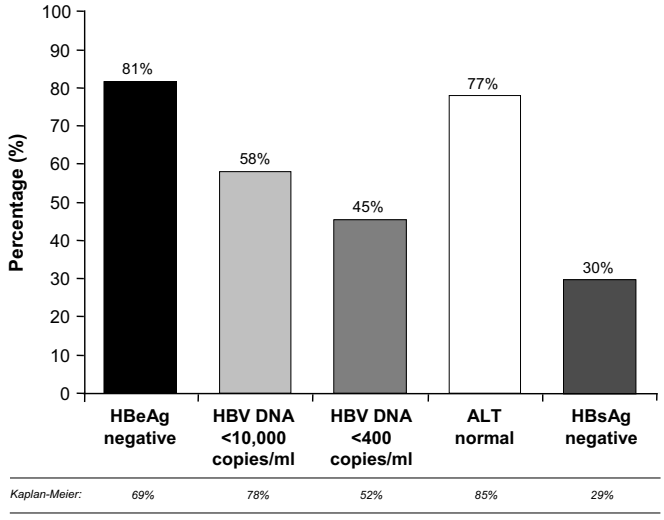


Figure 3: Long-term virological and biochemical response in 64 patients with initial HBeAg response.

This figure shows the proportion of initial responders (patients with HBeAg loss at 26 weeks post-treatment) who had negative HBeAg, HBV DNA <400 copies/ml and negative HBsAg at long-term follow-up. The 4-year cumulative response rate by Kaplan-Meier analysis is given for each of the outcome measures in the table below the figure.

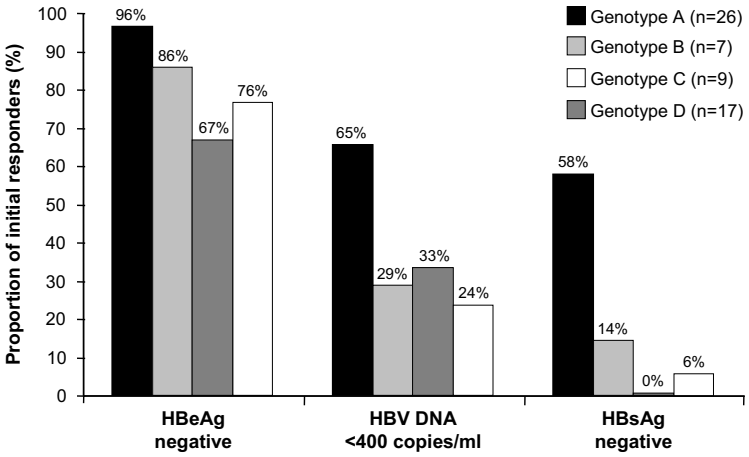


Figure 4: Long-term outcome in initial responders depending on HBV genotype.
This figure shows the rates of HBeAg negativity, HBV DNA <400 copies/ml and HBsAg negativity across HBV genotypes in 64 initial responders (patients with HBeAg loss at 26 weeks post-treatment). A significantly higher rate of HBV DNA <400 copies/ml and HBsAg loss was observed in genotype A infected patients as compared to those with genotype non-A (p<0.003).

Core promoter and precore analysis

Of 64 initial responders, 8 (13%) had a mutation in the core promoter region (A1762T/G1764A) and 12 (19%) had a mutation in precore region (G1896A). Mutations in either the core promoter or precore region were observed in 11 of 27 HBeAg negative patients (41%) who had serum HBV DNA above 1.0 x 10^4 copies/ml at LTFU and in 9 of 37 (24%) patients with HBV DNA below 1.0 x 10^4 copies/ml (p=0.16). The A1762T/G1764A core promoter mutation and the G1896A precore mutation were observed in 19% and 4%, 0% and 43%, 22% and 11%, and 6% and 41% of HBeAg negative patients with genotype A (n=26), B (n=7), C (n=9) and D (n=17), respectively (p=0.04 for the difference of occurrence of these mutations across genotypes).

Predictors of HBeAg relapse

For the prediction of HBeAg relapse, the initial study baseline characteristics and outcome measures at 26 weeks post-treatment were included in a univariate Cox Regression model. Baseline factors associated with an increased risk of HBeAg relapse after the initial study were younger age (0.93 per year increase in age, CI-95% 0.87-0.99; p=0.02) and HBV genotype-non-A (HR 11.84, CI-95%1.50-93.70; p=0.02). Elevated ALT (HR 5.10, CI-95% 1.53-17.03; p=0.008) and higher HBV DNA (HR 1.57 per 1log10 increase, CI-95% 1.21-2.04; p=0.001) at the end of the initial study were also associated with an increased risk of HBeAg relapse. Absence of anti-HBe in serum at the end of the initial study was not associated with HBeAg relapse (HR 1.36, CI-95% 0.37-5.04; p=0.65). Multivariate analysis was not possible due to the limited number of events.

DISCUSSION

This is the first study documenting long-term outcome after treatment with PEG-IFN alone or in combination with lamivudine in chronic hepatitis B patients. We found that 81% of patients with loss of HBeAg at 26 weeks post-treatment remained HBeAg negative after a mean of 3 years. About two-third of sustained HBeAg responders maintained an HBV DNA level <10,000 copies/ml and normal ALT, reflecting inactive disease. An increase in HBsAg negativity was observed in the LTFU study, with 30% of the initial responders being HBsAg negative at long-term follow-up. The highest rates of both sustained HBeAg negativity (96%) as well as HBsAg negativity (58%) were observed in genotype A infected initial responders. Although not all patients from the initial study participated in the long-term study, the LTFU cohort was representative for the entire study population. Duration of follow-up in the LTFU study was calculated from the end of the initial study (26 weeks post treatment), since HBeAg loss within 6 months post-treatment is generally considered to be PEG-IFN induced.[7,8]

Studies on the long-term benefits of treatment with conventional IFN have shown that response is durable in the majority of HBeAg positive patients, with reactivation occurring in only 10-20% after 4-8 years.[12-14] The observed sustained response rate of 81% in our study is thus comparable to those observed in previous studies of conventional IFN in HBeAg positive patients. A preliminary report on sustainability of HBeAg loss after treatment with PEG-IFN alpha-2a showed that 91% of patients with HBeAg loss at 6 months post-treatment maintained this response during another six months of follow-up.[15] Although the sustained response rate in that preliminary study, with only six months of additional follow-up, seems higher than in our study, with three years of follow-up, differences in duration of follow-up between the two studies should be taken into account. Although the rate of HBeAg negativity is stable over time, this group of responders changed over time due to a limited numbers of relapses, as well as a few additional late HBeAg responders. In HBeAg positive patients treated with the nucleoside analogues entecavir or telbivudine HBeAg seroconversion was observed in 29-31% after 2 years of therapy,[16,17] with sustained response in 75-80% of patients at 6 months post-treatment.[18,19] These high sustained response rates appear promising, but long-term studies have to elucidate whether response is durable over prolonged periods since lamivudine-induced HBeAg seroconversion was found to be durable in 46% of patients after 3 years compared to 68% for standard IFN.[20]

We found that in 27 of 64 (42%) responders, PEG-IFN induced HBeAg loss did not result in a transition to the "inactive carrier state", as defined by HBeAg negativity in combination with HBV DNA $<1.0 \times 10^4$ copies/ml and normal ALT.[21] Only 11 of these 27 patients (41%) harbored HBV variants in the precore or core promoter region, which abolish HBeAg production despite persistent active viral replication.[22] Interestingly, the incidence of mutations in the core promoter or precore region was not significantly different in initial responders with HBV DNA $>1.0 \times 10^4$ copies/ml at LTFU and in those with HBV DNA below this level.

We previously showed that anti-HBe negativity at the end of treatment was the strongest predictor

of HBeAg relapse within six months post-treatment.[23] In this study however we found no relation between anti-HBe status at 6 months post-treatment and the risk of HBeAg relapse during LTFU. In patients who still have undetectable HBeAg after a 6-month treatment-free interval, presence of anti-HBe seems thus of less importance for the long-term sustainability of HBeAg negativity.

Studies comparing the outcome of responders to standard IFN versus non-responders found that patients who cleared HBeAg had better survival free of hepatic complications, in particular patients with cirrhosis.[13,14,24,25] In our study, the limited number of hepatic complications precluded valid analysis for these endpoints. Previous studies in IFN treated patients from our group and others that demonstrated improved long-term outcome in responders compared to non-responders typically had a longer duration of follow-up after therapy than our study.[13,15]

Delayed clearance of HBsAg has been observed in 12-65% of patients within 5 years after IFN-induced HBeAg loss, although this seems to occur particularly in non-Asian populations.[12,14,24-29] In HBeAg negative patients treated with PEG-IFN alpha-2a, 6% of responders had loss of HBsAg and appearance of anti-HBs at 2 years post-treatment.[30] In these HBeAg negative patients, loss of HBsAg occurred significantly more often in genotype A infected patients (28%) than those with genotype D (0%; p=0.05).[30] In our study, HBsAg loss was observed in 11% of the overall group and in 30% of the initial HBeAg responders. High HBsAg loss rates were observed in genotype A infected patients (28%), while HBsAg loss hardly occurred in those with genotype B, C or D infection (3%). PEG-IFN should therefore particularly be considered as first line therapy in genotype A infected HBeAg positive patients, while for the other genotypes the potential risks and benefits should be more carefully balanced.

A caveat of the current study was the fact that patients had their additional LTFU visit at different time points after the initial study. The observed response rates at long-term follow-up may therefore overestimate or underestimate actual response rates. However, Kaplan-Meier estimates of 4 year cumulative HBeAg and HBsAg response seem comparable to observed response rates at LTFU. Furthermore, in retrospect a relatively low dose of PEG-IFN alpha-2b was may have been used in this study (100µg/week starting dose, reduced to 50µg/week after 32 weeks).

Adding lamivudine to PEG-IFN therapy has been reported to result in higher response rates at the end of therapy, but not after treatment discontinuation.[7,8] We also found that HBeAg loss occurred equally in patients treated with PEG-IFN alone and those with added lamivudine, However, patients in the combination therapy group were more likely to have undetectable HBV DNA compared to patients treated with PEG-IFN alone at LTFU. Adding lamivudine to PEG-IFN therapy might thus be beneficial in the long-term. Future studies with long duration of follow-up after treatment with PEG-IFN and newer (more potent) nucleos(t)ide analogues may clarify this observation. Since lamivudine was discontinued after one year of therapy both in this study as in other large randomized studies investigating the effects of added lamivudine to PEG-IFN therapy,[7,8,30] it is unknown whether continuing a nucleos(t)ide analogue after PEG-IFN may be beneficial in the long-term.

We conclude that HBeAg response to PEG-IFN alpha-2b alone or in combination with lamivudine is durable in the majority of patients and is associated with an increase in HBsAg loss. This study

further emphasizes the importance of HBV genotype in PEG-IFN therapy, also on the long-term, with clearance of HBsAg in more than a quarter of genotype A infected patients but rarely in those with other genotypes.

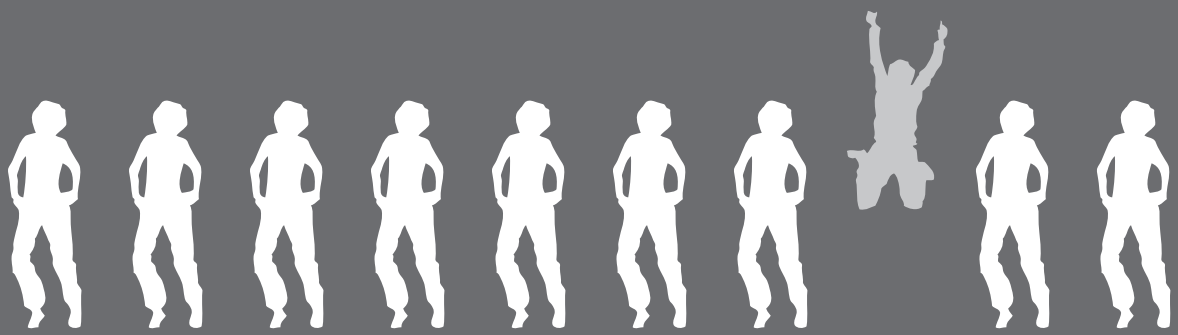
ACKNOWLEDGEMENTS

Members of the HBV99-01 LTFU study group include: Belgium - F Nevens, E de Wit (University Hospital Gasthuisberg, Leuven); PP Michielsen, R Braspenning (University Hospital, Antwerp); H van Vlierberghe, A de Geyter (University Hospital, Gent). Canada - EJ Heathcote, D Kaznowski (Toronto Western Hospital University Health Network, Toronto); S Bojarski (Liver Disease Unit, Mount Sinai Hospital, Toronto). Denmark - LR Mathiesen, G Kronborg, L Rosenørn (Copenhagen University Hospital, Hvidovre). Germany - G Gerken, S Bein (University Hospital, Essen). Greece - G Kitis (George Papanikolaou General Regional Hospital,Thessaloniki). Italy - GB Gaeta (Ospedale Gesù e Maria, Napoli); F D'Antona, G Montalto (Università di Palermo, Palermo). Malaysia - S Sharmila (Hospital Selayang, Institute for Medical Research, Kuala Lumpur). Netherlands - E Verhey, G Ramdjan, LA van Santen, A Heijens, M Ouwendijk, J Francke, S Diepstraten-Pas (Erasmus MC University Medical Center, Rotterdam); CMJ van Nieuwkerk, C van Doorn (VU University Medical Center, Amsterdam); J Drenth (University Medical Centre Radboud, Nijmegen); JW den Ouden-Muller (St Franciscus Hospital, Rotterdam); RA de Vries, P van Embden (Rijnstate Hospital, Arnhem). Poland - R Flisiak (Medical University of Bialystok, Bialystok). Singapore - R Guan (Mount Elizabeth Medical Center, Singapore). Spain - M Buti (Hospital General Universitari Pall d'Hebron, Barcelona); M Rodriguez, (Hospital Central de Asturias, Oviedo). Turkey - H Simsek (Hacettepe University Faculty of Medicine, Ankara).

REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
2. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-45.
3. Bosch FX, Ribes J, Cleries R, et al. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005;9:191-211.
4. Escorsell A, Mas A, de la Mata M. Acute liver failure in Spain: Analysis of 267 cases. Liver Transpl 2007;13:1389-95.
5. Di Bisceglie AM, Waggoner JG, Hoofnagle JH. Hepatitis B virus deoxyribonucleic acid in liver of chronic carriers. Correlation with serum markers and changes associated with loss of hepatitis B e antigen after antiviral therapy. Gastroenterology 1987;93:1236-41.
6. Gigi E, Lalla T, Orphanou E, et al. Long Term Follow-Up of a Large Cohort of Inactive HBsAg (+)/ HBeAg (-)/ anti-HBe (+) Carriers in Greece. J Gastrointestin Liver Dis 2007;16:19-22.
7. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-9.
8. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-95.
9. Wong DK, Cheung AM, O'Rourke K, et al. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312-23.
10. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-901.
11. Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard

method is misleading. Lancet 1995;346:1085-7.
12. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-97.
13. van Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-10.
14. Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422-7.
15. Lau GK, Piratvisuth T, Luo KX, et al. Durability of response and occurrence of late response to peginterferon alpha-2a (40KD) one year post-treatment in patients with HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.
16. Gish RG, Chang TT, De Man RA, et al. Entecavir results in substantial virologic and biochemical improvement and HBeAg seroconversion through 96 weeks of treatment in HBeAg(+) chronic hepatitis B patients (study ETV-022). Hepatology 2005;42:267A.
17. Lai CL, Gane E, Hsu CW, et al. Two-year results from the GLOBE trial in patients with hepatitis B: greater clinical and antiviral efficacy for telbivudine (LdT) vs. lamivudine. Hepatology 2006;44:222A.
18. Colonno RJ, Rose RE, Levine S, et al. Entecavir two year resistance update: no resistance observed in nucleoside naive patients and low frequency resistance emergence in lamivudine refractory patients. Hepatology 2005;42:573A.
19. Poynard T, Chutaputti A, Hwang SG, et al. Sustained off-treatment HBeAg response in telbivudine and lamivudine treated HBeAg-positive patients from the GLOBE study. J Hepatol 2007;46:S27.
20. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-4.
21. Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2001;34:617-24.
22. Grandjacques C, Pradat P, Stuyver L, et al. Rapid detection of genotypes and mutations in the pre-core promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity. J Hepatol 2000;33:430-9.
23. Flink HJ, Buster EH, Merican I, et al. Relapse after treatment with peginterferon alpha-2b alone or in combination with lamivudine in HBeAg positive chronic hepatitis B. Gut 2007;56:1485-6.
24. Lau DT, Everhart J, Kleiner DE, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;113:1660-7.
25. Fattovich G, Giustina G, Realdi G, et al. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1997;26:1338-42.
26. Carreno V, Castillo I, Molina J, et al. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. J Hepatol 1992;15:102-6.
27. Korenman J, Baker B, Waggoner J, et al. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.
28. Lin SM, Sheen IS, Chien RN, et al. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-5.
29. Lok AS, Chung HT, Liu VW, et al. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. Gastroenterology 1993;105:1833-8.
30. Marcellin P, Bonino F, Lau GKK, et al. The majority of patients with HBeAg-negative chronic hepatitis B treated with peginterferon alfa-2a (40KD) [Pegasys®] sustain responses 2 years post-treatment. J Hepatol 2006;44:S275.



Early HBeAg loss during peginterferon alpha-2b therapy predicts HBsAg loss - Results of a long-term follow-up study in chronic hepatitis B.

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American Journal of Gastroenterology, in press.

ABSTRACT

Background and aim

Treatment with peginterferon alpha-2b results in HBeAg loss in 36% of patients at 6 months post-treatment. The aim of this study is to determine whether long-term response to peginterferon (PEG-IFN) is dependent on the timing of HBeAg loss.

Methods

Ninety-one patients treated with PEG-IFN alpha-2b alone (100 µg/week) and 81 patients treated with PEG-IFN alpha-2b and lamivudine (100 mg/day) for 52 weeks were enrolled in this study. Patients were initially followed at 4 week intervals and had one additional long-term follow-up (LTFU) visit (mean 3.03 ± 0.77 years after 26 weeks post-treatment).

Results

Of 172 patients included, 78 patients (46%) did not have loss of HBeAg, 47 (27%) lost HBeAg within 32 weeks and 47 patients (27%) had loss of HBeAg after week 32. At LTFU, patients with HBeAg loss <=32 weeks significantly more often had HBV DNA <400 copies/ml than those who lost HBeAg after week 32 (47% vs. 21%, p=0.009). HBsAg negativity was also observed significantly more often in patients with early HBeAg loss (36% vs. 4%, p<0.001). Early HBeAg loss tended to occur more often in patients treated with PEG-IFN and lamivudine combination therapy than in those treated with PEG-IFN alone (35% vs. 21%, p=0.10), as well as HBsAg loss (15% vs. 8%; p=0.14).

Conclusion

Early PEG-IFN induced HBeAg loss results in a high likelihood of HBsAg loss and may be associated with more profound viral suppression during the first 32 weeks of therapy in patients with added lamivudine.

INTRODUCTION

Worldwide more than 2 billion people have evidence of infection with hepatitis B virus (HBV) and chronic hepatitis B affects about 400 million people.[1, 2] It is estimated that between 500,000 and one million people die annually due to HBV associated liver disease, largely because of cirrhosis and hepatocellular carcinoma. Despite the availability of safe and effective vaccines for more than two decades, HBV infection still is a global health problem.[3]

Treatment of chronic HBV infection has considerably improved over the last decade, with currently seven antiviral drugs available.[4] Treatment strategies can be divided into those providing sustained off-treatment response after a finite course of therapy (immunomodulatory drugs) and those aiming at maintaining on-treatment remission (direct antivirals). The effects of interferon (IFN) are predominantly immunomodulatory, but it also has limited direct antiviral effect on HBV. Nucleos(t)ide analogues such as lamivudine, adefovir, entecavir, telbivudine and tenofovir on the other hand are potent inhibitors of HBV replication. IFN induced HBeAg loss is sustained in the majority of responders due to the drug's immunomodulatory effects.[5-7]

Treatment with standard IFN or pegylated IFN (PEG-IFN) results in loss of HBeAg in about one-third of patients.[8-10] Twenty-nine percent of patients treated with PEG-IFN alpha-2b lost HBeAg by the end of an one-year treatment course, increasing to 36% at 6 months post-treatment.[8] Sustained HBeAg loss and HBsAg loss were observed in 81% and 30% of these responders, respectively, after three years of additional follow-up. HBsAg loss was observed significantly more often in genotype A infected responders compared to those with genotype-non-A infection (58% vs. 6%, $p<0.001$).[11] Other factors associated with an increased likelihood of response to PEG-IFN therapy include low initial viral load, high ALT concentrations, absence of previous interferon therapy and low HBeAg level.[8, 12] The aim of this study is to determine whether long-term virological and biochemical outcome is dependent on the timing of HBeAg loss in HBeAg positive patients treated with PEG-IFN alpha-2b.

PATIENTS AND METHODS

Participants and study design

One-hundred-seventy-two of 266 patients (65%) enrolled in the HBV99-01 study were enrolled in this LTFU study.[8, 11] The majority of the 94 patients who were not enrolled in the LTFU study did not participate because the local study site did, for variable reasons, not take part in this study (n=52, 55%) or because the patient was lost to follow-up (n=23, 24%). Informed consent was obtained from each participant and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the human research committee of all participating centers. Patients were treated with PEG-IFN alpha-2b 100µg weekly (PegIntron, Schering-Plough, Kenilworth, NJ, USA) in combination with placebo or lamivudine 100mg daily (Zeffix, GlaxoSmithKline, Greenford, UK) for 52 weeks. After 32 weeks, PEG-IFN a-2b dosage was lowered to 50µg per

week in order to prevent side effects and early treatment discontinuation.

The inclusion and exclusion criteria were reported previously[8, 11] In short, patients were eligible if they had been HBsAg positive for more than 6 months, were HBeAg positive on two occasions within 8 weeks prior to randomisation, had elevated serum ALT of at least twice the upper limit of normal (ULN), and had serum HBV DNA >1.0 x 10^5 copies/ml. Major exclusion criteria were: antiviral therapy within 6 months prior to randomisation, viral coinfections, pre-existent cytopenia or decompensated liver disease.

Patients were followed at four week intervals during the initial study. For the LTFU study, patients were re-evaluated by one additional visit at the local participating center. The local investigator assessed clinical signs and symptoms of liver disease, complications of liver disease (hepatocellular carcinoma, ascites, variceal bleeding, encephalopathy or jaundice), liver transplantation, mortality and administration of (other) antiviral therapy after the initial study according to predefined criteria on standardized questionnaires. If the patient had been retreated, local baseline data prior to retreatment were also collected. Blood samples were obtained for hematology, biochemistry and virology testing. Follow-up time was calculated from the end of the initial study (week 78) until the visit for the LTFU study. Patients were re-evaluated at a mean interval of 3.0 ± 0.8 years (range 1.6 – 5.0) after the end of the initial study (week 78). Patients who were retreated after the initial study were considered non-responders for all categorical outcome measures, except for HBeAg response for which the local test result at the time of restarting antiviral therapy was taken into account. Patients were classified as non-responders if they remained HBeAg positive throughout follow-up. Responders were those with HBeAg loss and were subdivided depending on timing of first evidence of HBeAg loss; within the first 32 weeks of therapy or after week 32. ALT flares were defined as serum ALT of at least two times the nadir value and more than 10 x ULN.[13]

Laboratory testing

During therapy and post treatment follow-up of the initial study, patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally in accordance with standardized procedures and therefore expressed as times upper limits of normal (ULN). Serology and quantification of serum HBV DNA was performed at Erasmus MC in Rotterdam by staff unaware of treatment allocation and outcome at the end of treatment (EOT) and end of the initial study (EOS). For the initial study, serum HBV DNA levels were measured monthly using an in-house developed Taqman polymerase chain reaction (PCR) assay (lower limit of detection 373 copies/ml) based on the EuroHep standard.[14] Quantification of serum HBV DNA levels for the LTFU study was performed with the Cobas TaqMan HBV assay (Roche Molecular Systems, Branchburg, NJ, USA), with a dynamic range of quantification of 30 - 1.1 x 10^8 IU/ml (174 – 6.4 x 10^8 copies/ml). Since serum HBV DNA was expressed in copies/ml in the initial study, all HBV DNA measurements for the LTFU study were recalculated to copies/ml (1 IU/ml = 5.8 copies/ml). The two HBV DNA quantification assays were comparable in the dynamic range.[11] Testing for markers of HBV infection (HBeAg, anti-HBe, HBsAg and anti-HBs) was

performed with the AxSYM radioimmunoassay (Abbott, Abbott Park, IL, USA) at week 0, 32, 52 and 78, and with the commercially available Enzyme-Linked Immuno Sorbent Assays (ELISA) of DiaSorin (DiaSorin S.p.A., Saluggia, Italy) at LTFU. HBV genotype analysis was performed by INNO-LiPA assay (Innogenetics, Gent, Belgium). All initial responders who had serum HBV DNA >5.0 x 10^3 copies/ml after HBeAg loss were tested for the presence of core promoter (A1762T/G1764A) and precore mutations (G1896A) by sequence analysis. Histological scoring was performed according to the modified histological activity index (HAI) by one experienced pathologist, who was unaware of the chronological order of biopsies, treatment allocation and outcome measures.[15]

Statistical analysis

For the comparison of frequencies between or within groups, X^2 test and Fisher's Exact test were used where appropriate. Student's t-test and Mann-Whitney test were used to compare means between groups. The positive predictive value (% response if the test is positive), negative predictive value (% non-response if test is negative), sensitivity (% responders identified by test) and specificity (% non-responders identified by test) of various patient characteristics and outcome measures for long-term HBsAg negativity was determined. The area under the receiver-operating characteristic curve (AUC) was used to assess the predictive value of these factors. Cox regression analysis was used for the identification of factors influencing HBsAg negativity at LTFU. Patients with missing data and patients who were retreated were considered non-responders. A p-value of 0.05 was considered to be statistically significant (all 2-sided). Statistical analysis was performed with the SPSS14.0 program (SPSS Inc., Chicago, IL).

RESULTS

Patients and baseline characteristics

At LTFU, HBeAg and HBsAg negativity were observed in 63 (37%) and 19 (11%) of 172 patients. HBeAg loss was observed in 1%, 2%, 11% and 27% of patients after 4, 12, 24 and 32 weeks of therapy respectively. For HBeAg seroconversion these rates were 1%, 2%, 9% and 17%, respectively. Patients were subdivided by timing of HBeAg loss; 78 (46%) did not have loss of HBeAg at any time point, 47 (27%) had loss of HBeAg within 32 weeks and 47 patients (27%) had loss of HBeAg after 32 weeks of therapy. Why the patients were subdivided like this will be discussed later on. Baseline characteristics of these patient groups are shown in table 1. Twenty-nine patients (17%) had HBeAg seroconversion within 32 weeks and 44 patients (26%) had HBeAg seroconversion after week 32.

Patients with loss of HBeAg within 32 weeks were older, more often acquired HBV infection via sexual or parenteral transmission, had higher baseline ALT levels and more often harboured genotype A than patients who lost HBeAg after week 32 ($p<0.05$). Duration of follow-up was comparable in the three patient groups (3.09 ± 0.77, 3.02 ± 0.84 and 2.95 ± 0.70 years for groups I, II and III, respectively; $p>0.32$). Retreatment with other antiviral drugs was needed in 26% of patients with

Table 1: Baseline characteristics

Characteristic	No (I) (n=78)	HBeAg loss <=32 weeks (II) (n=78)	HBeAg loss >32 weeks (II) (n=47)
Age (mean ± SD)	34.9 ± 14.5	41.2 ± 11.8†	30.9 ± 10.7
Sex (male)	80%	85%	70%
ALT (x ULN; mean ± SD)	3.6 ± 2.5	6.5 ± 5.6‡	4.5 ± 3.1
HBV DNA $\log_{10}$ copies/ml (mean ± SD)	9.1 ± 1.1	8.9 ± 0.8	8.9 ± 1.0
Route of transmission			
Perinatal	32%	13%	34%
Sexual or parenteral	21%	38%	19%
Unknown	47%	49%	47%
Genotype			
A	22%	60%†	17%
B	8%	6%	9%
C	26%	11%	15%
D	41%	21%	51%
Other	4%	2%	9%
Necroinflammation (mean ± SD)	4.5 ± 2.1	6.0 ± 2.3	4.6 ± 1.8
Fibrosis (mean ± SD)	2.4 ± 1.6	3.1 ± 1.4	2.6 ± 1.4
Combination therapy	40%	60%	47%

ULN = upper limit of normal; † p<0.001 for group II vs. III; ‡ p<0.05 for group II vs. III

HBeAg loss within 32 weeks, in 30% of those with HBeAg loss after week 32 and in 71% of patients without HBeAg loss (p<0.001). Lamivudine resistance was observed in 9 of 81 (11%) of patients in the combination therapy group (with a rate of 4%, 9% and 19% among patients with HBeAg loss <=32 weeks, HBeAg loss >32 weeks and no HBeAg loss, respectively [p=0.15])

HBeAg

The proportion of patients in each of the patient groups that was negative for serum HBeAg at various time points is shown in figure 1A. At LTFU, serum HBeAg was still undetectable in 36 of 47 patients (77%) who were HBeAg negative within 32 weeks compared to 27 of 47 patients (57%) who lost HBeAg after week 32 (p=0.05). At LTFU, HBeAg negativity with seroconversion to anti-HBe was observed in 43% and 30% of patients with HBeAg loss within or after 32 weeks of therapy, respectively (p=0.20). HBeAg seroconversion was sustained in 16 of 29 patients (55%)

who seroconverted within 32 weeks compared to 18 of 44 patients (41%) with HBeAg seroconversion after week 32 (p=0.23). Early HBeAg loss tended to occur more often in patients treated with PEG-IFN and lamivudine combination therapy than in those treated with PEG-IFN alone (35% vs. 21%, p=0.10), while HBeAg loss after week 32 occurred equally in both treatment groups (27% vs. 28%, respectively). Among patients who were HBeAg negative at 26 weeks post-treatment, core promoter and precore mutations were observed equally among patients who lost HBeAg within 32 weeks compared to those with HBeAg loss after week 32 (31% vs. 32%, p=0.89).

HBsAg

In figure 1B, the proportion of patients with loss of HBsAg at various time points is shown for the three patient groups. At LTFU, HBsAg negativity was observed in 17 of 47 (36%) patients with loss of HBeAg within 32 weeks compared to 2 of 47 (4%) patients who lost HBeAg after week 32 and none of 78 (0%) without HBeAg loss (p<0.001). Twelve of 19 patients (63%) with HBsAg loss at LTFU also developed anti-HBs and 18 of them (95%) had undetectable HBV DNA. There was no significant difference in HBsAg loss rate between patients with HBeAg loss after week 32 and those who remained HBeAg positive (p=0.14). HBsAg loss was observed in 12 of 29 patients (41%) and 6 of 44 patients (14%) with HBeAg seroconversion within or after 32 weeks, respectively (p=0.007). Anti-HBs was detectable in 12 of 19 patients (63%) with HBsAg loss. All but one of the HBsAg negative patients had HBV DNA <400 copies/ml at LTFU (the remaining patient had serum HBV DNA of 1.3×10^3 copies/ml). At LTFU, HBsAg loss was observed in 15% of patients treated with PEG-IFN and lamivudine compared to 8% of those treated with PEG-IFN alone (p=0.14). In both treatment groups virtually all patients who were HBsAg negative at LTFU lost HBeAg within 32 weeks of therapy (92% and 86% of patients with HBsAg loss receiving combination therapy or monotherapy, respectively).

HBV DNA

Figure 1C shows the proportion of patients with HBV DNA <400 copies/ml for the three patient groups at week 32, 52 and 78, and at LTFU. At all time points, patients with loss of HBeAg within 32 weeks significantly more often had HBV DNA <400 copies/ml compared to patients with loss of HBeAg after week 32 (p<0.03). At LTFU, HBV DNA <10,000 copies was observed in 5% of patients without HBeAg loss, 55% of patients with HBeAg loss within 32 weeks and 38% of patients with HBeAg loss after week 32 (p<0.005 for HBeAg loss within or after week 32 vs. no HBeAg loss).
Mean HBV DNA levels over time in the three patient groups are shown in figure 2. At the start of treatment, mean serum HBV DNA was comparable in the three patient groups (see table 1). While mean serum HBV DNA was significantly lower in patients with HBeAg loss within 32 weeks than in those with loss of HBeAg after week 32 during therapy (3.14 ± 0.96 vs. 5.82 ± 2.41 and 3.11 ± 1.03 vs. 5.82 ± 2.41 $\log_{10}$ copies/ml at week 32 and 52, respectively; p<0.001), the difference was no longer observed at week 78 (5.07 ± 2.65 vs. 6.07 ± 2.33 $\log_{10}$ copies/ml; p=0.08).
In both treatment groups decline in HBV DNA at week 32 was significantly more profound in patients

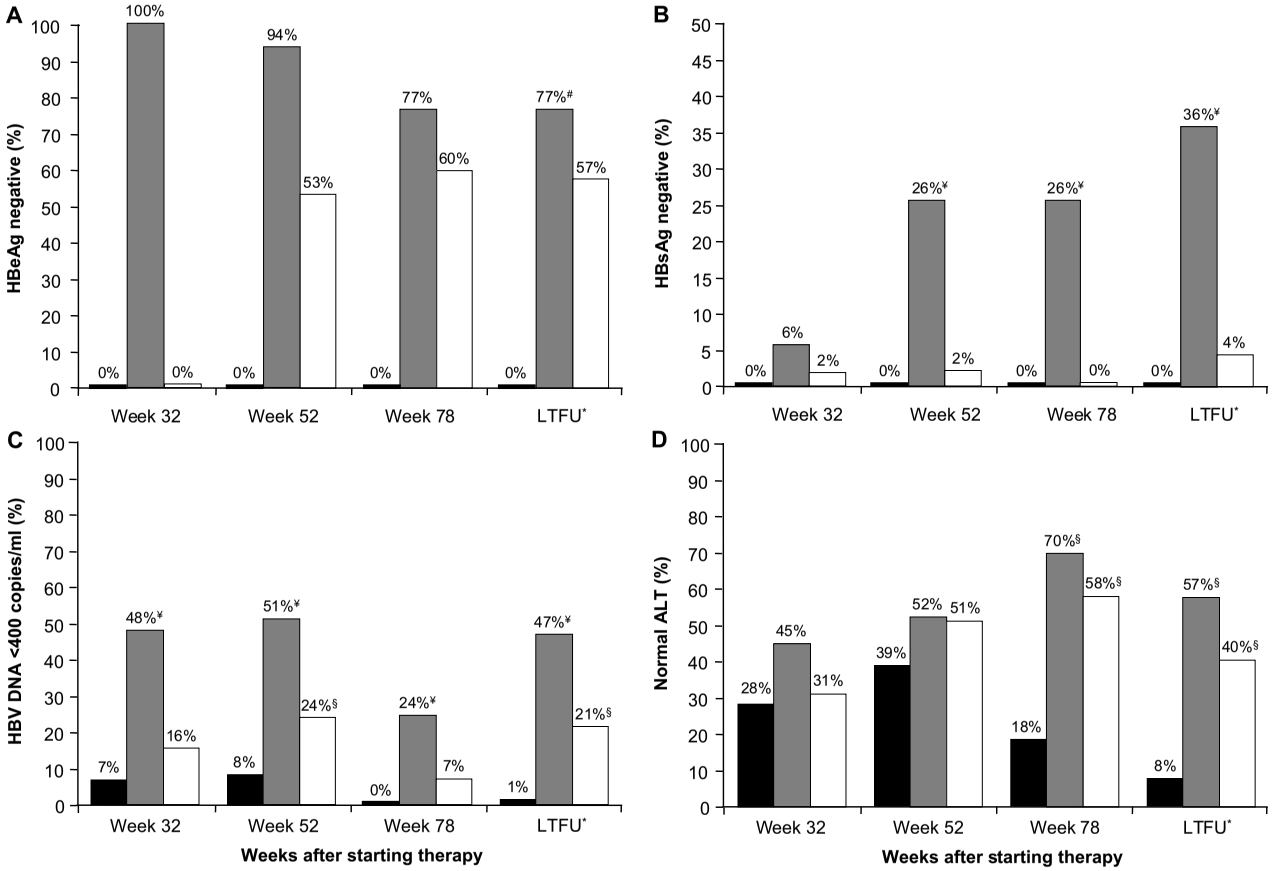


Figure 1: Outcome after 32 and 52 weeks of therapy, at 26 weeks post-treatment and at long-term follow-up.

This figure shows the proportion of patients with negative HBeAg (A), negative HBsAg (B), HBV DNA <400 copies/ml (C) and normal ALT (D) in patients without HBeAg loss (black, group I; n=78), patients with HBeAg loss within 32 weeks of therapy (grey, group II; n=47) and in patients with HBeAg loss after 32 weeks of therapy (white, group III; n=47). §p<0.02 versus group I; ¥p<0.03 vs. group I or III; #p=0.05 vs. group III.

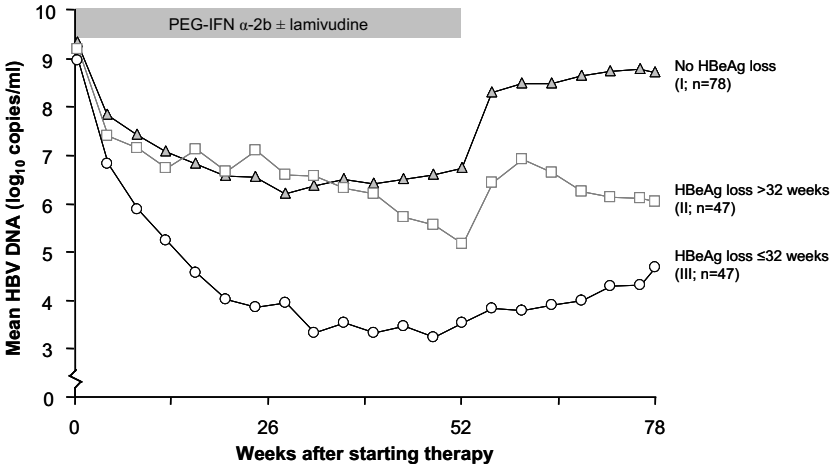


Figure 2: Mean HBV DNA levels over time depending on time of HBeAg loss.
Baseline serum HBV DNA was comparable in patients without HBeAg loss (group I), patients with HBeAg loss within 32 weeks of therapy (group II) and in patients with HBeAg loss after 32 weeks of therapy (group III). Patients in group II had significantly lower HBV DNA than patients in group I at week 32, 52 and 78 (p<0.001). Patients in group III had significantly lower HBV DNA than patients in group I at week 52 and 78 only (p<0.001). At week 32 and 52, patient in group II had significantly lower HBV DNA than those in group III (p<0.001), while mean HBV DNA tended to different in these patient groups at week 78 (p=0.08).

who lost HBeAg within 32 weeks than in those with delayed HBeAg loss, although the difference was most striking in patients receiving PEG-IFN alone (-6.36 vs. -5.02 $\log_{10}$ and -4.95 vs. -1.12 $\log_{10}$ copies/ml in patients receiving combination therapy or monotherapy, respectively; p<0.001).

ALT

The proportion of patients with normal ALT at week 32, 52 and 78, and at LTFU is shown in figure 1D for the three patients groups. During therapy (week 32 and 52), normal ALT was observed equally in patients with HBeAg loss (within or after 32 weeks) and in those who did not have loss of HBeAg. At 26 weeks post-treatment (week 78) and at LTFU, normal ALT was observed significantly more often in patients with loss of HBeAg within 32 weeks or after week 32 compared to those without HBeAg loss (p<0.02). However, there was no difference in the proportion of patients with normal ALT in patients with loss of HBeAg within 32 weeks and those with HBeAg loss after week 32 at any time point. The occurrence of ALT flares was not associated with early HBeAg loss (p=0.63).

Predictors of HBsAg negativity at LTFU

On Cox regression analysis older age, HBV genotype A infection, HBeAg negativity at week 4 and week 32, and HBV DNA clearance at week 24 and week 32 were found to be predictors of HBsAg negativity at LTFU (table 2). The occurrence of ALT flares did not predict HBsAg loss at LTFU (p=0.15). When correcting for age, HBV genotype and week 32 HBV DNA level by multivariate Cox

Table 2: Factors influencing long-term HBsAg negativity on Cox regression analysis

Variable	Hazard ratio	95% CI lower	95% CI upper	p
Baseline:				
Age (per 10 year increase)	1.63	1.20	2.24	0.002
ALT (x ULN)	1.00	0.90	1.13	0.91
HBV DNA (log_{10} copies/ml)	1.29	0.75	2.24	0.36
HBV genotype				
A	1.00			
B	0.22	0.03	1.71	0.15
C	0.00	0.00	-	0.97
D	0.06	0.01	0.44	0.006
Treatment allocation				
PEG-IFN monotherapy	1.00			
Combination therapy	1.53	0.58	4.02	0.39
Week 4:				
HBeAg loss	12.43	1.63	95.07	0.02
HBeAg seroconversion	12.43	1.63	95.07	0.02
HBV DNA <400 copies/ml	0.05	0.00	-	0.84
Week 12:				
HBeAg loss	2.42	0.32	18.29	0.39
HBeAg seroconversion	2.42	0.32	18.29	0.39
HBV DNA <400 copies/ml	0.05	0.00	-	0.85
Week 24:				
HBeAg loss	1.51	0.43	5.25	0.52
HBeAg seroconversion	1.69	0.48	5.90	0.41
HBV DNA <400 copies/ml	5.44	1.99	14.90	0.001
Week 32:				
HBeAg loss	27.38	6.28	119.34	<0.001
HBeAg seroconversion	9.36	3.62	24.17	<0.001
HBV DNA <400 copies/ml	10.09	3.49	29.20	<0.001
Timing of HBeAg loss				
>32 weeks	1.00			
>=32 weeks	9.19	2.09	40.36	<0.001
No HBeAg loss	0.00	0.00	-	0.92

Table 3: Predictive value of baseline factors and outcome at week 32 for HBsAg negativity in the long-term

Outcome measure	Positive Predictive Value	Negative Predictive Value	Sensitivity	Specificity	AUC
Baseline:					
Genotype A	28%	97%	79%	75%	0.77
Timing of HBeAg loss:					
Week 4	100%	90%	5%	100%	0.53
Week 12	25%	89%	5%	98%	0.52
Week 24	17%	90%	16%	90%	0.53
Week 32	36%	98%	89%	80%	0.85
Timing of HBeAg seroconversion:					
Week 4	100%	90%	5%	90%	0.53
Week 12	25%	89%	5%	89%	0.52
Week 24	20%	90%	16%	92%	0.54
Week 32	41%	95%	63%	89%	0.76
Timing of HBV DNA clearance:					
Week 4	0%	89%	0%	99%	0.50
Week 12	0%	89%	0%	99%	0.50
Week 24	30%	91%	32%	91%	0.61
Week 32	32%	95%	61%	84%	0.73

AUC = area under the (receiver operating characteristic) curve

regression analysis, HBeAg at week 32 was found to independently predict HBsAg loss (HR 13.15, 95%-CI 1.32-131.09).

We investigated the predictive value of HBV genotype and of various outcome measures at week 32 for achieving HBsAg negativity at LTFU (table 3). HBeAg negativity at week 32 was found to be the best predictor of HBsAg negativity at LTFU (AUC 0.85, 95%-CI 0.76 - 0.94), with a sensitivity, specificity, positive predictive value and negative predictive value of 89%, 80%, 36% and 98%, respectively. As indicated by the negative predictive value of 98%, virtually all patients who were HBeAg positive at week 32 remained HBsAg positive throughout follow-up.

DISCUSSION

In this long-term prospective study of PEG-IFN alpha-2b alone or in combination with lamivudine in HBeAg positive patients we showed that early loss of HBeAg is associated with a higher rate of undetectable HBV DNA by PCR assay and a higher likelihood of HBsAg loss compared to HBeAg loss later during therapy or after therapy. About one-third of patients who lost HBeAg within 32 weeks of therapy was negative for serum HBsAg at LTFU, thereby having a 9-fold higher chance of HBsAg loss compared to patients with HBeAg loss after week 32. Prediction of long-term outcome based on either HBeAg status or HBV DNA level at an earlier time point was not possible.This is the first study which shows that early HBeAg loss during PEG-IFN therapy is associated with an increased likelihood of HBsAg loss. HBV genotype A was common among patients with early HBeAg loss, but accounted for only 60% of the cases with early response. A recent study by Hou et al. also showed that genotype A infected patients were more likely to clear HBeAg from serum early during IFN therapy than patients harbouring other genotypes.[16] Other baseline factors associated with early HBeAg loss in our study were high ALT levels and older age. High ALT is a well known predictor of response to PEG-IFN.[8, 12] Age above 50 years has been described to be associated with HBsAg loss in lamivudine-treated patients.[17] Results of a study investigating outcome after PEG-IFN alpha-2a in HBeAg positive patients showed that patients with loss of HBeAg within 24 weeks of therapy were more likely to have serum HBV DNA <10,000 copies/ml at 24 weeks post-treatment compared to patients with loss of HBeAg after week 24 (79% vs. 59%).[18] Interpretation of this finding was hampered by the fact that patients with HBeAg loss after week 24 had a relatively shorter duration of follow-up after HBeAg loss. Our study, which has a longer duration of follow-up compared to this previous study, shows that also in the long-term, patients with early HBeAg loss still have a higher chance of HBeAg negativity, undetectable HBV DNA (<400 copies/ml) and HBsAg negativity than those with delayed HBeAg loss. Obviously, duration of HBeAg negativity was somewhat shorter for patients with HBeAg loss after week 32 as compared to those with early HBeAg loss in our study as well. However, since the difference in response rates between patients with HBeAg loss after week 32 and those with early HBeAg loss remained stable, these differences will most likely prevail if duration of follow-up after first evidence of HBeAg loss was comparable. In addition, in retrospect, a relatively low dosage of PEG-IFN alpha-2b may have been used in our study (100 ìg/wk starting dosage, reduced to 50 ìg/wk after 32 weeks).HBeAg loss was more durable in patients who lost HBeAg within 32 weeks compared to those with HBeAg loss after week 32. Among patients with HBeAg loss after week 32, only 57% were still HBeAg negative at LTFU. Immune control over the virus may therefore be more profound in patients with early HBeAg loss, of whom 77% was still HBeAg negative at LTFU. Interestingly, the incidence of mutations in the core promoter or precore region in early responders who remained HBeAg negative was comparable to that observed in patients with HBeAg loss after week 32. Patients with added lamivudine tended to have higher rates of early HBeAg loss and HBsAg loss compared to those treated with PEG-IFN alone. Decline in HBV DNA was most profound in these patients. In previous studies of PEG-IFN in HBeAg positive chronic hepatitis B with a shorter duration of follow-up,

these benefits of added lamivudine were not observed.[8, 9, 19] In nucleoside analogue treated HBeAg positive patients, low serum HBV DNA after six months of therapy was also associated with higher rates of long-term virological response and a lower risk of antiviral resistance.[20] Patients who had HBV DNA below 300 copies/ml after 24 weeks of telbivudine or lamivudine therapy had a significantly higher likelihood of achieving HBeAg seroconversion, normalization of ALT and undetectable HBV DNA at year 2 of therapy compared to patients with higher HBV DNA levels. In HBeAg positive patients treated with standard IFN, a profound decline in serum HBV DNA during the first weeks of therapy was associated with a higher likelihood of HBeAg loss.[21] We also found that low HBV DNA at week 32 of PEG-IFN therapy was strongly associated with long-term HBsAg negativity on Cox regression analysis. The magnitude of early HBV suppression by antiviral drugs is thus associated with long-term virological response in chronic hepatitis B infected patients treated with either IFN-based therapy or nucleos(t)ide analogues. A recent study in PEG-IFN treated patients showed that decline in HBeAg level more accurately predicted HBeAg loss than decline in HBV DNA.[22] However, so far quantitative HBeAg measurement is not routinely available in many institutions.

We found that early HBeAg loss independently predicted long-term HBsAg negativity. The overall test performance for the prediction of HBsAg negativity was best for HBeAg loss at week 32 compared to baseline factors and other outcome measures at week 32, although negative predictive value was good for these other factors as well. With a negative predictive value of 98%, testing for HBeAg at week 32 is particularly useful to identify those who will most likely not achieve HBsAg negativity after a course of PEG-IFN therapy. The rate of HBsAg negativity may still increase with longer duration of follow-up, as has been previously observed in studies of standard IFN in chronic HBV.[5, 6, 23-28] Delayed clearance of HBsAg has been observed in up to 65% of patients within 5 years after IFN-induced HBeAg loss. In conclusion, early HBeAg loss during PEG-IFN therapy was associated with higher rates of sustained HBeAg and HBV DNA negativity in the long-term. We found that HBsAg loss occurred in about one-third of patients with early HBeAg los, virtually all patients who lost HBeAg after week 32 remained HBsAg positive. These findings are of interest for the therapeutic management of HBV infection, since they may contribute to enhance predictive values of early outcome measures during PEG-IFN therapy in chronic hepatitis B. In addition, we showed that more profound viral suppression during the first 32 weeks of therapy in patients with added lamivudine may have led to higher rates of early HBeAg loss and HBsAg loss compared to treatment with PEG-IFN alone. These findings may reopen the perspective of combination therapy of PEG-IFN and other nucleos(t)ide analogues in chronic hepatitis B.

ACKNOWLEDGEMENTS

Other members of the HBV99-01 LTFU study group include: Belgium - E de Wit (University Hospital Gasthuisberg, Leuven); PP Michielsen, R Braspenning (University Hospital, Antwerp); H van Vlierberghe, A de Geyter (University Hospital, Gent). Canada – D Kaznowski (Toronto Western Hospital University Health Network, Toronto); SV Feinman, S Bojarski (Liver Disease Unit, Mount Sinai Hospital, Toronto). China – TMK So (Princess Margaret Hospital, Hong Kong). Denmark - LR Mathiesen, G Kronborg, L Rosenørn (Copenhagen

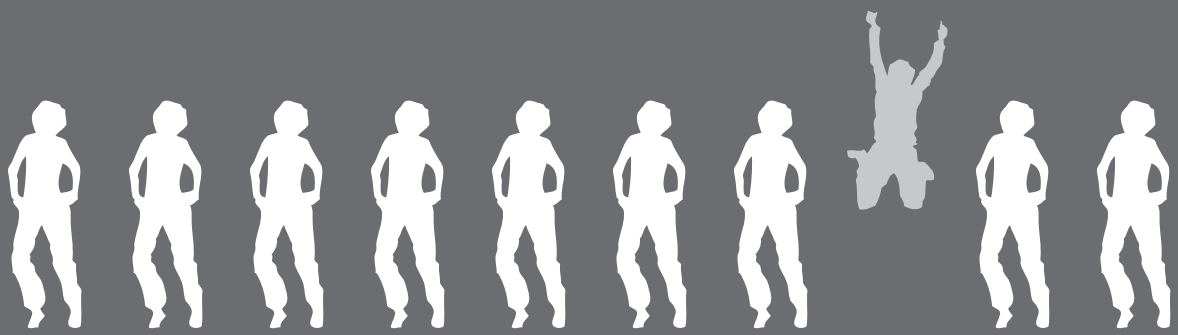
University Hospital, Hvidovre). Germany – S Bein (University Hospital, Essen); J Trojan (Johann Wolfgang Goethe University Medical Center, Frankfurt a.M.). Italy - GB Gaeta (Ospedale Gesù e Maria, Napoli); F D'Antona, G Montalto (Università di Palermo, Palermo). Netherlands – W Tielemans, G Ramdjan, LA van Santen, A Heijens, M Ouwendijk, J Francke (Erasmus MC University Medical Center, Rotterdam); CMJ van Nieuwkerk, C van Doorn (VU University Medical Center, Amsterdam); J Drenth (University Medical Centre Radboud, Nijmegen); JW den Ouden-Muller (St Franciscus Hospital, Rotterdam); P van Embden (Rijnstate Hospital, Arnhem). Poland – K Simon (Medical University, Wroclaw); T Mach (Jagiellonian University School of Medicine, Kraków); R Flisiak (Medical University of Bia³ystok, Bia³ystok). Singapore - R Guan (Mount Elizabeth Medical Center, Singapore). Spain – M Rodriguez, (Hospital Central de Asturias, Oviedo). Turkey – US Akarca (Ege University Faculty of Medicine, Izmir); Y Cakaloglu (Istanbul University Medical School, Istanbul); F Tabak, H Senturk (Istanbul University Cerrahpasa Medical School, Istanbul).

REFERENCES

1. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335-352.
2. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
3. Poland GA, Jacobson RM. Clinical practice: prevention of hepatitis B with the hepatitis B vaccine. N Engl J Med 2004;351:2832-2838.
4. Papatheodoridis GV, Manolakopoulos S, Dusheiko G, Archimandritis AJ. Therapeutic strategies in the management of patients with chronic hepatitis B virus infection. Lancet Infect Dis 2008;8:167-178.
5. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-397.
6. 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.
7. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, Schalm SW, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-810.
8. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.
9. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.
10. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312-323.
11. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459-467.
12. Cooksley G, Lau GKK, Liaw YF, Marcellin P, Chow WC, Thongsawat S, Gane E, et al. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol 2005;42:S30.
13. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
14. Pas SD, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time

detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-2901.

15. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.
16. Hou J, Schilling R, Janssen HL, Hansen BE, Heijtink R, Sablon E, Williams R, et al. Genetic characteristics of hepatitis B virus genotypes as a factor for interferon-induced HBeAg clearance. J Med Virol 2007;79:1055-1063.
17. Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Yatsuji H, et al. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol 2007;79:1472-1477.
18. Piratvisuth T, Lau GKK, Marcellin P, Chow WC, Cooksley G, Fried MW, Paik SW, et al. Association between HBeAg seroconversion and sustained HBV DNA suppression in patients treated with peginterferon alfa-2a (40KD) for HBeAg-positive chronic hepatitis B. J Hepatol 2006;44:S23.
19. Cooksley WG, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, et al. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.
20. Di Bisceglie A, Lai CL, Gane E, Chen YC, Thongsawat S, Wang Y, Chen Y, et al. Telbivudine GLOBE trial: Maximal early HBV suppression is predictive of optimal two-year efficacy in nucleoside-treated hepatitis B patients. Hepatology 2006;44:230A-231A.
21. van der Eijk AA, Niesters HG, Hansen BE, Heijtink RA, Janssen HL, Schalm SW, de Man RA. Quantitative HBV DNA levels as an early predictor of nonresponse in chronic HBe-antigen positive hepatitis B patients treated with interferon-alpha. J Viral Hepat 2006;13.
22. Fried MW, Piratvisuth T, Lau GK, Marcellin P, Chow WC, Cooksley G, Luo KX, et al. HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. Hepatology 2008;47:428-434.
23. Carreno V, Castillo I, Molina J, Porres JC, Bartolome J. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. J Hepatol 1992;15:102-106.
24. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1997;26:1338-1342.
25. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-634.
26. 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.
27. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-975.
28. Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. Gastroenterology 1993;105:1833-1838.



HBV immunohistochemistry in HBeAg positive patients treated with peginterferon alpha-2b alone or its combination with lamivudine.

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Submitted.

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ABSTRACT

Background and aims

It is unknown whether peginterferon (PEG-IFN) and lamivudine combination therapy is superior to PEG-IFN monotherapy in reducing intrahepatic expression of hepatitis B virus core and surface antigen (HBcAg and HBsAg), and how this correlates to regular treatment endpoints.

Methods

Patients who had paired liver biopsies taken before and directly after 52 week of treatment with PEG-IFN alpha-2b alone (n=51) or its combination with lamivudine (n=48) were studied. The degree of expression of HBcAg and HBsAg in hepatocytes was expressed as a proportion of the immunolabelled cells.

Results

In patients receiving PEG-IFN plus lamivudine there was a significant reduction in the proportion of HBcAg and HBsAg positive hepatocytes after therapy compared to baseline ($p<0.001$), but not in those treated with PEG-IFN alone ($p>0.09$). After therapy intrahepatic HBcAg and HBsAg were undetectable in 67% and 75%, and 14% and 29% of patients in the monotherapy and combination therapy group, respectively ($p=0.67$ and $p=0.16$). Baseline and post-treatment HBsAg expression significantly correlated with serum HBV DNA level (Spearman's rho 0.38 and 0.49, respectively; $p<0.001$). Absence of HBsAg expression in hepatocytes after therapy was strongly associated with both HBeAg and HBsAg loss. HBeAg and HBsAg loss occurred in 76% and 29% of patients who did not have intrahepatic HBsAg expression after therapy.

Conclusion

PEG-IFN and lamivudine combination therapy results in a significant decline in intrahepatic HBcAg and HBsAg expression. Clearance of HBsAg from the liver on immunohistochemical staining was strongly associated with loss of HBeAg and HBsAg from serum.

INTRODUCTION

Immunodetection of HBcAg and HBsAg in hepatocytes may provide helpful information about the replicative status of the hepatitis B virus (HBV) and is usually performed as part of a histopathological diagnosis of patients with chronic hepatitis B. The presence of hepatitis B core antigen (HBcAg) in the liver of chronic HBV infected patients detected by immunohistochemical techniques is generally assumed to indicate high viral replication.[1-4] Liver HBcAg can be present in the hepatocyte nuclei and/or cytoplasm. Predominant cytoplasmic localisation of HBcAg has been associated with an active and severe ongoing hepatitis.[1] Pure nuclear HBcAg expression, almost always associated with positive serum HBeAg, on the other hand was rarely associated with chronic active hepatitis.[5] In addition, the degree of expression of HBcAg in hepatocyte nuclei correlated with the level of viral replication.[6]

HBsAg can be detected by immunohistochemistry in liver tissue of both asymptomatic HBV carriers and in persons with chronic active hepatitis B.[7-9] Three patterns of staining, namely, membranous, submembranous, and cytoplasmic, have been described.[10] Expression of HBsAg on the membrane of hepatocytes has been associated with high serum HBV DNA,[11] while cytoplasmic expression was mainly seen in patients with inactive disease.[7]

The aim of this study was to investigate the expression of HBcAg and HBsAg in hepatocytes of HBeAg-positive chronic hepatitis B patients before and after treatment with peginterferon alpha-2b (PEG-IFN alpha-2b) alone or its combination with lamivudine.

METHODS

Patients and study design

Ninety-nine patients were included in this study, all patients were HBeAg positive and had ALT levels of at least two times the upper limit of normal (ULN).[12] Serum HBV DNA was >1.0 x 10^5 copies/ml. Fifty-one patients were treated with PEG-IFN alpha-2b alone (100 µg/week) and 48 patients were treated with PEG-IFN alpha-2b and lamivudine (100 mg/day) combination therapy, all for 52 weeks. Post-treatment follow-up lasted 26 weeks. Major exclusion criteria were antiviral therapy within 6 months prior to randomization, serum antibodies against hepatitis C virus (HCV), hepatitis delta virus (HDV) or human immunodeficiency virus (HIV), pre-existent leucopenia (white blood cell count <3,000/mm3, neutrophil count <1,800/mm3) or thrombocytopenia (platelets <100,000/mm3), or decompensated liver disease.

Laboratory testing

Patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally in accordance with standardized procedures and therefore expressed as times the ULN. Serum HBV DNA levels were measured monthly using an in-house developed Taqman PCR assay (lower limit of detection 373 copies/ml) based on the

Eurohep standard.[13] HBeAg and HBsAg (AxSYM, Abbott, Abbott Park, IL, USA) were assessed at week 0, 32, 52 (end of treatment) and week 78 (end of follow-up). HBV genotype and YMDD mutation analysis were performed by INNO-LiPA assay (Innogenetics, Gent, Belgium).

Liver histology

Liver biopsies were taken in all patients before the start of therapy and directly after therapy (week 52). If a liver biopsy was taken less than 1 year before the start of therapy, no new pre-treatment biopsy was required. All biopsies were blinded and scored by one experienced liver pathologist. Necroinflammation and fibrosis were scored according to the Ishak system.[14] Expression of HBcAg and HBsAg in hepatocytes was studied by the avidin-biotin immunoperoxidase method. The degree of expression of HBcAg and HBsAg in the hepatocyte nucleus or cytoplasm was expressed as a proportion of the immunolabelled cells, without consideration for the staining intensity of individual hepatocytes. The results were scored on a scale of 0 to 5, with values corresponding to positivity rates of 0%, 1% to 10%, 11% to 25%, 26% to 50%, 51 to 75% and >75%, respectively.[6] The patterns of the intracellular distribution of HBcAg were classified as follows: pure nuclear, pure cytoplasmic and mixed nuclear and cytoplasmic expression. Three patterns of HBsAg staining were identified: pure cytoplasmic, pure (peri)membranous and mixed cytoplasmic and (peri)membranous expression. Negative control experiments were carried out by substituting the primary antibody with phosphate-buffered saline.

Statistical analysis

Differences in degrees of expression of HBcAg and HBsAg in hepatocytes before and after therapy was analyzed by the Wilcoxon rank sum test. The difference in the pattern of HBsAg and HBsAg expression before and after therapy was analyzed by the marginal homogeneity test. Correlation of levels of HBV DNA and ALT in sera with the degrees of expression of HBcAg and HBsAg in hepatocytes was assessed by Spearman rank correlation. A p-value of 0.05 was considered statistically significant (all two-sided).

RESULTS

Of 99 patients included in this study, 51 were treated with PEG-IFN alone and 48 with PEG-IFN and lamivudine combination therapy. Baseline characteristics of the two treatment groups are shown in table 1. The groups were comparable, except for baseline fibrosis score, which was higher in patients treated with PEG-IFN and lamivudine combination therapy.

HBcAg expression

The degree of HBcAg expression before and after PEG-IFN therapy is shown in figure 1a. There was a significant reduction in the proportion of HBcAg positive hepatocytes after therapy compared to baseline in the combination therapy group ($p<0.001$), but not in patients treated with PEG-IFN

alone (p=0.19). There was no significant difference in the proportion of patients without HBcAg expression in the liver between the two treatment groups after therapy (figure 1a). The pattern of HBcAg expression did also not differ between the two treatment groups (table 2).

Table 1: Baseline characteristics

Parameter	PEG-IFN + placebo (n=51)	PEG-IFN + lamivudine (n=48)
Male sex	77%	81%
Age (mean ± SD)	32.1 ± 10.2	35.2 ± 12.5
ALT x ULN (mean ± SD)	4.5 ± 2.9	4.5 ± 2.9
HBV DNA $\log_{10}$ copies/ml (mean ± SD)	9.2 ± 0.8	9.1 ± 0.9
Genotype		
A	32%	33%
B	10%	9%
C	18%	17%
D	40%	41%
Necroinflammation (median, range)	5 (1-9)	5 (2-9)
Fibrosis (median, range)	2 (0-5)	3 (0-6)#

#p<0.05

HBsAg expression

The degree of HBsAg expression before and after PEG-IFN therapy is shown in figure 1b. Overall, there was a significant decline in intrahepatic HBsAg expression after therapy compared to baseline (p<0.001 for the trend); absence of intrahepatic HBsAg expression was observed in 6% of patients at baseline and 21% of patients after therapy. There was a significant increase in the proportion of patients without intrahepatic HBsAg expression among those receiving combination therapy (p<0.001). A similar trend was observed in patients receiving PEG-IFN alone (p=0.09). There were no significant differences in the degree or pattern of HBsAg expression between the two treatment groups before and after therapy (figure 1b and table 2).

HBV immunohistochemistry and serum HBV DNA

The mean decline in serum HBV DNA at week 52 was 2.2 ± 2.3 $\log_{10}$ copies/ml in patients treated with PEG-IFN alone and 5.7 ± 2.4 $\log_{10}$ copies/ml in those with added lamivudine (p<0.001). At baseline, the degree of HBsAg expression weakly correlated with serum HBV DNA level (Spearman's rho 0.38 for the entire group, p<0.001), but the degree of HBcAg expression did not (Spearman's rho 0.07 for the entire group, p=0.50). After therapy, there was a strong correlation between the degree of HBsAg expression and serum HBV DNA levels in patients treated with combination of PEG-IFN and lamivudine (Spearman's rho 0.51, p<0.001), and to a lesser extend in patients receiving PEG-IFN alone (Spearman's rho 0.35, p=0.01). Post-treatment HBcAg expression did

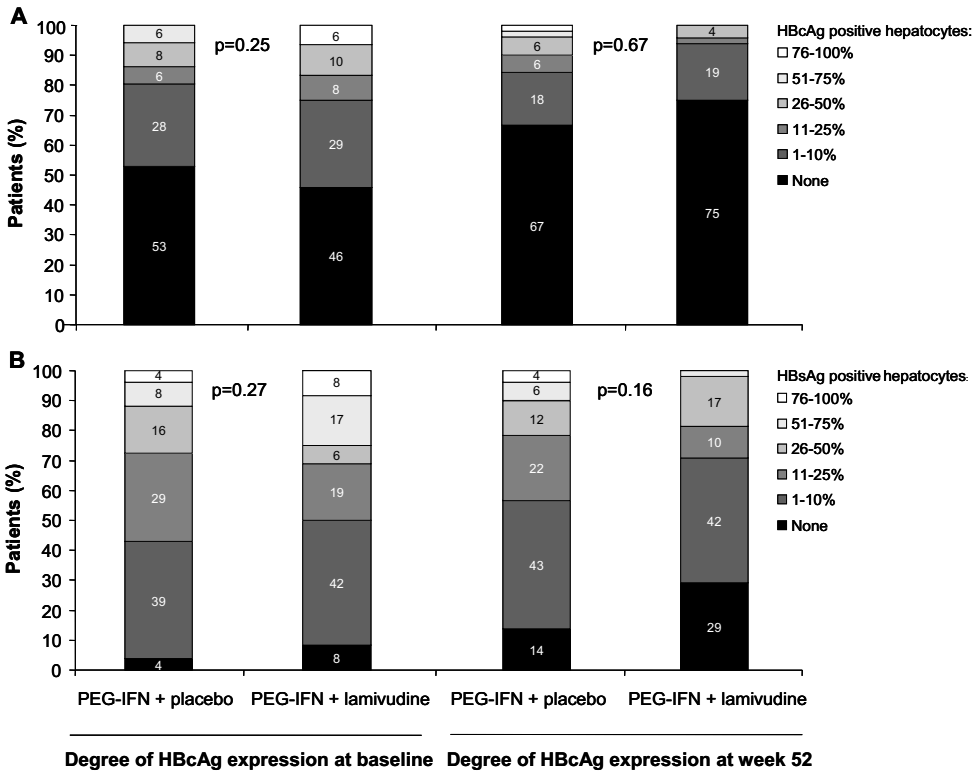


Figure 1: Degree of intrahepatic HBcAg and HBsAg expression at baseline and after therapy.
This figure shows the degree of HBcAg (A) and HBsAg (B) expression at baseline and after therapy in 51 patients treated with peginterferon alpha-2b (PEG-IFN) alone and 48 patients treated with PEG-IFN and lamivudine combination therapy. There were no significant differences between the two treatment groups.

not significantly correlate with serum HBV DNA level (Spearman's rho 0.13 for the monotherapy group and ñ 0.26 for the combination therapy group, respectively, p=0.08 and p=0.36).

HBV immunohistochemistry and HBeAg loss

At the end of treatment HBeAg loss was observed in 28% of patients treated with PEG-IFN alone and 50% of those with added lamivudine (p=0.02). There was a weak correlation between the degree of HBcAg expression at baseline and HBeAg loss at week 52 among patients receiving PEG-IFN monotherapy (Spearman's rho 0.25, p=0.05), but not in the combination therapy group (Spearman's rho 0.11, p=0.47). Baseline degree of intrahepatic HBsAg expression did not correlate with clearance of HBeAg from serum (Spearman's rho 0.16 for the entire group, p=0.11). Intrahepatic HBsAg expression after therapy did correlate with clearance of HBeAg from serum (Spearman's rho 0.36 for the entire group, p<0.001). HBeAg loss was observed in 16 of 21 patients (76%) who cleared intrahepatic HBsAg and in 22 of 78 patients (28%) without clearance of HBsAg from the liver (p<0.001).

Table 2 : Pattern of HBcAg and HBsAg expression before and after treatment

	PEG-IFN + placebo (n=51)	PEG-IFN + lamivudine (n=51)	P
Pattern of HBcAg expression			
Pre-treatment			0.91
None	53%	46%	
Pure nuclear	16%	19%	
Pure cytoplasmic	12%	13%	
Mixed	20%	23%	
Post-treatment			0.45
None	67%	75%	
Pure nuclear	14%	17%	
Pure cytoplasmic	16%	2%	
Mixed	14%	6%	
Pattern of HBsAg expression			
Pre-treatment			0.77
None	4%	8%	
Pure cytoplasmic	59%	52%	
Pure (peri)membranous	16%	15%	
Mixed	22%	25%	
Post-treatment			0.18
None	14%	29%	
Pure cytoplasmic	41%	25%	
Pure (peri)membranous	22%	19%	
Mixed	24%	27%	

HBV immunohistochemistry and HBsAg loss

HBsAg loss was observed in 4% of patients treated with PEG-IFN alone and 8% of patients with added lamivudine (p=0.43). Baseline degree of HBcAg and HBsAg expression in the liver did not correlate with clearance of HBsAg from serum at week 52 (Spearman's rho 0.03 and ñ 0.15 for the entire group, p=0.74 and p= 0.14). Intrahepatic HBsAg expression at week 52 significantly correlated with loss of HBsAg from serum (Spearman's rho 0.40, p<0.001). HBsAg loss was observed in 6 of 21 patients (29%) who cleared intrahepatic HBsAg and in none of 78 patients (0%) without clearance of HBsAg from the liver (p<0.001).

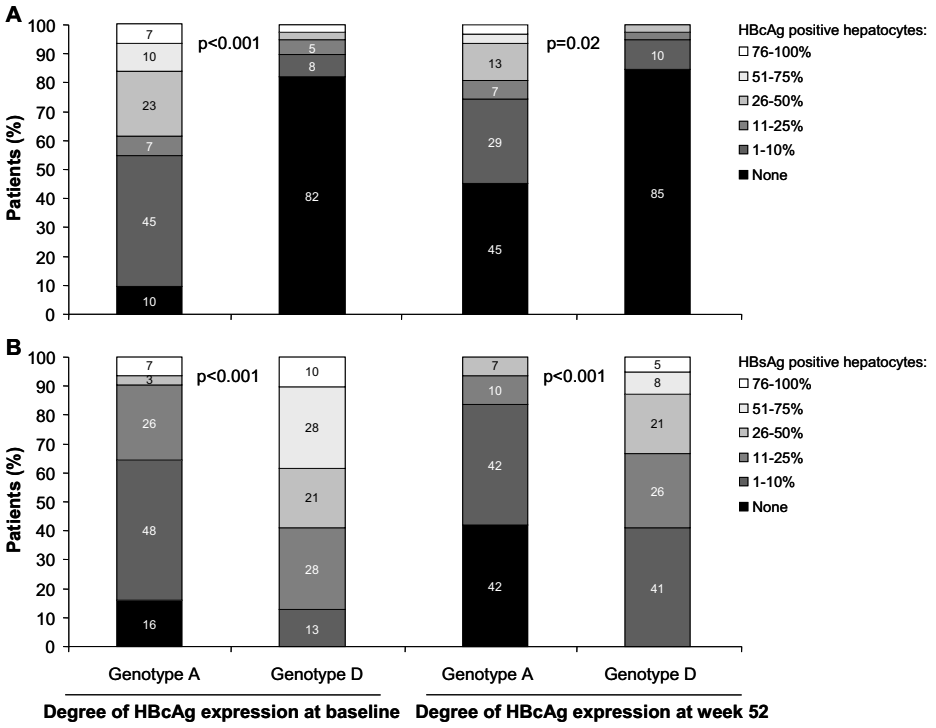


Figure 2: Degree of HBcAg and HBsAg expression at baseline and after therapy in genotype A and D infected patients.

This figure shows the expression of HBcAg (A) and HBsAg (B) before and after treatment with peginterferon alpha-2b alone or its combination with lamivudine in patients with HBV genotype A (n=31) of D (n=39). Because of their limited numbers, patients with HBV genotypes B (n=9) and C (n=17) were not included. HBcAg was more extensively expressed in hepatocytes of HBV genotype A than D infected patients (p<0.001 at baseline and after therapy), while for HBsAg expression it was the other way around (p<0.001 at baseline and after therapy).

HBV genotypes and HBV immunohistochemistry

There were significant differences in immunohistochemical findings between the two most prevalent HBV genotypes in our population (A and D). At baseline, HBcAg was expressed less extensively in HBV genotype D than A infected patients (figure 2a). In contrast, HBV genotype D infected patients had more HBsAg positive hepatocytes in the liver compared to patients with HBV genotype A (figure 2b). HBcAg expression declined in HBV genotype A infected patients after therapy compared to baseline (p=0.01 for the trend), but not in those with HBV genotype D (p=0.25 for the trend). After therapy expression of HBcAg was significantly less extensive in HBV genotype D than A infected patients (figure 2a). There was a decline in the proportion of HBsAg positive hepatocytes among both HBV genotype A and D infected patients after therapy compared to baseline (p<0.04 for the trend). HBsAg expression was still less extensive expressed in HBV genotype A infected patients compared to those with HBV genotype D after therapy (figure 2b), with absent intrahepatic HBsAg in 42% and 0% of patients, respectively (p<0.001).

DISCUSSION

This is first detailed report on HBV immunohistochemistry in patients treated with PEG-IFN. HBcAg was not expressed in the hepatocytes of about half of these HBeAg-positive patients at baseline, while the majority of patients had detectable HBsAg in the liver. Intrahepatic HBcAg expression significantly declined after therapy compared to baseline in patients treated with PEG-IFN and lamivudine therapy, but not in those receiving PEG-IFN monotherapy. Although not statistically significant, clearance of intrahepatic HBsAg expression was observed twice as often in patients receiving PEG-IFN and lamivudine combination therapy than in those receiving PEG-IFN alone. This is important because clearance of HBsAg from the liver was strongly associated with loss of HBeAg and HBsAg from serum.

A recent study in nucleoside analogue treated patients suggested that clearance of intrahepatic HBsAg expression could not be achieved despite profound HBV DNA suppression.[15] In 30 HBeAg negative patients, serum HBV DNA and intrahepatic HBcAg expression significantly decreased after five years of nucleoside analogue therapy compared to baseline. However, no significant reduction in intrahepatic HBsAg expression was observed. Absence of HBsAg expression in hepatocytes was observed in only three patients, all of whom cleared HBsAg from serum. Antiviral agents that directly suppress the activity of the polymerase may thus not directly affect intrahepatic HBsAg, while serum HBV DNA is generally promptly reduced.[16] We found a higher rate of intrahepatic HBsAg clearance in patients treated with PEG-IFN and lamivudine combination therapy than in those treated with PEG-IFN alone (29% vs. 14%), although the difference was not significantly different. We recently also found that patients treated with PEG-IFN and lamivudine seemed to have a higher chance of HBsAg loss from serum compared to patients receiving PEG-IFN alone in the long term.[17] There may thus be an additive effect of lamivudine, although the immunomodulatory effect of PEG-IFN seem crucial to achieve HBsAg clearance at all.

A shift of intracellular HBcAg from nucleus to cytoplasm has been described in the natural course of HBeAg positive chronic hepatitis B. Chu et al. found that HBcAg was expressed at a relatively higher level in the nucleus than in the cytoplasm during the immune tolerance phase, in which there is little or no inflammatory activity in the liver. Pure nuclear HBcAg thus seems to reflect an early phase of chronic HBV infection with little or mild host immune response.[5] In the immune clearance phase there was a shift to cytoplasmic HBcAg or negative HBcAg, which was frequently accompanied by active hepatitis and a high HBeAg seroconversion rate.[6] Hepatocytes with cytoplasmic (or membranous) HBcAg have been stated to be possible targets for immune hepatocytolysis,[8] thereby resulting in more active hepatic inflammation. We found mixed nuclear and cytoplasmic expression of HBcAg in the majority of patients at baseline, while most patients were negative for HBcAg in the liver after PEG-IFN therapy. Pure nuclear expression was most frequently observed in those positive for HBcAg after treatment with PEG-IFN. These findings thus are in concordance with previous findings in similar patient groups.

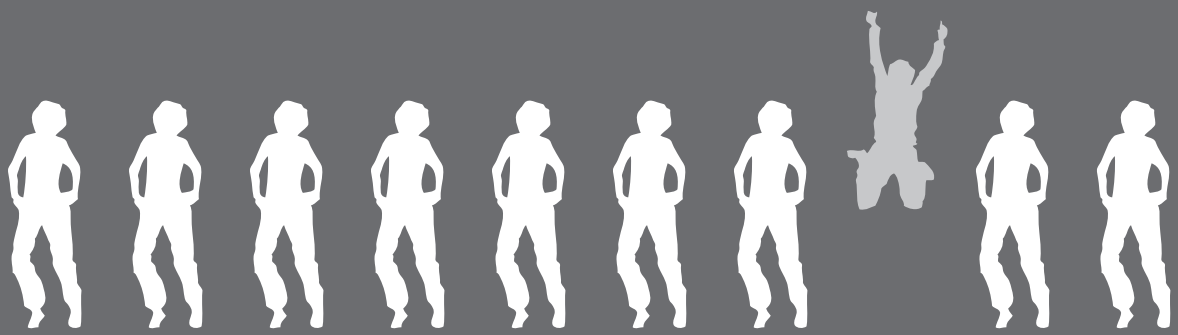
After HBeAg seroconversion, in the immune control phase, HBcAg is usually undetectable,[5] although loss of detectable HBcAg may also be associated with escape mutations occurring during periods of cytotoxic T-cell clearance of virus-infected hepatocytes. The occurrence of these core gene mutations is associated with the presence of precore stop-codon mutations, HBeAg-negative chronic hepatitis B and active liver disease.[18] We previously showed that precore and core-promoter mutations occur more often in HBV genotype D than A infected patients treated with PEG-IFN.[17] Our finding of lower HBcAg expression in hepatocytes of HBV genotype D infected patients is thus in concordance with these findings. In addition, the greater reduction in intrahepatic HBsAg expression in HBV genotype A than D infected patients is also in line with previously reported serum HBsAg clearance rates in these HBV genotype.[17, 19]

In conclusion, we showed that PEG-IFN significantly reduced the proportion of hepatocytes stained positive for HBcAg and HBsAg in patients with HBeAg positive chronic hepatitis B, although there seemed to be a beneficial effect of added lamivudine in terms of intrahepatic HBsAg clearance. Clearance of HBsAg from the liver is important because it was strongly associated with loss of HBeAg and HBsAg from serum.

REFERENCES

1. Chu CM, Lin SM, Liaw YF. Hepatocyte expression of HBcAg and serum HBeAg in hepatitis B: comparison of polyclonal and monoclonal antibodies during a trial of interferon. J Clin Pathol 1991;44:21-24.
2. Hadziyannis SJ, Lieberman HM, Karvountzis GG, Shafritz DA. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg Vs. anti-HBe positive carriers of hepatitis B virus. Hepatology 1983;3:656-662.
3. Perrillo RP, Schiff ER, Davis GL, Bodenheimer HC, Jr., Lindsay K, Payne J, Dienstag JL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. N Engl J Med 1990;323:295-301.
4. Ramakrishna B, Mukhopadhya A, Kurian G. Correlation of hepatocyte expression of hepatitis B viral antigens with histological activity and viral titer in chronic hepatitis B virus infection: An immunohistochemical study. J Gastroenterol Hepatol 2008.
5. Hsu HC, Su IJ, Lai MY, Chen DS, Chang MH, Chuang SM, Sung JL. Biologic and prognostic significance of hepatocyte hepatitis B core antigen expressions in the natural course of chronic hepatitis B virus infection. J Hepatol 1987;5:45-50.
6. Chu CM, Yeh CT, Chien RN, Sheen IS, Liaw YF. The degrees of hepatocyte nuclear but not cytoplasmic expression of hepatitis B core antigen reflect the level of viral replication in chronic hepatitis B virus infection. J Clin Microbiol 1997;35:102-105.
7. Ray MB, Desmet VJ, Bradburne AF, Desmyter J, Fevery J, De Groote J. Differential distribution of hepatitis B surface antigen and hepatitis B core antigen in the liver of hepatitis B patients. Gastroenterology 1976;71:462-469.
8. Chu CM, Liaw YF. Intrahepatic distribution of hepatitis B surface and core antigens in chronic hepatitis B virus infection. Hepatocyte with cytoplasmic/membranous hepatitis B core antigen as a possible target for immune hepatocytolysis. Gastroenterology 1987;92:220-225.
9. Hsu HC, Lai MY, Su IJ, Chen DS, Chang MH, Yang PM, Wu CY, et al. Correlation of hepatocyte HBsAg expression with virus replication and liver pathology. Hepatology 1988;8:749-754.

10. Wee A, Yap I, Guan R. Hepatocyte hepatitis B surface antigen expression in chronic hepatitis B virus carriers in Singapore: correlation with viral replication and liver pathology. J Gastroenterol Hepatol 1991;6:466-470.
11. Chu CM, Liaw YF. Membrane staining for hepatitis B surface antigen on hepatocytes: a sensitive and specific marker of active viral replication in hepatitis B. J Clin Pathol 1995;48:470-473.
12. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.
13. Pas SD, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-2901.
14. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.
15. Hadziyannis SJ, Costamena A, Katsikadelli E, Hadziyannis E. HBV surface and core protein expression in the liver in chronic hepatitis B under long term antiviral therapy. Hepatology 2006;44:546A.
16. Chan HL, Wong VW, Tse AM, Tse CH, Chim AM, Chan HY, Wong GL, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol 2007;5:1462-1468.
17. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459-467.
18. Locarnini S. Molecular virology of hepatitis B virus. Semin Liver Dis 2004;24 Suppl 1:3-10.
19. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol 2006;101:297-303.



Hepatitis B virus genotype is an important predictor of sustained off-treatment response to both peginterferon alpha-2b and entecavir in HBeAg positive chronic hepatitis B.

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Submitted.

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ABSTRACT

Background

Choice of initial antiviral therapy is becoming increasingly important in chronic hepatitis B. Knowledge of the predictors of sustained off-treatment response may facilitate choice of therapy in individual patients.

Methods

Grouped data from 354 patients treated with entecavir and 266 patients treated with peginterferon alpha-2b (PEG-IFN alpha-2b) for one year were evaluated in this retrospective study. Sustained virological response was defined as HBeAg loss and HBV DNA $<7.0 \times 10^5$ copies/ml at the end of treatment and at 6 months post-treatment. Predictors of response were identified by logistic regression analysis.

Results

Sustained virological response was observed in 20% and 17% of patients treated with PEG-IFN alpha-2b and entecavir, respectively. HBV genotype was a predictor of sustained virological response to both PEG-IFN alpha-2b and entecavir (p=0.001 and p=0.03, respectively). In addition, baseline necroinflammatory score predicted response to PEG-IFN alpha-2b (p=0.009), while baseline ALT and HBV DNA were associated with sustained virological response after entecavir therapy (p=0.02 and p=0.02, respectively). Multivariate logistic regression analysis showed that PEG-IFN alpha-2b resulted in higher rates of sustained virological response in genotype A and B infected patients than entecavir. Response rates in genotype C infected patients were comparable, while in genotype D infected patients sustained virological response rates were higher with entecavir.

Conclusion

HBV genotype is an important predictor of sustained response to both PEG-IFN alpha-2b and entecavir. When aiming for sustained off-treatment response, determination of HBV genotype is important in order to choose the optimal antiviral agent.

INTRODUCTION

The introduction of nucleos(t)ide analogues in the 1990's heralded a new era in the treatment of chronic hepatitis B virus (HBV) infection. These drugs provided a safe, effective, and well-tolerated alternative for interferon (IFN). Treatment with IFN-based therapy also significantly improved; the addition of a polyethylenglycol (PEG) molecule to interferon allowed for a more convenient dosing interval of once per week with response rates equal to or higher than those observed with standard IFN.[1] However, treatment with PEG-IFN is still associated with significant side-effects in a large proportion of patients.[2]

A major advantage of IFN-based therapy in HBeAg positive chronic hepatitis B is the long-term sustainability of response of about 80-90% among initial responders due to its immunomodulatory effects.[3-5] Antiviral potency of peginterferon (PEG-IFN) on the other hand is inferior to that of nucleos(t)ide analogues. Nucleos(t)ide analogues directly target the HBV polymerase and treatment with these drugs usually results in a rapid decline of serum HBV DNA levels. Although treatment with nucleos(t)ide analogues profoundly suppresses serum HBV DNA levels and response can be maintained over prolonged periods with ongoing therapy, response to lamivudine does not sustain in a large proportion of patients after discontinuation of therapy. This implicates the necessity of long-term, and maybe indefinite, nucleos(t)ide analogue treatment.[6, 7]

In a recent study of entecavir in HBeAg positive chronic hepatitis B 21% of patients, who had HBeAg loss and HBV DNA <0.7MEq/ml at week 48, discontinued antiviral therapy. At 24 weeks post-treatment, 82% of these patients had a sustained response.[8, 9] A study investigating PEG-IFN alpha-2b alone or in combination with lamivudine in HBeAg positive chronic hepatitis B showed loss of HBeAg in 29% of patients at the end of a one-year treatment course with sustained off-treatment response in most patients.[10]

It is unknown whether the same baseline factors predict sustained response to both PEG-IFN and entecavir. If differences would exist, this may help in choosing what treatment to start in an individual patient.

METHODS

Patients

Data from 354 patients treated with entecavir in the BEHoLD AI463022 study,[11] as well as data from 266 patients treated with PEG-IFN alpha-2b (alone or in combination with lamivudine) in the HBV99-01 study[10] were evaluated in this retrospective analysis. The inclusion and exclusion criteria for both studies have been described previously.[10, 11] In short, patients were eligible for participating in the BEHoLD study if they were 16 years or older, positive for hepatitis B surface antigen (HBsAg) for at least 24 weeks, hepatitis B e antigen (HBeAg) positive, had HBV DNA >=3.0 MEq/ml (about 3.0 x 10^6 copies/ml]), serum ALT 1.3-10 x ULN and evidence of chronic hepatitis on a liver biopsy specimen obtained within 52 weeks prior to randomization. Patients were eligible for participation

in the HBV99-01 study if they were HBsAg positive for at least 6 months, hepatitis B e antigen (HBeAg) positive on two occasions within 8 weeks prior to randomization, had elevated serum ALT of >2-10 x the upper limit of normal (ULN) and had serum HBV DNA above 1.0 x 10^5 copies/ml. Major exclusion criteria for both studies were: antiviral therapy with activity against HBV within 6 months prior to randomization, coinfection with hepatitis C virus (HCV), hepatitis delta virus (HDV) or human immunodeficiency virus (HIV), or signs of decompensated liver disease.

Study design

A retrospective stratified analysis of grouped patient was performed. Sustained virological response was defined as HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml, according the definition of virological response used in the BEHoLD study, both at the end of treatment and at six months post-treatment. Data were stratified by HBV genotype in combination with one of the following baseline patient and disease characteristics: ALT (<2 x ULN, 2-5 x ULN, >=5 x ULN), HBV DNA (<8 $\log_{10}$ copies/ml, 8-9 $\log_{10}$ copies/ml, 9-10 $\log_{10}$ copies/ml, >=10 $\log_{10}$ copies/ml), previous antiviral treatment with interferon alpha or lamivudine (yes, no), weight (<55 kg, 50-80 kg, >80 kg), BMI (<20 kg/m^2, 20-25 kg/m^2, 25-30 kg/m^2, >=30 kg/m^2), and necroinflammatory score (<3, 3-6, 6-9, >=9) and fibrosis score (1-2, 3-4 or 5-6) according to the histological activity index.[12]

Statistics

Statistical analysis was performed using the SPSS 15.0 program (SPSS Inc. Chicago, IL). Predictors of sustained virological response after treatment with PEG-IFN alpha-2b or entecavir were investigated by univariate logistic regression analyses. A direct comparison of sustained response rates to both therapies by Chi-square test could not be performed because the groups were not comparable regarding baseline characteristics. Therefore, response rates after PEG-IFN alpha-2b and entecavir therapy were compared by multivariate logistic regression analysis using HBV genotype together with either baseline ALT, HBV DNA, previous antiviral treatment with interferon alpha or lamivudine, weight, BMI, necroinflammatory score or fibrosis score, as well as the type of antiviral therapy (PEG-IFN alpha-2b or entecavir) and the interactions between the respective factors.

RESULTS

Baseline characteristics of patients treated with PEG-IFN alpha-2b and entecavir are shown in table 1. Although inclusion and exclusion criteria of the two studies were quite similar, there were significant differences in baseline weight, body mass index (BMI), ALT, HBV DNA, necroinflammatory score, fibrosis score and HBV genotypes between the two patient groups. At the end of treatment, virological response, defined as HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml at the end of treatment, was observed in 33.6% and 21.0% of patients treated with PEG-IFN alpha-2b and entecavir, respectively. At 6 months post-treatment, these rates were 20.2% and 17.2%, respectively.

Table 1: Baseline characteristics

Baseline factor	PEG-IFN (n=266)	Entecavir (n=354)	p
Weight			0.001
<55 kg	9%	16%	
55-<80kg	62%	68%	
>=80 kg	29%	17%	
Body mass index			0.04
<20 kg/m2	12%	14%	
20-<25 kg/m2	49%	53%	
25-<30 kg/m2	29%	28%	
>=30 kg/m2	11%	5%	
Alanine aminotransferase			<0.001
<2 x ULN	16%	39%	
2-5 x ULN	59%	45%	
>=5 x ULN	26%	16%	
HBV DNA			<0.001
<8 log10 copies/ml	11%	17%	
8-<9 log10 copies/ml	26%	22%	
9-<10 log10 copies/ml	47%	28%	
>=10 log10 copies/ml	15%	33%	
Genotype			<0.001
A	34%	27%	
B	9%	20%	
C	15%	32%	
D	38%	10%	
Other	4%	11%	
Necroinflammation			<0.001
<3	10%	8%	
3-<6	50%	12%	
6-<9	31%	29%	
>=9	8%	51%	
Fibrosis			<0.001
1-2	37%	64%	
3-4	52%	28%	
5-6	11%	8%	
Previous IFN or lamivudine	27%	14%	<0.001

Baseline ALT

Rates of sustained virological response with different baseline ALT levels are shown in figure 1 for patients treated with PEG-IFN alpha-2b and entecavir separately. Among patients treated with PEG-IFN alpha-2b, higher baseline ALT levels tended to be associated with a higher chance of sustained virological response compared to lower baseline ALT levels (table 2, p=0.09). In entecavir treated patients, sustained virological response rates significantly increased with increasing baseline ALT levels (table 2, p=0.02).

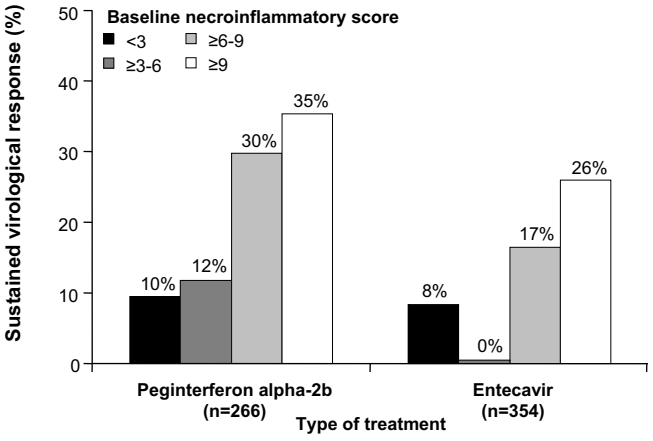


Figure 1:
This figure shows the rate of sustained virological response (HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml at six months post-treatment) depending on baseline ALT level in patients treated with peginterferon alpha-2b or entecavir.

Baseline HBV DNA

Figure 2 shows the rates of sustained virological response after treatment with PEG-IFN alpha-2b or entecavir depending on baseline serum HBV DNA. Although higher rates of sustained virological response after PEG-IFN alpha-2b were observed with lower baseline HBV DNA levels, the differences were not statistically significant (table 2, p=0.24). Lower HBV DNA levels were associated with a higher chance of sustained virological response to entecavir (table 2, p=0.02).

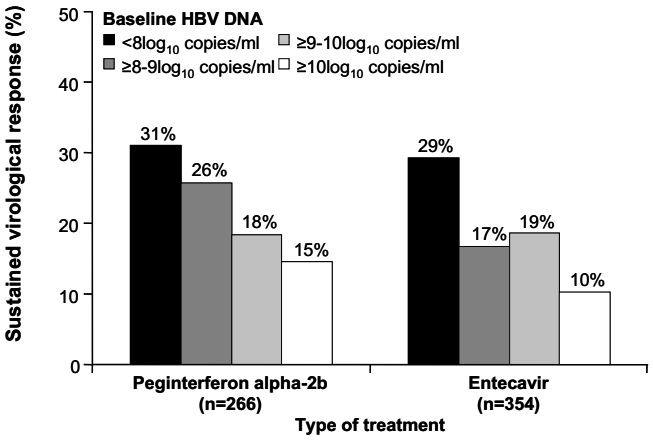


Figure 2:
This figure shows the rate of sustained virological response (HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml at six months post-treatment) depending on baseline HBV DNA level in patients treated with peginterferon alpha-2b or entecavir.

HBV genotype

Sustained virological response rates were different across genotypes in both PEG-IFN alpha-2b and entecavir treated patients (figure 3). Genotype A infected patients had a higher chance of achieving sustained virological response after treatment with PEG-IFN alpha-2b compared to those with genotype C or D infection (table 2, p=0.001). In entecavir treated patients, patients with genotype B had a lower chance of achieving sustained virological response compared to those with genotype A, C or D (table 2, p=0.03). All other comparisons between the different genotypes were not statistically significant in both PEG-IFN alpha-2b and entecavir treated patients.

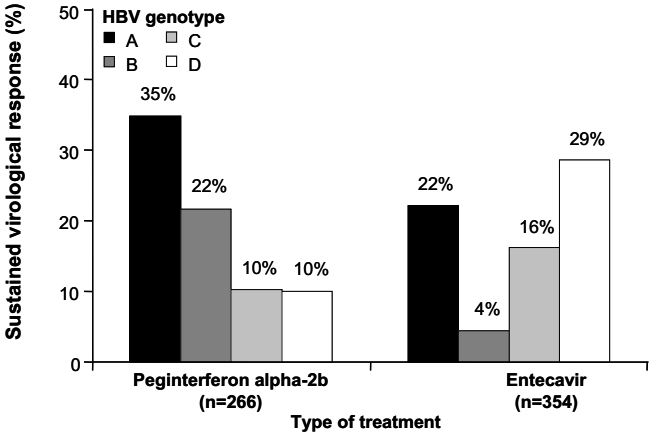


Figure 3:
This figure shows the rate of sustained virological response (HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml at six months post-treatment) depending on HBV genotype in patients treated with peginterferon alpha-2b or entecavir.

Baseline liver histology

Figure 4 shows the rate of sustained virological response depending on baseline necroinflammatory score. In patients treated with PEG-IFN alpha-2b, high baseline necroinflammatory scores were asociated with higher rates of sustained virological response compared to low necroinflammatory activity (table 2, p=0.009). There was no statistically significant difference in sustained virological response rates depending on baseline necroinflammation in entecavir treated patients (table 2,

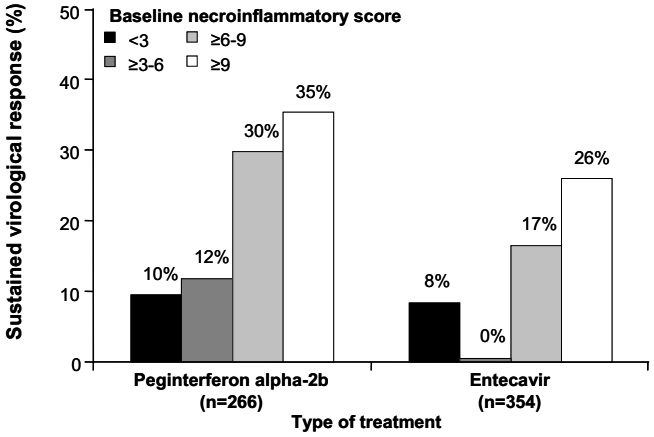


Figure 4:
This figure shows the rate of sustained virological response (HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml at six months post-treatment) depending on baseline necroinflammatory score in patients treated with peginterferon alpha-2b or entecavir.

Table 2: Predictors of sustained virological response after treatment with PEG-IFN alpha-2b or entecavir by univariate logistic regression analysis

	PEG-IFN			Entecavir		
Baseline factor	**OR**	**Lower**	**Upper**	**OR**	**Lower**	**Upper**
ALT						
<2 x ULN	1.00			1.00		
>=2 - <5 x ULN	3.86	1.13	13.26	1.41	0.73	2.73
>=5 x ULN	3.35	0.90	12.46	2.98	1.39	6.37
HBV DNA (copies/ml)						
<8 $\log_{10}$	1.00			1.00		
>=8 - <9 $\log_{10}$	0.77	0.30	2.00	0.48	0.21	1.10
>=9 - <10 $\log_{10}$	0.50	0.20	1.23	0.55	0.26	1.18
>=10 $\log_{10}$	0.38	0.12	1.23	0.28	0.12	0.63
HBV genotype						
A	1.00			6.15	1.75	21.56
B	0.52	0.18	1.53	1.00		
C	0.21	0.07	0.66	4.19	1.19	14.82
D	0.21	0.10	0.46	8.67	2.20	34.11
Other	0.70	0.17	2.84	5.59	1.39	25.54
Previous therapy						
None	1.00			1.00		
Interferon / lamivudine	0.80	0.40	1.60	1.71	0.83	3.51
Weight (kg)						
<55	1.00			1.00		
>=55 - <80	0.73	0.27	1.99	1.37	0.60	3.10
>=80	0.91	0.32	2.65	0.79	0.27	2.35
Body mass index (kg/m²)						
<20	1.00			1.00		
>=20 - <25	1.11	0.42	2.99	1.05	0.45	2.45
>=25 - <30	0.71	0.24	2.11	1.14	0.46	2.85
>=30	2.19	0.68	7.10	0.31	0.04	2.64
Necroinflammation						
<3	1.00			1.00		
>=3 - <6	1.27	0.26	6.13	0.00	0.00	
>=6 - <9	4.01	0.85	18.95	2.17	0.46	10.23
>=9	5.18	0.89	30.25	3.85	0.87	17.08
Fibrosis						
1 or 2	1.00			1.00		
3 or 4	1.30	0.65	2.60	1.65	0.89	3.06
5 or 6	1.09	0.35	3.35	1.59	0.59	4.28

p=0.14). Liver fibrosis stage did not influence the chance of achieving sustained virological response to either antiviral drug, with sustained virological response in 20% and 17%, 24% and 25%, and 21% and 24% of patients with Ishak fibrosis score 1-2, 3-4, and 5-6 treated with PEG-IFN alpha-2b or entecavir, respectively (p=0.76 and 0.24 for PEG-IFN alpha-2b and entecavir, respectively).

Other baseline factors

Baseline weight, body mass index, and previous treatment with interferon or lamivudine were not associated with sustained virological response rates in patients treated with PEG-IFN alpha-2b (table 2, p>0.16), nor in those receiving entecavir (table 2, p>0.14).

Comparison of sustained virological response rates after PEG-IFN alpha-2b and entecavir

Multivariable logistic regression analyses including the type of antiviral therapy (PEG-IFN alpha-2b or entecavir) and HBV genotype plus either ALT, HBV DNA or necroinflammation, and the interaction between these factors showed that the only significant interaction term was that of HBV genotype and antiviral therapy. Odds ratios for the chance of achieving sustained virological response after treatment with PEG-IFN alpha-2b compared to entecavir in the different genotypes after correction for ALT, HBV DNA or necroinflammation are shown in table 3.

Table 3: Likelihood of sustained virological response after treatment with PEG-IFN alpha-2b compared to entecavir

Genotype	Baseline factor corrected for	Odds Ratio¥	95% CI lower	95% CI upper
A	ALT	1.56	0.80	3.05
	HBV DNA	1.75	0.88	3.48
	Necroinflammation	3.19†	1.41	7.19
B	ALT	4.73†	1.02	22.00
	HBV DNA	4.30	0.86	21.51
	Necroinflammation	12.76†	2.55	63.91
C	ALT	0.52	0.16	1.67
	HBV DNA	0.50	0.15	1.60
	Necroinflammation	1.04	0.27	4.05
D	ALT	0.20‡	0.07	0.55
	HBV DNA	0.31‡	0.11	0.83
	Necroinflammation	0.54	0.18	1.58

CI; confidence interval; ˚Sustained virological response; HBeAg negative and HBV DNA <7.0 x 10 ^{5} copies/ml at six months post-treatment; ¥Odds ratio for PEG-IFN alpha-2b compared to entecavir; †Higher chance (p<0.05) of achieving sustained virological response with PEG-IFN compared to entecavir; ‡Higher chance (p<0.05) of achieving sustained virological response with entecavir compared to PEG-IFN

DISCUSSION

This is the first study investigating predictors of sustained response to both PEG-IFN and entecavir. We found that HBV genotype was an important predictor of sustained virological response (HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml at 6 months post-treatment) to treatment with both drugs. In addition, baseline necroinflammatory score predicted response to PEG-IFN alpha-2b and baseline ALT and HBV DNA level were significantly associated with sustained virological response after entecavir therapy.

The inclusion criteria of the two studies involved were quite similar. Still, the baseline characteristics of the two patient groups were different. A direct comparison of response rates in two treatment groups and in subgroups was therefore not possible. Ideally, multivariate logistic regression analysis including all baseline characteristics and type of antiviral treatment would have been performed to correct for differences in baseline factors. However, this was not possible because individual patient data from the entecavir treated patients were unfortunately not made available to us. We therefore performed logistic regression analysis with grouped patient data.

It is well known that HBV genotype is associated with response to IFN-based therapy, with the highest response rates in genotype A infected patients.[10, 13, 14] Although one study showed a higher rate of HBeAg seroconversion in genotype B than genotype C infected lamivudine treated patients, [15] other studies did not find such a relation between HBV genotype and response to either lamivudine, adefovir or entecavir.[16-18] It should be noted that other definitions of response to therapy were used in these studies than the endpoint we used in this study. Off-treatment sustained response is rarely used as an endpoint of nucleos(t)ide analogue therapy since prolonged treatment regimes are generally used. We found a significant interaction between HBV genotype and type of antiviral therapy on multivariate logistic regression analysis, indicating that the chance of sustained response to PEG-IFN alpha-2b or entecavir was different across genotypes. Genotype A and B infected patients had a higher likelihood of sustained virological response to PEG-IFN alpha-2b than entecavir. Patients infected with genotype C responded equally to PEG-IFN alpha-2b and entecavir, while genotype D infected patients had a higher chance of achieving sustained virological response with entecavir than PEG-IFN alpha-2b.

One may question whether the long-term sustainability of response to PEG-IFN alpha-2b and entecavir is comparable. A recent study investigating long-term outcome after treatment with PEG-IFN alpha-2b showed that HBeAg loss was sustained in 81% of initial responders after a mean period of 3 years.[19] The long-term off-treatment sustainability of entecavir-induced HBeAg loss is however still unknown. It has already been shown that the long-term sustainability of HBeAg loss after IFN therapy was significantly higher than of HBeAg loss occurring during lamivudine therapy, with HBeAg relapse in about 40% of lamivudine treated and 20% of IFN treated patients after one year.[4] However, extending lamivudine therapy for several months after HBeAg seroconversion significantly increased long-term sustainability of response compared to stopping lamivudine therapy shortly after HBeAg seroconversion.[15, 20] Therefore, in a recent study comparing telbivudine and

lamivudine for the treatment of HBeAg positive chronic hepatitis B, treatment could be discontinued only if HBeAg seroconversion had been achieved and maintained for at least six months.[21] In that study, 86% of patients with telbivudine induced HBeAg loss were still HBeAg negative at one year post-treatment.[22] These findings suggest that long-term sustained response can be achieved with nucleos(t)ide analogues in HBeAg positive patients, given that an treatment is continued for several months after HBeAg loss. Investigating treatment strategies aimed at sustained off-treatment response to either PEG-IFN or nucleos(t)ide analogues is in our opinion of particular interest because many young HBV infected patients have an indication for antiviral therapy and a significant proportion of them may otherwise unnecessarily receive indefinite nucleos(t)ide analogue maintenance therapy. We conclude that HBV genotype is an important predictor of sustained response after both PEG-IFN alpha-2b and entecavir. Since response to PEG-IFN alpha-2b and entecavir was significantly different across genotypes we recommend to determine HBV genotype prior to antiviral therapy in all patients in whom inducing sustained off-treatment response is pursued. Knowledge of the HBV genotype can then facilitate the choice of optimal antiviral therapy in individual patients. The results of this study warrant a head-to-head comparison of sustained virological response after treatment with PEG-IFN and one of the newest, potent, nucleos(t)ide analogues.

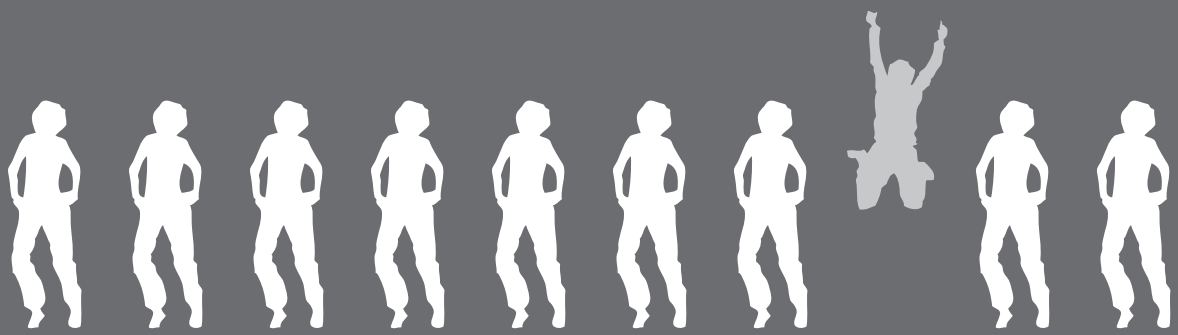
ACKNOWLEDGMENT

We thank Dr. Keith Aizen (Bristol-Myers Squibb, New York, NY, USA) for his support for this study.

REFERENCES

1. Cooksley WG, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, et al. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.
2. van Zonneveld M, Flink HJ, Verhey E, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. Aliment Pharmacol Ther 2005;21:1163-1171.
3. 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.
4. van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, Heathcote J, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-424.
5. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, Schalm SW, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-810.
6. Dienstag JL, Cianciara J, Karayalcin S, Kowdley KV, Willems B, Plisek S, Woessner M, et al. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology 2003;37:748-755.
7. Song BC, Suh DJ, Lee HC, Chung YH, Lee YS. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 2000;32:803-806.
8. Gish RG, Chang TT, De Man RA, Gadano A, Sollano J, Han KH, Zhu J, et al. Entecavir results in

substantial virologic and biochemical improvement and HBeAg seroconversion through 96 weeks of treatment in HBeAg(+) chronic hepatitis B patients (study ETV-022). Hepatology 2005;42:267A.
9. Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, Han KH, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. Gastroenterology 2007;133:1437-1444.
10. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-129.
11. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-1010.
12. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.
13. Hadziyannis S, Lau GKK, Marcellin P, Piratvisuth T, Cooksley G, Bonino F, Chutaputti A, et al. Sustained HBsAg seroconversion in patients with chronic hepatitis B treated with peginterferon alpha-2a (40kDa) (Pegasys®). J Hepatol 2005;42:S178.
14. Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. Hepatology 2002;36:1425-1430.
15. Chien RN, Yeh CT, Tsai SL, Chu CM, Liaw YF. Determinants for sustained HBeAg response to lamivudine therapy. Hepatology 2003;38:1267-1273.
16. Buti M, Cotrina M, Valdes A, Jardi R, Rodriguez-Frias F, Esteban R. Is hepatitis B virus subtype testing useful in predicting virological response and resistance to lamivudine? J Hepatol 2002;36:445-446.
17. Lurie Y, Manns MP, Gish RG, Chang TT, Yurdaydin C, Lai CL, Shouval D, et al. The efficacy of entecavir is similar regardless of disease-related baseline subgroups in treatment of nucleoside-naive, HBeAg(+) and HBeAg(-) patients with chronic hepatitis B. J Hepatol 2005;42:184.
18. Yuen MF, Wong DK, Sablon E, Yuan HJ, Sum SM, Hui CK, Chan AO, et al. Hepatitis B virus genotypes B and C do not affect the antiviral response to lamivudine. Antivir Ther 2003;8:531-534.
19. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. Hepatology 2007;46:667A.
20. Ryu SH, Chung YH, Choi MH, Kim JA, Shin JW, Jang MK, Park NH, et al. Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospective study. J Hepatol 2003;39:614-619.
21. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576-2588.
22. Poynard T, Hou JL, Chutaputti A, Manns M, Naoumov N. Sustained durability of HBeAg seroconversion in chronic hepatitis B patients after treatment with telbivudine. J Hepatol 2008;48:S263-S264.



Doctor to patient transmission of hepatitis B virus: implications of HBV DNA levels and potential new solutions.

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Antiviral Research 2003; 60(2): 79-85.

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SUMMARY

Hepatitis B virus (HBV) infected health care workers (HCWs) can infect patients undergoing exposure prone procedures. Until now reviews have focussed on the problem of the HBeAg positive HCWs. After transmission of HBV by HBeAg negative surgeons, the focus of Public Health policy in the UK and the Netherlands has changed from HBeAg status to serum HBV DNA level.

Viral load and the volume of blood transmitted determine the transmission risk of HBV. We have estimated the number of infectious particles transmitted by needlesticks, in comparison with those attributed in maternal-fetal transfusion. The blood-volume transmitted by needlestick is roughly 1-30% of that of delivery. As vertical transmission with maternal HBV DNA levels below 10^7 copies/ml is rarely documented, HBV transmission by needlesticks is according to our assumptions unlikely to occur with HBV DNA levels below 10^7 copies/ml.

Sera of transmitting HCWs contained HBV DNA levels between 5.0×10^9 and 6.35×10^4 copies/ml, interpretation of these levels is hampered as the sera were taken at least 3 months after transmission. To prevent both loss of expertise and nosocomial transfer, highly viremic HCWs can be offered antiviral therapy. Lamivudine and alpha-interferon can now be complemented with adefovir, tenofovir and entecavir to provide effective new solutions for chronic HBV infected HCWs.

INTRODUCTION

Hepatitis B virus (HBV) is known to have been transmitted by infected health care workers (HCW) to patients undergoing exposure prone procedures (EPP). Worldwide 45 HCW, who transmitted HBV to their patients, have been identified since 1970.[1-8] These 45 cases of doctor-to-patient transmission resulted in 437 hepatitis B infected patients.

The CDC recommendation states that HCWs who are infected with HBV should not perform exposure-prone procedures unless they have sought counsel from an expert review panel and have been advised under what circumstances, if any, they may continue to perform these procedures. Such circumstances would include notifying prospective patients of the HCW's seropositivity before they undergo EPPs.[9] Until 1997 all described cases of HBV transmission to patients involved hepatitis B e-antigen (HBeAg) positive HCWs. Therefore, to prevent patients from being infected, Public Health measures focussed on excluding HBeAg positive HCWs from performing EPPs.

Transmission by HBeAg negative surgeons was first described in 1997.[10] All described cases of HBeAg negative HCWs, who infected patients, involved surgeons bearing precore mutants.[6-8,11] HBV carriers with this variant form of HBV do not produce HBeAg. After the identification of these transmitting HBeAg negative HCWs a more direct measure of infectivity, based on HBV DNA levels, was required.

The 2000 NHS Health Service Circular defines criteria necessary for the conduct of exposure prone procedures by HBV carriers whose serum does not contain HBeAg. For a HBeAg negative

HCW to be permitted to perform EPP their HBV DNA level must be below 10^3 copies/ml. The UK and Eire exclude all HBeAg positive HCW.[12,13] In the Netherlands a maximum HBV DNA level of 10^5 copies/ml is used to allow for the conduct of EPPs, irrespective of HBeAg status.[14] In the US exclusion from performing EPPs is still based only on the presence of HBeAg.[15]
In this paper we review doctor-to-patient transmission of HBV with main focus on quantitative HBV DNA levels.

DOCTOR TO PATIENT TRANSMISSION OF HEPATITIS B VIRUS

Since the early 1970s HBV infected HCWs have been identified to be the source of infection for patients who underwent surgical procedures. Retrospective investigations were performed to identify all possibly infected patients of HBV infected HCWs. Hasselhorn and Hofmann reported 40 cases of hepatitis B transmission by health care workers.[6] We found that at least five other cases of HBV transmission have been described, which are summarised here, pointing to a more widespread problem:

- 11 patients infected by a nurse[3]
- 7 infected patients and 4 cases of secondary transmission by a cardiac surgeon[5]
- 6 dental patients infected by a HBeAg positive dentist[4]
- 4 patients infected by a HBeAg positive oral surgeon[1]
- 2 patients infected by a HBeAg negative cardiothoracic surgeon bearing a precore mutant[7]

In our registry 45 described incidents of HBV transmission from HCW to patients resulted in 437 infected patients. The risk of transmission is proven and real, but still small. For example, in the Netherlands with a compulsory reporting of viral hepatitis, 3 cases of doctor to patient transmission have been described since the 1970s and about 500,000 surgical procedures are performed each year.

RISK OF TRANSMISSION OF HBV

The risk for patients to become infected during surgical procedures depends on several factors (table 1). Most infections occur during high-risk procedures. Characteristics of procedures associated with higher risk of transmission include: blind digital palpation of a needle tip,[16-18] digital guidance or handling of the needle tip while suturing,[18] simultaneous presence of fingers and instrument in the operating area[19] and interrupted vision during a surgical procedure.[20]
Although various routes of transmission of HBV have been described, most HBV infections are caused by contact with infected blood. Sharp injuries with needles or other sharp devices can occur during the treatment of patients. Percutaneous injuries occur in 6.9% of operative procedures, in 32% of the observed injuries to surgeons the sharp object recontacted the patient's open wound. The risk for contact between the HCW's and patient's blood is therefore 2.21%.[21] A comparable risk for blood contact of 2.02% during operations was found by Tokars et al.[22]

Table 1: Factors associated with the risk of hepatitis B virus transmission.

Factors associated with transmission risk:
- Serum HBV DNA level[21,23,30]
- HBeAg positivity[20,23,27]
- Duration of surgery[20,21,27,33]
- Volume of blood transmitted[21,23,30]
- Route of transmission: percutaneous vs. mucosal[23,30]
- Skill and medical condition of HCW [20,21]

The amount of infected blood transmitted affects the risk of transmission .[21,23] The volume of blood inoculated in a needlestick injury from a suture needle without the use of gloves varies from 11 nl (0.33mm needle, 2mm penetration) to 366 nl (1.12mm needle, 5mm penetration). The volume of blood inoculated in a needlestick with a phlebotomy needle is higher and varies between 133 nl (0.71mm needle, 2 mm penetration) and 683 nl (1.12mm needle, 5 mm penetration).[24] The volume of blood increases significantly with increasing depth of penetration, increasing needle diameter and the use of a phlebotomy needle instead of a suturing needle.[24] Napoli found the mean volume of blood inoculated using a 0.71 mm (22 gauge) phlebotomy needle to be on the order of 1 μl.[25] About 75% of sharp injuries is related to suturing.[21-23] Injuries with solid-bore needles (suture needles) carry a lower risk of transmission than hollow-bore, blood-filled needles.[26] Double gloving is effective in reducing the risk of inner glove puncture and decreases the volume of blood transmitted by suture needles.[24,27] During delivery infected maternal blood can be transmitted to the unborn child. Maternal-fetal transfusion during delivery with blood volumes greater than 1 μl is infrequent.[28]

The number of HBV particles transmitted by a needlestick and during delivery depends on the viral load and the volume of blood transmitted. We have estimated the number of infectious HBV particles transmitted by maternal-fetal transfusion and needlesticks (table 2). Because HBV DNA levels are measured in serum, a factor of 0.64 has been used to calculate the volume of serum in the blood transmitted (assuming a hematocrite value of 0.36; the lower limit of normal in women). Multiplying the serum volume by the HBV DNA concentration gives an estimate of the number of viral particles in the serum volume. Heermann et al. stated that almost 10% of detected HBV particles is infectious.[29] Therefore, the estimated number of viral particles in the serum volume has been devided by ten. The number of infectious particles transmitted during delivery and by needlesticks with HBV DNA levels ranging from 10^3 to 10^9 copies/ml is shown in table 2.

IMPLICATION OF HBEAG STATUS

HBeAg is considered a marker for viral replication and infectivity. High levels of HBV DNA usually correlate with the presence of HBeAg,[30] serum of HBeAg positive persons is likely to contain up to

Table 2. Estimation of the number of infectious HBV particles transmitted in needlestick accidents and delivery.

		Exposure to infectious particles after transmission of serum with different HBV DNA						
Event	**µl serum transmitted**	**10^3 copies/ml**	**10^4 copies/ml**	**10^5 copies/ml**	**10^6 copies/ml**	**10^7 copies/ml**	**10^8 copies/ml**	**10^9 copies/ml**
Delivery: maternal-fetal transfusion[28]	0.64	<1	<1	6	64	640	6400	64,000
Needlestick with suture needle[24]								
- 0.33mm needle, 2mm penetration	~0.007	<1	<1	<1	<1	7	70	700
- 0.33mm needle, 5mm penetration	~0.03	<1	<1	<1	3	30	300	3,000
- 1.12mm needle, 2mm penetration	~0.06	<1	<1	<1	6	60	600	6,000
- 1.12mm needle, 5mm penetration	~0.23	<1	<1	2	23	230	2,300	23,000
Needlestick with hollow needle[24]								
- 1.07mm needle, 2mm penetration	~0.14	<1	<1	1	14	140	1,400	14,000
- 1.07mm needle, 5mm penetration	~0.44	<1	<1	4	44	440	4,400	44,000

* Calculation infectious particles: volume of serum in ml (a) x HBV DNA concentration in copies/ml x 0.10 (b)

a) Percentage serum in whole blood is 0.64, assuming a hematocrite value of 0.36 (lower limit of normal in women)

b) Number of infectious HBV particles 10% of total number of HBV particles [29]

10^9 HBV particles per ml of serum.[23,27] The presence of anti-HBe is thought to indicate a low level or lack of viral replication and often the absence of virus in the blood.[31] However, in HBV carriers with precore mutants HBeAg is not expressed despite continuing viral replication. The most common mutation, a G-to-A transition at nucleotide 1896 of codon 28 introduces a stop-codon, preventing expression of hepatitis B e-antigen. About half of all HBeAg negative, anti-HBe positive virus carriers carry a precore mutant. These mutants can be associated with highly productive infection in HBeAg negative HBV carriers.[11,12,30,31]

Martinot-Peignoux et al. performed a study to quantify HBV DNA levels in inactive HBsAg carriers using the Cobas Amplicor HBV Monitor (Roche) with a sensitivity of 200 copies/ml.[32] The mean HBV DNA concentration in this group of patients was found to be 1300 copies/ml and 98% of sera of inactive HBeAg negative carriers contained HBV DNA levels below 10^5 copies/ml. Tedder et al. found evidence for fluctuations in HBV DNA levels in HBeAg negative HBV carriers. The variations in HBV DNA level of several orders of magnitude occurred over relative short time periods and indicate a dynamic host-parasite relationship.[12]

HBeAg / anti-HBe status is often used as a marker of infectivity. However, serum HBeAg is at best an indirect measure of hepatitis B viremia because of possible mutations in the precore region. Measurement of both HBV DNA level and HBeAg status gives a more reliable estimate of infectivity.[30]

HBV DNA CONCENTRATION AND TRANSMISSION RATE

The infection risk after exposure to HBV infected blood depends on the viral load. Although transmission of HBV from health care workers to patients has repeatedly been described since 1970, the viral load of the HCW involved has only been determined in five investigations (table 3). Sera of transmitting surgeons were found to contain HBV DNA levels between 5.0×10^9 and 6.35×10^4 copies/ml. The lowest measured HBV DNA level in serum from a transmitting surgeon was 4.0×10^4 copies/ml in a sample taken at least 3 months after transmission.[11]

The proportion of patients infected with HBV after treatment by an infected HCW varies between 0.5 % and 13.1% in different investigations.[6] The study performed by Spijkerman et al. describes a retrospective analysis to identify infected patients.[33] All patients operated on by the surgeon, sexual partners and household contacts of patients with evidence of HBV were offered serological testing for anti-HBc and HBsAg after the incubation period. Cases were considered confirmed cases if the patients' sera patients contained HBV markers and the same HBV DNA sequence as the surgeon. Probable cases were positive for anti-HBc and anti-HBs and provided clinical evidence of HBV infection within six months after surgery. Possible cases were positive for anti-HBc and anti-HBs and provided no epidemiological evidence of other sources of HBV infection. Using these definitions 8 confirmed cases, 2 probable cases and 18 possible cases were identified among 1564 tested patients. The proportion of infected patients (transmission rate) lies between 0.5% (confirmed cases only) and 1.8% (all cases). Because many different calculations and definitions

Table 3. Cases of doctor to patient transmission of HBV with HCW's profession, transmission rate and HBV DNA level of each case.

Author	HCW's profession	Published transmission rate[1]	Recalculated transmission rate[2]	HBV DNA (copies/ml)	Quantification technique	Time sample taken (after transmission)
Harpaz[17]	Thoracic surgeon	13.1%	5.3-11.2%	1.00×10^9	Semiquantitative PCR dot-blot hybridization‡	4 months
Anonymous[10]	Genral surgeon	N.A.[3]	N.A.	1.00×10^7	Liquid hybridization and enzyme linked oligonu-cleotide assay	12 weeks
	Gynaecologist	3.22%	1.08-3.22%	4.40×10^6		Unknown
	Gynaecologist	0.90%	0.90%	5.50×10^6		Unknown
	General surgeon	4.76%	4.76%	2.50×10^5		12 weeks
Molyneaux [7]	Surgeon	1.6%	1.6%	1.03×10^6	Lightcycler PCR	Unknown
Spijkerman[33]	Surgeon	0.5-1.8%	0.5-1.8%	5.00×10^9	Limited dilution PCR	1 year
Corden[11]	Surgeon	N.A.	N.A.	1.12×10^8	Chiron Quantiplex Branched DNA assay and Roche Amplicor HBV DNA Monitor assay	at least 3 months
	Surgeon	N.A.	N.A.	2.55×10^5		at least 3 months
	Surgeon	N.A.	N.A.	6.72×10^5		at least 3 months
	Surgeon	N.A.	N.A.	6.35×10^4		at least 3 months
	Surgeon	N.A.	N.A.	4.20×10^8		at least 3 months
	Surgeon	N.A.	N.A.	9.47×10^8		at least 3 months

[1]Transmission rate: proportion of patients infected during medical procedures

[2]Recalculated transmission rate: transmission rates are recalculated according one definition[33]

‡With serum containing 10^8 Chimpanzee infectious particles as comparison

have been used in the studies of doctor-patient transmissions the transmission rates were recalculated using the definitions stated by Spijkerman et al.[33]

The highest transmission rate is found by Harpaz et al., in this study 19 of 144 susceptible patients operated on were found to be infected by a surgeon (13.2%).[17] Sequence analysis confirmed infection in 9 cases. The total number of tested patients was actually 170, therefore the infection rate varies between 5.3% and 11.2%. In the study performed by Welch et al. a transmission rate of 8.9% was found.[19] The transmission rate is based on 22 infected patients of 247 tested patients. In six patients with presence of HBsAg subtyping confirmed infection by the surgeon, resulting in a transmission rate between 2.4% and 8.9%. Prentice found that a surgical trainee infected 6.1% of patients treated.[34] Two hundred eighty patients thought to be at risk were tested, 17 were found to have acquired HBV after the operation and 9 patients had the same HBV subtype as the surgeon. The transmission rate in study varies between 3.2% and 6.1%. The actual rate is probably lower because patients undergoing procedures with minimal risk of transmission were excluded from the study. Hadler found that 6 of 764 patients of a HBV infected dentist had signs of HBV infection.[4] The proportion of infected patients lies between 0.3% and 0.8%. In two patients HBsAg subtyping could be performed and was found to be the same as the surgeon. In the studies performed by Molyneaux, Sundkvist, Haerem and Lettau all patients were found to have the same subtype as their surgeons.[5,7,8,18]

DISCUSSION

Public Health policy to prevent transmission of HBV to patients in different countries was based on serum HBeAg status. After incidents of transmission by HBeAg negative surgeons a more reliable estimate of non-infectivity was needed. Serum HBV DNA level may be more reliable to estimate non-infectivity than anti-HBe status alone. Current Public Health policy in the Netherlands does not allow the conduct of EPPs by HCWs with HBV DNA levels above 10^5 copies/ml, irrespective of HBeAg status.[14] The use of this HBV DNA level of 10^5 copies/ml as a cut-off minimises the risk of transmission and allows most high-educated HBV infected HCWs to continue practice. Setting the cut off below this level would exclude the majority of HBeAg negative HCWs from performing EPPs in the Netherlands. The UK and Eire exclude all HBeAg positive HCW and a HBV DNA cut off level of 10^3 copies/ml is used for HBeAg negative HCWs.[13] In the US excluding HCWs from performing EPPs is based on the presence of HBeAg only.[15]

Transmission of HBV is not likely to occur with HBV DNA levels below 10^7 copies/ml according to vertical transmission studies. No cases of mother to child transmission were observed with maternal HBV DNA levels below 6,0 x 10^5 copies/ml, whereas 25% of children from women with HBV DNA levels above 10^7 copies/ml were infected.[35] The maximum HBV DNA level of 10^5 copies/ml in the Netherlands is based on 10^7 copies/ml, below which vertical transmission is not likely to occur. A safety margin of 2 log is used to account for natural fluctuations in viral load and variations in the assay used for quantifying HBV DNA. In the UK the lower cut off level of 10^3 copies/ml is based on

a safety margin of 3 log as the viral load is then unlikely to rise above 10^6 copies/ml.

Most percutaneous injuries during surgical procedures involve suture needles. As can be seen in table 2 the estimated number of particles transmitted with suture needles is less than the number transmitted by maternal-fetal transfusion. HBV transmission by needlesticks is according to our assumptions unlikely to occur with HBV DNA levels below 10^7 copies/ml.

Recent investigations of transmissions of HBV to patients involved determining HBV DNA levels of the HCWs. Transmission rate does not seem to depend on serum HBV DNA level only. Serum of the surgeon described by Harpaz et al. contained 1.0 x 10^9 copies/ml, associated with a maximum transmission rate of 11.2%.[17] The surgeon described by Spijkerman et al. had a viral load of 5.0 x 10^9 copies/ml and infected 1.8% of his patients.[33] Although these surgeons were both HBeAg positive and had comparable HBV DNA levels the transmission rates vary greatly. A relation between HBV DNA level and transmission rate was not found in the HBeAg positive HCWs. A possible explanation could be that the surgeon described by Harpaz et al., as a thoracic surgeon, performed more high risk procedures as the general surgeon described by Spijkerman et al.

The lowest serum HBV DNA level in a transmitting surgeon was found to be 4.0 x 10^4 copies/ml.[11] This rises the question which HBV DNA level should be used to allow for the conduct of exposure prone procedures by HBV infected HCWs. However, in our opinion Public Health policy should not be based on the measurements of HBV DNA levels in these HCWs because all samples were taken at least 3 months after the actual transmission occurred. As described by Tedder et al., variations in HBV DNA levels in HBeAg negative carriers occur over relative short periods of time.[12] Therefore, HBV DNA levels might actually have been higher at the time of transmission.

Doctor to patient transmission is a complex issue due to legal and ethical factors. These factors include the hospital's policy, federal discrimination laws and issues of informed consent and disclosure of HBV infection. Restriction of infected HCWs is also complicated by the definition of disability. A surgeon might be restricted in practice but possibly not qualify for disability insurance compensation.[36]

HCWs not allowed to perform EPPs due to high HBV DNA levels can be offered antiviral therapy to prevent both exclusion from practice and transmission to patients. In most countries the registered treatment for HBV consists of alpha-interferon or lamivudine. In an experimental setting lamivudine is sometimes combined with interferon to accomplish higher HBeAg seroconversion rates, generally associated with low HBV DNA levels. Viral resistance to lamivudine with emergence of YMDD mutants in the c-region of the HBV polymerase gene during long-term lamivudine therapy is well described.[37,38] After 1 year in 15-30% and in up to 50% after 3 years of patients treated with lamivudine monotherapy a resistant virus emerges.[39] Recently new nucleoside analogues tenofovir disoproxil fumarate,[40] adefovir dipivoxil[41,42] and entecavir[43,44] have shown to be effective in suppressing both wild-type and YMDD-mutant HBV replication. Although these antiviral drugs have not yet been registered for the treatment of HBV in most countries, they can provide potential new solutions for the treatment of chronic hepatitis B infected health care workers.

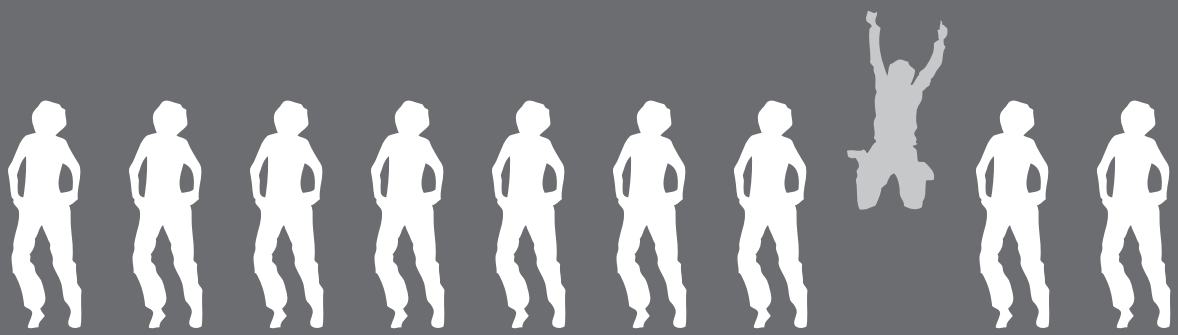
ACKNOWLEDGEMENTS

We would like to thank Dr. R.A. de Man and Dr. H.G.M. Niesters for helpful discussion.

REFERENCES

1. Anonymous. Outbreak of hepatitis B associated with an oral surgeon--New Hampshire. MMWR Morb Mortal Wkly Rep 1987;36(9):132-3.
2. Anonymous. Surgeons who are hepatitis B carriers. BMJ 1991;303(6795):184-5.
3. Garibaldi RA, Rasmussen CM, Holmes AW, et al. Hospital-acquired serum hepatitis. Report of an outbreak. JAMA 1972;219(12):1577-80.
4. Hadler SC, Sorley DL, Acree KH, et al. An outbreak of hepatitis B in a dental practice. Ann Intern Med 1981;95(2):133-8.
5. Haerem JW, Siebke JC, Ulstrup J, et al. HBsAG transmission from a cardiac surgeon incubating hepatitis B resulting in chronic antigenemia in four patients. Acta Med Scand 1981;210(5):389-92.
6. Hasselhorn HM, Hofmann F. [Transmission of HBV, HCV and HIV by infectious medical personnel--presentation of an overview]. Chirurg 2000;71(4):389-95.
7. Molyneaux P, Reid TM, Collacott I, et al. Acute hepatitis B in two patients transmitted from an e antigen negative cardiothoracic surgeon. Commun Dis Public Health 2000;3(4):250-2.
8. Sundkvist T, Hamilton GR, Rimmer D, et al. Fatal outcome of transmission of hepatitis B from an e antigen negative surgeon. Commun Dis Public Health 1998;1(1):48-50.
9. Rhodes RS. Hepatitis B virus, surgeons, and surgery. Bull Am Coll Surg 1995;80(9):32-42.
10. The Incident Investigation Teams. Transmission of hepatitis B to patients from four infected surgeons without hepatitis B e antigen. N Engl J Med 1997;336(3):178-84.
11. Corden S, Ballard AL, Ijaz S, et al. HBV DNA levels and transmission of hepatitis B by health care workers. J Clin Virol 2003;27(1):52-8.
12. Tedder RS, Ijaz S, Gilbert N, et al. Evidence for a dynamic host-parasite relationship in e-negative hepatitis B carriers. J Med Virol 2002;68(4):505-12.
13. Health Service Circular 2000/020. NHS Executive. Hepatitis B Infected Health Care Workers; 2000.
14. Inspectorate of Health. IGZ Bulletin: Prevention Iatrogenic Hepatitis B. The Hague; 2002.
15. Hofmann F, Hasselhorn HM. [European and North American regulations on employing HBV-, HCV- and HIV-infected persons in health care]. Chirurg 2000;71(4):396-403.
16. Anonymous. Acute hepatitis B associated with gynaecological surgery. Lancet 1980;1(8158):1-6.
17. Harpaz R, Von Seidlein L, Averhoff FM, et al. Transmission of hepatitis B virus to multiple patients from a surgeon without evidence of inadequate infection control. N Engl J Med 1996;334(9):549-54.
18. Lettau LA, Smith JD, Williams D, et al. Transmission of hepatitis B with resultant restriction of surgical practice. JAMA 1986;255(7):934-7.
19. Welch J, Webster M, Tilzey AJ, et al. Hepatitis B infections after gynaecological surgery. Lancet 1989;1(8631):205-7.
20. Hasselhorn HM, Hofmann F. [Nosocomial hepatitis B virus, hepatitis C virus and HIV infections by infectious medial personnel]. Gesundheitswesen 1998;60(10):545-51.
21. Bell DM, Shapiro CN, Ciesielski CA, et al. Preventing bloodborne pathogen transmission from health-care workers to patients. The CDC perspective. Surg Clin North Am 1995;75(6):1189-203.
22. Tokars JI, Bell DM, Culver DH, et al. Percutaneous injuries during surgical procedures. JAMA 1992;267(21):2899-904.
23. Beltrami EM, Williams IT, Shapiro CN, et al. Risk and management of blood-borne infections in health care workers. Clin Microbiol Rev 2000;13(3):385-407.

24. Bennett NT, Howard RJ. Quantity of blood inoculated in a needlestick injury from suture needles. J Am Coll Surg 1994;178(2):107-10.
25. Napoli VM, McGowan JE, Jr. How much blood is in a needlestick? J Infect Dis 1987;155(4):828.
26. Puro V, De Carli G, Scognamiglio P, et al. Risk of HIV and other blood-borne infections in the cardiac setting: patient-to-provider and provider-to-patient transmission. Ann N Y Acad Sci 2001;946:291-309.
27. Goldmann DA. Blood-borne pathogens and nosocomial infections. J Allergy Clin Immunol 2002;110(2 Suppl):S21-6.
28. Brossard Y, Pons JC, Jrad I, et al. Maternal-fetal hemorrhage: a reappraisal. Vox Sang 1996;71(2):103-7.
29. Heermann KH, Gerlich WH, Chudy M, et al. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol 1999;37(1):68-73.
30. Ballard AL, Boxall EH. Assessing the infectivity of hepatitis B carriers. Commun Dis Public Health 1999;2(3):178-83.
31. Knoll A, Rohrhofer A, Kochanowski B, et al. Prevalence of precore mutants in anti-HBe-positive hepatitis B virus carriers in Germany. J Med Virol 1999;59(1):14-8.
32. Martinot-Peignoux M, Boyer N, Colombat M, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. J Hepato. 2002;36(4):543-6.
33. Spijkerman IJ, van Doorn LJ, Janssen MH, et al. Transmission of hepatitis B virus from a surgeon to his patients during high-risk and low-risk surgical procedures during 4 years. Infect Control Hosp Epidemiol 2002;23(6):306-12.
34. Prentice MB, Flower AJ, Morgan GM, et al. Infection with hepatitis B virus after open heart surgery. BMJ 1992;304(6829):761-4.
35. Xu DZ, Yan YP, Choi BC, et al. Risk factors and mechanism of transplacental transmission of hepatitis B virus: a case-control study. J Med Virol 2002;67(1):20-6.
36. Rhodes RS, Telford GL, Hierholzer WJ, Jr., et al. Bloodborne pathogen transmission from healthcare worker to patients. Legal issues and provider perspectives. Surg Clin North Am 1995;75(6):1205-17.
37. Honkoop P, Niesters HG, de Man RA, et al. Lamivudine resistance in immunocompetent chronic hepatitis B. Incidence and patterns. J Hepatol 1997;26(6):1393-5.
38. Niesters HG, Honkoop P, Haagsma EB, et al. Identification of more than one mutation in the hepatitis B virus polymerase gene arising during prolonged lamivudine treatment. J Infect Dis 1998;177(5):1382-5.
39. Papatheodoridis GV, Dimou E, Papadimitropoulos V. Nucleoside analogues for chronic hepatitis B: antiviral efficacy and viral resistance. Am J Gastroenterol 2002l;97(7):1618-28.
40. Benhamou Y, Tubiana R, Thibault V. Tenofovir disoproxil fumarate in patients with HIV and lamivudine-resistant hepatitis B virus. N Engl J Med 2003 9;348(2):177-8.
41. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med 2003;348(9):800-7.
42. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348(9):808-16.
43. Lai CL, Rosmawati M, Lao J, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. Gastroenterology 2002;123(6):1831-8.
44. de Man RA, Wolters LM, Nevens F, et al. Safety and efficacy of oral entecavir given for 28 days in patients with chronic hepatitis B virus infection. Hepatology 2001;34(3):578-82.



Prolonged antiviral therapy for hepatitis B virus infected health care workers: a feasible option to prevent work restriction without jeopardizing patient safety.

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Journal of Viral Hepatitis 2007; 14(5): 350-354.

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SUMMARY

To prevent transmission of hepatitis B virus (HBV) from health care workers (HCWs) to patients, highly viremic HCWs are often advised to restrict performing exposure prone procedures (EPPs). To prevent loss of highly qualified medical personnel and simultaneously minimize transmission risk to patients, we offered highly viremic HCWs antiviral therapy and evaluated the effects of this strategy. Eighteen chronic HBV infected HCWs have been monitored every 3-6 months for a median period of 5.6 years (range 1.1-12.5 years). Antiviral therapy was offered if HBV DNA was above 10^5 copies/ml and EPPs were performed or active liver disease was present. Median HBV DNA levels, the percentage of days with HBV DNA above 10^3, 10^4 and 10^5 copies/ml, and reduction of HBV DNA during antiviral treatment have been analysed for HBeAg-positive and HBeAg-negative HCWs separately. Prolonged viral suppression was achieved in both HBeAg-positive, as well as HBeAg-negative HCWs. In HBeAg-negative HCWs treatment with interferon or lamivudine maintained HBV DNA levels below 10^5 copies/ml. For HBeAg-positive HCWs continuous treatment with tenofovir or entecavir was essential for reaching low viremia persistently. In 2004 median HBV DNA levels in both HBeAg-negative and HBeAg-positive HCWs were below 10^3 copies/ml and all HCWs executed their professional work full-range. For both HBeAg-positive and HBeAg-negative HCWs, antiviral treatment is effective in persistent suppression of virus levels below 10^5 copies/ml. This observation supports antiviral therapy as a viable management option instead of work restriction, with the provision of regular expert monitoring including quantification of HBV DNA.

INTRODUCTION

Transmission of hepatitis B virus (HBV) from infected health care workers (HCW) to patients has repeatedly been reported in the past 30 years. Since the 1970s at least 45 of such cases have been described, altogether resulting in 437 infected patients.[1] To prevent transmission of HBV from HCWs to patients, infected HCWs are often restricted in performing exposure prone procedures (EPP). The CDC defined exposure prone procedures to include "digital palpation of a needle tip in a body cavity or the simultaneous presence of the HCWs fingers and a needle or other sharp object in a poorly visualized anatomic site".[2]

In 1991, the CDC stated that HCWs who are infected with HIV or HBeAg-positive HBV should not perform exposure-prone procedures unless they have sought counsel from an expert review panel.[2] In 2003, a European consensus panel proposed that HBsAg-positive HCWs undergo annual HBV DNA testing and that those with HBV DNA levels above 10^4 copies/ml are restricted, as this would provide a balance between risk of transmission and loss of specialist HCWs.[3]

The transmission risk of HBV is determined by the number of infectious particles transmitted and thus the HCW's serum HBV DNA level and the volume of infectious blood transmitted. Transmission is rarely documented with HBV DNA levels below 10^5 copies/ml, according to several studies on vertical transmission.[4-6] Only one case of transmission of HBV by a HCW with a HBV DNA level below 10^5 copies/ml has been described; in that case, interpretation is difficult because the sample was taken more than three months after the actual transmission occurred.[7] The fact that the level of viremia is an important factor for the risk of transmission leads to the concept that suppression of viremia by antiviral therapy may almost completely reduce the risk of transmission, as has been proven in the setting of mother-to-infant transmission both for HIV and HBV.[8,9]

Punitive policies may provide disincentives for HCWs to determine their virus status. Gostin addressed this topic in 2000 and suggested a new policy, focused on management of the workplace environment, injury prevention and antiviral therapy for infected HCWs as this would achieve patient safety without discrimination and invasion of HCW privacy.[10] At that time, registered treatment of HBV consisted of interferon (IFN) and lamivudine. Three new nucleos(t)ide analogues provide increased potential for prolonged effective treatment of chronic HBV infected HCWs. Adefovir dipivoxil, entecavir and tenofovir have activity against both wild-type and lamivudine resistant HBV.[11-13] The former two have recently been licensed for the treatment of chronic hepatitis B.

In this paper we analyse the effectiveness of current antiviral treatment in suppressing viral replication in HBV infected HCWs and provide evidence supporting the concept of antiviral therapy as a feasible option that allows continuation of a full range of work without jeopardizing patient safety.

METHODS

Study participants, treatment and monitoring

All eighteen chronic HBV infected HCWs monitored in the Erasmus MC University Medical Center

Rotterdam, The Netherlands between 1994 and 2004 were included in this study; ten were HBeAg-positive and eight HBeAg-negative at inclusion. They were tested at least every 3 to 6 months for serum ALT, HBV DNA, HBeAg, anti-HBe, HBsAg and anti-HBs.

HCWs were offered antiviral therapy if they had serum HBV DNA levels above 10^5 copies/ml and performed EPPs or had elevated serum ALT above twice the upper limit of normal.[14] Patients were initially treated with (pegylated)-IFN (various treatment regimes) and/or lamivudine (100mg daily). In case of non-response, relapse or resistance the new nucleos(t)ide analogues adefovir (10mg daily), entecavir (0.5-1mg daily) and tenofovir (245mg daily) were used depending on availability in the Netherlands. For patients with antiviral resistance sequential nucleos(t)ide analogue monotherapy with an overlap period was used.

Median HBV DNA levels, the percentage of days with HBV DNA levels above various cut-off levels and speed of reduction of HBV DNA levels during antiviral treatment have been analysed for HBeAg-positive and HBeAg-negative HCWs separately. Transition in HBV DNA level was assumed to occur halfway between two samples.

HBV DNA measurement

For the accurate measurement of HBV DNA in serum over a ten year period several assays were used including the commercially available assays, Digene Hybrid Capture II microplate assay (Digene Diagnostics; dynamic range from 1.4 x 10^5 - 1.7 x 10^9 copies/ml) and HBV Monitor assay (Roche Diagnostics; dynamic range from 400 - 4.0 x 10^7 copies/ml), as well as an in-house developed HBV DNA TaqMan assay (dynamic range 373 - 10^{10} copies/ml).[15] All assays were calibrated using EUROHEP HBV DNA standards, and yearly validated by participation in a quality control program.[16,17]

Mutations in the polymerase gene were detected using a line probe assay (INNO-LiPA, Innogenetics, Ghent, Belgium).[18] Where the INNO-LiPA assay was indeterminate a sequence analysis was used.[19]

Statistical analysis

Statistical analysis was performed using the functions of the SPSS 11.5 software. Statistical analysis of differences between groups was performed with the Mann-Whitney U-test or Student's *t*-test, where appropriate. A p-value of <0.05 was considered significant.

RESULTS

Baseline characteristics of the HBV infected HCWs are shown in table 1. Median HBV DNA level at inclusion was significantly higher for HBeAg-positive HCWs compared to HBeAg-negative HCWs (7.5 x 10^8 and 1.9 x 10^4 copies/ml, $p<0.001$). The HCWs were monitored for a median period of 5.6 years (range 1.1-12.5 years). At the end of follow-up 9 of 10 HBeAg-positive HCWs and 4 of 8 HBeAg-negative HCWs had been treated with antiviral drugs.

From 1998 - 2003 median serum HBV DNA remained above 10^5 copies/ml in HBeAg-positive

Table 1: Baseline characteristics of the health care workers (HCWs).

	HBeAg-positive (n=10)	HBeAg-negative (n=8)	Total (n=18)
Sex			
- Male	60%	62%	67%
- Female	40%	38%	33%
Mean age (years)	30.4 (range 18-44)	41.8 (range 32-63)*	35.4 (range 18 - 63)
Race			
- Caucasian	50%	75%	61%
- Asian	40%	25%	33%
- Other	10%	0%	6%
Precore genotype			
- G1896G	NA	63%	NA
- G1896A	NA	37%	NA
Median HBV DNA (copies/ml)	7.5×10^8 (range 7.1×10^6 - 3.6×10^9)	1.9×10^4 (range $<1.0 \times 10^3$ - 1.6×10^8)*	1.0×10^7 (range $<1.0 \times 10^3$ - 3.6×10^9)

*HBeAg-positive HCWs compared to HBeAg-negative HCWs: $p<0.05$

HCWs. In 2004 median serum HBV DNA for HBeAg-positive HCWs was below 10^3 copies/ml. Only one untreated HBeAg-positive HCW continuously had HBV DNA above 10^5 copies/ml, this patient received no treatment since ALT was normal and EPPs were not performed. One HCW showed a temporary rise in HBV DNA from 1.5×10^3 to 6.8×10^5 copies/ml after switching from experimental lamivudine-tenofovir combination therapy to licensed adefovir monotherapy. In HBeAg-negative HCWs median serum HBV DNA was continuously below 10^5 copies/ml, and below 10^4 copies/ml since 2001. In 2004 a median HBV DNA level below 10^3 copies/ml was observed (range <373 - 2.2×10^4 copies/ml).

The percentage of days HCWs had HBV DNA levels above 10^3, 10^4, and 10^5 copies/ml is given in figure 1 for HBeAg-positive and HBeAg-negative HCWs separately. In HBeAg-positive HCWs continuous effective viral suppression was not observed in IFN-alfa and/or lamivudine treated HCWs. A decline in percentage of days with high serum HBV DNA was observed with the use adefovir, entecavir and tenofovir. Almost all HBeAg-negative HCWs could effectively be treated with lamivudine and/or IFN-alfa, non-responders with individually tailored nucleos(t)ide analogue treatment.

Furthermore, we analyzed the time required for serum HBV DNA to reach levels below the maximum level described in public health guidelines. Figure 2 shows the percentage of HBeAg-positive HCWs with HBV DNA levels below 10^3, 10^4 and 10^5 copies/ml after one, three, six and 12 months of antiviral treatment. HBV DNA levels were below 10^5 copies/ml in all treated HBeAg-positive HCWs after 12 months of adefovir, tenofovir or entecavir treatment. In all HBeAg-negative HCWs

HBV DNA levels below 10^3 copies/ml were observed 6 months after starting lamivudine and/or IFN therapy and as soon as 1 month after starting treatment with adefovir, entecavir or tenofovir.

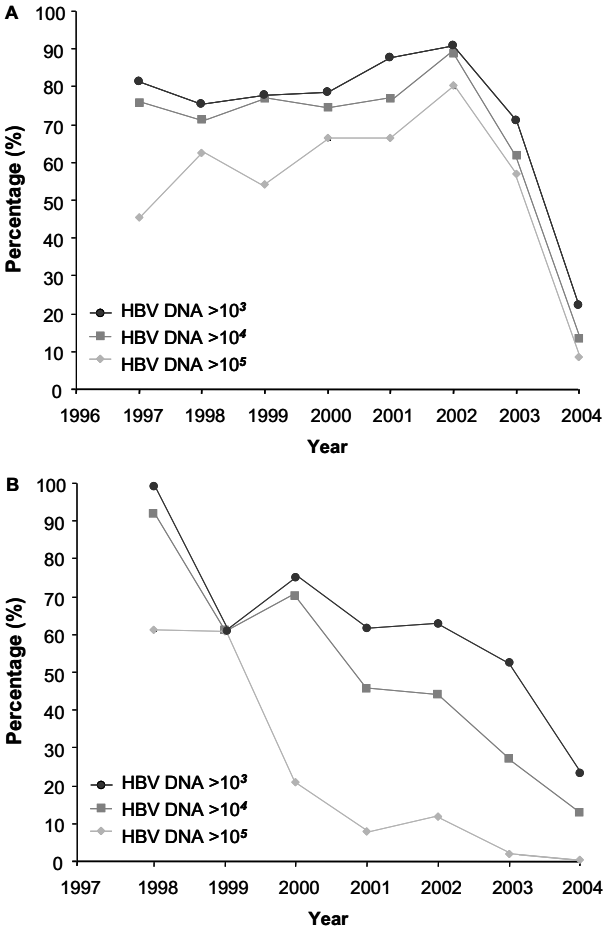


Figure 1: Percentage of days with HBV DNA levels above various cut-off levels.

In HBeAg-positive HCWs (n=10; A) continuous effective viral suppression was not observed with IFN and/or lamivudine treatment from 1997-2002. In 6 lamivudine treated HBeAg-positive HCWs a mutation in the YMDD motif had occurred. With the successive availability of tenofovir, adefovir and entecavir from 2002 onwards a decline in percentage of days with high serum HBV DNA was observed in 2003 and 2004.

Almost all HBeAg-negative HCWs (n=8; B) could effectively be treated with lamivudine and/or IFN from 1998-2002. Lamivudine resistance has not been found in any of these HBeAg-negative HCWs. Since the start of individually tailored nucleos(t)ide analogue treatment, that was continuous since 2003, HBV DNA levels above 10^5 copies/ml have not been observed.

DISCUSSION

Prolonged antiviral treatment in HBV infected HCWs has rarely been documented.[20,21] This study documents in small number of HCWs that both HBeAg-negative, as well as HBeAg-positive HCWs can effectively maintain HBV DNA levels below 10^5 copies/ml for prolonged periods. This requires an arsenal of antiviral drugs, which fortunately is readily available since 2004. In this patient population compliance with antiviral medication was excellent, be it in a setting of HBV DNA monitoring every 3-6 months. Our observations may give further support to solving the HBV infected HCW problem in a new way.

Although the number of HCWs studied is small, the uniformity of the findings at several time periods point to confidence in the reliability of the observations. Even with this small number of patients, many foreseeable complications, like resistance to lamivudine, adefovir or entecavir,

were observed and quickly amended. One of the HCWs is now receiving antiviral medication for 9 years with prolonged control of HBV DNA suppression.

In 2003, an expert panel advised referral of all HBV infected HCWs to a hepatologist for specialist advice on antiviral treatment.[3] In order to return to performing EPPs, infected HCWs receiving treatment should have HBV DNA levels below the maximum cut-off level according to local public health policy, and should be retested every 3 months while receiving antiviral therapy. A maximum HBV DNA level of 10^4 copies/ml was proposed. In HCWs receiving antiviral therapy reduction of HBV DNA to levels below 10^4 copies/ml is now feasible (figure 1).

We used a maximum level of 10^5 copies/ml, as currently used in The Netherlands, as indication for antiviral therapy.[14] Based on data of studies on vertical transmission and the virtual absence of reports on HBV transmission by HCWs with HBV DNA levels below 10^5 copies/ml, doctor to patient transmission of HBV seems unlikely to occur with serum HBV DNA below this level.[4,6,7] Gerlich estimated a risk below 1:100,000 that one case of HBV transmission caused by viremia below 10^5 copies/ml occurs in a period of 15 years.[22]

We remain reluctant to initiate antiviral therapy in individuals with repeated HBV DNA between 10^3 and 10^5 copies/ml. Regular quantification of HBV DNA is required to account for naturally occurring fluctuations in serum HBV DNA in these untreated patients.[23] Further data with validated HBV DNA assays are needed to guide public health measures on this topic. Use of standardised assays for quantification of HBV DNA is essential when using HBV DNA in the decision process of work restriction.

It is still a question of debate whether to strive for a zero-risk strategy or whether a strategy aimed at reducing the risk of transmission to a level comparable to other accepted risks associated with surgical procedures, such as anaesthesia or wound infection, can be chosen. Acceptance of antiviral therapy as a management option for HCWs by public health policies may result in increased willingness of HCWs to undergo voluntary testing. This would increase infections to be detected and treated, and thereby decrease risks to patients.[24]

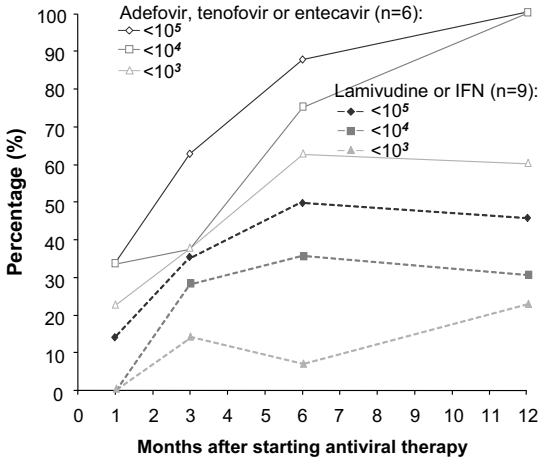


Figure 2: Percentage of HBeAg-positive HCWs with confirmed HBV DNA below various cut-off levels after starting antiviral treatment.

Four of 9 HBeAg-positive HCWs (44%) treated with standard IFN and/or lamivudine therapy had serum HBV DNA levels below 10^5 copies/ml 12 months after starting treatment. Four of 6 HBeAg-positive HCWs (75%) treated with adefovir, entecavir or tenofovir had serum HBV DNA below 10^4 copies/ml after 6 months of treatment, all of them had HBV DNA below this level 12 months after starting treatment with these drugs.

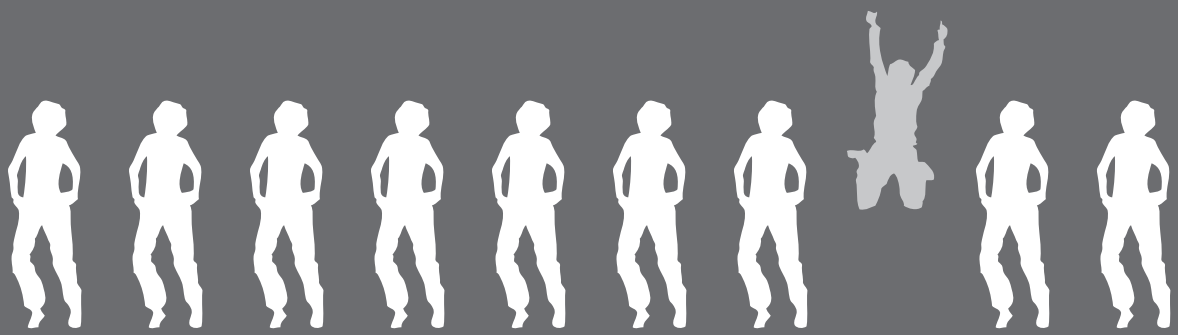
This study shows promising results for maintaining persistent minimal risk viremia in both HBeAg-positive, as well as HBeAg-negative HCWs with the use of antiviral drugs. Based on these data we suggest general application of prolonged antiviral therapy as a new management option for chronic HBV infected HCWs with high viremia, with the provision of regular expert monitoring including quantification of HBV DNA.

REFERENCES

1. Buster EH, van der Eijk AA, Schalm SW. Doctor to patient transmission of hepatitis B virus: implications of HBV DNA levels and potential new solutions. Antiviral Res 2003;60:79-85.
2. Recommendations for preventing transmission of human immunodeficiency virus and hepatitis B virus to patients during exposure-prone invasive procedures. MMWR Morb Mortal Wkly Rep. 1991;40(RR-8):1-9.
3. Gunson RN, Shouval D, Roggendorf M, et al. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in health care workers (HCWs): guidelines for prevention of transmission of HBV and HCV from HCW to patients. J Clin Virol 2003;27:213-230.
4. Ip HM, Lelie PN, Wong VC, et al. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989;1(8635):406-410.
5. Zaaijer HL, ter Borg F, Cuypers HT, et al. Comparison of methods for detection of hepatitis B virus DNA. J Clin Microbiol 1994;32:2088-2091.
6. Xu DZ, Yan YP, Choi BC, et al. Risk factors and mechanism of transplacental transmission of hepatitis B virus: a case-control study. J Med Virol 2002;67:20-26.
7. Corden S, Ballard AL, Ijaz S, et al. HBV DNA levels and transmission of hepatitis B by health care workers. J Clin Virol 2003;27:52-58.
8. Zonneveld M, Nunen AB, Niesters HG, et al. Lamivudine treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. J Viral Hepat 2003;10(4):294-297.
9. Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pedriatic AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 1994;331(18):1173-1180.
10. Gostin LO. A proposed national policy on health care workers living with HIV/AIDS and other blood-borne pathogens. JAMA 2000;284:1965-1970.
11. Lai CL, Rosmawati M, Lao J, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. Gastroenterology 2002;123:1831-1838.
12. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.
13. van Bommel F, Wunsche T, Mauss S, et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40:1421-1425.
14. Inspectorate of Health. IGZ Bulletin: Prevention Iatrogenic Hepatitis B. The Hague; 2002
15. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-2901.
16. Heermann KH, Gerlich WH, Chudy M, et al. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol 1999;37:68-73.
17. Valentine-Thon E, van Loon AM, Schirm J, et al. European proficiency testing program for molecular detection and quantitation of hepatitis B virus DNA. J Clin Microbiol 2001;39:4407-4412.
18. Lok AS, Zoulim F, Locarnini S, et al. 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.

19. Osterhaus AD, Vos MC, Balk AH, et al. Transmission of hepatitis B virus among heart transplant recipients during endomyocardial biopsy procedures. J Heart Lung Transplant 1998;17:158-166.
20. Schalm SW, van Wijngaarden JK. Doctor-to-patient transmission of viral hepatitis B: is it a problem, is there a solution? J Viral Hepat 2000;7:245-249.
21. Buster EH, van der Eijk AA, de Man RA, et al. Doctor-to-patient transmission of hepatitis B virus: the potential of antiviral therapy for prevention. Scand J Gastroenterol Suppl 2004:45-49.
22. Gerlich WH. [Hepatitis B and C. Risk of transmission from infected health care workers to patients]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 2004;47:369-378.
23. Tedder RS, Ijaz S, Gilbert N, et al. Evidence for a dynamic host-parasite relationship in e-negative hepatitis B carriers. J Med Virol 2002;68:505-512.
24. Summers T. Public policy for health care workers infected with the human mmunodeficiency virus. JAMA 2001;285:882.



Summary and Discussion

Antiviral treatment for chronic hepatitis B virus infection: Immune modulation or viral suppression?

Adapted from: *The Netherlands Journal of Medicine* 2006; 64(6):175-185.

INTRODUCTION

Hepatitis B virus (HBV) infection is still an important health problem, it is estimated that about one-third of world's population has evidence of infection with HBV and that 400 million people are chronically infected.[1, 2] Without antiviral therapy, ongoing hepatic inflammation will lead to progression of liver disease and eventually to the development of decompensated cirrhosis and hepatocellular carcinoma (HCC) in 25-40% of patients.[3] It is estimated that over 500,000 people die annually due to HBV associated liver disease, largely due to complications of cirrhosis and/or HCC.[4] With the availability of multiple new antiviral agents the treatment of chronic hepatitis B has become more effective and a large proportion of future deaths could potentially be prevented.

ANTIVIRAL THERAPY FOR HBEAG POSITIVE CHRONIC HEPATITIS B

In hepatitis B e antigen (HBeAg) positive patients with high serum HBV DNA levels (>1.0 x 10^5 copies/ml) and elevated ALT levels, antiviral therapy can be postponed for 3-6 months to await spontaneous HBeAg seroconversion. Among 3,063 Chinese patients, spontaneous HBeAg seroconversion occurred in 46% of those with peak ALT levels over five times the upper limit of normal (ULN) within three months.[5] HBeAg positive patients with elevated ALT levels for longer periods should be considered for antiviral therapy. In addition, antiviral therapy can also be considered in those with histological evidence of significant hepatic necroinflammation and/or significant liver fibrosis. In HBeAg positive patients, successful antiviral treatment with loss of HBeAg, decline in serum HBV DNA and normalization of ALT is associated with favorable long-term outcome, independent of the antiviral drug used. The practicing clinician is currently faced with a number of treatment options for chronic hepatitis B, including two formulations of IFN (standard IFN and pegylated IFN) and five nucleos(t)ide analogues (lamivudine, adefovir, entecavir, telbivudine and tenofovir). As a result, the complexity of decision-making has increased and the question arises whether a finite course of pegylated IFN (PEG-IFN) or nucleos(t)ide analogue therapy, with the possibility of long-term maintenance therapy, is better as first-line therapy.

FACTORS INFLUENCING THE CHOICE OF ANTIVIRAL THERAPY

The added complexity of treating chronic hepatitis B has led to the development of national and international guidelines for the treatment of chronic HBV infection in the past few years.[4, 6-9] Most of these guidelines do however not provide specific recommendations as to whether PEG-IFN or nucleos(t)ide analogues should be used as preferred first-line therapy, because both approaches have proved effective, and both have advantages and limitations (Table 1). In addition, numerous other factors including serum HBV DNA and ALT levels, HBeAg status, HBV genotype, the severity of liver disease and the patient's preference play an important role when deciding on which drug to start in an individual patient. Ideally, antiviral therapy should be directed toward achieving the high-

est rate of viral clearance with the shortest duration of treatment.

SIDE EFFECTS OF TREATMENT

PEG-IFN therapy is often complicated by the occurrence of side-effects such as flu-like symptoms, myelosuppression and depression.[10] Nucleos(t)ide analogues are very well tolerated by comparison. Because of presumed lack of efficacy and fear for hepatic flares and other toxicity of PEG-IFN, it is often not given to HBV infected patients with advanced fibrosis or cirrhosis. It is well known that IFN-based therapy can induce severe complications of bacterial infection and exacerbation of liver disease in patients with Child's class B or C cirrhosis.[11,12] Although we found that patients with advanced fibrosis more often required dose adjustment and more often prematurely discontinued PEG-IFN alpha-2b therapy, response rates were comparable to those observed in patients without advanced fibrosis (Chapter 3).

Retinopathy is a relatively rare adverse reaction which can be observed during PEG-IFN therapy. In chronic hepatitis C virus infected patients retinopathy is probably more common, it was observed in 24% of patients who underwent routine fundoscopic examination.[13] We found retinopathy in only one of 24 (4%) chronic hepatitis B patients during treatment with PEG-IFN alpha-2b (Chapter 4). Routine screening for retinopathy seems therefore not necessary in chronic hepatitis B patients undergoing PEG-IFN therapy in the absence of risk factors for retinopathy such as diabetes mellitus and hypertension.

SUSTAINED RESPONSE TO PEG-IFN

Of currently licensed drugs for the treatment of chronic hepatitis B, PEG-IFN seems to results in the highest rate of sustained off-treatment response after a one-year course of therapy.[14-17] At the end of treatment, HBeAg loss was observed in 29% of patients treated with PEG-IFN alpha-2b alone and in 44% of patients treated with combination therapy with lamivudine.[15] Relapse occurred in 13% and 39% of these patients within 6 months post-treatment (Chapter 5). Post-treatment HBeAg loss was observed in 9% of patients, resulting in an overall HBeAg loss rate of 36% at six months after discontinuation of PEG-IFN. After an additional mean 3.0 years of follow-up, 37% of patients treated with PEG-IFN alpha-2b were HBeAg negative and clearance of HBsAg from serum was observed in 11% (Chapter 7). Among patients who were HBeAg negative at six months post-treatment (initial responders), the HBsAg clearance rate was 30%. It is well known that the rate of HBsAg loss increases during prolonged follow-up to 12-65% of virological responders.[18-26] We showed that PEG-IFN significantly reduced the proportion of hepatocytes stained positive for HBcAg and HBsAg in patients with HBeAg positive chronic hepatitis B, although there seemed to be a beneficial effect of added lamivudine in terms of intrahepatic HBsAg clearance. Clearance of HBsAg from the liver is important because it was strongly associated with loss of HBeAg and HBsAg from serum (Chapter 9). In addition we found that HBsAg loss particularly occurred in patients who

cleared HBeAg from serum within 32 weeks of PEG-IFN alpha-2b therapy (Chapter 8). Since early HBeAg loss and long-term HBsAg loss may be associated with more profound viral suppression during the first weeks of therapy in patients with added lamivudine, adding a nucleos(t)ide analogue to PEG-IFN therapy might increase HBsAg loss rates in the long-term.

PREDICTORS OF RESPONSE TO PEG-IFN

Most studies investigating IFN-based therapy in HBeAg-positive chronic hepatitis B found that high baseline ALT, low baseline HBV DNA and HBV genotype A or B infection were associated with a higher chance of response to IFN-based therapy.[15,27-29] In addition to these factors, we identified sex, age and previous IFN therapy as predictors of response to PEG-IFN (Chapter 2). We found that the influence of sex, age, HBV DNA and previous IFN therapy was significantly different across HBV genotypes. Based on these findings we developed a practical tool to calculate the predicted probability of sustained response to PEG-IFN in individual HBeAg-positive patients. This treatment index can be easily used in clinical practice to select the optimal candidates for PEG-IFN therapy. Based on our finding we recommend to consider PEG-IFN therapy in all genotype A infected patients, except for those with both low ALT (<2 x ULN) and high HBV DNA levels (>=9$\log_{10}$ copies/ml). In addition, genotype B and C infected patients with both favorable ALT (>=2 x ULN) and HBV DNA (<9$\log_{10}$ copies/ml) values are good candidates for PEG-IFN therapy. Genotype D infected patients are generally not candidates for treatment with PEG-IFN, because they have a low likelihood of sustained response irrespective of ALT and HBV DNA levels.

SUSTAINED RESPONSE TO NUCLEOS(T)IDE ANALOGUES

Although treatment with nucleos(t)ide analogues profoundly suppresses serum HBV DNA levels and response can be maintained over prolonged periods with ongoing therapy, response to lamivudine was less sustainable after discontinuation of therapy compared to IFN.[30] We found combined response of HBeAg loss and HBV DNA <7.0 x 10^5 copies/ml in 34% of patients treated with PEG-IFN alpha-2b and 21% of patients treated with entecavir at the end of a one-year course (Chapter 10). At 6 months post-treatment, these rates were 20% and 17%, respectively. Predictors of sustained response after entecavir therapy include high baseline ALT, low baseline HBV DNA, high baseline necroinflammatory score and infection with HBV genotype A or D (Chapter 10).
Relapse after discontinuation of therapy poses a risk for hepatitis flares. Flares are a well known and potentially dangerous phenomenon after discontinuation nucleos(t)ide analogues and in patients developing antiviral resistance, particularly in case of advanced liver disease.[31,32] We however found that severe flares also occur frequently after discontinuation of PEG-IFN therapy, with a rise in ALT above 10 x ULN in 14% of PEG-IFN treated patients (Chapter 6). In order to reduce the risk of relapse, nucleos(t)ide analogue therapy can be extended for several months after HBeAg seroconversion as this significantly reduces relapse rates.[33,34] HBeAg seroconversion was sus-

tained in 86% of patients treated with telbivudine or lamivudine who discontinued therapy after at least six months of maintenance therapy.[35,36] These findings suggest that sustained off-treatment response can be achieved with nucleos(t)ide analogues, given that treatment is continued for several months after HBeAg loss.

THE IMPORTANCE OF HBV GENOTYPE

It is well known that HBV genotype is associated with response to IFN-based therapy, with the highest response rates in genotype A infected patients.[15,37,38] We found that HBV genotype also was a strong predictor of long-term sustainability of response to PEG-IFN alpha-2b, with significantly higher rates of HBeAg negativity in genotype A infected initial responders compared to those with genotype-non-A (96% vs. 71%, p=0.02), as well as HBsAg loss (58% vs. 11%, p<0.001) after a mean of 3.5 years of follow-up (Chapter 7). Although a higher rate of lamivudine-induced HBeAg seroconversion has been reported in genotype B as compared to genotype C infected patients,[33] other studies did not find such a relation between HBV genotype and on-treatment response to either lamivudine, adefovir or entecavir.[39-41] We did however find an association of HBV genotype with sustained off-treatment response to entecavir (Chapter 10). In addition, we found that the chance of sustained response to either PEG-IFN alpha-2b or entecavir was significantly different across genotypes. Genotype A and B infected patients had a higher chance of sustained response to PEG-IFN alpha-2b than entecavir. Patients infected with genotype C responded equally to PEG-IFN alpha-2b and entecavir, while genotype D infected patients had a higher chance of sustained response to entecavir than PEG-IFN alpha-2b. HBV genotype can thus guide the choice of therapy in HBeAg positive chronic hepatitis B.

MAINTAINED VIRAL SUPPRESSION WITH NUCLEOS(T)IDE ANALOGUE THERAPY

Because of the modest rates of sustained response, long-term, and possibly indefinite, nucleos(t)ide analogue treatment seems necessary in a large proportion of patients.[42,43] The excellent tolerability of nucleoside analogs has made it relatively easy to use them for prolonged periods in order to maintain viral suppression. This strategy proved effective in reducing disease progression and the development of hepatocellular carcinoma in patients with advanced fibrosis.[44] A drawback however is the risk of developing antiviral resistance in the long-term, particularly when using drugs with a low genetic barrier and/or low potency.[45] In chronic HBV infected health care workers (HCWs) who perform exposure prone procedures there is even more at stake. According to a European expert panel, HBV infected HCWs should have HBV DNA levels below 1.0×10^4 copies/ml in order to be allowed to continue the full range of work.[46] Those receiving antiviral treatment should be retested every three instead of six months while receiving therapy. Based on data of studies on vertical transmission and the virtual absence of reports on HBV transmission by HCWs with HBV DNA levels below 1.0×10^5 copies/ml, doctor to patient transmission of HBV seems highly unlikely

to occur with serum HBV DNA below this level (Chapter 11). We showed that both HBeAg positive and HBeAg negative HCWs can now effectively maintain HBV DNA levels below this level for prolonged periods (Chapter 12). This requires an arsenal of antiviral agents, which fortunately is readily available.

CONCLUSION

In conclusion, choice of antiviral therapy has become more important and more complex at the same time in the past decade. Both treatment with PEG-IFN therapy and nucleos(t)ide analogue therapy have proven effective and can improve long-term outcome. Since HBV genotype has such great influence on choice of antiviral therapy in HBeAg positive chronic hepatitis B, determination of HBV genotype is essential in all patients in whom sustained off-treatment response is pursued. In general, PEG-IFN therapy is recommended in all HBV genotype A infected HBeAg positive patients, while nucleos(t)ide analogue therapy is preferred in those with HBV genotype D. In patients harbouring the HBV genotype B and C, the pros and cons of the available drugs as well as patient-specific characteristics should be carefully balanced.

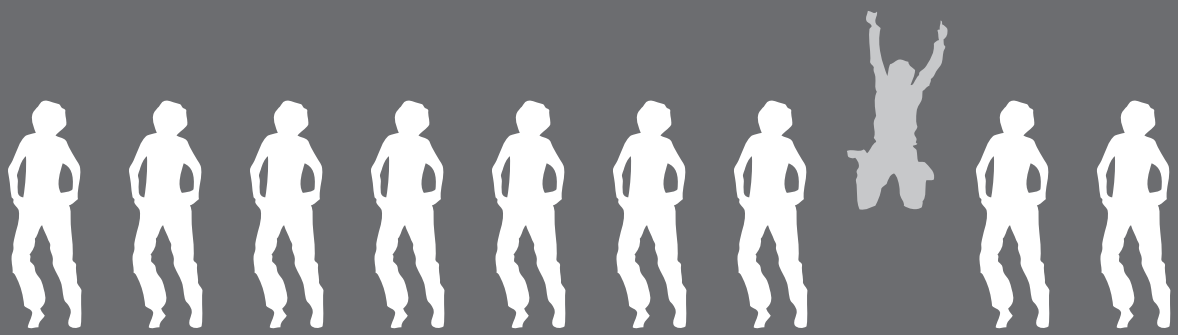
REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
2. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-45.
3. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis 2005;25 Suppl 1:3-8.
4. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, Mele A, Paumgartner G, Pietrangelo A, Rodes J, Rosenberg W, Valla D. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39:S3-25.
5. Yuen MF, Yuan HJ, Hui CK, Wong DK, Wong WM, Chan AO, Wong BC, Lai CL. A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut 2003;52:416-9.
6. Cornberg M, Protzer U, Dollinger MM, Petersen J, Wedemeyer H, Berg T, Jilg W, Erhardt A, Wirth S, Schirmacher P, Fleig WE, Manns MP. The German guideline for the management of hepatitis B virus infection: short version. J Viral Hepat 2008;15 Suppl 1:1-21.
7. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008:DOI 10.1007/s12072-008-9080-3.
8. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
9. Buster EH, van Erpecum KJ, Schalm SW, Zaaijer HL, Brouwer JT, Gelderblom HC, de Knegt RJ, Minke Bakker C, Reesink HW, Janssen HL. Treatment of chronic hepatitis B virus infection - Dutch national guidelines. Neth J Med 2008;66:292-306.
10. van Zonneveld M, Flink HJ, Verhey E, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Hansen BE, Schalm SW, Janssen HL. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. Aliment Pharmacol Ther 2005;21:1163-71.
11. Hoofnagle JH, Di Bisceglie AM, Waggoner JG, Park Y. Interferon alfa for patients with clinically apparent

cirrhosis due to chronic hepatitis B. Gastroenterology 1993;104:1116-21.
12. Perrillo R, Tamburro C, Regenstein F, Balart L, Bodenheimer H, Silva M, Schiff E, Bodicky C, Miller B, Denham C, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. Gastroenterology 1995;109:908-16.
13. d'Alteroche L, Majzoub S, Lecuyer AI, Delplace MP, Bacq Y. Ophthalmologic side effects during alpha-interferon therapy for viral hepatitis. J Hepatol 2006;44:56-61.
14. 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;354:1001-10.
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. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-9.
16. 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;352:2682-95.
17. 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 B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-16.
18. Carreno V, Castillo I, Molina J, Porres JC, Bartolome J. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. J Hepatol 1992;15:102-6.
19. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1997;26:1338-42.
20. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.
21. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-97.
22. 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;113:1660-7.
23. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-5.
24. Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. Gastroenterology 1993;105:1833-8.
25. 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;334:1422-7.
26. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, Feinman SV, Mach T, Akarca US, Schutten M, Tielemans W, van Vuuren AJ, Hansen BE, Janssen HL. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459-467.
27. Cooksley G, Lau GKK, Liaw YF, Marcellin P, Chow WC, Thongsawat S, Gane E, Fried MW, Zahm FE. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol 2005;42:S30.
28. Bonino F, Lau GKK, Marcellin P, Hadziyannis S, Kitis G, Jin R, Yao GB, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, McCloud P, Brunetto MR, Farci P. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized,

partially double-blind study of peginterferon-alfa-2a (4-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone. Hepatology 2004;40:A1142.
29. Craxi A, Di Bona D, Camma C. Interferon-alpha for HBeAg-positive chronic hepatitis B. J Hepatol 2003;39 Suppl 1:S99-105.
30. van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, Heathcote J, Song BC, Janssen HL, de Man RA, Schalm SW. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-4.
31. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. Hepatology 2000;32:635-9.
32. Janssen HL, Brouwer JT, Nevens F, Sanchez-Tapias JM, Craxi A, Hadziyannis S. Fatal hepatic decompensation associated with interferon alfa. European concerted action on viral hepatitis (Eurohep). BMJ 1993;306:107-8.
33. Chien RN, Yeh CT, Tsai SL, Chu CM, Liaw YF. Determinants for sustained HBeAg response to lamivudine therapy. Hepatology 2003;38:1267-73.
34. Ryu SH, Chung YH, Choi MH, Kim JA, Shin JW, Jang MK, Park NH, Lee HC, Lee YS, Suh DJ. Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospective study. J Hepatol 2003;39:614-9.
35. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, Bzowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576-88.
36. Poynard T, Hou JL, Chutaputti A, Manns M, Naoumov N. Sustained durability of HBeAg seroconversion in chronic hepatitis B patients after treatment with telbivudine. J Hepatol 2008;48:S263-S264.
37. Hadziyannis S, Lau GKK, Marcellin P, Piratvisuth T, Cooksley G, Bonino F, Chutaputti A, Diago M, Jin R, Pluck N. Sustained HBsAg seroconversion in patients with chronic hepatitis B treated with peginterferon alpha-2a (40kDa) (Pegasys®). J Hepatol 2005;42:S178.
38. Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. Hepatology 2002;36:1425-30.
39. Buti M, Cotrina M, Valdes A, Jardi R, Rodriguez-Frias F, Esteban R. Is hepatitis B virus subtype testing useful in predicting virological response and resistance to lamivudine? J Hepatol 2002;36:445-6.
40. Lurie Y, Manns MP, Gish RG, Chang TT, Yurdaydin C, Lai CL, Shouval D, Brown Jr. RS, Apelian D, Fernandes L, Klesczewski KS, Cross A, Wilber R. The efficacy of entecavir is similar regardless of disease-related baseline subgroups in treatment of nucleoside-naive, HBeAg(+) and HBeAg(-) patients with chronic hepatitis B. J Hepatol 2005;42:184.
41. Yuen MF, Wong DK, Sablon E, Yuan HJ, Sum SM, Hui CK, Chan AO, Wang BC, Lai CL. Hepatitis B virus genotypes B and C do not affect the antiviral response to lamivudine. Antivir Ther 2003;8:531-4.
42. Dienstag JL, Cianciara J, Karayalcin S, Kowdley KV, Willems B, Plisek S, Woessner M, Gardner S, Schiff E. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology 2003;37:748-55.
43. Song BC, Suh DJ, Lee HC, Chung YH, Lee YS. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 2000;32:803-6.
Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON,
44. Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-31.
45. Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, Liaw YF, Mizokami M, Kuiken C. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007;46:254-65.
46. Gunson RN, Shouval D, Roggendorf M, Zaaijer H, Nicholas H, Holzmann H, de Schryver A, Reynders D,

Connell J, Gerlich WH, Marinho RT, Tsantoulas D, Rigopoulou E, Rosenheim M, Valla D, Puro V, Struwe J, Tedder R, Aitken C, Alter M, Schalm SW, Carman WF. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in health care workers (HCWs): guidelines for prevention of transmission of HBV and HCV from HCW to patients. J Clin Virol 2003;27:213-30.



Samenvatting en discussie

Antiviral behandeling bij chronische hepatitis B virus infectie: Immuun-modulatie of virale suppressie?

Bewerking van: *The Netherlands Journal of Medicine* 2006; 64(6):175-185.

INLEIDING

Hepatitis B virus (HBV) infectie is nog steeds een belangrijk gezondheidsprobleem, naar schatting zijn er aanwijzingen voor contact met HBV bij eenderde van de wereldbevolking. Het aantal mensen met chronische HBV infectie wordt geschat op 400 miljoen.[1, 2] Indien onbehandeld zal persisterende onsteking van de lever leiden tot progressive van fibrose en uiteindelijk tot gedecompenseerde cirrose en hepatocellulair carcinoom (HCC) bij 25-40% van de patiënten.[3] Naar schatting sterven er wereldwijd elk jaar 500.000 personen aan de gevolgen van HBV geassocieerde leverziekte, voornamelijk door de complicaties van levercirrose en HCC.[4] Met de beschikbaarheid van meerdere nieuwe antivirale middelen is de behandeling van chronische hepatitis B effectiever geworden en kan er in potentie een aanzienlijke reductie in sterfte aan deze chronische leverziekte bereikt worde..

ANTIVIRALE THERAPIE VOOR HBEAG POSITIEVE CHRONISCHE HEPATITIS B

Er is een indicatie voor antivirale behandeling bij hepatitis B e antigen (HBeAg) positieve patiënten met hoog HBV DNA (>1.0 x 10^5 kopieën/ml) en verhoogd alanine aminotransferase (ALAT). Wel kan behandeling 3-6 maanden worden uitgesteld om eventuele spontane HBeAg seroconversie af te wachten. Spontane HBeAg seroconversie trad binnen drie maanden op bij 46% van 3063 Chinese patiënten met ALAT waarden boven vijf maal de bovenwaarde van normaal.[5] Indien er geen spontane HBeAg seroconversie is opgetreden na deze periode komt de patiënt in aanmerking voor behandeling. Daarnaast kan behandeling overwogen worden indien er bij histologisch onderzoek aanwijzingen zijn voor significante necroinflammatie of fibrose. Bij HBeAg positieve patiënten is succesvolle antivirale behandeling met verlies van HBeAg, daling van HBV DNA en normalisatie van ALAT geassocieerd met verbeterde uitkomst op lange termijn. Dit is onafhankelijk van het gebruikte antivirale medicijn. Behandelaars hebben op dit moment keuze uit een tweetal vormen van IFN (standaard IFN en gepegyleerd IFN [PEG-IFN]) en een vijftal nucleos(t)ide analogen (lamivudine, adefovir, entecavir, telbivudine en tenofovir). Met de beschikbaarheid van nieuwe antivirale middelen is de keuze van optimale behandeling complexer geworden. Indien een patiënt in aanmerking komt voor behandeling dient er allereerst een keuze gemaakt te worden tussen behandeling met PEG-IFN met een vaste behandelduur of langdurige behandeling met een nucleos(t)ide analog.

FACTOREN DIE DE KEUZE VAN BEHANDELING BEINVLOEDEN

De toegenomen complexiteit van behandeling van chronische hepatitis B heeft geleid tot de ontwikkeling van meerdere nationale en internationale richtlijnen in de afgelopen jaren.[4, 6-9] De meeste van de richtlijnen geven echter geen specifieke aanbevelingen betreffende de keuze tussen PEG-IFN of een nucleos(t)ide analoog als eerstelijns behandeling. Beide strategieën zijn effectief gebleken en hebben ieder hun voor- en nadelen (tabel 1). Daarnaast spelen factoren als HBV

DNA, ALAT, HBeAg status, HBV genotype, ernst van leverziekte en de voorkeur van de patiënt een belangrijke rol bij de keuze van antivirale behandeling in een individuele patiënt. Optimale antvirale behandeling is geassocieerd met de hoogste kans op klaring van het virus met een beperkte behandelduur.

BIJWERKINGEN VAN DE BEHANDELING

Behandeling met PEG-IFN gaat vaak gepaard met bijwerkingen als griep-achtige klachten, myelosuppressie en depressive.[10] Nucleos(t)ide analogen worden daarentegen zeer goed verdragen. PEG-IFN wordt vaak niet gegeven aan patiënten met gevorderde fibrose of cirrose in verband met de veronderstelde verminderde effectiviteit en het verhoogde risico op acute exacerbatie van ontstekingsactiviteit. Het is bekend dat behandeling met IFN ernstige compllicaties als bacteriële infectie en decompensatie kan geven bij patiënten met Child B of C levercirrose.[11, 12] Hoewel we inderdaad vonden dosisreductie en vroegtijdig staken van PEG-IFN behandeling vaker nodig waren bij patiënten met dan zonder gevorderde cirrose, waren de responspercentages vergelijkbaar in beide groepen *(Hoofdstuk 3*).

Retinopathie is een relatief zeldzame afwijkinng die kan worden gezien tijdens PEG-IFN behandeling. Bij patiënten met chronische hepattis C virus infectie retinopathie waarschijnlijk vaker voor, het werd gezien bij 24% van patiënten die behandeld werden met PEG-IFN en een fundoscopie ondergingen.[13] Wij vonden slecht bij één van de 24 (4%) met chronische hepatitis B aanwijzingen voor retinopathie tijdens behandeling met PEG-IFN. (*Hoofdstuk 4*). Routinematige screening op retinopathie voorafgaand aan en tijdens PEG-IFN lijkt daarom niet noodzakelijk bij patiënten met chronische hepatitis B bij afwezigheid van andere risicofactoren voor retinopathie als diabetes mellitus en hypertensie.

BLIJVENDE RESPONS OP PEG-IFN

Eén jaar na het staken van de behandeling lijkt PEG-IFN te resulteren in de hoogste kans op blijvende respons in vergelijking met de overige beschikbare antivirale middelen voor de behandeling van chronische hepatitis B.[14-17] Aan het einde van de behandeling werd HBeAg verlies gezien bij 29% van de patiënten die behandeld werden met alleen PEG-IFN alpha-2b en bij 44% van de patiënten die hierbij ook lamivudine kregen.[15] Verlies van de behaalde response trad op bij 13% en 39% van deze patiënten binnen 6 maanden na behandeling (*Hoofdstuk 5*). Bij 9% van de patiënten trad HBeAg verlies pas op nadat de behandeling reeds gestaakt was, hiermee komt 6 maanden na behandeling het responspercentage op 36%. Na gemiddeld 3 jaar was 37% van de patiënten die behandeld waren met PEG-IFN á-2b HBeAg negatief en was 11% ook HBsAg negatief (*Hoofdstuk 7*). Het percentage HBsAg verlies was 30% onder patiënten die 6 maanden na behandeling HBeAg negatief waren (initiële responders). Het is bekend dat het percentage HBsAg verlies toeneemt bij

een langere follow-up duur. Dit neemt op de lange termijn toe toe tot 12-65% van de patiënten met virologische respons.[18-26]

We vonden dat het percentage hepatocyten dat positief was voor HBcAg en HBsAg significant verminderd was na PEG-IFN behandeling in vergelijking met voorafgaand aan behandeling. Er leek een gunstig effect te zijn van het toegevoegen van lamivudine. Verdwijnen van HBsAg expressie in de lever is belangrijk omdat het sterk geassocieerd is met klaring van HBeAg en HBsAg uit het serum (*Hoofdstuk 9*). Daarnaast bleek HBsAg verlies voornamelijk op te treden bij patiënten die binnen 32 weken na aanvang van PEG-IFN behandeling HBeAg negatief waren geworden (*Hoofdstuk 8*). Een sterke daling van de virusconcentratie tijdens de eerste maanden van behandeling, wat met name bereikt werd in patiënten met toegevoegde lamivudine, leek geassocieerd met een hogere kans op vroeg HBeAg verlies en HBsAg verlies op lange termijn *(Hoofdstuk 9)*.

VOORSPELLERS VAN RESPONS OP PEG-IFN

De meeste studies met IFN voor HBeAg positieve chronische hepatitis B hebben gevonden dat hoog ALAT, laag HBV DNA en HBV genotype A of B zijn geasscocieerd met een hogere kans op respons.[15, 27-29] Naast deze factoren vonden wij ook dat geslacht, leeftijd en voorgaande behandeling met IFN voorspellers van respons op PEG-IFN waren (*Hoofdstuk 2*). Wel bleek de invloed van geslacht, leeftijd, HBV DNA en voorgaande IFN behandeling significant verschillend tussen de HBV genotypes. Op basis van deze bevindingen hebben we een practisch hulpmiddel opgesteld om de voorspelde kans op respons op PEG-IFN voor HBeAg positieve patiënten te berekenen. Deze behandelindex kan in de klinische praktijk het selecteren van optimale HBeAg positieve kandidaten voor PEG-IFN behandeling vereenvoudigen. Op basis van het model zouden we PEG-IFN willen aanbevelen voor genotype A patiënten met hoog ALAT (>=2 x ULN) of laag HBV DNA (<9$\log_{10}$ kopieën/ml) en voor genotype B of C patiënten met hoog ALAT en laag HBV DNA. Patiënten met genotype D hebben in het algemeen een lage kans op respons op PEG-IFN en zijn daarom meestal geen goede kandidaten voor behandeling met PEG-IFN, onafhankelijk van HBV DNA en ALAT concentraties.

BLIJVENDE RESPONS NA BEHANDELING MET NUCLEOS(T)IDE ANALOGEN

Behandeling met nucleos(t)ide analogen resulteert in een sterke daling van de serum HBV DNA concentratie, wat behouden kan worden middles langdurige onderhoudsbehandeling. Het percentage blijvende respons na een jaar behandeling met lamivudine was echter lager dan met IFN.[30] Wij vonden dat gecombineerde respons van HBeAg verlies en HBV DNA <7.0 x 10^5 kopieën/ml na één jaar behandeling was opgetreden bij 34% van de patiënten behandeld met PEG-IFN (en lamivudine) en bij 21% van degenen met entercavir behandeling (*Hoofdstuk 10*). Zes maanden na behandeling waren de percentages blijvende respons vergelijkbaar, namelijk 20% en 17%.

Voorspellers van blijvende respons op entecavir zijn hoog ALAT, laag HBV DNA, hoge necroinflammatiescore en infectie met HBV genotype A of D *(Hoofdstuk 10).*
Verlies van respons na het staken van behandeling geeft een risico op acute verergering van de hepatitis (flare). Flares kunnen met name optreden in patiënten die stoppen met nucleos(t)ide analogen of antitivrale resistentie ontwikkelen, ze zijn met name berucht in patiënten met gevorderde leverziekte.[31, 32] Wij vonden dat ernstige flares ook optreden na het stoppen van behandeling met PEG-IFN, de ALAT concentratie steeg tot boven de 10 maal de bovenwaarde van normaal bij 14% van de met PEG-IFN behandelde patiënten (*Hoofdstuk 6*).
Bij nucleos(t)ide analog behandeling kan het risico op verlies van respons worden gereduceerd door de behandeling tenminste enkele maanden te continueren na het optreden van HBeAg seroconversie.[33, 34] Bij patiënten die volgens dit principe behandeld werden met lamivudine of telbivudine was HBeAg seroconversie 6 maanden na behandeling blijvend bij 86% van de patiënten.[35, 36] Deze bevindingen suggereren dat blijvende respons bij HBeAg positieve patiënten ook kan worden bereikt met nucleos(t)ide analogen mits er gedurende enkele maanden zogenaamde consolidatiebehandeling wordt gegeven.

HET BELANG VAN HBV GENOTYPE

Het is al geruime tijd bekend dat patiënten met HBV genotype A de hoogste kans op respons hebben op behandeling met IFN.[15, 37, 38] We vonden dat HBV genotype ook een sterke voorspeller is van blijvende respons op lange termijn (gemiddeld 3,5 jaar na behandeling). HBeAg negatitiviteit werd significant vaker gezien bij patiënten met genotype A dan in degenen met een ander genotype (96% vs. 71%, p=0.02), net als HBsAg verlies (58% vs. 11%, p<0.001) (*Hoofdstuk 7*). Met lamivudine is er is een hogere kans op HBeAg seroconversie gerapporteerd in genotype B dan genotype C,[33] al hebben andere studies dit niet kunnen bevestigen zowel lamivudine, adefovir en entecavir.[39-41] Wij vonden echter wel een relatie tussen genotype en blijvende respons na behandeling met entecavir (*Hoofdstuk 10*). Verder bleken er significante verschillen te zijn in de kans op blijvende respons op behandeling met PEG-IFN of entecavir tussen de verschillende genotypes. Zo hadden patiënten met genotype A of B een hogere kans op blijvende respons met PEG-IFN, terwijl genotype D patiënten een hogere kans op blijvende respons met entecavir hadden. Bij patiënten met genotype C werden geen verschillen in blijvende respons gezien. HBV genotype kan dus de keuze van optiamel antivirale behandeling vereenvoudigen.

LANGDURIGE VIRALE SUPPRESSIE MET NUCLEOS(T)IDE ANALOGEN

Door de beperkte kans op blijvende respons moet behandeling met nucleos(t)ide analogen meestal langdurig en wellicht levenslang gegeven worden in een groot deel van de patiënten.[42, 43] De voortrefffelijk verdraaglijkheid van deze middelen is het relatief makkelijk ze voor langere tijd te gebruiken om zo optimale virale suppressie te behouden. Deze strategie is effectief gebleken bij

patiënten met gevordere fibrose, waarbij ere en verlaagde kans op ziekte progressive en HCC was.[44] Een nadeel van langdurige behandeling met nucleos(t)ide analogen is echter het ontwikkelen van antivirale resistentie, voornamelijk bij gebruik van middelen met een lage genetische barrière of weinig potente middelen.[45]

Bij gezondheidszorgwerkers met chronische hepatitis B staat er zelfs nog meer op het spel. Volgens een panel van Europese experts op dit gebied moeten gezondheidszorgwerkers met HBV infectie een HBV DNA concentratie hebben van minder dan 1.0 x 10^4 kopieën/ml om het verrichten van invasieve ingrepen te mogen voortzetten.[46] Indien zij antivirale behandeling krijgen moeten er elke 3 maanden bepaling van de HBV DNA concentratie plaatsvinden, anders volstaat eenmaal per 6 maanden. Op basis van onderzoek naar moerder-kind transmissie en de afwezigheid van gedocumenteerde casus van transmissie bij een HBV DNA lager dan 1.0 x 10^5 kopieën/ml lijkt overdracht van behandelaar naar patiënt erg onwaarschijnlijk bij een HBV DNA concentratie onder dit niveau (*Hoofdstuk 11*). We toonden aan dat het bij zowel HBeAg positieve als HBeAg negatieve gezondheidszorgwerkers mogelijk is de HBV DNA concentratie langdurig onder dit niveau te houden met de huidige nucleos(t)ide analogen *(Hoofdstuk 12)*.

CONCLUSIE

Concluderend in het kiezen van de optimale behandelstrategie belangrijker maar tegelijk ook complexer geworden in de afgelopen 10 jaar. Zowel behandeling met PEG-IFN als met nucleos(t)ide analogen is effectief kan waarschijnlijk de uitkomst op lange termijn gunstig beïnvloeden. Omdat HBV genotype zo'n belangrijke invloed heeft op de keuze van behandeling is het van belang om het genotype bepalen bij alle patiënten bij wie gestreefd wordt naar blijvende respons. In het algemeen heeft PEG-IFN de voorkeur bij genotype A patiënten, terwijl bij patiënten met genotype D betere resultaten behaalt worden met nucleos(t)ide analogen. Bij patiënten met genotype B en C kunnen niet zonder meer aanbevelingen gedaan worden en moeten nog meer ook andere factoren alsmede de voor- en nadelen van beide strategieën nauwkeurig worden afgewogen voor een individuele patiënt.

REFERENTIES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
2. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-45.
3. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis 2005;25 Suppl 1:3-8.
4. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, Mele A, Paumgartner G, Pietrangelo A, Rodes J, Rosenberg W, Valla D. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39:S3-25.
5. Yuen MF, Yuan HJ, Hui CK, Wong DK, Wong WM, Chan AO, Wong BC, Lai CL. A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut 2003;52:416-9.

6. Cornberg M, Protzer U, Dollinger MM, Petersen J, Wedemeyer H, Berg T, Jilg W, Erhardt A, Wirth S, Schirmacher P, Fleig WE, Manns MP. The German guideline for the management of hepatitis B virus infection: short version. J Viral Hepat 2008;15 Suppl 1:1-21.
7. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008:DOI 10.1007/s12072-008-9080-3.
8. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
9. Buster EH, van Erpecum KJ, Schalm SW, Zaaijer HL, Brouwer JT, Gelderblom HC, de Knegt RJ, Minke Bakker C, Reesink HW, Janssen HL. Treatment of chronic hepatitis B virus infection - Dutch national guidelines. Neth J Med 2008;66:292-306.
10. van Zonneveld M, Flink HJ, Verhey E, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Hansen BE, Schalm SW, Janssen HL. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. Aliment Pharmacol Ther 2005;21:1163-71.
11. Hoofnagle JH, Di Bisceglie AM, Waggoner JG, Park Y. Interferon alfa for patients with clinically apparent cirrhosis due to chronic hepatitis B. Gastroenterology 1993;104:1116-21.
12. Perrillo R, Tamburro C, Regenstein F, Balart L, Bodenheimer H, Silva M, Schiff E, Bodicky C, Miller B, Denham C, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. Gastroenterology 1995;109:908-16.
13. d'Alteroche L, Majzoub S, Lecuyer AI, Delplace MP, Bacq Y. Ophthalmologic side effects during alpha-interferon therapy for viral hepatitis. J Hepatol 2006;44:56-61.
14. 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;354:1001-10.
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. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-9.
16. 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;352:2682-95.
17. 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 B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-16.
18. Carreno V, Castillo I, Molina J, Porres JC, Bartolome J. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. J Hepatol 1992;15:102-6.
19. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1997;26:1338-42.
20. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.
21. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-97.
22. 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;113:1660-7.
23. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-5.

24. Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. Gastroenterology 1993;105:1833-8.
25. 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;334:1422-7.
26. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, Feinman SV, Mach T, Akarca US, Schutten M, Tielemans W, van Vuuren AJ, Hansen BE, Janssen HL. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459-467.
27. Cooksley G, Lau GKK, Liaw YF, Marcellin P, Chow WC, Thongsawat S, Gane E, Fried MW, Zahm FE. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol 2005;42:S30.
28. Bonino F, Lau GKK, Marcellin P, Hadziyannis S, Kitis G, Jin R, Yao GB, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, McCloud P, Brunetto MR, Farci P. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized, partially double-blind study of peginterferon-alfa-2a (4-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone. Hepatology 2004;40:A1142.
29. Craxi A, Di Bona D, Camma C. Interferon-alpha for HBeAg-positive chronic hepatitis B. J Hepatol 2003;39 Suppl 1:S99-105.
30. van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, Heathcote J, Song BC, Janssen HL, de Man RA, Schalm SW. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420-4.
31. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. Hepatology 2000;32:635-9.
32. Janssen HL, Brouwer JT, Nevens F, Sanchez-Tapias JM, Craxi A, Hadziyannis S. Fatal hepatic decompensation associated with interferon alfa. European concerted action on viral hepatitis (Eurohep). BMJ 1993;306:107-8.
33. Chien RN, Yeh CT, Tsai SL, Chu CM, Liaw YF. Determinants for sustained HBeAg response to lamivudine therapy. Hepatology 2003;38:1267-73.
34. Ryu SH, Chung YH, Choi MH, Kim JA, Shin JW, Jang MK, Park NH, Lee HC, Lee YS, Suh DJ. Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospective study. J Hepatol 2003;39:614-9.
35. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, Bzowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576-88.
36. Poynard T, Hou JL, Chutaputti A, Manns M, Naoumov N. Sustained durability of HBeAg seroconversion in chronic hepatitis B patients after treatment with telbivudine. J Hepatol 2008;48:S263-S264.
37. Hadziyannis S, Lau GKK, Marcellin P, Piratvisuth T, Cooksley G, Bonino F, Chutaputti A, Diago M, Jin R, Pluck N. Sustained HBsAg seroconversion in patients with chronic hepatitis B treated with peginterferon alpha-2a (40kDa) (Pegasys®). J Hepatol 2005;42:S178.
38. Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. Hepatology 2002;36:1425-30.
39. Buti M, Cotrina M, Valdes A, Jardi R, Rodriguez-Frias F, Esteban R. Is hepatitis B virus subtype testing useful in predicting virological response and resistance to lamivudine? J Hepatol 2002;36:445-6.
40. Lurie Y, Manns MP, Gish RG, Chang TT, Yurdaydin C, Lai CL, Shouval D, Brown Jr. RS, Apelian D, Fernandes L, Klesczewski KS, Cross A, Wilber R. The efficacy of entecavir is similar regardless of disease-related baseline subgroups in treatment of nucleoside-naive, HBeAg(+) and HBeAg(-) patients

with chronic hepatitis B. J Hepatol 2005;42:184.
41. Yuen MF, Wong DK, Sablon E, Yuan HJ, Sum SM, Hui CK, Chan AO, Wang BC, Lai CL. Hepatitis B virus genotypes B and C do not affect the antiviral response to lamivudine. Antivir Ther 2003;8:531-4.
42. Dienstag JL, Cianciara J, Karayalcin S, Kowdley KV, Willems B, Plisek S, Woessner M, Gardner S, Schiff E. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology 2003;37:748-55.
43. Song BC, Suh DJ, Lee HC, Chung YH, Lee YS. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 2000;32:803-6.
44. 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;351:1521-31.
45. Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, Liaw YF, Mizokami M, Kuiken C. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007;46:254-65.
46. Gunson RN, Shouval D, Roggendorf M, Zaaijer H, Nicholas H, Holzmann H, de Schryver A, Reynders D, Connell J, Gerlich WH, Marinho RT, Tsantoulas D, Rigopoulou E, Rosenheim M, Valla D, Puro V, Struwe J, Tedder R, Aitken C, Alter M, Schalm SW, Carman WF. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in health care workers (HCWs): guidelines for prevention of transmission of HBV and HCV from HCW to patients. J Clin Virol 2003;27:213-30.

Dankwoord

Allereerst moet ik zeggen dat ik mijn promotieonderzoek als een erg leuke tijd heb ervaren. Het onderzoek zelf, maar zeker de samenwerking met alle collega's waren daarvoor doorslaggevend.

Ik wil mijn promotor, prof.dr. Harry Janssen, bedankten voor de begeleiding tijdens het promotieonderzoek. Harry, je werkwijze vond ik erg prettig en motiverend. Ondanks je drukke agenda wist je altijd tijd te maken als dat nodig was. Ook enorm bedankt voor alle mogelijkheden die je me naast het promotieonderzoek geboden. Ik denk hierbij aan presentaties op congressen en symposia en natuurlijk de mogelijkheid om deel uit te maken van de commissie voor de ontwikkeling van de Nederlandse richtlijnen voor de behandeling van hepatitis B en C.

Uiteindelijk is alles begonnen met een afstudeeronderzoek onder supervisie van prof.dr. Solko Schalm naar de mogelijkheden voor antivirale behandeling bij gezondheidszorgwerkers met hepatitis B. Solko, je enthausiasme voor wetenschappelijk onderzoek is ongekend en heb je zeker over weten te brengen. Ook wil ik je bedanken voor de alle vrijheid om bij ExpertDoc te werken aan de beslisbomen voor de behandeling van hepatitis B en andere leverziekten.

Mijn opleider dr. Rob de Man en ook prof.dr. Ernst Kuipers wil bedanken voor het in mij gestelde vertrouwen voor de opleiding tot Maag-Darm-Leverarts.

De samenwerking met de collega's van de hepatitis B groep, Martijn ter Borg, Marjolein op den Brouw, Annemiek van der Eijk, Wim Leemans, Jurriën Reijnders, Eric Tjwa, Hanneke van Vuuren, Andrea Woltman en Vincent Rijckborst was super. Altijd was het mogelijk om bij het HBV-overleg of tussendoor samen wat te bespreken of door te nemen. Natuurlijk mogen ook mijn voorgangers, en met name Hajo Flink, niet onbenoemd blijven omdat er al zo veel werk was gedaan voor de LTFU studie.

Statistiek is het onderdeel waar de meeste beginnende promovendi denk ik het meest tegenop zien. Als je echter terecht kunt bij jou Bettina, weet je dat er altijd wat moois uitkomt. Zonder jouw hulp was veel van het statistisch onderzoek niet gelukt ben ik bang. Wat een geweldige publicatie hebben we van het predictiemodel gemaakt, wie had dat gedacht. Ewout ook erg veel dank voor je advies bij dit artikel wat natuurlijk helemaal aansluit bij jouw expertise.

De meeste tijd heb ik doorgebracht op de al vaak geroemde en vervloekte dakpoli. Warm en gezellig was het in ieder geval altijd. Met mijn kamergenoten Els, Geert, Paul en Daphne heb ik een zeer goede tijd gehad in dat kleine kamertje. We hebben veel gelachen samen, nog meer koffie gehaald, maar ook op zijn tijd de perikelen rondom het onderzoek besproken. Ook de overige collega-onderzoekers: Aafke, Arjun, Claudia, Désirée, Edith, Femme, Gijs, Jildou, Jilling, Joyce, Judith, Lieke, Madeleen, Manon, Margot, Mark, Nicoline, Rachel, Robert, Sarwa, Suzanne en alle anderen heel erg bedankt voor de leuke tijd, de koffiepauzes, lunches, etentjes, borrels, skireis en niet te vergeten de congressen.

De secretaresses van de sectie Hepatologie, Marion en Margriet, stonden werkelijk altijd klaar om te helpen. Jullie hulp en kennis bij de gang van zaken rondom de promotie en in het ziekenhuis is echt onmisbaar! Marion in het bijzonder bedankt voor je hulp bij de correspondentie rondom de promotie en natuurlijk bij het (re-re-re-) submitten van alle artikelen tijdens de opleiding...

De dames van het clinical research bureau, Wanda, Elke, Claudia, Gaalda en Irene wil ik enorm bedanken voor de hulp bij het schrijven van de protocollen, het invoeren en controleren van de data en natuurlijk het feit dat jullie ervoor gezorgd hebben dat alles ook gebeurt zoals het behoort te gaan (er is natuurlijk vaak een makkelijkere weg...)!

Promovendi komen en gaan, maar de research verpleegkundigen Cokki, Anneke, Heleen en Lucille zorgen er al jaren voor dat altijd het hele traject van inclusie, behandeling, follow-up en alles daaromheen strak werd gepland. Bedankt voor al jullie hulp.

Een zeer leuke afwisseling tijdens het onderzoek was de HBV-poli op de dinsdagmiddag, met de polidames (Ester, Wilma, Nermin, Lakshmie, Ellen en Esther) en heer (Ronald) was het er altijd gezellig. Ook kon ik er altijd terecht voor vermiste voorwerpen (lees statussen).

Ook met de collega's van andere afdeling van het Erasmus MC heb ik met veel plezier samengewerkt. Vooral bij Suzan en Martin van de afdeling Virologie heb ik regelmatig aangeklopt voor aanvullende analyses. Bedankt voor jullie snelle hulp altijd. Aardig wat uurtje heb ik achter de microscoop gezeten. Pieter, bedankt voor de tijd die je hiervoor uittrok en je koffie is natuurlijk onovertroffen.

Ingrid en Martijn, super dat jullie mijn paranimfen willen zijn! Ook al wonen we inmiddels wat verder uit elkaar, hopelijk blijven we elkaar veel zien. Dat geldt natuulijk ook voor mijn andere zus Mirjam, jullie zijn weliswaar Nederbelgen maar gelukkig altijd in de buurt. Martin en Martijn, we hebben veel meegemaakt samen! Hartstikke leuk dat we alledrie zo kort na elkaar een kleine krijgen, dat ze snel vriendjes en vriendinnetjes mogen worden.

Papa en mama, heel erg bedankt voor alle steun, maar vooral ook alle vrijheid die jullie me altijd hebben gegeven. Jullie staan echt altijd voor ons klaar en ik denk nog altijd terug aan een geweldige tijd bij jullie thuis in Rijen. Het is fantastisch om nu te zien hoe blij jullie zijn met jullie eerste kleinkind.

Het laatste plekje heb ik bewaard voor jou Ineke. Bedankt voor al je interesse in mijn onderzoek en natuurlijk voor de hulp bij het opmaken van mijn promotieboekje. Ik ben hartstikke trots op je, je bent een geweldige verloskundige. Ik kan echt niet beschrijven hoe blij je me hebt gemaakt met onze prachtige dochter Nine!

Curriculum Vitae

De auteur van dit proefschrift werd geboren op 23 juni 1980 te Goirle. Na het behalen van zijn V.W.O. diploma aan de Nassau Scholengemeenschap te Breda in 1998, studeerde hij Gezondheidswetenschappen aan de Universiteit Maastricht. Hij behaalde het propedeusediploma in 1999. Aansluitend startte hij met de opleiding Geneeskunde aan de Erasmus Universiteit te Rotterdam. Op de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC te Rotterdam (afdelingshoofd prof.dr. E.J. Kuipers) verrichtte hij zijn afstudeeronderzoek met als onderwerp “Doctor to patient transmission of hepatitis B virus” onder supervisie van prof.dr. S.W. Schalm. Hij ontving hiervoor de Gerrit Jan Mulderprijs voor veelbelovend wetenschappelijk onderzoek. Het doctoraalexamen werd behaald in juli 2003 en het artsexamen in november 2005. Aansluitend werkte hij als arts-onderzoeker onder supervisie van prof.dr. H.L.A. Janssen aan het onderzoek beschreven in dit proefschrift. Tijdens het promotieonderzoek was hij tevens secretaris van de Commissie *Richtlijnen behandeling chronische hepatitis B en C virusinfectie* van de Nederlandse Vereniging van Maag-Darm-Leverartsen. In januari 2009 werd begonnen met de opleiding tot Maag-Darm-Leverarts vanuit het Erasmus MC te Rotterdam (opleider dr. R.A. de Man) in het Albert Schweitzer ziekenhuis te Dordrecht (opleider Interne Geneeskunde: dr. E.F.H. van Bommel, opleider Maag-Darm-Leverziekten: dr. W. Lesterhuis). Hij is samenwonend met Ineke Beens in Breda, ze hebben een dochter Nine.

Bibliography

1. Schalm SW, Buster EH. Management of hepatitis B virus infected health care workers based on HBV DNA levels. *J Clin Virol.* 2003;27(3):231-234
2. Buster EH, van der Eijk AA, Schalm SW. Doctor to patient transmission of hepatitis B virus: Implications of HBV DNA levels and potential new solutions. *Antiviral Res.* 2003;60(2):79-85.
3. Buster EH, van der Eijk AA, de Man RA, Schalm SW. Doctor to patient transmission of hepatitis B virus: The potential of antiviral therapy for prevention. *Scand J Gastroenterol.* 2004;Suppl.(241):45-49.
4. Buster EH, ter Borg MJ, Vingerling JR, Janssen HL. Low incidence of retinopathy during peginterferon alpha-2b and lamivudine therapy for chronic hepatitis B. *J Hepatol.* 2006;45(1):160-161.
5. Buster EH, Janssen HL. Antiviral treatment for chronic hepatitis B virus infection - Immune modulation or viral suppression? *Neth J Med.* 2006;64(6):175-185.
6. ter Borg MJ, Buster EH, Janssen HL. Interferon and pegylated interferon in chronic hepatitis B. In: Buti M and Esteban R. *BC)VH Viral Hepatitis*. Barcelona, Spain 2006: 32-45.
7. Buster EH, ter Borg MJ, Janssen HL. Pegylated interferon alpha for chronic hepatitis B - alone or in combination with lamivudine. In: Negro F. *Hot Topics in Viral Hepatitis*. Treatment of chronic hepatitis B: an update. Modena, Italy 2007: 21-27 (ISBN 978-88-89881-28-6).
8. Buster EH, van der Eijk AA, de Man RA, Janssen HL, Schalm SW. Prolonged antiviral therapy for hepatitis B virus infected health care workers: A feasible option to prevent work restriction without jeopardizing patient safety. *J Viral Hepat.* 2007;14(5):350-354.
9. Buster EH, Hansen BE, Buti M, Delwaide J, Niederau C, Michielsen P, Flisiak R, Zondervan PE, Schalm SW, Janssen HL. Peginterferon alpha-2b is safe and effective in HBeAg positive chronic hepatitis B patients with advanced fibrosis. *Hepatology* 2007;46(2):388-394.
10. Flink HJ, Buster EH, Merican I, Nevens F, Kitis G, Cianciara J, de Vries RA, Hansen BE, Schalm SW, Janssen HL. Relapse after treatment with peginterferon alpha-2b alone or in combination with lamivudine in HBeAg positive chronic hepatitis B. *Gut* 2007;56(10):1485-1486.
11. Buster EH, van Vuuren AJ, Zondervan PE, Metselaar HJ, Tilanus HW, de Man RA. Thiopurine-methyltransferase and inosine triphosphate pyrophosphatase polymor-phism in a liver transplant recipient developing nodular regenerative hyperplasia on low-dose azathioprine. *Eur J Gastroenterol Hepatol.* 2008; 20(1): 68-72.
12. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TMK, Feinman SV, Mach T, Akarca US, Schutten M, Tielemans W, Van Vuuren AJ, Hansen BE, Janssen HL. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. *Gastroenterology* 2008;135(2):459-467.
13. Buster EH, van Erpecum KJ, Schalm SW, Zaaijer HL, Brouwer JT, Gelderblom HC, de Knegt RJ, Bakker CM, Reesink HW, Janssen HL; Netherlands Association of Gastroenterologists and Hepatologists. Treatment of chronic hepatitis B virus infection - Dutch national guidelines. *Neth J Med.* 2008;66(7):292-306.
14. de Bruijne J, Buster EH, Gelderblom HC, Brouwer JT, de Knegt RJ, van Erpecum KJ, Schalm SW, Bakker CM, Zaaijer HL, Janssen HL, Reesink HW; Netherlands Association of Gastroenterologists and Hepatologists. Treatment of chronic hepatitis C virus infection - Dutch national guidelines. *Neth J Med.* 2008;66(7):311-322.
15. Schalm SW, Buster EH. The practice guideline 'Viral hepatitis and other liver diseases' (second revision) from the Dutch College of General Practitioners; a response from the perspective of gastroenterology. *Ned Tijdschr Geneeskd.* 2008;152(49):2658-61.
16. Janssen HL, Buster EH. Comments on the EASL practice guidelines for the management of chronic hepatitis B: controversies in interferon-based therapy. *J Hepatol.* 2009;51(1):224-226.

17. Buster EH, Schalm SW, Janssen HL. Peginterferon for the treatment of chronic hepatitis B in the era of nucleos(t)ide analogues. *Best Pract Res Clin Gastroenterol.* 2008;22(6):1093-1108.
18. Buster EH, Flink HJ, Simsek H, Heathcote EJ, Sharmila S, Kitis GE, Gerken G, Buti M, de Vries RA, Verhey E, Hansen BE, Janssen HL. Early HBeAg Loss During Peginterferon alpha-2b Therapy Predicts HBsAg Loss: Results of a Long-Term Follow-Up Study in Chronic Hepatitis B Patients. *Am J Gastroenterol*. in press.
19. Buster EH, Hansen BE, Lau GK, Piratvisuth T, Zeuzem S, Steyerberg EW, Janssen HL. Predicting response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa. *Gastroenterology* in press.

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PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student:	Erik H.C.J. Buster	PhD Period:	2005 - 2008
Erasmus MC department:	Gastroenterology and Hepatology	Promotor:	prof.dr.H.L.A. Janssen

1. PhD training	Year	Workload
Presentations and workshops		
Doctor to patient transmission of hepatitis B virus. Twice annual meeting of the Netherlands Association of Hepatology. March 20, Veldhoven, The Netherlands.	2003	12 hours
Solutions for infected health care workers. International Erasmus workshop on chronic hepatitis B. June 9, Rotterdam, The Netherlands.	2006	12 hours
Early peginterferon alpha-2b induced HBeAg loss results in increased rates of HBsAg loss and undetectable HBV DNA. Twice annual meeting of the Netherlands Association of Hepatology. October 6, Veldhoven, The Netherlands.	2006	18 hours
Peginterferon alpha-2b is well tolerated and leads to high virological and histological response rates in chronic hepatitis B patients with advanced fibrosis. Twice annual meeting of the Netherlands Association of Hepatology. October 6, Veldhoven, The Netherlands.	2006	18 hours
Hepatitis B virus (AASLD update). 4th Post-AASLD symposium. Noverber 23, Rotterdam, The Netherlands.	2006	18 hours
ITPA-polymorphism in a liver transplant patient. XIth Winter meeting of the Belgian Association for the Study of the Liver (BASL). December 15, Bruges, Belgium.	2006	8 hours
Workshop - Hepatitis B and C: from virus to antiviral therapy. Hepatitis Week. February 16, Amersfoort, The Netherlands.	2007	8 hours
Withdrawal flares after treatment with peginterferon alpha-2b alone or in combination with lamivudine in HBeAg-positive chronic hepatitis B. Twice annual meeting of the Netherlands Association of Hepatology. March 23, Veldhoven, The Netherlands.	2007	12 hours
New insights in HBV antiviral therapy. Symposium Hepatitis B and C guidelines. Twice annual meeting of the Netherlands Association of Hepatology. October 5, Veldhoven, The Netherlands.	2007	18 hours
Predicting sustained HBeAg loss after treatment with peginterferon alpha-2b: development and validation of a practical model. Twice annual meeting of the Netherlands Association of Hepatology. October 5, Veldhoven, The Netherlands.	2007	12 hours
AASLD HBV practice guidelines. Roche Pharma International Hepatitis Meeting: face the challenges, maximise success. November 30th, Marrakech, Morocco.	2007	12 hours

Antiviral therapy for chronic hepatitis B: to combine or not? 5th post-AASLD symposium. December 6, Rotterdam, The Netherlands.	2007	12 hours
Hepatitis B: the GLOBE study. 11th international symposium Current Topics in Infectious Diseases (CTID). Novartis satellite symposium. January 30, Grindelwald, Switzerland.	2008	4 hours
Diagnosis and Treatment of Hepatitis B and C. Second Siemens HCV roundtable. March 27, Breda, The Netherlands.	2008	4 hours
Chronische hepatitis B virus infection - new guidelines for antiviral therapy. Meeting of the Laboratory for Infectious Diseases. March 31, Paterswolde, The Netherlands.	2008	12 hours
Chronic hepatitis B - should we aim for sustained response? Meeting of the Limburg working party of Hepatology. April 7, Eindhoven, The Netherlands.	2008	6 hours
Guidelines for the treatment of chronic hepatitis B virus infection. World Hepatitis Day. May 19, Bilthoven, The Netherlands.	2008	4 hours
HBV genotype is an important predictor of sustained off-treatment response to both peginterferon alpha-2B and entecavir in HBeAg positive chronic hepatitis B. Twice annual meeting of the Netherlands Association of Hepatology. October 2, Veldhoven, The Netherlands.	2008	12 hours
Early HBeAg loss during peginterferon alpha-2b therapy predicts HBsAg loss - Results of a long-term follow-up study in chronic hepatitis B. Twice annual meeting of the Netherlands Association of Hepatology. October 2, Veldhoven, The Netherlands.	2008	12 hours
Prediction of response to peginterferon-alfa in HBeAg positive chronic hepatitis B: a model based on 721 patients. Twice annual meeting of the Netherlands Association of Hepatology. October 2nd, Veldhoven, The Netherlands.	2008	12 hours
Belang van hepatitis serologie voor diagnose en therapie. Symposium "Uitdagingen bij virale hepatitis". Nunspeet, October 29.	2008	4 hours
Poster presentations		
Peginterferon alpha-2b is well tolerated and leads to high virological and histological response rates in chronic hepatitis B patients with advanced fibrosis. 57th Annual meeting of the American Association of the Study of Liver Diseases (AASLD), abstract 974. October 30, Boston, MA, United States of America.	2006	24 hours
Early peginterferon alpha-2b induced HBeAg loss results in increased rates of HBsAg loss and undetectable HBV DNA. 57th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 975. October 30, Boston, MA, United States of America.	2006	32 hours
Withdrawal flares after treatment with peginterferon alpha-2b alone or in combination with lamivudine in HBeAg-positive chronic hepatitis B. 42nd Annual meeting of European Association for the Study of the Liver (EASL), abstract 2083. April 11, Barcelona, Spain.	2007	32 hours
Predicting sustained HBeAg loss after treatment with peginterferon alpha-2b: development and validation of a practical model. 58th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 1002. November 5, Boston, MA, United States of America.	2007	24 hours
Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg positive patients treated with peginterferon alpha-2b. 58th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 965. November 5, Boston, MA, United States of America.	2008	24 hours
HBV genotype is an important predictor of sustained off-treatment response to both peginterferon alpha-2B and entecavir in HBeAg positive chronic hepatitis B. 59th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 915. November 5, San Francisco, CA, United States of America.	2008	32 hours

Early HBeAg loss during peginterferon alpha-2b therapy predicts HBsAg loss - Results of a long-term follow-up study in chronic hepatitis B. 59th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 928. November 5, San Francisco, CA, United States of America.	2008	32 hours
Prediction of Response to Peginterferon-Alfa in HBeAg Positive Chronic Hepatitis B: A Model Based on 721 Patients. 59th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 929. November 5, San Francisco, CA, United States of America.	2008	36 hours
International Conferences		
Management of Chronic Hepatitis B: 2006. National Institutes of Health. April 6-8, Bethesda, MD, United States of America.	2006	18 hours
41st Annual Meeting of the European Association for the Study of the Liver (EASL). April 26-30, Vienna, Austria.	2006	28 hours
The Liver Meeting 2006, 57th Annual Meeting of the American Association for the Study of Liver Diseases. October 27-31, Boston, MA, United States of America.	2006	28 hours
42nd Annual Meeting of the European Association for the Study of the Liver (EASL). April 11-15, Barcelona, Spain.	2007	28 hours
The Liver Meeting 2007, 58th Annual Meeting of the American Association for the Study of Liver Diseases. November 2-6, Boston, MA, United States of America.	2007	28 hours
Roche Pharma International Hepatitis Meeting: Face the challenges, maximise success. November 29-30, Marrakech, Morocco.	2007	14 hours
43rd Annual Meeting of the European Association for the Study of the Liver (EASL). April 23-27, Milan, Italy.	2008	28 hours
The Liver Meeting 2008, 59th Annual Meeting of the American Association for the Study of Liver Diseases. October 31 -November 4, San Francisco, CA, United States of America.	2008	28 hours
Attended seminars and workshops		
International Erasmus Workshop on Chronic Hepatitis B. June 8-9, Rotterdam, The Netherlands.	2006	8 hours
Post-EASL symposium. May 9, Utrecht, The Netherlands. 4e Post AASLD symposium. November 23, Rotterdam, The Netherlands.	2006	2 hours
De Sterkste Schakel Hepatitis B en C. September 13, Hilversum, The Netherlands.	2006	2 hours
De Sterkste Schakel Hepatitis B en C. September 26, Utrecht, The Netherlands.	2006	2 hours
5e Post AASLD symposium. December 6, Rotterdam, The Netherlands.	2007	2 hours
Refereeravond Laboratorium voor Infectieziekten: Chronische hepatitis B virusinfectie - Nieuwe richtlijnen voor diagnostiek en behandeling. March 31, Paterswolde, The Netherlands.	2007	2 hours
Refereeravond Limburgse Werkgroep voor Hepatitis: Chronische hepatitis B virusinfectie - Nieuwe richtlijnen voor diagnostiek en behandeling. April 7, Eindhoven, The Netherlands.	2008	2 hours
Studiemiddag HIV en hepatitis B: Maakt de dokter het verschil? March 19, Rotterdam, The Netherlands.	2008	2 hours
Eerste Lagerhuisdebat Hepatitis B en C. Amsterdam, September 25.	2008	2 hours
De 24-uur van De Vanenburg. Putten, September 26-27.	2008	6 hours

Other		
Secretary of the hepatitis B and C guidelines committee of the Netherlands Association of Gastroenterologists and Hepatologists	2006-2008	144 hours
2. Teaching activities		
Lecturing		
Hepatitis B. 3rd year Erasmus MC medical students participating in a 4-week Gastroenterology and Hepatology training program. January 25, Rotterdam, The Netherlands.	2007	8 hours
Hepatitis B. 2nd year Erasmus MC medical students participating in a 4-week Infectious Diseases training program. June 7, Rotterdam, The Netherlands.	2007	8 hours
Hepatitis B. 2nd year Erasmus MC medical students participating in a 4-week Infectious Diseases training program. June 5, Rotterdam, The Netherlands.	2008	4 hours
Supervising practicals and excursions		
Interactive workshops on strategies for treating difficult-to-cure patients: post-transplant patients with recurrent HCV. Roche Pharma International Hepatitis Meeting: face the challenges, maximise success. November 29, Marrakech, Morocco.	2007	8 hours
Supervising theses		
Undergraduate thesis students Turnhout school of Midwivery: "Management of hepatitis B in pregnancy - An important role for midwifes"	2008	40 hours