Immune Modulating Therapy and its Viral Kinetics in Chronic Hepatitis B

Martijn ter Borg

Immune Modulating Therapy and its Viral Kinetics in Chronic Hepatitis B

Martijn J. ter Borg

ISBN:
Layout by: Martijn J. ter Borg
Printed by: Optima Grafische Communicatie

This study was performed at the department of Gastroenterology and Hepatology of the Erasmus MC, University Medical Center Rotterdam, The Netherlands. Financial support for this theses was kindly given by: AstraZeneca B.V., Boehringer Ingelheim B.V., Janssen-Cilag B.V., Roche Nederland B.V., Schering-Plough B.V., Zambon Nederland B.V. and the Erasmus University Rotterdam.

Immune Modulating Therapy and its Viral Kinetics in Chronic Hepatitis B

Immuunmodulerende therapie en virale kinetiek in chronische hepatitis B

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 3 december 2008 om 13:45 uur.

door

Martijn Johannes ter Borg
geboren te Rotterdam

PROMOTIECOMMISSIE

Promotor:	Prof.dr. H.L.A. Janssen
Overige leden:	Prof.dr. C.A.B. Boucher
	Prof.dr. J.P.H. Drenth
	Prof.dr. H.J. Metselaar

Voor Marjolein,

TABLE OF CONTENTS

ABBREVIATIONS

ALT	alanine aminotransferase
anti-HBe	antibody to hepatitis B e antigen
anti-HBs	antibody to hepatitis B surface antigen
DNA	deoxyribonucleic acid
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
IFN	interferon alpha
HBV	hepatitis B virus
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
HIV	human immunodeficiency virus
PCR	polymerase chain reaction
PEG	polyethylene glycol
PEG-IFN	pegylated interferon alpha
ROC	receiver operating characteristic

CHAPTER 1

Interferon and pegylated interferon in chronic hepatitis B

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

Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands.

Adapted from "*Interferon and pegylated interferon in chronic hepatitis B*", Viral hepatitis B and C, Maria Buti & Rafael Esteban (editors). Barcelona: Grup 3, 2006: 32-45.

Introduction

Approximately 400 million people worldwide are chronically infected with the hepatitis B virus (HBV) and it is estimated that between 500,000 and 1 million people die annually from cirrhosis and hepatocellular carcinoma due to HBV infection.[1-3] Despite the availability of safe and effective vaccines for more than two decades, HBV infection still is one of the major global health problems.

Patients with chronic hepatitis B can present in one of four phases of infection.[4] In the immunotolerant phase, hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) are detectable, HBV DNA levels are high and there is minimal hepatic inflammation. When infected at childhood, this immunotolerant phase may last 10 to 30 years with a low rate of spontaneous HBeAg clearance.[5] In the immuno-active phase, HBsAg, HBeAg and high HBV DNA are still present, while an active immune response results in hepatic inflammation with elevation of serum alanine aminotransferase (ALT) levels. Spontaneous loss of HBeAg and seroconversion to anti-HBe can occur. The immune-control phase follows HBeAg-seroconversion, with minimal hepatic inflammation and low HBV DNA levels due to a continuous host immune response. There is however a subgroup of HBeAg-negative chronic hepatitis B patients where biochemical and histological activity recurs with higher HBV DNA levels compared to patients in the immune-control phase. These patients have HBV variants that hamper the production of the HBeAg. The most commonly described mutation is a G to A switch at position 1896 of the pre-core region of the hepatitis B genome. This mutation leads to a translational stop codon in the leader sequence of the HBeAg protein, resulting in the inhibition of the protein synthesis.

Many studies show the importance of HBV genotype on both natural course of HBV infection and response to antiviral treatment.[6-11] Hepatitis B virus has been classified into 8 genotypes (A to H), based on an intergroup divergence of the genomic nucleotide sequence of more than 8%.[12-14] Genotypes A and D are most frequently observed in Europe and North America, while genotypes B and C are common in Asia.

In the last years, treatment options for chronic hepatitis B have largely extended. Currently registered treatment for chronic hepatitis B consists of (pegylated) interferon, lamivudine, adefovir, entecavir and telbivudine. Tenofovir disoproxil fumarate, an acyclic nucleotide

analogue, appears to be an effective drug against HBV and is already approved for the treatment of human immunodeficiency virus (HIV) infection. Initiation of therapy requires consideration not only of the potency of individual drugs, but also the resistance profile of each agent.

Interferon-alpha (IFN-α) has been the mainstay of therapy for both HBeAg-positive and -negative chronic hepatitis B since the early 1980s, despite frequently occurring side-effects such as flu-like symptoms, depression and bone marrow suppression. A finite treatment course with (pegylated) interferon results in sustained off-treatment response in about one third of patients. Induction of an HBV specific immune response seems to be crucial for persistent control of HBV infection. It has been demonstrated that response to a course of IFN-α is durable in the majority of patients and leads to both improved survival and reduction of the incidence of hepatocellular carcinoma.[15, 16]

Nucleos(t)ide analogues are potent inhibitors of HBV replication. However, indefinite therapy is required in most patients. Another important drawback of nucleos(t)ide treatment is the risk for antiviral resistance, which occurs most frequently in lamivudine and telbivudine treatment and to a lesser extent during adefovir, entecavir and probably tenofovir therapy.

Antiviral treatment is generally recommended for chronically infected patients with high serum HBV DNA (above 10^5 and 10^4 copies/ml in HBeAg-positive and HBeAg-negative patients, respectively) and persistent elevated ALT levels over a 3-6 month period.[3, 17] In this chapter we will discuss the role of interferon alpha (IFN-α) and pegylated interferon alpha (PEG-IFN) in the treatment of chronic HBV.

Antiviral actions of interferon

Interferon was discovered as an antiviral agent during studies on virus interference in the late 1950s. Interferons are potent cytokines with immunomodulatory, anti-proliferative and antiviral properties. There are multiple naturally occurring forms of IFN in humans including IFN-α, which is produced by lymphocytes. IFNs are involved in the host's elimination or control of acute and chronic viral infections. The effects of IFN-α are predominantly immunoregulatory, but there is also a limited direct antiviral effect on HBV. IFN-α inhibits viral replication, degrades viral components, induces the production of interleukins and subsequently T-cell growth, augments lytic activity of natural killer cells and cytotoxic T-

cells, enhances the expression of antigens by the major histo-compatibility complex, and modulates the production of pro-inflammatory cytokines.

The addition of a polyethylene glycol (PEG) molecule to IFN-α significantly prolongs half-life, resulting in more sustained IFN activity and a more convenient once weekly dosing. Two pegylated IFNs have been studied for the treatment of HBV, a large branched 40kDa PEG linked to IFN-α2a (PEG-IFN alpha-2a) and a small linear 12kDa PEG linked to IFN-α2b (PEG-IFN alpha-2b).[18] PEG-IFN alpha-2a (40 kDa) has a longer half-life (approximately 80 hours), is mainly catabolized in the liver and has active breakdown products. The smaller PEG-IFN alpha-2b (12 kDa) has a shorter half-life (approximately 40 hour) and may act as a pro-drug depot, slowly releasing interferon.[19]

Both these IFNs were initially investigated for the treatment of chronic hepatitis C infection and have shown similar tolerability and higher rates of sustained viral response compared to conventional IFN-α.[20, 21] PEG-IFN alpha-2a has recently been licensed for the treatment of both HBeAg-positive and HBeAg-negative chronic HBV. PEG-IFN alpha-2b has yet only been licensed for the treatment of chronic HBV in specific countries.

HBeAg response

HBeAg-loss or HBeAg-seroconversion is often used as primary endpoint for treatment of HBeAg-positive chronic hepatitis B. Two studies have directly compared PEG-IFN to conventional IFN. One study shows that PEG-IFN is superior to conventional IFN-α in inducing HBeAg-loss (23% vs. 17% at the end of treatment and 24% vs. 14% at the end of follow up, p=0.04),[22] the other found a higher combined response rate (HBeAg-loss, HBV DNA <500,000 copies/ml and ALT normalization) in the PEG-IFN monotherapy group compared to the conventional IFN group (p=0.04).[23]

Several randomized trials of PEG-IFN for the treatment of HBeAg-positive chronic HBV have been reported. Treatment duration varied among these studies from 24 to 52 weeks with 24 to 26 weeks of treatment-free follow-up. By the end of treatment, loss of HBeAg occurs in 23-30% of PEG-IFN treated patients[10, 11, 22] and in 27-44% of patients on combination therapy with lamivudine.[10, 11] End of treatment HBeAg-loss rates are significantly higher in patients receiving combination therapy than PEG-IFN monotherapy in one study (44% vs. 29%, p=0.01),[11] while another study does not confirm this finding

(30% vs. 27%).[10] However, at the end of follow-up HBeAg-loss rates are comparable among treatment groups and vary between 24% and 36% (figure 1).[10, 11, 22, 23]

At the end of a 24 or 48-week course of PEG-IFN monotherapy, 22% to 27% of patients has seroconverted to anti-HBe compared to 24% to 60% of patients treated with combination therapy.[10, 11, 22] As for HBeAg-loss, HBeAg-seroconversion rates are higher in PEG-IFN and lamivudine treated patients compared to patients treated with PEG-IFN alone at the end of treatment but this difference in HBeAg-seroconversion rate is no longer observed at the end of follow-up.[10, 11, 22]

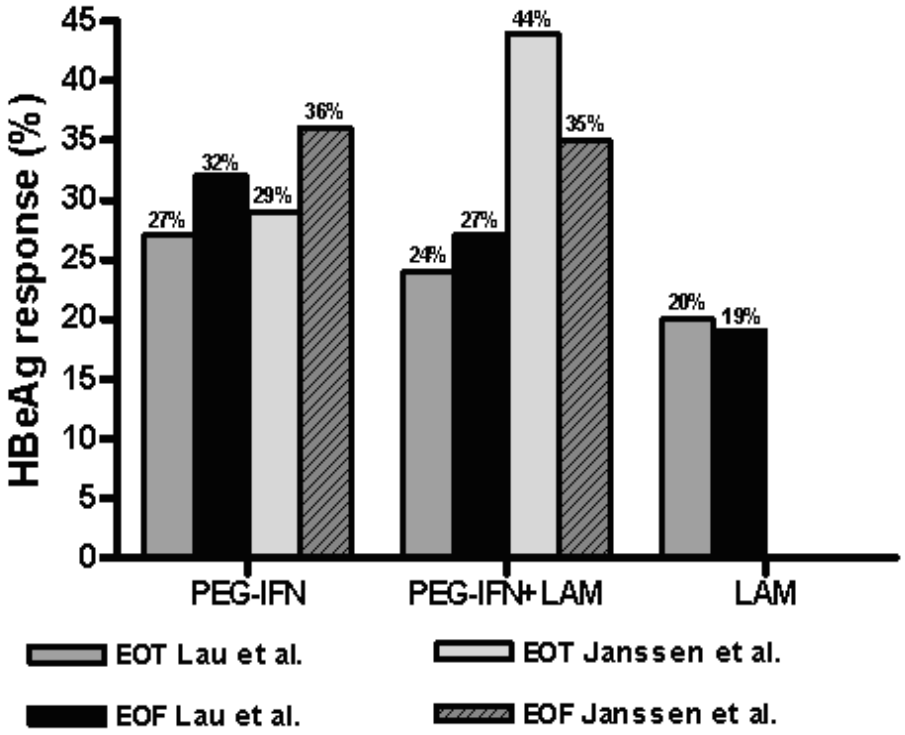


Figure 1: HBeAg-response rates for PEG-IFN with or without lamivudine compared to lamivudine monotherapy at the end of treatment and end of follow-up. *In the study by Janssen et al.[11], HBeAg-loss was used as primary endpoint and there was a significant difference in HBeAg-loss at the end of treatment between the two treatment arms (p=0.01). This difference was not observed at the end of follow-up (p=0.91). In the Lau study[10], HBeAg-seroconversion occurred significantly more often in PEG-IFN and PEG-IFN plus lamivudine treated patients compared to lamivudine monotherapy at the end of follow-up (p<0.001 and p=0.02, respectively). Combination therapy of PEG-IFN plus*

lamivudine did not lead to higher HBeAg-seroconversion rates compared to PEG-IFN monotherapy. (27% vs. 32%, p=0.23). EOT = end of treatment; EOF = end of follow-up.

HBV DNA response

Suppression of serum HBV DNA is an important outcome measure of antiviral treatment for chronic hepatitis B since recent studies show that lower HBV DNA levels are associated with lower rates of progression to cirrhosis and hepatocellular carcinoma and thereby improved long-term outcome.[24, 25]

In studies comparing conventional IFN-α with PEG-IFN in HBeAg-positive chronic hepatitis B, higher rates of HBV DNA suppression below 10^5 copies/ml at the end of follow-up were observed in the PEG-IFN treated patients (27-43%) compared to those who received conventional IFN (25-27%).[22, 23] These differences are however not significant. In one study, there was a significant higher mean reduction in HBV DNA levels at the end of treatment in the PEG-IFN group compared with the conventional IFN group (2.22 $\log_{10}$ copies/ml vs. 1.68 $\log_{10}$ copies/ml; p=0.02).[22]

In HBeAg-positive patients treated with PEG-IFN alone, there is a less pronounced decline in HBV DNA during treatment compared to combination therapy with PEG-IFN and lamivudine (figure 2). Suppression of HBV DNA levels below 10^5 copies/ml can be observed in 29-52% of PEG-IFN treated patients at the end of treatment,[10, 11, 22] compared to 74-86% of patients treated with PEG-IFN and lamivudine combination therapy.[10, 11] However, in the combination therapy group there is a marked rebound in HBV DNA after treatment discontinuation resulting in comparable proportions of patients with HBV DNA below 10^5 copies/ml in the two treatment groups of 27% to 39% at the end of follow-up.[10, 11, 22, 23] A decline in serum HBV DNA below the lower limit of detection by quantitative PCR assay occurs in 10-25% of PEG-IFN treated patients by the end of treatment. Addition of lamivudine significantly increases this rate to 33-69%. However, also for this endpoint, end of follow-up analysis show that the advantage of added lamivudine is not durable, with comparable rates of PCR negativity in both groups (6-14%).[10, 11, 26]

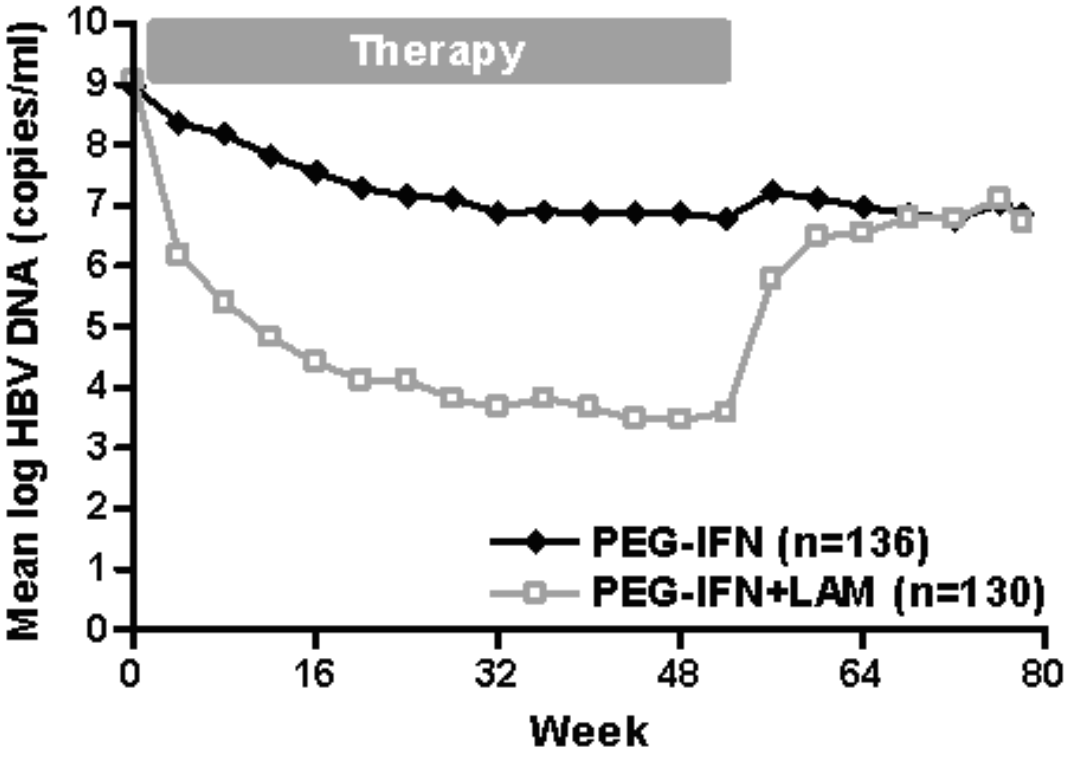


Figure 2: HBV DNA decline during treatment with PEG-IFN alone or in combination with lamivudine. *This figure shows decline in HBV DNA during treatment and follow-up in patients treated with PEG-IFN monotherapy (♦) and PEG-IFN plus lamivudine combination therapy (□) in the study by Janssen et al.[11] During treatment, there is a more pronounced decline in HBV DNA in the combination therapy group. Mean HBV DNA was significantly lower for combination therapy compared to monotherapy at the end of treatment (3.70 log10 copies/ml vs. 6.81 log10 copies/ml, p<0.001). This greater decline is however not durable and HBV DNA levels in the two treatment arms are comparable at the end of follow-up. In HBeAg-negative patients serum HBV DNA is below 20,000 copies/ml in 81% of PEG-IFN treated patients and 92% of patients receiving PEG-IFN and lamivudine combination therapy. At the end of follow-up these rates are 43% and 44%, respectively.[27] Suppression of HBV DNA below 400 copies/ml at the end of treatment occurs more frequently in patients on combination therapy (87% vs. 63% in the PEG-IFN monotherapy group), while this difference is not observed at the end of follow-up (19% vs. 20%).[27]*

Biochemical response

Elevated ALT levels are an indirect marker of hepatic inflammation. When comparing conventional IFN-α with PEG-IFN, similar rates of ALT normalization were observed in both treatment groups at the end of follow-up (25-44%).[22, 23] In HBeAg-positive patients, ALT normalization occurs in 32-51% of patients treated with either PEG-IFN monotherapy or combination therapy with lamivudine at the end of treatment.[10, 11, 22, 26] After 24 weeks of follow-up, a comparable rate of 32-50% can be observed, indicating a durable response to PEG-IFN treatment (figure 3A).[10, 11, 22, 23, 26] The proportion of HBeAg-negative patients with normalized ALT levels is 38% with PEG-IFN monotherapy compared to 49% with PEG-IFN and lamivudine at the end of treatment. After 24 weeks of follow-up, higher rates of 59% and 60% are observed, respectively.[27] Combined biochemical and virological response (HBV DNA < 20,000 copies/ml) is an important outcome measure in HBeAg-negative patients and occurs in 36% of PEG-IFN treated patients and 49% of patients receiving combination therapy at the end of treatment. This higher biochemical and virological response with combination therapy compared to PEG-IFN alone is not observed at the end of follow-up (figure 3B).[27]

Histological response

Histological response is usually defined as a 2-point decrease in necroinflammatory score (range 0-18) or 1-point decrease in fibrosis score (range 0-6), according to the histological activity index.[28] In HBeAg-positive patients, 49-53% of patients has improvement of liver histology after an one year course of PEG-IFN using the definition stated above (figure 4).[10, 11] Among patients with HBeAg-loss, 78% has decrease of necroinflammation and 39% decrease of fibrosis, compared to 43% and 15% of non-responders (p=0.01 and p=0.04 for responders compared to non-responders, respectively).[29] Patients with normalization of ALT after therapy also have an increased likelihood of improvement in necroinflammatory score compared to patients with persistence of elevated ALT (76% vs. 40%, p=0.01).[29] In HBeAg-negative patients, improvement of liver histology can be observed in 55% of PEG-IFN treated patients at the end of follow-up (figure 4), and a decreased fibrosis score in only 15%.[27] Among patients with HBV DNA <20,000 copies/ml

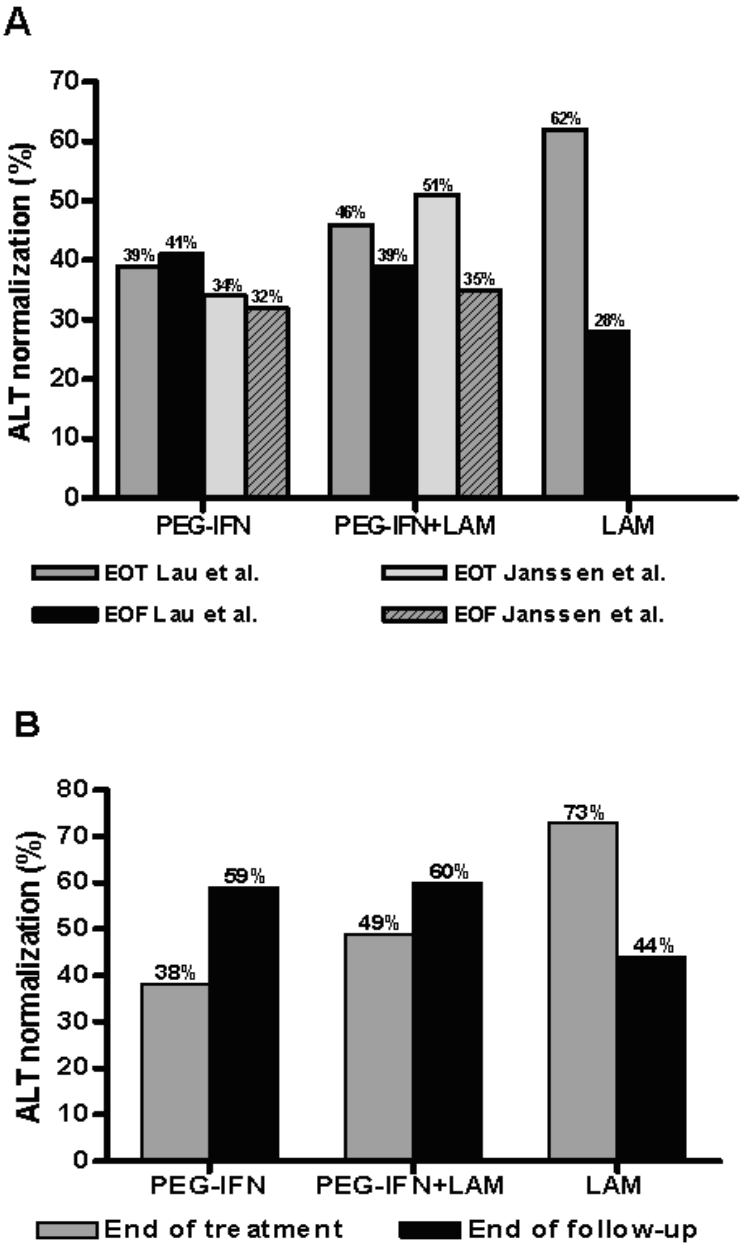


Figure 3: ALT normalization for PEG-IFN monotherapy, PEG-IFN plus lamivudine and lamivudine monotherapy in both HBeAg-positive (A) and HBeAg-negative (B) chronic hepatitis B at the end of treatment and end of follow-up. *In HBeAg-positive chronic hepatitis B, the percentage ALT normalization is higher in patients treated with lamivudine monotherapy compared to PEG-IFN treated patients at the end of treatment. During follow-up the ALT normalization rate remains stable in PEG-IFN treated patients but there is a marked decrease in the lamivudine monotherapy treated arm. In the study from Marcellin et al.[27] in HBeAg-negative chronic hepatitis B, ALT normalization rates in*

PEG-IFN treated patients tends to be higher at the end of follow-up compared to the end of treatment. As in HBeAg-positive chronic hepatitis B, there is a decrease in ALT normalization in patients treated with lamivudine monotherapy. EOT = end of treatment; EOF = end of follow-up.

at week 72, the combined histological response rate was 73%, compared to 49% in non-responders (p<0.001). As in HBeAg-positive patients, normalization of ALT is associated with higher rates of histological response.[27] Reversal of cirrhosis has been observed in 35% of cirrhotic HBeAg-negative patients (13/37 patients) after PEG-IFN therapy.[30] No additional benefit on histological response was observed from the addition of lamivudine in either HBeAg-positive or HBeAg-negative patients.[10, 11, 27, 29, 30]

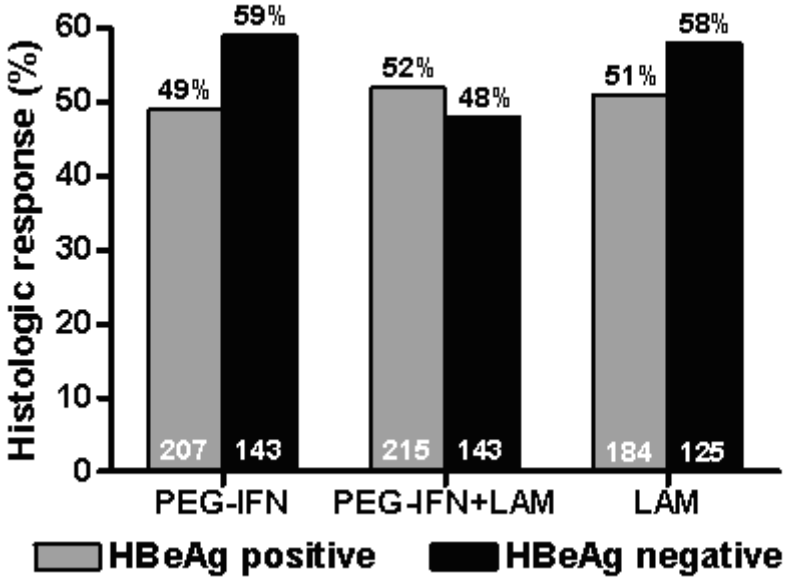


Figure 4: Histological response after PEG-IFN monotherapy, PEG-IFN plus lamivudine and lamivudine monotherapy in HBeAg-positive[10] and HBeAg-negative[27] chronic hepatitis B. *The histological response rate, defined as a reduction of at least two points in the modified Histological Activity Index score, was similar among the three treatment groups in both HBeAg-positive and HBeAg-negative chronic hepatitis B patients. The number of patients with paired biopsy samples is given in the boxes.*

HBsAg response

Loss of HBsAg with seroconversion to anti-HBs is the ultimate goal of antiviral therapy since this represents complete control of the virus by the host's immune system. In HBeAg-positive patients, treatment with PEG-IFN monotherapy results in HBsAg-seroconversion in 3-5% of patients at the end of follow-up.[10, 11] HBeAg-responders are more likely to achieve HBsAg-seroconversion with rates of about 10-19%.[31, 32] Further, higher rates of HBsAg-seroconversion have been observed in Caucasian patients compared to Asians (17% vs. 2%, $p<0.0001$).[32] Combination therapy with PEG-IFN and lamivudine does not increase HBsAg-seroconversion rates.

In HBeAg-negative chronic hepatitis B patients, HBsAg-seroconversion occurs in 3% treated with PEG-IFN alone and in 2% of patients with added lamivudine. Achievement of combined response (HBV DNA < 20,000 and ALT normalization) increases the HBsAg-seroconversion rate in HBeAg-negative patients to 6%.[32]

Predictors of response

In previous studies in chronic hepatitis B with standard IFN, several baseline factors have been identified which positively influence the chance of response. These baseline factors include high ALT, low HBV DNA, high necroinflammation score and infection on adult age.[3] HBV genotype also influences response rates, with genotype A and B resulting in higher response rates than genotype C and D.[8, 9] The role of HBV genotype as a predictor of response to PEG-IFN will be discussed separately below. In HBeAg-positive chronic hepatitis B, baseline factors that are predictive of response to PEG-IFN therapy include low viral load,[11, 33] high ALT concentrations,[11, 33] absence of previous interferon therapy,[11] low HBeAg level,[33] and HBV genotype.[11] In HBeAg-negative patients, low baseline HBV DNA and high baseline ALT levels also independently predict biochemical and virological response after 24 weeks post-treatment follow-up ($p=0.005$ and $p=0.001$).[34]

Hepatitis B virus genotype and response to PEG-IFN

The frequency of the four most prevalent HBV genotypes (A-D) differs across the large trials of PEG-IFN for chronic HBV as these studies were performed in different geographic

regions. Among HBeAg-positive patients, almost all patients in the study by Lau et al. were infected with HBV genotype B (28%) or C (59%),[10] while genotypes A and D were predominant in the Janssen study (33% and 39%, respectively).[11] In the HBeAg-negative study, genotype B (24%), C (37%) and D (31%) were most prevalent.[35]

As previously shown in treatment with conventional IFN,[8, 9] HBV genotype also seems to influence response to PEG-IFN. In the study by Janssen et al. in HBeAg-positive chronic hepatitis B, there was a significant difference in response rate across HBV genotypes. In patients treated with PEG-IFN with or without lamivudine, HBeAg-loss occurs significantly more often in patients with genotype A compared to genotype D (47% vs. 25%, p=0.01), while genotype B infection tends to result in higher response rates than genotype C (figure 5).[11] Not only HBeAg-loss, but also loss of HBsAg is more likely in patients with genotype A than in those with genotype D (14% vs. 2%, p=0.006).[31] In contrast to the results from the Janssen study, HBeAg-seroconversion rates are comparable between genotype B and C in the study from Lau et al.[10] Furthermore, in the limited number of patients with genotype A (41) or D (20) treated with PEG-IFN with or without lamivudine in this study, HBeAg-seroconversion seems more likely to occur in patients with genotype A (39%) than D (20%) (figure 5). HBsAg-seroconversion rates significantly differ across genotypes in this study.[32] Patients with genotype A have higher rates of HBsAg-seroconversion than patients with genotype B (17% vs. 0.6%, p<0.001) and tend to have higher HBsAg seroconversion rates compared to genotype D (17% vs. 0%, p=0.08).[32]

In HBeAg-negative patients, sustained response at week 72 (HBV DNA <20,000 copies/ml) occurs more often in patients with genotype C (58%) compared to genotype A (33%), B (43%) or D (29%) in patients treated with PEG-IFN or its combination with lamivudine (p<0.04 for genotype C vs. A , B or D).[34, 35] Virological response rates are higher in genotype B infection compared to genotype D (p=0.05). HBeAg-negative chronic hepatitis B patients with genotype A (14%) are more likely to achieve HBsAg-seroconversion than those with genotype B (4%), C (2%) or D (0%) (p<0.02 for genotype A compared to other genotypes).[32] HBsAg-seroconversion rates tend to be higher in genotype B infection than genotype D (p=0.08).

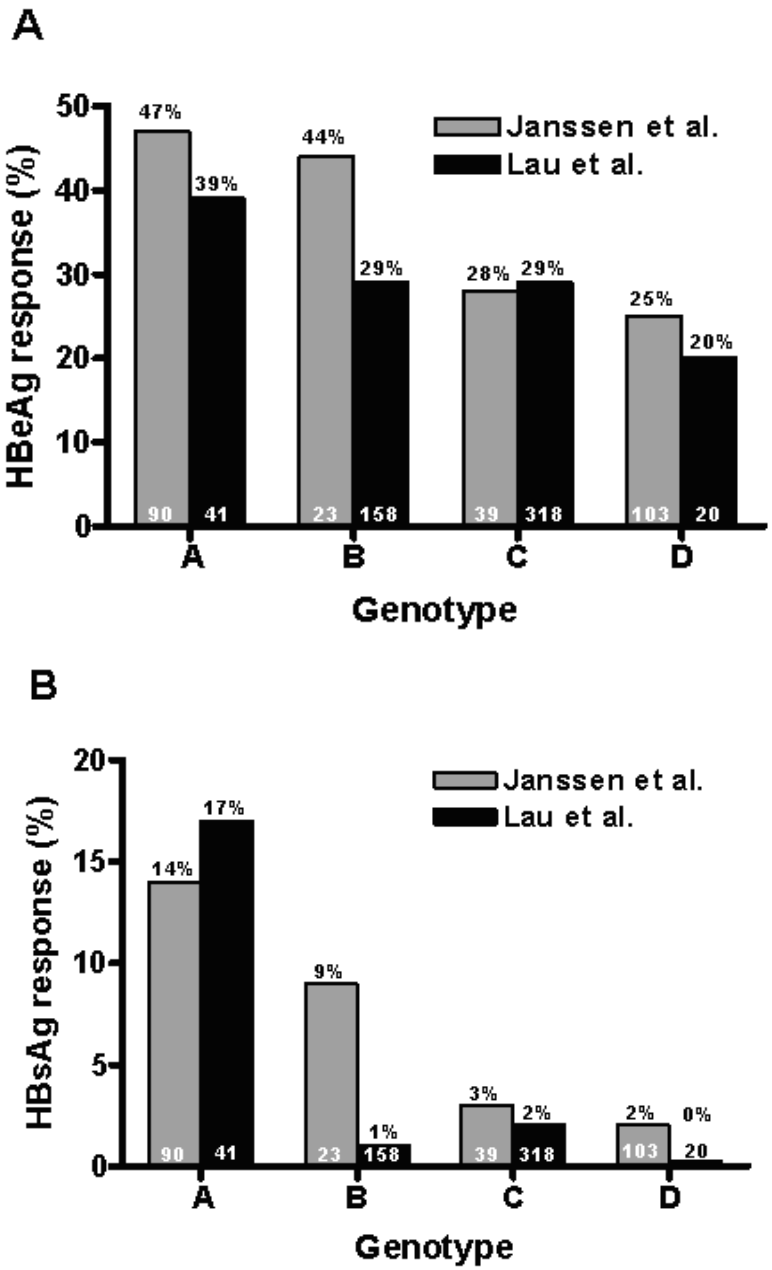


Figure 5: HBeAg-response (A) and HBsAg-response (B) according to genotype in patients treated with PEG-IFN with or without lamivudine in two studies in HBeAg-positive chronic hepatitis B. *In the study by Janssen et al.[11], loss of HBeAg and HBsAg was used as endpoint of treatment, whereas seroconversion to anti-HBe and anti-HBs was used in the Lau study.[10] In the Janssen study, patients with HBV genotype A had a significant higher chance of HBeAg-loss and HBsAg-loss compared to genotype D (p=0.01 and p=0.006, respectively).*

Long term outcome

Several studies have been performed on the long term outcome of conventional IFN treatment for chronic hepatitis B, while these studies are pending for PEG-IFN treated patients.[15, 16, 36] In the most recent study in HBeAg-positive chronic hepatitis B, patients were treated with a median IFN-α dose of 30 MU/week with a median treatment duration of 16 weeks. HBeAg-loss occurred in 33% of patients within 12 months after stopping therapy[16]. The long term results of these patients with HBeAg-loss after IFN therapy are very promising, with sustained HBeAg-loss in 87% and HBsAg-loss in 52% of responders after 8.8 years of follow-up. HBsAg-loss was observed in 9% of patients without initial HBeAg-loss after IFN treatment. Another outcome of this study was a significantly reduced risk of developing HCC and improved survival in patients with initial HBeAg-loss.

Conclusion

The approach to treatment of chronic hepatitis B has rapidly changed over the past decades. Treatment options have advanced with the availability of new nucleos(t)ide analogues. Treatment with PEG-IFN results in sustained HBeAg or virological response in more than one third of HBeAg-positive and HBeAg-negative patients. Response is associated with improvement of liver enzyme abnormalities, liver histology, and even with an increased likelihood of HBsAg-loss. Long term follow-up studies in IFN-α treated patients also show improved survival and a reduced risk of developing HCC in patients with HBeAg-loss

The addition of lamivudine to PEG-IFN therapy has been investigated in several studies. Although this combination provided greater viral suppression compared to PEG-IFN monotherapy at the end of treatment, it did not improve response rates at the end of follow-up. Combination with other nucleos(t)ide analogues and different treatment regimens may lead to higher sustained response rates and should be studied in future trials.

Despite its side effects, PEG-IFN therapy still offers the highest chance of sustained off-treatment response for a large group of chronic hepatitis B patients. Treatment with this drug is therefore preferable as first line-therapy in eligible patients with a high likelihood of response. This includes in particular HBeAg-positive patients with high baseline ALT, moderate HBV DNA levels and genotype A or B.

References

1. Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13 Suppl 1:S47-9.
2. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337(24):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 Suppl 1:S3-25.
4. Wong SN, Lok AS. Treatment of hepatitis B: who, when, and how? Arch Intern Med 2006;166(1):9-12.
5. Chang MH. Natural history of hepatitis B virus infection in children. J Gastroenterol Hepatol 2000;15 Suppl:E16-9.
6. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118(3):554-9.
7. Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterology 2002;123(6):1848-56.
8. Erhardt A, Blondin D, Hauck K, et al. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut 2005;54(7):1009-13.
9. 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(6):1425-30.
10. 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(26): 2682-95.
11. 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.
12. Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J Gen Virol 2002;83(8):2059-73.
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. Stuyver L, De Gendt S, Van Geyt C, et al. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 2000;81(1):67-74.
15. 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(22):1422-7.
16. 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(3): 804-10.
17. Manesis EK, Papatheodoridis GV, Sevastianos V, Cholongitas E, Papaioannou C, Hadziyannis SJ. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis B e antigen-negative chronic hepatitis B virus infection. Am J Gastroenterol 2003;98(10):2261-7.
18. Craxi A, Cooksley WG. Pegylated interferons for chronic hepatitis B. Antiviral Res 2003;60(2):87-9.
19. Kozlowski A, Charles SA, Harris JM. Development of pegylated interferons for the treatment of chronic hepatitis C. BioDrugs 2001;15(7):419-29.
20. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358(9286):958-65.
21. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.
22. Zhao H, Si CW, Wei L, et al. A multicenter, randomized. open-label study of peginterferon alfa-2b vs interferon alfa-2b for the treatment of Chinese patients with HBeAg+ve chronic hepatitis B. J Hepatol 2006;44(Suppl. 2):S20-1.
23. 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(4):298-305.
24. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295(1):65-73.
25. 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(3):

678-86.

26. 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(4):240-50.
27. 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(12):1206-17.
28. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1(5):431-5.
29. van Zonneveld M, Zondervan PE, Cakaloglu Y, et al. Peg-interferon improves liver histology in patients with HBeAg-positive chronic hepatitis B: no additional benefit of combination with lamivudine. Liver Int 2006;26(4):399-405.
30. Cooksley G, Lau GKK, Marcellin P, et al. Peginterferon alpha-2a (40kDa) (Pegasys®) reverses cirrhosis, improves fibrosis and slows progression of fibrosis in patients with HBeAg-positive of HBeAg-negative chronic hepatitis B (CHB). J Hepatol 2006;44(Suppl. 2):S182.
31. 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.
32. 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(Suppl 2):S178.
33. Cooksley G, Lau GKK, Liaw YF, et al. Effects of genotype and other baseline factors on response to peginterferon α-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol 2005;42(Suppl.2):S30.
34. Bonino F, Lau GKK, Marcellin P, et al. The first detailed analysis of pedictors 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(S4):A1142.

35. Marcellin P, Lau GKK, Bonino F, et al. Peginterferon alfa-2a (40KD) (Pegasys®) monotherapy is more effective than lamivudine monotherapy in the treatment of HBeAg-negative chronic hepatitis B: 72-week results from a phase III, partially double-blind study of Pegasys® alone vs Pegasys® plus lamivudine vs lamivudine. J Hepatol 2004;40(Suppl. 1):S34.
36. 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(8):629-34.

CHAPTER 2

Patterns of viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B. Relation to treatment response

Martijn J ter Borg[1], Monika van Zonneveld[1], Stefan Zeuzem[2], Hakan Senturk[3], Ulus S Akarca[4], Christopher Simon[5], Bettina E Hansen[1,6], Bart L Haagmans[7], Robert A de Man[1], Solko W Schalm[1], Harry LA Janssen[1] for the HBV 99-01 study group*

1. Department of Gastroenterology and Hepatology, Erasmus Medical Center Rotterdam, The Netherlands
2. Medizinische Klinik und Poliklinik, Innere Medizin II, Universitätskliniken des Saarlandes, Homburg/Saar, Germany
3. Department of Internal Medicine, Cerraphasa Medical Faculty, University of Istanbul, Istanbul, Turkey
4. Department of Gastroenterology, Ege University Hospital, Izmir, Turkey
5. Department and Clinic of Infectious Diseases, Medical University, Wroclaw, Poland
6. Department of Epidemiology and Biostatistics, Erasmus Medical Center Rotterdam, The Netherlands
7. Department of Virology, Erasmus Medical Center Rotterdam, The Netherlands

* Other members of the HBV 99-01 Study Group are listed in the appendix

Hepatology 2006;44(3): 721-7

Summary

In chronic hepatitis B, it is difficult to predict response early during therapy. We investigated the viral decline during therapy with pegylated interferon alpha-2b (PEG-IFN) with or without lamivudine in 266 HBeAg-positive chronic hepatitis B patients.

In patients treated with PEG-IFN and lamivudine, a uniform biphasic viral decline pattern was found during therapy and there were no marked differences in viral load between those who lost HBeAg at the end of follow-up (response) or not. In contrast, those treated with PEG-IFN monotherapy exhibited different viral decline patterns. A delayed decline of at least two log from baseline HBV DNA after week 4 but before week 32 was associated with the highest response rate (63%). In comparison, response was 52% for patients with an early decline (week 0-4), 38% for a late decline (week 32-52), 27% for a post-treatment decline (week 52-78) and 11% for patients with no substantial decline. The HBsAg loss was 22% in the delayed decline pattern compared to 4% for those with early decline and none for other decline patterns.

In conclusion, different patterns of decline in viral load during treatment with PEG-IFN monotherapy were associated with different rates of HBeAg and HBsAg loss at the end of follow-up. Since there was a considerable response, even in patients with a late or post-treatment decline pattern, prediction of response based on viral decline during the first months of therapy was difficult.

Introduction

Nucleos(t)ide analogues, such as lamivudine or adefovir dipivoxil strongly suppress viral replication and induce hepatitis B e antigen (HBeAg) loss in 12-33% of the patients.[1-5] However the response of these agents may not be durable after discontinuation of therapy and prolonged therapy leads to the emergence of resistant hepatitis B virus (HBV) mutants in an increasing proportion of patients.[6-10]

Interferon alpha (IFN) has been shown to induce HBeAg seroconversion in approximately one third of patients,[11] but has considerable side effects. The efficacy of IFN therapy is improved by using its pegylated form, resulting in higher response rates in patients with chronic hepatitis B.[12-15] The most important predictive baseline factors for response to pegylated interferon therapy are HBV genotype A or B, low HBV DNA and elevated alanine aminotransferase (ALT) levels.[13,14] In the current consensus statements for the management of chronic hepatitis C stopping rules are used according to early viral decline.[16] Until now, there are no stopping rules used in the management of chronic hepatitis B.[17,18] The side effects and costs associated with the treatment of pegylated interferon make it worthwhile to investigate the possibility of a model to predict non-response at an early stage of treatment.

To evaluate whether viral decline during therapy can predict a sustained off-treatment response (HBeAg loss) we analyzed viral decline in HBeAg-positive chronic hepatitis B patients treated with pegylated interferon alpha-2b (PEG-IFN) alone or in combination with lamivudine.

Patients and methods

Patients

A total of 266 patients were evaluated in an international multicenter randomized double-blinded study reported previously.[13] Detailed in- and exclusion criteria are also reported elsewhere.[13] Eligible patients were men and women over 16 years of age with HBeAg-positive chronic hepatitis B. All patients were HBV DNA positive (above 10^5 copies/ml) and had ALT levels of at least 2 times the upper limit of normal on two occasions within eight weeks before randomization.

Study design

Patients received pegylated interferon alpha-2b (PegIntron, Schering-Plough, Kenilworth, NJ, USA) 100 µg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN was reduced to 50 µg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and followed for 26 weeks post-treatment. Serum samples for HBV DNA measurement were taken at the start of therapy, and monthly thereafter until the end of follow-up. HBV DNA levels were measured using an in-house developed TaqMan real-time PCR assay (dynamic range $4x10^2$-10^{10} copies/ml).[19] The Eurohep HBV DNA standard was used for validation of HBV DNA levels.[20] The assessment of HBV genotypes was done by Inno-Lipa assay (Innogenetics, Ghent, Belgium). Hepatitis B surface antigen (HBsAg), HBeAg, antibodies to hepatitis B surface (anti-HBs) and e antigen (anti-HBe) were measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, Il). Response was defined as serum HBeAg negativity at the end of follow-up.

Statistical analysis:

Comparisons between groups were done using the chi-square test or Fisher's exact test for categorical variables, and the Mann-Whitney test for continuous variables. To investigate whether response could be predicted during therapy, HBV DNA decline from baseline to the time points week 4, week 16 and week 32 were assessed as possible predictors of response. For each test, areas under the receiver operating characteristic (ROC) curves were calculated and compared. Also for each time point, different levels (in half-log steps) of HBV DNA decline from baseline were assessed to determine which cut-off level best excluded patients who failed to respond. All data were analyzed using SPSS (version 12.0.1 SPSS Inc., Chicago, IL). Where appropriate, mean values are given ± standard deviation. All tests for significance and resulting P values were two-sided, with a level of significance of 0.05.

Results

Patients

Patient characteristics have been described in detail previously.[13] In short, 205 men (77%) and 61 women (23%) with a mean age of 35 ± 13 years were included. Ninety patients

(34%) had genotype A, 23 (9%) genotype B, 39 (15%) genotype C, 103 (38%) genotype D, and eleven patients (4%) had other genotypes. The PEG-IFN monotherapy and PEG-IFN/lamivudine combination therapy groups were comparable with respect to all baseline parameters. HBeAg loss at the end of follow-up (response) was achieved in 36% of the PEG-IFN monotherapy therapy group and 35% of the combination group (p=0.91). Of the 95 patients who lost HBeAg at the end of follow-up, 77 (81%) patients were also anti-HBe positive. This was 80% in the PEG-IFN monotherapy group and 83% in the combination therapy group. HBsAg loss was achieved in 9 patients (7%) of the PEG-IFN monotherapy group and in 9 patients (7%) of the combination group (p=0.92).[13] Sixteen out of those 18 patients (89%) with HBsAg loss were also anti-HBs positive. Fifty-five patients (21%) had been treated previously with standard IFN, and 33 patients (12%) with lamivudine. In patients previously treated with IFN, 25% responded; in the treatment naïve population the response rate was 39% (p=0.17).

Viral dynamics in PEG-IFN monotherapy or combined with lamivudine

The decline of HBV DNA during PEG-IFN monotherapy and combination therapy is shown in figure 1A. In the PEG-IFN monotherapy group, HBV DNA decline after 52 weeks was 2.27 ± 2.32 log; in the combination therapy group HBV DNA decline after 52 weeks was 5.34 ± 2.18 log. In individual patients, the decline of HBV DNA during monotherapy with PEG-IFN treatment and follow-up had a variable pattern whereas nearly all patients in the combination therapy group showed a biphasic decline pattern during treatment. HBV DNA decline was significantly slower in the monotherapy group than in the combination therapy group during the first 16 weeks (p<0.01). Patients in the combination therapy group had significantly lower mean HBV DNA levels at all time points during therapy. Due to a relapse in HBV DNA during follow-up in the combination therapy group HBV DNA levels became comparable for both treatment groups at the end of follow-up (figure 1A).

In the combination treatment group, viral decline after 16 weeks was 5.06 ± 1.27 log for responders (HBeAg loss at the end of follow-up) and 4.44 ± 1.22 log for non-responders (p=0.01). The large overlap in viral load reduction between responders and non-responders (0.62 log) precluded the use of on-treatment HBV DNA decline as a predictor for HBeAg loss to combination therapy. In contrast, in the PEG-IFN monotherapy group marked differences in viral decline between responders and non-responders were

observed. The HBV DNA decline after 16 weeks was 2.26 ± 2.11 log for responders and 0.89 ± 1.03 log for non-responders (p<0.001) (figure 1 B and C).

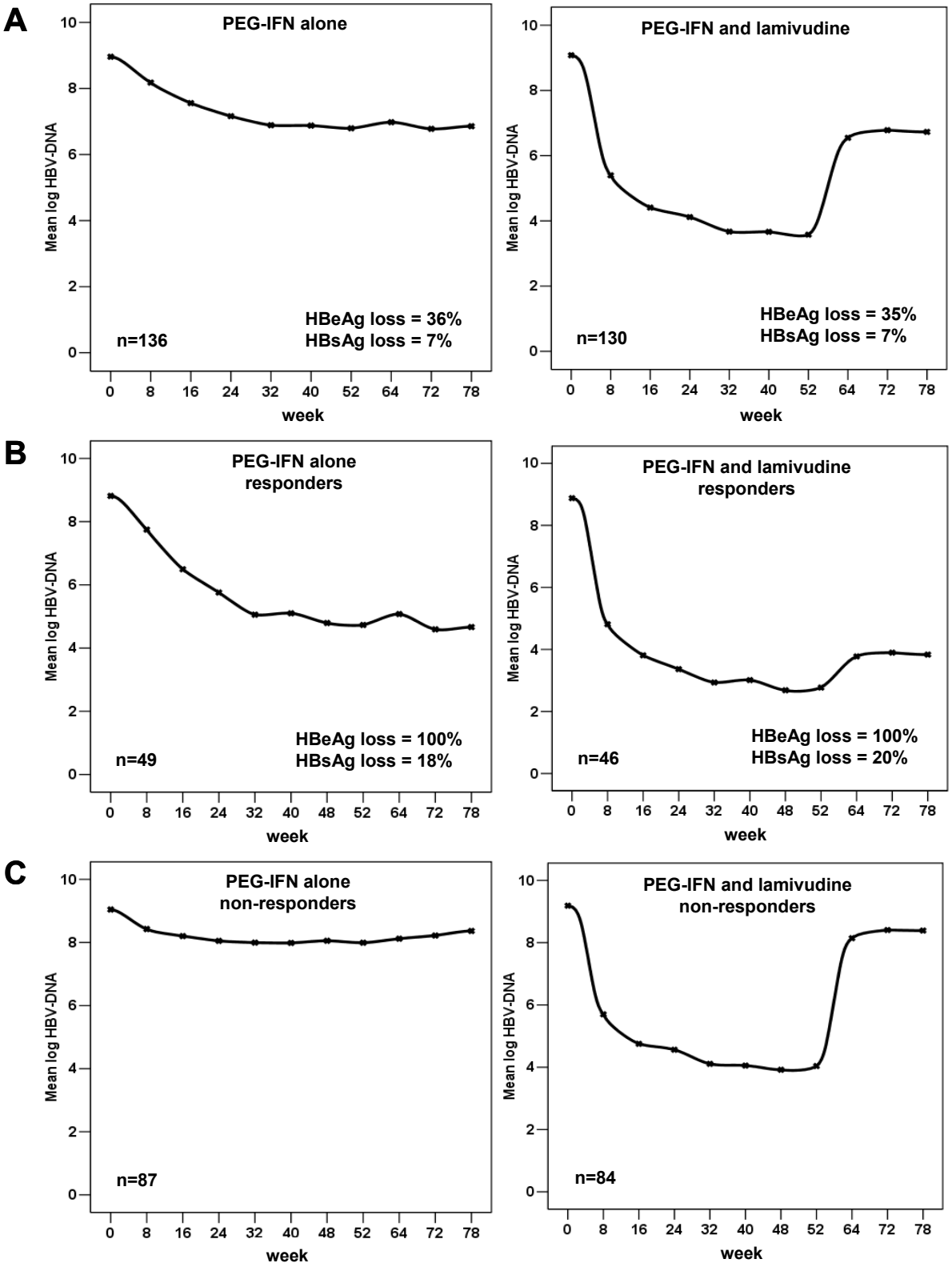


Figure 1: Decline of HBV DNA during 52 weeks of therapy and 24 weeks of follow-up in the PEG-IFN monotherapy group and PEG-IFN-lamivudine combination therapy group in all patients (A); in responders (defined as HBeAg negative at the end of follow-up) (B); and in non-responders (C). *The percentages of HBeAg and HBsAg loss at the end of follow-up are given for the overall group (A). All responders (B) had, per definition, HBeAg loss and HBsAg loss was observed in 18% in the PEG-IFN monotherapy group and in 20% in the combination therapy group. The non-responders (C) had no HBeAg loss and HBsAg loss was also not observed.*

Different patterns of viral decline in PEG-IFN monotherapy

To investigate whether response could be predicted at an early time point during monotherapy with PEG-IFN (n=136), HBV DNA decline from baseline to week 4, 16 and 32 were assessed as possible predictors of response. In this group of patients, the areas under the ROC curve for week 4, 16 and 32 were 0.63, 0.70 and 0.80, respectively. Response could be predicted best by a 1-log HBV DNA decline at week 32 of therapy, which included 82% of responders but excluded only 64% of the non-responders. The positive and negative predictive values were not more than 58% and 86%, respectively.

Patterns of viral decline. We analyzed the different patterns of viral decline in patients treated with PEG-IFN monotherapy. A specific predefined pattern of viral decline could be assessed in 123 patients; 11 patients discontinued the therapy early due to side effects and of two patients insufficient HBV DNA measurements were available to assess HBV DNA decline patterns. According to earlier studies on viral kinetics we used week 4 as cutoff for early decline.[21,22] Five different patterns of viral decline could be recognized: I. *early decline* defined as more than 1 log reduction in HBV DNA during week 0-4 of therapy (n=23); II. *delayed decline* of at least 2 log from baseline HBV DNA during week 4-32 without early decline (n=32); III. *late decline* of at least 2 log from baseline HBV DNA between week 32 and 52 without previous decline patterns (n=13); IV. *post-treatment decline* of 2 log from baseline HBV DNA after week 52 without previous decline patterns (n=11); V. *no substantial decline* at any time point (n=44). Figure 2 shows the longitudinal HBV DNA reduction and the percentages of HBeAg (response) and HBsAg loss at the end of follow-up for each decline pattern. Response rate was 52% for patients with an early

decline, 63% for a delayed decline, 38% for a late decline, 27% for a post-treatment decline and 11% for patients with no substantial decline.

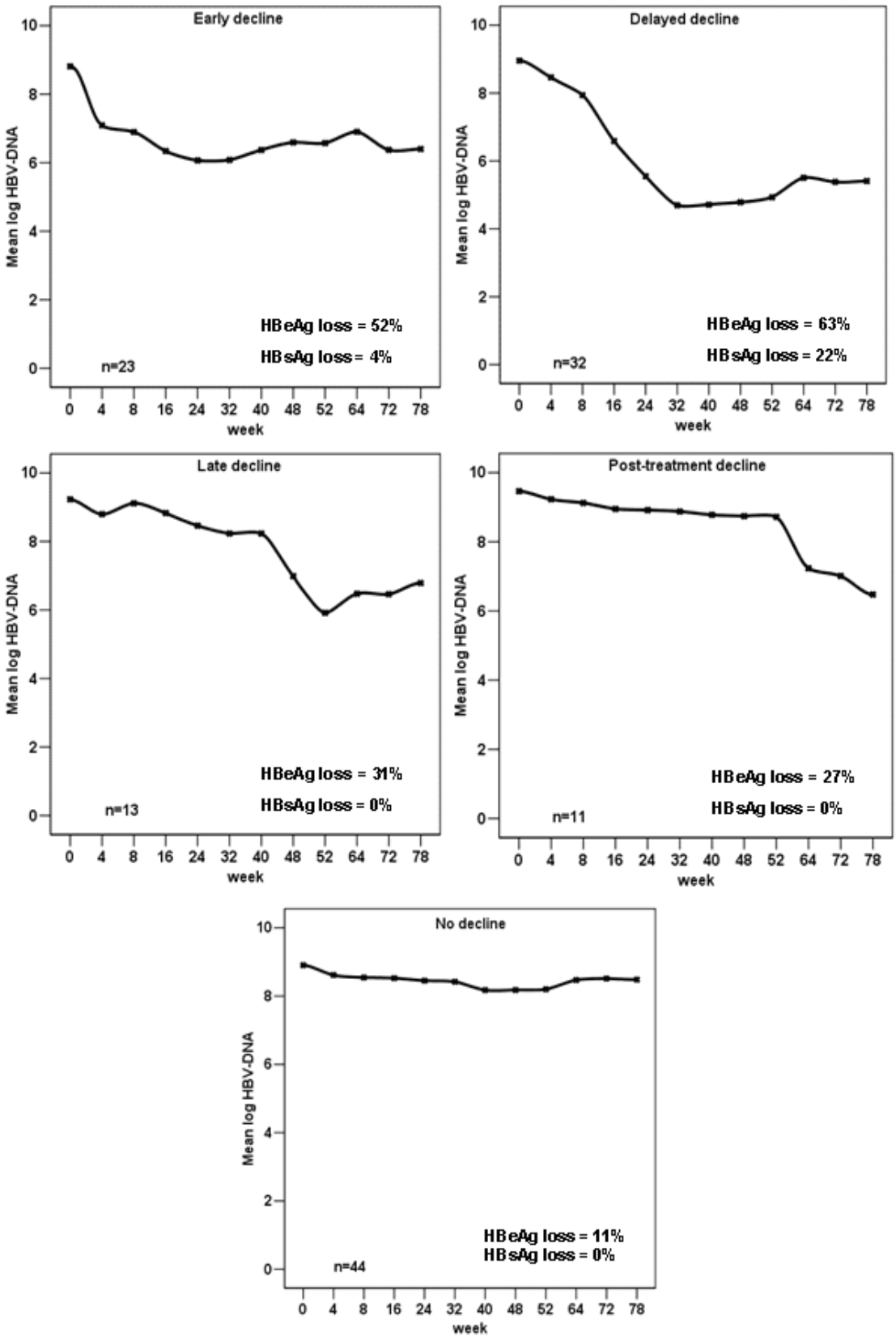


Figure 2: Decline of mean log HBV DNA and the percentages of HBeAg and HBsAg loss in the five determined patterns of viral decline during PEG-IFN monotherapy.

The difference in HBeAg loss among patients with an early or delayed decline compared to patients with a late, post-treatment or no decline pattern was significant (p=0.001). Among the patients with a delayed decline (n=32), 14 (44%) patients had genotype A, 2 (6%) genotype B, 4 (13%) genotype C and 10 (31%) genotype D. These differences were not significant, particularly because of the limited number of patients with HBV genotype B and C. During treatment and follow-up, 37 patients exhibited ALT flares, defined as a threefold increase in serum ALT compared with baseline levels.[23] There was a clear and nearly significant difference in the amount of ALT flares between patients with a delayed decline (41%) and those with no decline (20%) (p=0.056). ALT flares occurred in 17% of the patients with an early decline, in 23% of the patients with a late decline and in 18% of the patients with a post-treatment decline. In the group of patients with a delayed viral decline, the high HBeAg response was accompanied by a substantial loss of HBsAg (22%) and reduction of HBV DNA below 400 copies/ml (25%). In fact, 7 out of 8 patients (88%) with HBsAg loss and all patients with HBV DNA below 400 copies/ml at the end of follow-up (n=8) exhibited a delayed decline pattern. Only one patient with early viral decline and none with late or post-treatment decline became serum HBsAg negative.

Relation between viral dynamics and response in different hepatitis B virus genotypes

In patients treated with PEG-IFN monotherapy, marked differences were found between responders and non-responders according to HBV genotype (figure 3). For patients with genotype A (n=47), responders had a pronounced decline of HBV DNA whereas non-responders remained flat during the treatment period and follow-up. In contrast to other HBV genotypes, a 1 log decline of HBV DNA at week 32 of treatment was highly predictive for response in genotype A; the area under the ROC curve was 0.96, with a sensitivity of 94%, a specificity of 92%, a positive predictive value of 89% and a negative predictive value of 96%. Using this 1 log HBV DNA decline at week 32 as a stopping rule would thus lead to exclusion of 4% of potential sustained responders.

In the other genotypes, i.e. B (n=12), C (n=21) and D (n=51), both responders and non-responders showed an on-treatment decline in HBV DNA, which persisted post-treatment only in responders (figure 4). The area under the ROC curve for a 1 log decline at week 32 was 0.83, 0.71 and 0.59, respectively for genotypes B, C and D. Five patients with other genotypes were not analyzed.

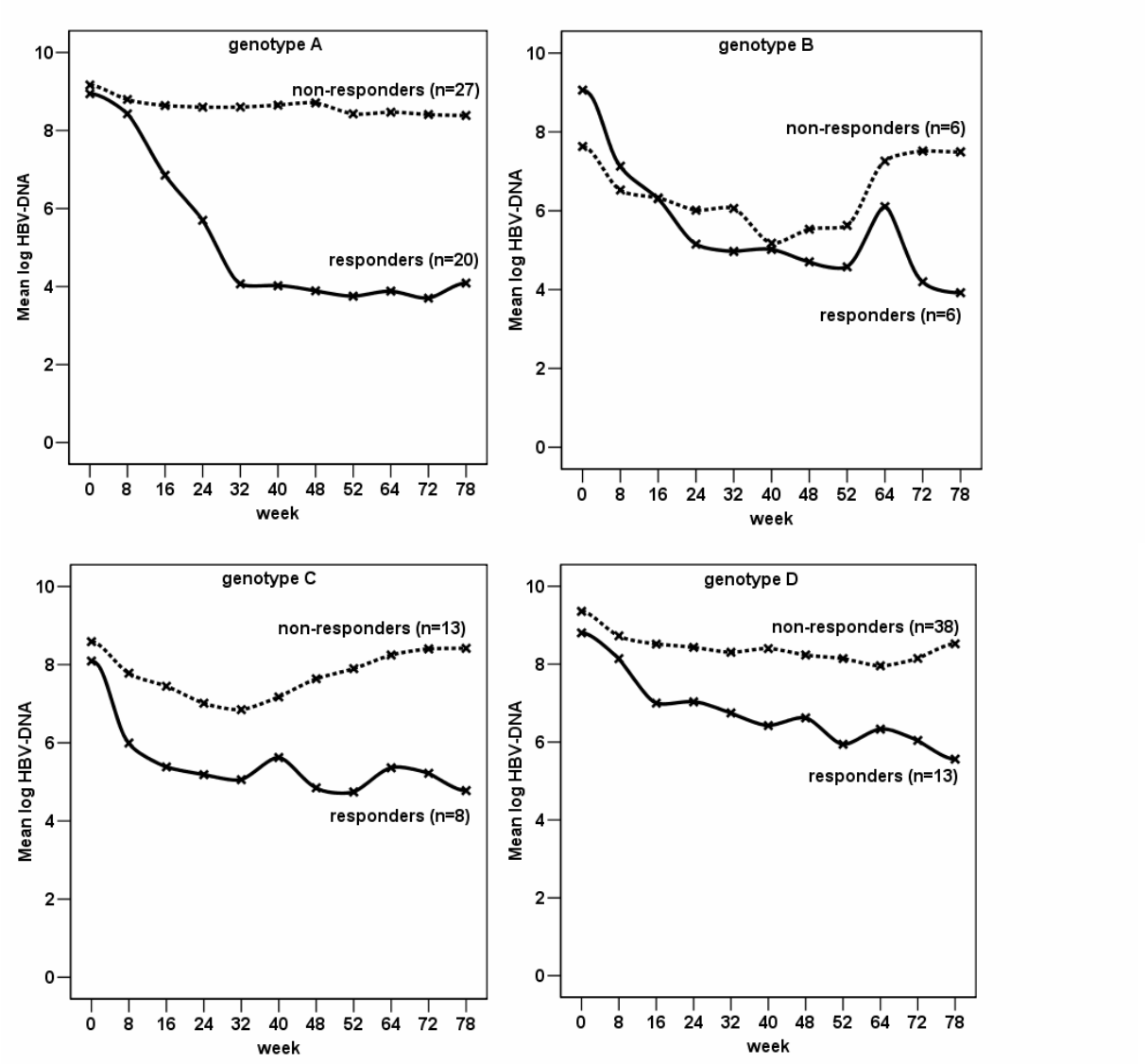


Figure 3: Decline of mean log HBV DNA for different HBV genotypes in responders (defined as HBeAg negative at the end of follow-up) and non-responders to PEG-IFN monotherapy.

Discussion

In the present study, different patterns of viral decline in 266 HBeAg-positive chronic hepatitis B patients were analyzed during treatment with pegylated interferon alpha-2b with or without lamivudine. HBV DNA showed a biphasic decline in the combination therapy group, as has previously been described for nucleoside analogues in chronic hepatitis B.[24-28] For PEG-IFN monotherapy we found significantly less decline of HBV DNA throughout the treatment period as compared to combination therapy. Despite these differences in HBV DNA decline, loss of HBeAg and HBsAg at the end of follow-up were similar in both treatment groups. We observed a pronounced HBV DNA relapse during post-treatment follow-up in the combination therapy group.

Patients with a delayed HBV DNA decline (>2 log HBV DNA decline between week 4 and 32) rather than those with an early HBV DNA decline, had the highest chance of response at the end of follow-up. Moreover, nearly all patients who became HBsAg negative (7 out of 8; 88%) or HBV DNA negative by PCR (8 out of 8; 100%) exhibited a delayed viral decline pattern. Both these findings suggest that early and vigorous suppression of HBV DNA is not always sufficient to tip the balance in favor of the host's immune response. It is assumed that an immunomodulatory effect of PEG-IFN rather than its direct antiviral effect leads to a sustained off-treatment response with high chances of HBsAg seroconversion after a long-term follow-up.[29] In the delayed viral decline pattern, one could hypothesize that moderate viral decline in the first month of therapy reflects the direct but partial antiviral effect of PEG-IFN, whereas the strong viral load reduction thereafter is induced by a combined immunomodulatory and antiviral effect.

Patients with a late decline pattern between weeks 32-52 of treatment or even a post treatment decline still had a considerable chance (31% and 27%, respectively) to lose HBeAg. It thus remains difficult to establish a good predictor of response early during PEG-IFN treatment in our total chronic hepatitis B population. This concurs with other studies on IFN or PEG-IFN treatment where primarily baseline factors (low viral load and high ALT) but not on-treatment factors could predict treatment response.[14,30]

When analyzing the results according to HBV genotype we found that only for genotype A viral decline of responders (defined as HBeAg negative at the end of follow-up) and non-responders diverged apparently during early PEG-IFN monotherapy. Responders showed a pronounced decline in HBV DNA compared to non-responders. Therefore, in genotype A

response could be predicted by a 1 log HBV DNA decline at week 32 with a high sensitivity and specificity. Earlier predictions were less accurate and not sufficient to be used in clinical practice. In patients with genotype B, C and D, both responders and non-responders show a decline in HBV DNA during PEG-IFN treatment. This precluded response prediction. However, this stopping rule was based on a relatively small number of patients and it may not work with PEG-IFN-alpha-2a or with PEG-IFN-alpha-2b at a different or constant dose, therefore it needs to be validated in other studies.

Although this study is the first to describe that prediction of response in patients with chronic HBeAg-positive hepatitis B treated with PEG-IFN is possible, there are two limitations. First, a relatively small number of patients with genotypes B and C were included in our study. Second, after 32 weeks a dose reduction of PEG-IFN from 100 µg to 50 µg once weekly was scheduled to limit early treatment discontinuation. This dose reduction could have led to suboptimal response and thus influence response prediction based on viral dynamics.

Recently, for patients treated with pegylated interferon alfa-2a sustained response could not be predicted sufficiently on the basis of viral decline during therapy. Farci et al.[31] studied on-treatment predictors for sustained response in HBeAg-negative chronic hepatitis B treated with pegylated interferon alfa-2a. They found a 1 log drop in HBV DNA or HBV DNA reduction below 7 log by week 12 of some predictive value for response and concluded that these data are insufficient to be used as a stopping rule. In this study with pegylated interferon alfa-2a, the number of patients with genotype A was small.

In conclusion, combination therapy with PEG-IFN and lamivudine is more effective in suppressing HBV replication than PEG-IFN alone during treatment. However, this did not result in enhanced response (HBeAg loss) at the end of follow-up. Different patterns of viral decline during PEG-IFN monotherapy could be identified: a delayed decline pattern was associated with the highest HBeAg- and HBsAg loss. Furthermore, viral decline in responders and non-responders were different across HBV genotypes during PEG-IFN monotherapy. To reduce unnecessary prolonged exposure to costly and toxic PEG-IFN stopping rules are needed. In our population, such a stopping rule could only be retrieved for patients harboring genotype A. In those patients one log HBV DNA decline after 32 weeks of PEG-IFN monotherapy was a satisfactory predictor for sustained off-treatment response. This stopping rule needs to be validated in other studies.

References

1. Schalm SW, Heathcote J, Cianciara J, Farrell G, Sherman M, Willems B, Dhillon A, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. Gut 2000;46:562-568.
2. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256-1263.
3. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998;339:61-68.
4. 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.
5. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, Jeffers L, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.
6. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, Lim SG, 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;33:1527-1532.
7. Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE, et al. Long-term therapy of chronic hepatitis B with lamivudine. Hepatology 2000;32:828-834.
8. 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.
9. Locarnini S, Qi X, Arteburn S, Snow A, Brosgart CL, Currie G, Wulfsohn M, et al. Incidence and predictors of emergence of adefovir resistant HBV during four years of adefovir dipivoxil therapy for patients with chronic hepatitis B. Journal of Hepatology 2005;42:17.
10. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative

chronic hepatitis B. N Engl J Med 2005;352:2673-2681.

11. 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.
12. 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.
13. 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.
14. 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.
15. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, 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;351:1206-1217.
16. NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements 2002;19:1-46.
17. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, Mele A, et al. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39 Suppl 1:S3-25.
18. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001;34:1225-1241.
19. 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.
20. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol 1999;37:68-73.
21. Neumann AU. Hepatitis B viral kinetics: a dynamic puzzle still to be resolved.

Hepatology 2005;42:249-254.

22. Sypsa VA, Mimidis K, Tassopoulos NC, Chrysagis D, Vassiliadis T, Moulakakis A, Raptopoulou M, et al. A viral kinetic study using pegylated interferon alfa-2b and/or lamivudine in patients with chronic hepatitis B/HBeAg negative. Hepatology 2005;42:77-85.
23. 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.
24. Nowak MA, Bonhoeffer S, Hill AM, Boehme R, Thomas HC, McDade H. Viral dynamics in hepatitis B virus infection. Proc Natl Acad Sci U S A 1996;93:4398-4402.
25. Tsiang M, Rooney JF, Toole JJ, Gibbs CS. Biphasic clearance kinetics of hepatitis B virus from patients during adefovir dipivoxil therapy. Hepatology 1999;29:1863-1869.
26. Lau GK, Tsiang M, Hou J, Yuen S, Carman WF, Zhang L, Gibbs CS, et al. Combination therapy with lamivudine and famciclovir for chronic hepatitis B-infected Chinese patients: a viral dynamics study. Hepatology 2000;32:394-399.
27. Lewin SR, Ribeiro RM, Walters T, Lau GK, Bowden S, Locarnini S, Perelson AS. Analysis of hepatitis B viral load decline under potent therapy: complex decay profiles observed. Hepatology 2001;34:1012-1020.
28. Wolters LM, Hansen BE, Niesters HG, Zeuzem S, Schalm SW, de Man RA. Viral dynamics in chronic hepatitis B patients during lamivudine therapy. Liver 2002;22:121-126.
29. 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.
30. Perrillo RP, Lai CL, Liaw YF, Dienstag JL, Schiff ER, Schalm SW, Heathcote EJ, et al. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36:186-194.
31. Farci P, Marcellin P, Lu Z, Diago M, Lai M, Gurel S, Kitis G, et al. On-treatment predictors of sustained biochemical and virological response in patients with

HBeAg-negative chronic hepatitis B treated with peginterferon alfa-2a (40KD) (PEGASYS®). Journal of Hepatology 2005;42:175.

Appendix

In addition to the authors, the HBV 99-01 Study Group includes the following investigators: The Netherlands: 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: 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: 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: 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), 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)

CHAPTER 3

Modelling of early viral kinetics and pegylated interferon-alpha-2b pharmacokinetics in patients with HBeAg-positive chronic hepatitis B

Martijn J ter Borg[1], Bettina E Hansen[1,2], Eva Herrmann[3], Stefan Zeuzem[4], Yilmaz Cakaloglu[5], Selim Karayalcin[6], Robert Flisiak[7], Annemarie van 't Veen[8], Robert A de Man[1], Solko W Schalm[1], Harry LA Janssen[1], Bart L Haagmans[8] for the HBV 99-01 study group

1. Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
2. Department of Epidemiology and Biostatistics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
3. Faculty of Medicine, Department of Internal Medicine, Saarland University, Homburg/Saar, Germany
4. Department of Medicine I, J.W. Goethe-University Hospital, Frankfurt, Germany
5. Department Gastroenterohepatology, Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey
6. Department of Gastroenterology, Medical School Cebeci Kampusu, Ankara, Turkey
7. Department of Infectious Diseases, Medical University of Bialystok, Bialystok, Poland
8. Department of Virology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

* Other members of the HBV 99-01 Study Group are listed in the appendix

Antiviral Therapy 2007; 12(8):1285-94.

Summary

Treatment with pegylated interferon (PEG-IFN) α-2b is effective for HBeAg-positive chronic hepatitis B although its mechanism of action remains unclear. HBeAg loss is achieved in 36% of patients after one year of PEG-IFN α-2b treatment and combination therapy with lamivudine is not superior to PEG-IFN α-2b monotherapy. In this study, we analyzed early pharmaco- and viral kinetics in patients treated for 52 weeks with PEG-IFN α-2b with or without lamivudine. After 4 weeks of treatment, there was a median viral decline of 2.94 $\log_{10}$ copies/ml in those treated with PEG-IFN α-2b and lamivudine and only 0.45 $\log_{10}$ copies/ml in the PEG-IFN α-2b monotherapy group. Peak IFN levels were reached approximately one day after administration and subsequently declined exponentially consistent with a viral load rebound near to baseline levels at the end of the dosing period in most patients receiving PEG-IFN α-2b monotherapy. Modelling of pharmaco- and viral kinetics data in this group revealed that viral load was minimal 3.6 days after PEG-IFN α-2b administration, the mean maximal and mean antiviral effectiveness was 70% and 48% with a mean infected cell loss rate of 0.07 per day, while no significant biphasic decline was observed. We conclude that PEG-IFN α-2b induces a sustained response in a considerable number of patients despite limited direct antiviral activity during the first weeks of antiviral therapy.

Introduction

Patients with HBeAg-positive chronic hepatitis B often have high levels of circulating virus and immune responses directed against the virus cause inflammation which in turn may lead to cirrhosis and hepatocellular carcinoma.[1] Although treatment with nucleos(t)ide analogues, like lamivudine, adefovir and entecavir, is effective for viral load reduction, long-term treatment is often necessary and carries the risk of viral resistance.[2-4] Using interferon therapy, a durable treatment response can be achieved in 35-45% of HBeAg-positive and HBeAg-negative chronic hepatitis B patients.

Pegylated interferons induce HBeAg seroconversion in approximately one third of HBeAg-positive patients.[5-9] In a recent trial, a durable loss of HBeAg was achieved in 36% of patients after a 52 week course of PEG-IFN α-2b treatment with a 26 week follow-up period.[6] The decline in viral load during PEG-IFN α-2b therapy was not uniform and different patterns of viral decline could be recognized both during treatment and follow-up.[10] Remarkably, a marked viral decline between weeks 4 and 32 of treatment resulted in the highest rate of HBeAg-loss.[10] In general, there was only minimal decline in viral load in the first month of treatment. Until now, no viral kinetics data are available during PEG-IFN treatment in HBeAg-positive chronic hepatitis B.[11] Therefore, we analyzed the relation between viral kinetics and pharmacokinetics of PEG-IFN α-2b in HBeAg-positive chronic hepatitis B. To our knowledge, this is the first analysis fitting data from both pharmacokinetics and viral kinetics during treatment in patients with chronic hepatitis B.

Material and methods

Patients

A total of 96 patients who participated in an international multicenter randomized double-blinded study reported previously,[6] underwent frequent blood sampling in the first month of therapy. Eligible patients were men and women over 16 years of age with chronic hepatitis B, documented by liver biopsy and HBsAg positivity for over six months, and positive serum HBV DNA levels. All patients were HBeAg-positive and had ALT levels of at least 2 times the upper limit of normal on two occasions within eight weeks before randomization. Patients received PEG-IFN α-2b 100 μg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN α-2b was reduced to 50 μg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and

followed for 6 months post-treatment.

HBV DNA quantification

HBV DNA levels were measured frequently during the first month of therapy (at days 0, 1, 2, 3, 4, 7, 14, 21 and 28) in a randomly selected subgroup of 38 patients (19 patients in the monotherapy group and 19 patients in the combination therapy group) using an in-house developed TaqMan real-time PCR test with a dynamic range of $4x10^2$-10^{10} copies/ml.[12] Monthly HBV DNA measurements were available in all 96 patients.

PEG-IFN α-2b concentration

PEG-IFN α-2b serum concentrations were also measured at days 0, 1, 2, 3, 4, 7, 14, 21 and 28 using a quantitative sandwich interferon enzyme-linked immuno-sorbent assay (ELISA, Bender MedSystems Diagnostics GmbH, Vienna, Austria) in all 96 patients. Binding of (pegylated) interferon to a murine monoclonal antibody directed against interferon adsorbed onto micro wells was detected by an HRP-conjugated monoclonal anti-interferon antibody. Following 2 hours of incubation unbound complexes were removed by washing (three times) after which tetramethyl-benzidine was used to determine the amount of interferon in the sample. Absorbency was read using a spectro-photometer using 450nm as the primary wave length. Standards were prepared from diluted series of pegylated interferon in normal human serum obtained from healthy volunteers. Patient sera and standards were tested in triplicate, on the same plate. Although optical densities obtained were related to a standard of pegylated interferon, the ELISA also may detect free recombinant interferon-2b molecules and natural interferon. The detection limit of the assay is 35 pg/ml and is linear up to a concentration of 2000 pg/ml.

Modelling of pharmacokinetics

For modelling of the pharmacokinetics of PEG-IFN α-2b we used the absorption and elimination model recently applied by Powers et al. and Talal et al.[13,14] This model describes the concentration of drug in the blood (*C*) following a single injection at time *t=0* as follows:

$$C(t)= \frac{k_a D(F/V_d)}{(k_e-k_a)} (e^{-k_a t}-e^{-k_e t}) \quad (1)$$

where t is the time after injection, k_a is the rate of absorption, k_e is the rate of elimination, F is the bioavailability, D is the drug dose and V_d is the volume of distribution. We used a more general model for multiple weekly injections of PEG-IFN α-2b that accounts for random variability effects between subjects. The PEG-IFN α-2b concentration in the blood for individual i at the time point t is then described as the sum of the individual contributions of each injection d until time t, i.e. $t_d < t$ is the injection day (i.e. t_d = 0, 7, 14, 21, …,) and D_d is the dose per injection d:

$$C_i(t) = \sum_{d:t_d<t} \frac{(FD_d)_i}{V_{di}} \frac{k_{a,i}}{(k_{e,i}-k_{a,i})} \left(e^{-k_{a,i}*(t-t_d)} - e^{-k_{e,i}*(t-t_d)}\right) \quad (2)$$

$k_{a,i}$, $k_{e,i}$ *and* $(F/V_d)_i$ consist of both a fixed-effect as well as a individual random effect parameter. Using this formula, the area under the curve (AUC) of the PEG-IFN α-2b concentration could be calculated. Furthermore, these changes in PEG-IFN α-2b concentration over time have an effect on the effectiveness of PEG-IFN in contrast to a constant effect. Assume that the effectiveness of PEG-IFN α-2b for individual i is given by:

$$\varepsilon_i(t) = \frac{C_i(t-t_0)^n}{IC_{50}{}^n + C_i(t-t_0)^n} \quad (3)$$

where IC_{50} is the concentration at which the drug's effectiveness is half its maximum, and n is the Hill coefficient, a parameter that determines the steepness of the rise of the effectiveness with increasing PEG-IFN concentration, and t_0 is a possible time delay.[14]

Modelling of viral kinetics

Using the pharmacodynamic efficacy model (3), the viral kinetics for the first week of PEG-IFN α-2b monotherapy can be described by a model originally applied by Nowak et al.[15] and modified by Sypsa et al.[16] and Powers et al.[13] In our approach the constant e_i is substituted by $e_i(t)$ in the differential equation system modelling viral kinetics:

$$\frac{d}{dt} V_i(t) = (1-\varepsilon_i(t)) p_i I_i(t) - c_i V_i(t) \quad (4)$$

and

$$\frac{d}{dt} I_i(t) = \beta_i V_i(t) T_i(t) - \delta_i V_i(t) \quad (5)$$

The resulting model function $V_i(t)$ describes the viral load of individual *i* at time point *t* and depends on the virion clearance rate c_i and the infected cell loss rate δ_i. The total number of cells (i.e. infected target cells, I_i, and uninfected target cells, T_i) are assumed to remain constant in each individual during treatment motivated by a fast liver regeneration. As usual, the infection rate β_i and the viral production rate p_i were substituted by the other parameters assuming that they remained unchanged from the steady state situation.

Modelling and data fitting

The PROC NLMIXED procedure of SAS 9.1 (SAS Institute Inc., Cary, NC) was used to fit the first month pharmacokinetic data of all 96 patients with a non-linear mixed modelling approach. The NLME procedure of R (R Foundation for Statistical Computing) yield highly comparable results (data not shown). The prediction of the PEG-IFN α-2b concentration (equation 2) and the model for effectiveness (equation 3) was thereafter incorporated in the model of the viral load as solution from equations 4 and 5 of the patients treated with PEG-IFN α-2b monotherapy. The viral load was hereafter fitted with non-linear mixed modelling with the NLME procedure of R including the ordinary differential equation solver LSODA from the ODESOLVE package in a nested way to estimate the infected cell loss rate δ, the baseline levels of viral load as well as the IC_{50} levels and the time delay t_0. Because interindividual variation could already be modelled by baseline viral load and IC_{50} levels, the other parameters were set constant between patients (fixed effects). Furthermore, relatively few data points can lead to biased estimates of the viral clearance rate *c*.[16] Therefore, we fixed *c* to 1.3 per day. Different Hill coefficients (n=1, n=2, n=3 and n=4) were checked and we used a coefficient of 1 because this gave the best results.

SPSS (version 14.0.1, SPSS Inc., Chicago, IL) was used for further data analyses. All tests for significance and resulting P values were two-sided, with a level of significance of 0.05.

Results

Patient characteristics

Demographic and baseline characteristics of the 96 included patients in this study are shown in table 1. Forty-eight patients received PEG-IFN α-2b monotherapy; the other 48 patients received combination therapy consisting of PEG-IFN α-2b and lamivudine. There were no significant differences between the two groups with respect to ALT, viral load, age, sex, weight and race. PEG-IFN α-2b concentration was measured in all 96 patients whereas frequent HBV DNA measurements were obtained in a representative subset of 38 patients (19 in each treatment arm).

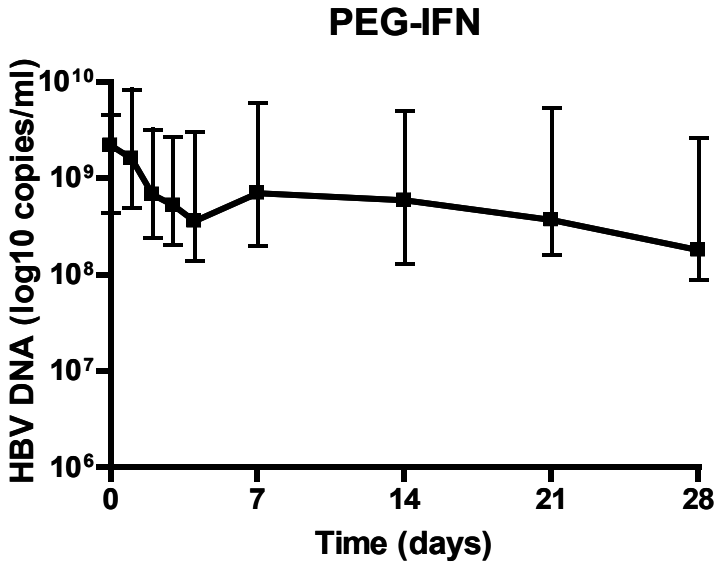
Viral kinetics

In the PEG-IFN α-2b monotherapy group (n=19), the median viral decline after one month of treatment was 0.45 log10 copies/ml (range, -0.03 – 1.56) (Figure 1) and 0.40 $\log_{10}$ copies/ml (range, -0.28 – 2.30) at week 8 of treatment. The median viral decline was 0.028 log10 copies/mL per day (range, -0.069 – 0.165) for the first week and 0.017 $\log_{10}$ copies/ mL per day (range, -0.006 – 0.046) between week 1 and 4. In the first week of treatment, there was a median decline in viral load of 0.20 $\log_{10}$ copies/ml (range, -0.48 – 1.15). There was an initial decline in viral load until 4 days after drug administration in all patients in the PEG-IFN α-2b monotherapy group. Thereafter there was a rebound towards the end of the week. The median slope of viral rebound at the end of the first week (day 4 to day 7) was 0.060 $\log_{10}$ copies/ml per day (range, -0.117 – 0.393). There was no effect of the baseline viral load level on the amount of viral decline in the first month of treatment.

	PEG-IFN + lamivudine (n=48)	PEG-IFN + placebo (n=48)
Age (years)*	33 ± 12	32 ± 12
Sex M/F (% male)	32/16 (67%)	37/11 (77%)
Weight (kg)*	72 ± 16	71 ± 13
Race (%)		
Caucasian	42 (88%)	43 (90%)
Asian	2 (4%)	3 (6%)
Other	4 (8%)	2 (4%)
Genotype (%)		
A	13 (27%)	15 (31%)
B	1 (2%)	2 (4%)
C	2 (4%)	2 (4%)
D	31 (65%)	29 (61%)
E	1 (2%)	0 (0%)
ALT (U/L)*	175 ± 193	167 ± 130
HBV DNA (log_{10} copies/mL)*	9.2 ± 1.1	9.3 ± 0.7

Table 1: Baseline characteristics. * Mean ± standard deviation

Figure 1:
A



B

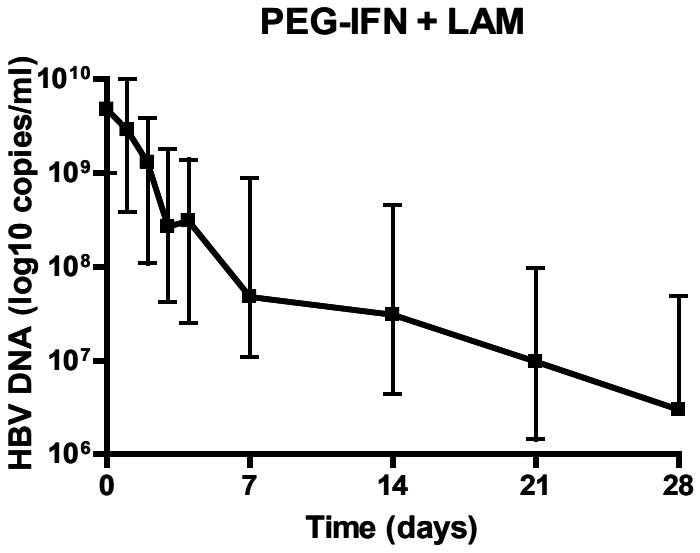


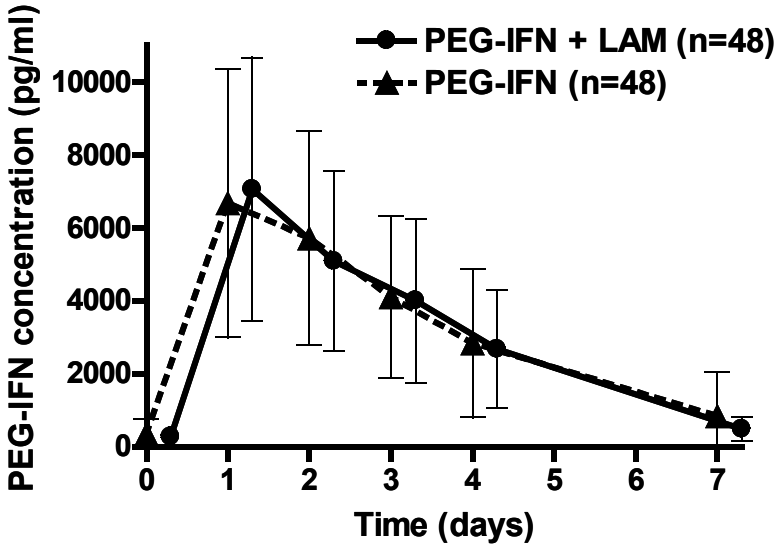
Figure 1: Median HBV DNA (log_{10} copies/ml) in patients with HBeAg-positive chronic hepatitis B in the first month of treatment with PEG-IFN alone (A) or in combination with lamivudine (B).

When viral decline was analyzed in the PEG-IFN α-2b and lamivudine combination therapy group (n=19) on the other hand, a median decline in viral load of 2.94 $\log_{10}$ copies/ml (range, 0.55 – 5.02) after one month of treatment was observed (Figure 1). There was a viral decline of 3.43 $\log_{10}$ copies/ml (range, 0.71 – 6.25) at week 8 of treatment. The median viral decline was 0.228 $\log_{10}$ copies/ml per day (range, -0.037 – 0.337) for the first week of treatment and 0.055 $\log_{10}$ copies/ml per day (range, 0.010 – 0.127) between week 1 and 4. All patients treated with combination therapy showed a biphasic HBV DNA decline pattern. The median decline in viral load was 1.59 $\log_{10}$ copies/ml (range, -0.26 – 2.36) in the first week of treatment. The median slope of viral decline at the end of the first week (day 4 to day 7) was 0.083 $\log_{10}$ copies/ml (range, -0.297 – 0.250) per day in the combination therapy group.

Pharmacokinetics of pegylated interferon-alpha-2b

In a first attempt to understand why HBV DNA levels showed a minimal decline during the first month, we analyzed PEG-IFN α-2b levels in all 96 patients. Maximum levels of PEG-IFN α-2b concentration were reached one day after administration. Thereafter, a decline in the PEG-IFN α-2b levels was seen in all patients (Figure 2A). No significant differences in PEG-IFN α-2b levels between patients treated in the PEG-IFN α-2b monotherapy and the PEG-IFN α-2b plus lamivudine combination therapy group were observed. In 52 out of 96 patients (54%), the PEG-IFN α-2b concentration had returned to undetectable levels 7 days after drug administration; this was still the case in 24/96 (25%) patients at day 28, 7 days after the fourth injection. In those with detectable PEG-IFN α-2b levels at day 7 and 28, these concentrations were in general low with a mean of 1175 pg/mL and 1645 pg/mL, respectively.

Figure 2:
A



B

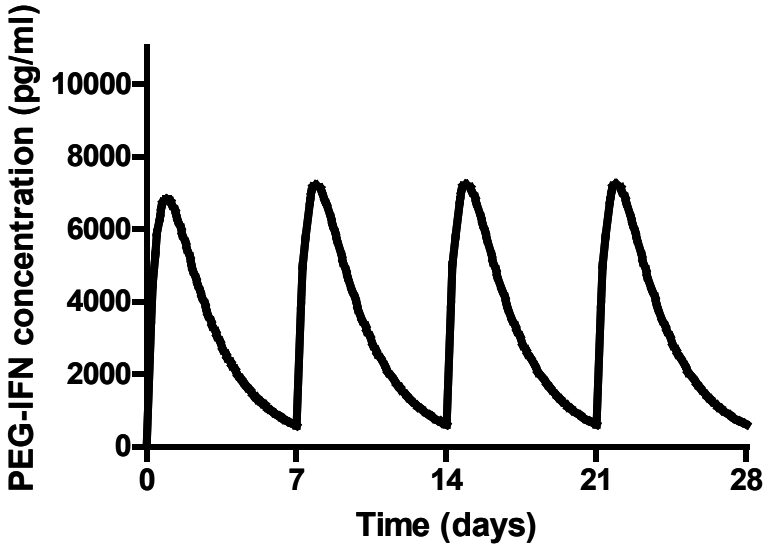


Figure 2: Pharmacokinetics of PEG-IFN in patients treated with PEG-IFN with or without lamivudine in the first week (A) and the modelled pharmacokinetics in the first month (B) of treatment.

The pharmacokinetics were modelled using a non-linear mixed model. The fitted non-linear mixed model resulted in a population mean of the pharmacokinetic parameters k_a,

k_e and F/V_d of all 96 patients as well as an individual fit of these parameters (Figure 2B). The estimated population mean of k_a was 2.363 d^{-1}(SE 0.461), of k_e 0.420 d^{-1} (SE 0.029) and of F/V_d 1.023 pg/mL (SE 0.084) (Table 2 gives per patient data). The modelled interval between PEG-IFN α-2b administration and the maximum modelled drug concentration (t_{max}) was 0.89 day (0.71-1.24). There was a significant negative correlation between the per patient AUC of the PEG-IFN α-2b concentration for the first week of treatment and the body mass index (BMI) (p=.024) as well as a significant relation between the AUC and sex; AUC was higher in females than in males (p=.002).

Patient	Pharmacokinetic parameters							Viral kinetic parameters[1]			
	k_e (day^{-1})	k_a (day^{-1})	F/V_d (pg/mL)	t_{max} (days)	C_{max} (pg/mL)	EC_{50}	AUC (pg.wk/ mL)	v_0 (log_{10}cp/mL)	decl. wk 1 (log_{10}cp/mL)	c (day^{-1})	ε
1	0.42	0.60	1.30	1.98	5630	5852	26415	9.30	0.20	0.97	0.49
3	0.48	2.73	1.80	0.77	12410	6485	35918	9.49	0.35	0.96	0.66
5	0.48	1.02	1.55	1.40	7940	6713	30267	10.04	-0.48	0.90	0.54
8	0.47	4.47	1.62	0.56	12430	1400	33228	9.06	0.30	0.80	0.89
9	0.55	2.93	1.86	0.70	12610	1480	32823	8.68	0.34	0.91	0.89
11	0.46	0.91	1.43	1.52	7160	3782	28890	8.69	0.53	0.77	0.65
12	0.43	1.34	1.31	1.25	7630	3969	28079	8.36	0.24	0.87	0.66
22	0.56	4.29	1.63	0.54	12020	276	28295	9.57	1.15	0.97	0.98
24	0.50	2.07	1.93	0.91	12300	2017	37259	8.65	0.71	0.55	0.86
27	0.37	2.08	0.88	1.01	6020	5881	21372	9.03	-0.28	1.43	0.50
29	0.55	1.14	2.43	1.24	12320	3717	42477	8.36	0.10	1.58	0.77
32	0.58	0.69	3.37	1.57	13500	9634	54171	10.26	0.07	1.09	0.58
39	0.39	4.43	0.65	0.60	5140	570	15541	9.30	0.69	1.05	0.90
41	0.36	1.09	0.76	1.52	4420	3844	18654	9.94	-0.07	1.09	0.54
43	0.45	1.23	1.43	1.29	8030	12684	29735	9.53	0.11	1.08	0.39
45	0.37	1.56	0.87	1.21	5540	1078	21063	9.13	0.42	1.26	0.84
50	0.58	3.34	1.83	0.64	12660	5325	31017	9.91	0.03	0.89	0.70
51	0.58	0.71	3.29	1.56	13360	5282	53428	8.85	-0.16	1.03	0.71
73	0.43	4.09	0.87	0.61	6690	3228	19105	10.03	0.17	0.87	0.67
Median	0.47	1.56	1.55	1.21	8030	3844	29735	9.30	0.20	0.97	0.67
Q 25	0.42	1.02	0.88	0.64	6020	1480	21372	8.69	0.06	0.87	0.54
Q 75	0.55	3.34	1.86	1.52	12430	5881	35918	9.91	0.39	1.09	0.86

decl. wk 1 = the decline in viral load in the first week of treatment, Q = quartile

[1]Identical estimates for all patients (fixed effects) were obtained for the pharmacokinetic time delay t_0 (0.9 day), the infected cell loss rate δ (0.07 per day) and the Hill coefficient (n=1).

Table 2: Pharmacokinetic and viral kinetic parameters for the first week of treatment for the 19 patients treated with PEG-IFN monotherapy.

Modelling of viral kinetics and its relation to pharmacokinetics and response

Using the non-linear mixed model it was possible to fit the first month viral kinetics data in the PEG-IFN α-2b monotherapy arm (n = 19) using the results of the modelled pharmacokinetics for the first month of treatment with e dependant on time (Figure 3, Table 2). Thus, a clear biphasic viral load decline is not observed using PEG-IFN α-2b monotherapy in HBeAg-positive chronic hepatitis B patients.

In the first week, the modelled viral load was minimal at 3.6 days (2.8-4.5) after administration of PEG-IFN α-2b. The mean and maximum estimated population antiviral effectiveness ε_{mean} and ε_{max} in patients receiving PEG-IFN α-2b monotherapy 48% and 70% (24-80% and 39 - 98%), respectively. The infected cell loss rate δ was estimated as 0.07 per day and the time delay of pharmacokinetics t_0 as 0.9 days. No clear association was found between the estimated maximum antiviral effectiveness and baseline HBV DNA levels, ALT levels, sex and BMI. Maximal effectiveness but not mean effectiveness was significantly smaller in older patients (p=0.046).

HBeAg loss at the end of follow-up was observed in 9 out of 19 patients. Despite the correlation between the AUC of the PEG-IFN α-2b concentration and BMI and sex, no significant difference was observed between the AUC in relation to treatment response (HBeAg loss at the end of follow-up) or viral decline at the end of treatment and follow-up. Furthermore, viral decline in the first month of treatment was 0.45 $\log_{10}$ copies/ml (range -0.12 – 1.56) in patients with a lower than median AUC and also 0.45 $\log_{10}$ copies/ml (range -0.03 – 1.87) in those with a higher than median AUC of the PEG-IFN α-2b concentration.

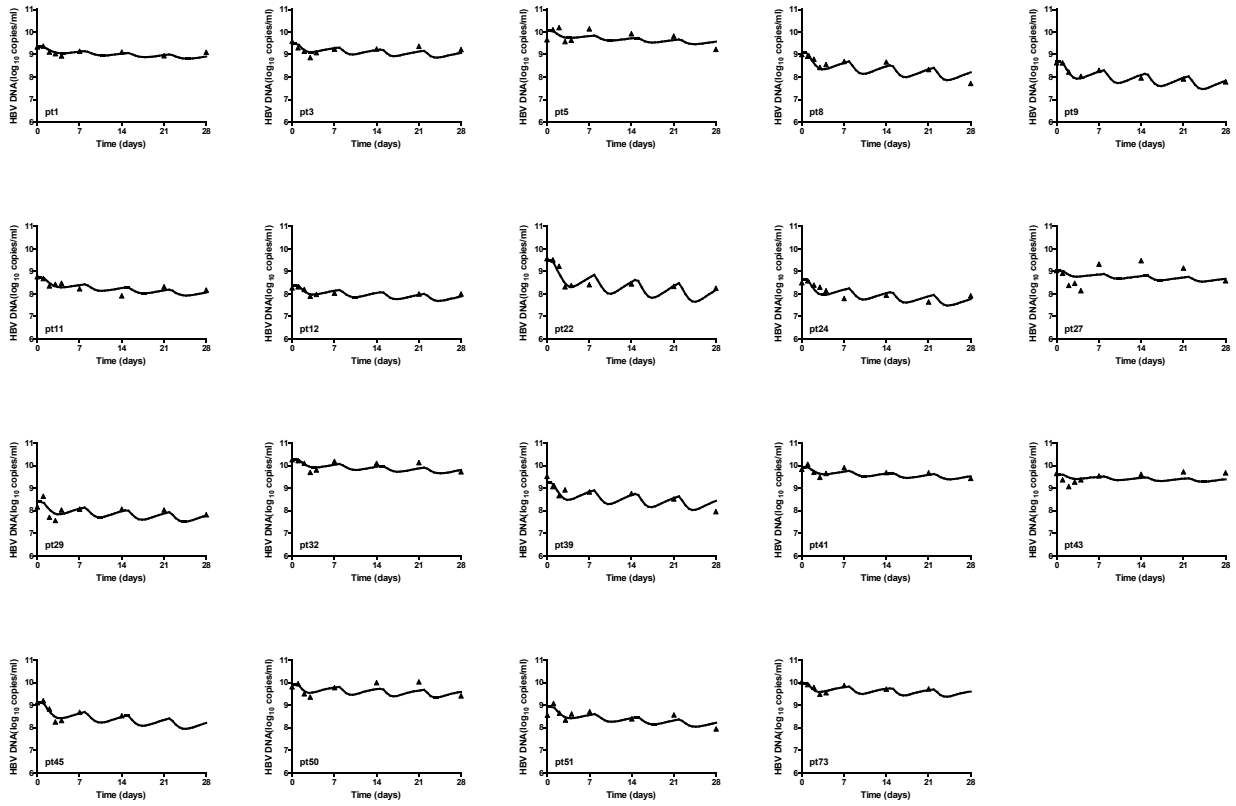


Figure 3: Modelled viral decline and observed viral load in all 19 patients treated with PEG-IFN monotherapy in the first month of treatment.

Discussion

In this study, we analyzed early pharmacokinetics and HBV viral kinetics in HBeAg-positive chronic hepatitis B patients during the first 4 weeks of treatment with PEG-IFN α-2b and used the PEG-IFN α-2b pharmacokinetics to model viral decline. We observed only a minimal decline in viral load during the first month of PEG-IFN α-2b monotherapy, without a clear biphasic pattern. Given the fact that a significant number of patients is able to control the infection after 52 week of PEG-IFN α-2b treatment, immunomodulatory effects rather then direct antiviral activities of PEG-IFN-2b may explain its beneficial effect.

In the first week of PEG-IFN α-2b treatment, we found highest drug concentrations one day after drug administration followed by a pronounced decline over time until the end of the week. At the end of the week, the PEG-IFN α-2b concentration returned to undetectable levels in the majority of patients. This is in accordance with previous PEG-IFN α-2b pharmacokinetic studies in patients with chronic hepatitis C.[14, 17-19] Based on

these pharmacokinetic data, one could consider twice-weekly administration of PEG-IFN α-2b. In chronic hepatitis C patients treated with twice weekly administration for 28 days, there were high PEG-IFN α-2b concentrations in the blood at all days during the week and there was no rebound in HCV-RNA at the end of the week as was seen with once weekly injections.[18] Nevertheless, despite these suboptimal pharmacokinetic characteristics for PEG-IFN α-2b, the end of treatment and follow-up results of PEG-IFN α-2b and PEG-IFN α-2a - which has a prolonged higher concentration in blood - are comparable in chronic hepatitis B.[6,7,9]

We analyzed the pharmacokinetics during PEG-IFN α-2b therapy in all 96 patients using a model proposed by Powers et al. and Talal et al. for chronic hepatitis C infection.[13,14] This model takes the decreasing efficacy of PEG-IFN α-2b at the end of the week into account during once-weekly administration. We observed a significant correlation between the AUC of the PEG-IFN α-2b concentration and body mass index (BMI) and a correlation between sex and the AUC of PEG-IFN α-2b. Based on these findings, weight-based PEG-IFN α-2b dosing should also be considered in the treatment of chronic hepatitis B to optimize drug availability as is the standard in hepatitis C treatment.[20,21] However, despite the influence of BMI on the pharmacokinetic constants of PEG-IFN α-2b, no clear effect of the PEG-IFN α-2b concentration was observed on treatment outcome or decline in viral load, as previously shown for PEG-IFN α-2a.[22] Furthermore, treatment of chronic hepatitis B patients with escalating doses of both PEG-IFN α-2a and α-2b did not lead to a better treatment outcome in chronic hepatitis B.[5,16]

Next we incorporated the pharmacokinetic model for multiple weekly PEG-IFN α-2b injections proposed recently[13,14] in a combined pharmacokinetic-pharmacodynamic model. Viral kinetics were modelled using equations 3-5 We were able to use per patient PEG-IFN α-2b pharmacokinetics as well as viral kinetics data in 19 patients of the PEG-IFN α-2b monotherapy group. With this approach, it was possible to fit the viral decline during the first month of PEG-IFN α-2b monotherapy in patients with HBeAg-positive chronic hepatitis B. The maximum antiviral effectiveness of PEG-IFN α-2b monotherapy, ε_{max}, was 70% and this is slightly lower than the antiviral effectiveness (83%) of PEG-IFN α-2b 100/200 µg in HBeAg negative chronic hepatitis B patients in the study by Sypsa et al., probably due to the lower PEG-IFN dose given.[16] There was no clear association between the antiviral effectiveness and several baseline factors, only older patients

showed a slightly reduced maximal antiviral effectiveness (p=0.046). This antiviral effectiveness is lower compared to the estimated antiviral effectiveness of approximately 92-99% for nucleos(t)ide analogues.[23-26] In the combination therapy group, viral load showed a biphasic decline pattern as a result of the addition of lamivudine. This pattern has already been extensively described in chronic hepatitis B patients treated with nucleos(t)ide analogues and therefore we did not model viral decline in the combination therapy group.[15,24-26]

In the first week, there was a pronounced decline in viral load in the combination therapy group and after one month of treatment there was a 2.94 $\log_{10}$ copies/ml decline in viral load. In the monotherapy group, probably as a result of the decline in drug concentration associated with once-weekly administration of PEG-IFN α-2b, we observed only a minimal decline in viral load with a rise towards the end of the week as also recently reported by Sypsa et al. in HBeAg-negative chronic hepatitis B[16] Therefore, there was only a limited decrease in viral load at the end of the first week of treatment in the monotherapy group and no clear biphasic decline pattern was observed as seen during PEG-IFN α-2a treatment in HBeAg-negative chronic hepatitis B.[27] After one month of PEG-IFN α-2b monotherapy there was still only a marginal decline of 0.45 $\log_{10}$ copies/ml in viral load. Regardless of this minimal decline in viral load early during treatment, treatment outcome was comparable in both treatment arms.[6] This emphasizes that a rapid early antiviral effect of PEG-IFN α-2b is not necessary for a sustained response 24 weeks post-treatment in HBeAg-positive chronic hepatitis B as it is in chronic hepatitis C infection. In line with these results, we previously showed that patients with a delayed rather than with an early viral load decline pattern exhibited the highest rates of HBeAg loss after PEG-IFN α-2b treatment.[10]

In conclusion, the pharmacokinetics during the first week of therapy with PEG-IFN α-2b alone showed a peak one day after the administration with a rapid decline thereafter. Concurrently, after an initial decline an increase in HBV DNA was found during the second half of the week. Using the PEG-IFN α-2b pharmacokinetic data it was possible to model the HBV viral dynamics during the first month of treatment. Despite the minimal viral decline in the first weeks of PEG-IFN α-2b treatment, a sustained HBeAg-response was achieved in a considerable proportion of patients.

Acknowledgements

In addition to the authors, the HBV 99-01 Study Group includes the following investigators: The Netherlands: H.G.M. Niesters, P.E. Zondervan (Erasmus MC, 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: 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: J. Cianciara, J. Jablonska, J Kozlowska (Medical Academy of Warsaw), D. Prokopowicz (Medical Academy of Bialystok), T. Mach (Collegium Medicum UJ Kraków), C. Simon (Medical University Wroclaw); 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), C. Yurdayin, H. Bozkaya (Medical School Cebeci Kampusu Ankara), H. Simsek, Y. Balaban (Hacettepe University Faculty of Medicine Ankara), F. Tabak, H. Senturk (Istanbul University Cerrahpasa Medical School, 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).

This study was supported in part by the German Network of Competence of Viral Hepatitis Hep-Net (Federal Ministry of Education and Research, BMBF), and the clinical research unit KFO 129 (German Research Foundation, DFG).

References

1. Kane M. Global programme for control of hepatitis B infection. *Vaccine* 1995; 13 Suppl 1:S47-49.

2. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD & Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; 125:1714-1722.

3. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, Hussain M & Lok AS. Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; 44:283-290.

4. Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw YF, Cianciara J, Boron-Kaczmarska A, Martin P, Goodman Z, Colonno R, Cross A, Denisky G, Kreter B & Hindes R. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006; 130:2039-2049.

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

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

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

8. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P & Pluck N. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in

patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004; 351:1206-1217.

9. Chan HL, Leung NW, Hui AY, Wong VW, Liew CT, Chim AM, Chan FK, Hung LC, Lee YT, Tam JS, Lam CW & Sung JJ. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alpha2b and lamivudine with lamivudine alone. *Ann Intern Med* 2005; 142:240-250.

10. ter Borg MJ, van Zonneveld M, Zeuzem S, Senturk H, Akarca US, Simon C, Hansen BE, Haagmans BL, de Man RA, Schalm SW & Janssen HL. Patterns of viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B: Relation to treatment response. *Hepatology* 2006; 44:721-727.

11. Neumann AU. Hepatitis B viral kinetics: a dynamic puzzle still to be resolved. *Hepatology* 2005; 42:249-254.

12. 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.

13. Powers KA, Dixit NM, Ribeiro RM, Golia P, Talal AH & Perelson AS. Modeling viral and drug kinetics: hepatitis C virus treatment with pegylated interferon alfa-2b. *Semin Liver Dis* 2003; 23 Suppl 1:13-18.

14. Talal AH, Ribeiro RM, Powers KA, Grace M, Cullen C, Hussain M, Markatou M & Perelson AS. Pharmacodynamics of PEG-IFN alpha differentiate HIV/HCV coinfected sustained virological responders from nonresponders. *Hepatology* 2006; 43:943-953.

15. Nowak MA, Bonhoeffer S, Hill AM, Boehme R, Thomas HC & McDade H. Viral dynamics in hepatitis B virus infection. *Proc Natl Acad Sci U S A* 1996; 93:4398-4402.

16. Sypsa VA, Mimidis K, Tassopoulos NC, Chrysagis D, Vassiliadis T, Moulakakis A, Raptopoulou M, Haida C & Hatzakis A. A viral kinetic study using pegylated interferon alfa-2b and/or lamivudine in patients with chronic hepatitis B/HBeAg negative. *Hepatology* 2005; 42:77-85.

17. Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M & Esteban R. Viral

kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. *Hepatology* 2002; 35:930-936.

18. Formann E, Jessner W, Bennett L & Ferenci P. Twice-weekly administration of peginterferon-alpha-2b improves viral kinetics in patients with chronic hepatitis C genotype 1. *J Viral Hepat* 2003; 10:271-276.

19. Zeuzem S, Welsch C & Herrmann E. Pharmacokinetics of peginterferons. *Semin Liver Dis* 2003; 23 Suppl 1:23-28.

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

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

22. Chow WC, Manns M, Paik SW, Berg T, Piratvisuth T, Chang WY, Lau GKK, Marcellin P, Gane E & Pluck N. Effect of ethnicity, genotype, gender, age and bodyweight on sustained response in a large, randomised study of peginterferon (40KD) +/- lamivudine for HBeAG-positive chronic hepatitis B. *Hepatology* 2005; 42 (Suppl. 1):576A.

23. Wolters LM, Hansen BE, Niesters HG, Levi-Drummer RS, Neumann AU, Schalm SW & de Man RA. The influence of baseline characteristics on viral dynamic parameters in chronic hepatitis B patients treated with lamivudine. *J Hepatol* 2002; 37:253-258.

24. Tsiang M, Rooney JF, Toole JJ & Gibbs CS. Biphasic clearance kinetics of hepatitis B virus from patients during adefovir dipivoxil therapy. *Hepatology* 1999; 29:1863-1869.

25. Wolters LM, Hansen BE, Niesters HG, DeHertogh D & de Man RA. Viral dynamics during and after entecavir therapy in patients with chronic hepatitis B. *J Hepatol* 2002; 37:137-144.

26. van der Eijk AA, Hansen BE, Niesters HG, Janssen HL, van de Ende M, Schalm SW & de Man RA. Viral dynamics during tenofovir therapy in patients infected with lamivudine-resistant hepatitis B virus mutants. *J Viral Hepat* 2005; 12:364-372.

27. Colombatto P, Civitano L, Bizzarri R, Oliveri F, Choudhury S, Gieschke R, Bonino F & Brunetto MR. A multiphase model of the dynamics of HBV infection in HbeAg-negative patients during pegylated interferon-alpha2a, lamivudine and combination therapy. *Antivir Ther* 2006; 11:197-212.

CHAPTER 4

ALT and viral load decline during PEG-IFN alpha -2b treatment for HBeAg-positive chronic hepatitis B

M.J. ter Borg[1], B.E. Hansen[1,2], G. Bigot[1,2], B.L. Haagmans[3], H.L.A. Janssen[1].

Departments of [1]Gastroenterology & Hepatology, [2]Epidemiology & Biostatistics, and [3]Virology, Erasmus MC, University Medical Centre Rotterdam, Rotterdam, The Netherlands

Journal of Clinical Virology, 2008; 42(2):160-4.

Summary

Alanine aminotransferase (ALT) is one of the main indicators for inflammatory activity in chronic hepatitis B. During interferon-based therapy, approximately 25-40% of patients exhibit an ALT flare.

To analyze the relation between ALT and HBV DNA during pegylated interferon alpha-2b (PEG-IFN) treatment and compare different patterns of on-treatment viral load decline with the occurrence of ALT flares.

Of the 123 patients included in this study 31 (25%) exhibited an ALT flare during treatment or follow-up. Six out of 8 (75%) host induced flares, i.e. ALT flares which were followed by a HBV DNA decrease associated with a favorable treatment outcome, occurred in patients with a delayed HBV DNA decline pattern (delayed vs. non-delayed decline, p=.022); 5 of these 8 patients exhibited HBeAg loss and 4 even HBsAg loss at the end of follow-up. Prediction of ALT normalization was possible using on-treatment viral load. Based on the difference from baseline, the evolution of viral load and ALT level were strongly interrelated during treatment and follow-up. With a joint model we estimated a correlation coefficient of 0.38 ($p<0.001$) during the first 4 weeks of the treatment and of 0.72 ($p<0.0001$) thereafter.

There was a strong relation between ALT and viral load in HBeAg-positive chronic hepatitis B patients treated with PEG-IFN alpha-2b, especially after 4 weeks of treatment. Patients with a delayed decline in viral load often exhibited a host induced flare associated with a favorable outcome.

Introduction

Chronic hepatitis B remains a major global health problem. Worldwide approximately 350 million persons are chronically infected.[1] Patients infected with the hepatitis B virus (HBV) are at increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma.[2]

Alanine aminotransferase (ALT) in serum is one of the main indicators for inflammatory activity in chronic hepatitis B. During treatment with nucleos(t)ide analogues there is no increased incidence of ALT flares, but flares do occur in 10-20% of patients after withdrawal of treatment.[3,4] In contrast, during interferon-based therapy, approximately 25-40% of patients exhibit an ALT flare. This is probably caused by the immunostimulatory effects of interferon.[5] These flares can lead to hepatic decompensation, but have also been associated with an increased response rate.[6] A previous study recognized that host-induced flares, i.e. an ALT flare followed by a decrease in HBV DNA, are associated with a favorable treatment response.[7]

The aim of this study was to determine the relation between the levels of ALT and HBV DNA and to compare different patterns of on-treatment viral load decline with the occurrence of ALT flares.

Patients & Methods

Study population

A total of 136 patients were included in an international multicenter randomized double-blinded trial and received pegylated interferon alpha-2b (PEG-IFN) (PegIntron, Schering-Plough, Kenilworth, NJ, USA) 100 µg monotherapy for 52 weeks with 26 weeks of post-treatment follow-up.[8] PEG-IFN dose was halved after 32 weeks of treatment. Eligible patients were men and women over 16 years of age with an HBeAg-positive chronic hepatitis B infection. All patients had an HBV-DNA above 10^5 copies/ml and ALT levels of at least 2 times the upper limit of normal on two occasions within eight weeks before randomization. Exclusion criteria have been reported previously.[8]

Virological and biochemical assays

Serum HBV-DNA levels were measured monthly during both treatment and follow-up and

were determined by a in-house developed quantitative real-time Taqman PCR assay with a dynamic range of $4x10^2$-10^{10} copies/ml.[9] The Eurohep HBV-DNA standard was used for validation of HBV DNA levels.[10] Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibodies to hepatitis B surface (anti-HBs) and e antigen (anti-HBe) were measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, IL, USA). The assessment of HBV genotypes was done by Inno-Lipa assay (Innogenetics, Ghent, Belgium). ALT levels were measured monthly during treatment and follow-up.

Definitions of flare types and HBV DNA decline patterns

A virus induced flare was defined as rise in ALT preceded by an increase of at least 1 log HBV DNA within four months. Patients had a host induced flare when a rise in ALT was followed by a more than 1 log decline in HBV DNA within four months thereafter.[7]

The definitions of different HBV DNA decline patterns were described in detail previously.[11] In short, patients had an 'early decline' when there was a more than 1 log decline in HBV DNA in the first month of treatment. A 'delayed decline' was defined as a more than 2 log decline between week 4 and 32. A 'late decline' as a more than 2 log decline between week 32 and 52, and a 'post-treatment' decline as a more than 2 log decline between week 52 and 78.

Statistical analysis

A multivariate mixed linear model (joint model) was used to determine the correlation between HBV DNA and ALT levels. The model for both HBV DNA and ALT was defined, as proposed by Thiebaut et al.[12]:

$$Y_i^k(t) = Y_0^k + \left(\beta_1^k t\right) + \left(\beta_2^k (t-T)\right) \cdot I_{t \geq T} + \left(\gamma_{1,i}^k t\right) + \left(\gamma_{2,i}^k (t-T)\right) \cdot I_{t \geq T} + \varepsilon_i^k$$

where $Y_i^1(t)$ is ALT and $Y_i^2(t)$ is HBV DNA at time t for individual i. For ALT (k=1) and HBV DNA (k=2) Y_0^k is the baseline value, b_1^k is the slope before time point T, $b_1^k+b_2^k$ is the slope after time point T, $\gamma_{1,i}^k$ is the individual random effect for subject i of the slope before time point T, $(\gamma_{1,i}^k + \gamma_{2,i}^k)$is the individual random effect for subject i of the slope after time point T and e_i^k is the is the residual error of measurement; I_{t^3T} = 1 if t³T and 0 if t<T. With

the joint structure of this model the correlation between ALT and HBVDNA can be described. The PROC MIXED procedure of SAS 9.1 (SAS Institute Inc., Cary, NC) was used to estimate the model . Further analyses were done with the statistical package SPSS version 14.0 (SPSS Inc. Chicago, IL, USA). The c2 or Fisher's exact test was used for categorical variables, and the Mann-Whitney U test was performed for continuous data. In all cases, a 2-tailed $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

From the 136 patients included in the PEG-IFN monotherapy arm of a large randomized controlled trial,[8] sufficient HBV DNA and ALT measurements were available in 123 patients. Table 1 gives an overview of the baseline characteristics of these 123 patients included in this study. Mean HBV DNA was 9.1±0.8 $\log^{10}$ copies/mL with a mean ALT of 4.3±3.1 times the upper limit of normal at baseline. Response was defined as HBeAg loss at week 78.

Age*	35.3 (13.2)
Male / female	96 (78%) / 27 (22%)
ALT xULN*	4.3 (3.1)
HBV DNA (log_{10} copies/ml)*	9.1 (0.8)
Race	
Caucasian	89 (72%)
Asian	28 (23%)
Other	6 (5%)
Genotype	
A	39 (32%)
B	12 (10%)
C	21 (17%)
D	47 (38%)
Other	4 (3%)

* Mean (SD)

Table 1: Baseline chacteristics of all PEG-IFN treated patients (n=123).

Relation viral load & ALT

Based on the difference from baseline, the evolution of viral load and ALT level was positively correlated both during the treatment and follow-up period. With a joint model (with T at 4 weeks) we estimated a correlation coefficient of 0.38 ($p<0.001$) during the first 4 weeks of the treatment and of 0.72 ($p<0.0001$) thereafter.

There was a decline in both ALT and HBV DNA in the first 4 weeks of treatment in virtually all patients irrespective of response (Figure 1). After week 4, the slope of both ALT and HBV DNA decline was not significant different from 0 in those without HBeAg-loss at week 78 (Figure 1). However, HBV DNA and ALT continued to decrease in patients with HBeAg-loss at week 78.

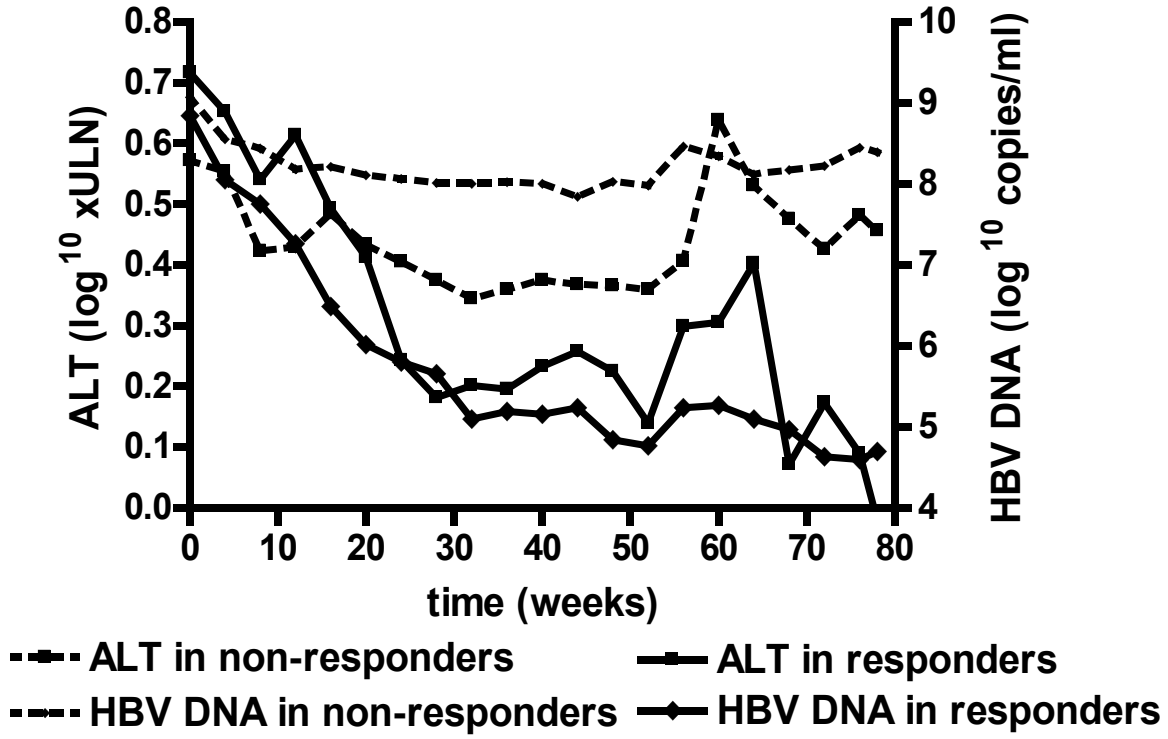


Figure 1: Relation between viral load and ALT according to response at the end of follow-up (defined as HBeAg loss at week 78).

Prediction of sustained ALT normalization

Sustained ALT normalization (i.e. a normal ALT both at the end of treatment and the end of follow-up) was achieved in 34 out of 123 patients (28%). Prediction of ALT normalization was possible using on-treatment viral load. The area under the ROC curves was 0.80, 0.80 and 0.83 for a viral load less than 10^3, 10^4 and 10^5 copies/ml, respectively. Patients with a viral load of less than 10^3 copies/ml at any time point during treatment had 88% chance to have a sustained ALT normalization; this was 72% and 69% for those with a viral load below 10^4 and 10^5 copies/ml respectively (Figure 2).

In patients with genotype A (n=39) and B (n=12), we observed higher rates of sustained ALT normalization than in genotypes C (n=21) and D (n=47) (33% for A, 33% for B, 19% for C and 23% for D). Four patients had other genotypes and were not analyzed.

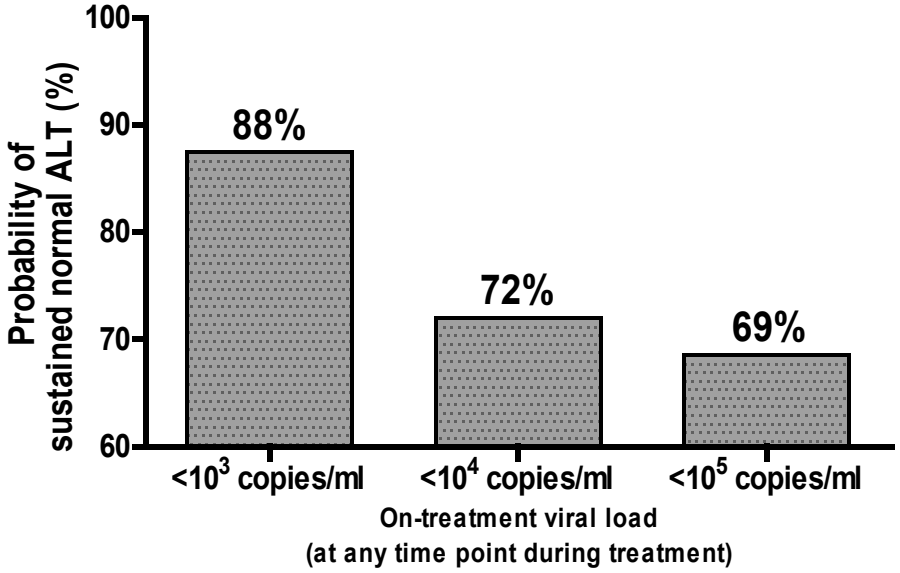


Figure 2: Probability of sustained ALT normalization (ALT normal at the end of treatment and follow-up) based on on-treatment viral load.

Relation between type of ALT flare and HBV DNA decline pattern

Of the 123 patients included in this study, 31 (25%) exhibited an ALT flare during treatment or follow-up. A host-induced flare was observed in 8 (26%) patients, a virus induced flare in 10 (32%) patients and an indeterminate flare in 13 (42%) patients. In a previous study, we described different patterns of viral decline during PEG-IFN monotherapy treatment.[11] Most ALT flares occurred in patients with a delayed and late decline pattern with a more than 2 $\log_{10}$ copies/ml decline in HBV DNA between week 4-32 and 32-52, respectively (Table 2). Furthermore, six out of 8 (75%) host-induced flares occurred in patients with a delayed HBV DNA decline pattern (delayed vs. non-delayed decline, p=.022) (Table 2). Five of these 8 patients with a host-induced flare exhibited HBeAg loss and 4 also HBsAg loss at the end of follow-up. Patients with a late HBV DNA decline pattern only exhibited virus-induced (n=1) or indeterminate flares (n=8) (Table 2).

	Type of ALT flare			
HBV DNA decline pattern	Host induced	Virus induced	Indeterminate	Total
Early decline (n=23)	1	3	0	4 (17%)
Delayed decline (n=32)	6	5	2	13 (41%)
Late decline (n=13)	0	1	8	9 (69%)
Post-treatment decline (n=11)	1	1	1	3 (27%)
No decline (n=44)	0	0	2	2 (5%)
Total (n=123)	8	10	13	31 (25%)

Table 2: Relation between ALT flares and pattern of viral decline.

Discussion

In this study, we found a strong relation between ALT and viral load during treatment with PEG-IFN alpha-2b in HBeAg-positive chronic hepatitis B patients, especially after 4 weeks of treatment. In patients without HBeAg-loss at the end of follow-up, the slope of both ALT and HBV DNA decline was not significant different from 0 after 4 weeks of treatment. ALT flares were observed in 25% of patients during treatment and follow-up. We previously reported that a delayed decline of at least two $\log_{10}$ copies/ml from baseline HBV DNA after week 4 but before week 32 was associated with the highest response rate of 63%.[11] In the present study, patients with a delayed decline in viral load also most often exhibited a host induced flare which is associated with a favorable outcome.[7]

During the first four weeks of treatment with PEG-IFN a significant decline in viral load and ALT was observed in both the responder and non-responder group with only a moderate correlation between ALT and HBV DNA (correlation coefficient of 0.38). Thereafter, viral load and ALT stabilized in the non-responders and continued to decline in responders with a good correlation between HBV DNA and ALT (overall correlation coefficient of 0.72). The immunological mechanism of action of PEG-IFN could be an explanation for this phenomenon. IFN-based therapy has a stimulating effect on cytotoxic T lymphocytes and natural killer cell function but has also a direct antiviral effect by inhibiting viral replication.[13] This direct antiviral effect of PEG-IFN could be responsible for the decline in

the first weeks of treatment in both groups, whereas the combined immunomodulatory and antiviral effect is only observed in responders thereafter. Due to the relation between viral load and ALT decline during treatment with PEG-IFN, it was possible to predict sustained ALT normalization (i.e. a normal ALT both at the end of treatment and follow-up) based on viral load. Patients with an on-treatment viral load below 10^3 copies/ml at any time point during treatment had an 88% to achieve sustained ALT normalization.

ALT flares are common during treatment with (PEG-)IFN for chronic hepatitis B most likely due to the stimulatory effect of IFN on the immune system. Approximately 25 to 40% of patients exhibit an IFN-induced flare during treatment. These flares may rarely cause decompensated liver disease but may also precede a favorable HBeAg-seroconversion.[3, 14-16] A recent study from our group in HBeAg-positive patients found ALT flares in 25% of cases during PEG-IFN with or without lamivudine treatment.[7] Flares during (PEG-)IFN treatment should therefore not be treated with nucleos(t)ide analogues and (PEG-)IFN treatment should only be discontinued in case of impending liver failure. Most of the beneficial host-induced flares[7] were observed in patients with a delayed or late HBV DNA decline pattern, i.e. a viral decline of more than 2 logs between week 4-32 and 32-52, respectively. Patients with a late HBV DNA decline pattern only exhibited an indeterminate or virus-induced flares. On the other hand, nearly all host-induced flares (6 out of 8) were observed in patients with a delayed HBV DNA decline pattern, which is associated with the highest HBeAg and HBsAg loss rates (63% and 22%, respectively).[11]

In conclusion, there is a strong relation between viral load and ALT during PEG-IFN treatment. In virtually all patients an HBV DNA and ALT decrease was observed in the first 4 weeks of treatment, with a significant decline thereafter only in responders. ALT flares, and especially host-induced flares associated with a favorable outcome, occurred most often in patients with a delayed HBV DNA decline pattern. This suggests that immune stimulation, rather than viral load decline in itself, is the most prominent factor leading to sustained off-treatment response.

References

1. 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.
2. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61(10):1942-56.
3. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology 2001;120(4):1009-22.
4. 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(3):635-9.
5. Tang TJ, Kwekkeboom J, Laman JD, et al. The role of intrahepatic immune effector cells in inflammatory liver injury and viral control during chronic hepatitis B infection. J Viral Hepat 2003;10(3):159-67.
6. 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(5):1021-6.
7. 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(11):1604-9.
8. 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.
9. 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(8):2897-901.
10. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol 1999;37(1):68-73.
11. ter Borg MJ, van Zonneveld M, Zeuzem S, et al. Patterns of viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B: Relation to treatment response. Hepatology 2006;44(3):721-7.
12. Thiebaut R, Jacqmin-Gadda H, Chene G, Leport C, Commenges D. Bivariate linear

mixed models using SAS proc MIXED. Computer methods and programs in biomedicine 2002;69(3):249-56.

13. Sprengers D, Janssen HL. Immunomodulatory therapy for chronic hepatitis B virus infection. Fundam Clin Pharmacol 2005;19(1):17-26.
14. Peters M, Davis GL, Dooley JS, Hoofnagle JH. The interferon system in acute and chronic viral hepatitis. Prog Liver Dis 1986;8:453-67.
15. 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(5):295-301.
16. Alexander GJ, Brahm J, Fagan EA, et al. Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. Lancet 1987;2(8550):66-9.

Appendix

In addition to the authors, the HBV 99-01 Study Group includes the following investigators: The Netherlands: 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: 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: 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: 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), 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)

CHAPTER 5

Effects of PEG-IFN α-2b treatment compared with placebo in patients with HBeAg-positive chronic hepatitis B

Martijn J ter Borg[1], Bettina E Hansen[1,2], Harry LA Janssen[1]

1. Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

2. Department of Epidemiology and Biostatistics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Submitted

Summary

During PEG-IFN treatment there is a considerable decline in hepatitis B virus (HBV) DNA. A good comparison of viral load and ALT decline during PEG-IFN treatment and placebo is however not available. A total of 136 patients were treated with PEG-IFN α-2b. This group was compared with 167 patients that received placebo therapy. All patients were HBeAg-positive with ALT level of at least 1.2 and 2 times the upper limit of normal, respectively.

In the PEG-IFN α-2b therapy group, the mean viral decline at the end of treatment was 2.3 $\log_{10}$ copies/ml (SD±2.3) compared to 1.0 $\log_{10}$ copies/ml (SD±1.3) in the placebo group. With mixed procedure analysis, the effect of PEG-IFN treatment on viral decline was estimated and compared to placebo. At week 8 of treatment, there was significant more viral decline in the PEG-IFN group compared to placebo. This difference remained significant during the whole treatment period. HBV DNA was below 10^3 copies/ml at the EOT in 13% of PEG-IFN treated patients and never occurred in the placebo group. This percentage was highest in patients with genotype A (26%).

PEG-IFN treatment is able to reduce HBV DNA in a considerable amount of patients compared to placebo. Especially in patients with genotype A, a significant viral load and ALT decline was observed compared to placebo.

Introduction

Approximately 400 million people worldwide are chronically infected with the hepatitis B virus (HBV). Furthermore, it is estimated that between 500,000 and 1 million patients die each year from cirrhosis and hepatocellular carcinoma (HCC) related to HBV infection.[1-3] Nucleos(t)ide analogues, such as lamivudine, telbivudine, adefovir dipivoxil and entecavir strongly suppress viral replication. After 1 year of treatment, these nucleos(t)ide analogues achieve a decline in viral load between 3.5-6.9 $\log_{10}$ copies/ml. They induce hepatitis B e antigen (HBeAg) seroconversion in 12-21% of HBeAg-positive chronic hepatitis B patients after one year of treatment.[4-6]

The mechanism of action of (pegylated) interferons is different from that of nucleos(t)ide analogues. The immunomodulatory effect of interferons seems to be the most important rather than a direct antiviral effect on the hepatitis B virus replication. Therefore, the decline in viral load is in general lower compared with nucleos(t)ide analogues. After 1 year pegylated interferon (PEG-IFN) treatment, there is a 2.3-4.5 $\log_{10}$ copies/ml drop in viral load in HBeAg-positive chronic hepatitis B patients[7, 8] and HBeAg seroconversion is observed in approximately 29-34% patients.[7, 9]

Even in non- or placebo treated patients, fluctuations in HBV DNA around 1 $\log_{10}$ copies/ml are observed over a 1 year period.[5] Especially genotype A chronic HBV patients exhibit a favourable natural disease course with a high rate of spontaneous disease remission. It is often questioned whether this group of patients needs antiviral therapy or whether one could wait until spontaneous remission occurs. Until now, extensive viral kinetics data are not available during PEG-IFN treatment in HBeAg-positive chronic hepatitis B and have never been compared to natural occurring fluctuations in viral load during placebo therapy.[10] Therefore, we compared viral decline in PEG-IFN α-2b treated HBeAg-positive chronic hepatitis B patients with a group of placebo treated patients. The differences in viral load and ALT decline over time during treatment were studied.

Material and methods

Patients

A total of 136 patients who participated in an international multicenter randomized double-blinded study reported previously[9] were treated with PEG-IFN α-2b. Eligible patients were men and women over 16 years of age with chronic hepatitis B, documented by liver biopsy and HBsAg positivity for over six months, positive serum HBV DNA levels. All patients were HBeAg-positive with an ALT level of at least 2 times the upper limit of normal. Patients received PEG-IFN α-2b 100 µg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN α-2b was reduced to 50 µg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and followed for 6 months post-treatment.

The placebo arm consists of 167 patients that participated in another international multicenter trial.[5] Eligible patients were men and women 16 to 65 years of age who were HBeAg-positive with a compensated liver disease and a documented HBsAg positivity for more than 6 months. HBV DNA level had to be at least 1 million copies/ml with an ALT level of over 1.2 times the upper limit of normal.

Virological and biochemical assessments

HBV DNA levels were measured monthly during treatment and follow-up using an in-house developed TaqMan real-time PCR test (dynamic range $4x10^2$-10^{10} copies/ml) in the PEG-IFN α-2b treated patients[11] and a Roche Amplicor Monitor polymerase-chain-reaction (PCR) assay) in the placebo therapy treated patients. Hepatitis B surface antigen (HBsAg), HBeAg, antibodies to hepatitis B surface (anti-HBs) and e antigen (anti-HBe) were measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, Il). A significant viral decline (SPD) was defined as a more than 1 log copies/ml viral decline on two occasion compared to the viral load directly before the viral decline.

Statistics

Comparisons between groups were done using the chi-square test or Fisher's exact test for categorical variables, and the Mann-Whitney test for continuous variables. SPSS (version 14.0.1 SPSS Inc., Chicago, IL) was used for data analyses. Where appropriate, mean values are given ± standard deviation (SD). All tests for significance and resulting P values were two-sided, with a level of significance of 0.05. Viral decline modeling was done by a mixed procedure analysis using SAS 9.1 (SAS Institute Inc., Cary, NC).

Results

Patient characteristics

Demographic and baseline characteristics of the 303 included patients in this study are shown in table 1. A total of 136 patients received PEG-IFN α-2b therapy; the remaining 167 patients received placebo therapy. There were no significant differences between the two groups with respect to ALT, viral load, age, sex and weight. There were differences in ethnic background with more Asians in the placebo treated group (Table 1). Furthermore, baseline HBV DNA levels where higher in the PEG-IFN treated group.

	PEG-IFN (n=136)	Placebo (n=167)	*p*
Age – yr*	36 ± 14	37 ± 12	*n.s.*
Male sex - no. (%)	107 (79)	119 (71)	*n.s*
Weight - kg*	72 ± 13	70 ± 15	*n.s*
Race – no. (%)			*0.01*
Caucasian	101 (74)	60 (36)	
Asian	29 (21)	101 (60)	
Other	6 (5)	6 (4)	
ALT (U/L)*	167 ± 128	148 ± 154	*n.s.*
HBV DNA (log_{10} copies/ ml)*	9.1 ± 0.8	8.4 ± 0.7	*0.01*

Table 1: Baseline characteristics. ** mean ± standard deviation*

Viral kinetics

At baseline, mean HBV DNA was 9.1±0.8 $\log_{10}$ copies/ml and 8.4±0.7 in the PEG-IFN α-2b and placebo therapy respectively (p=0.01). In the PEG-IFN α-2b therapy group, the mean viral decline at the end of treatment (EOT) was 2.3 $\log_{10}$ copies/ml (SD±2.3) compared to 1.0 $\log_{10}$ copies/ml (SD±1.3) in the placebo group. Patients with genotype A and B treated with PEG-IFN had the most pronounced decline in viral load (figure 1; table 2).

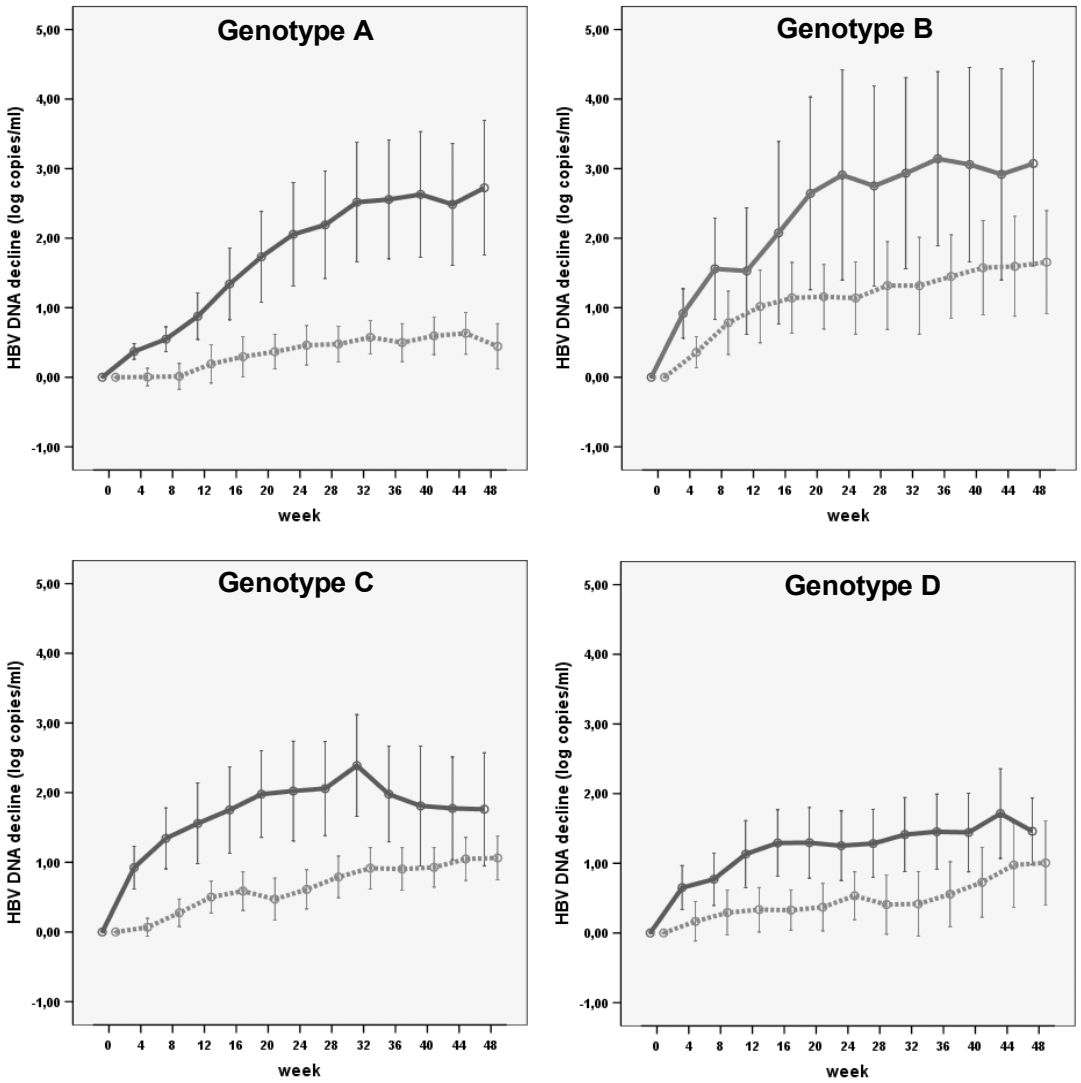


Figure 1: HBV DNA decline from baseline during treatment in the PEG-IFN group (solid line) and the placebo group (dashed line) per genotype.

With mixed procedure analysis, the effect of PEG-IFN treatment on viral decline was estimated and compared to placebo. At week 8 of treatment, there was significant more viral decline in the PEG-IFN group compared to placebo. This difference remained significant during the whole treatment period. In the subgroup of patients with HBV genotype A (n=85; 41 in the PEG-IFN α-2b group and 44 in the placebo group), the difference in viral load decline became significant at week 24 and remained significant thereafter. A sustained significant difference compared to placebo was not observed in the other genotypes. HBV DNA was below 10^3 copies/ml at the EOT in 13% of PEG-IFN treated patients and never occurred in the placebo group.

In genotype A, HBV DNA was below 10^3 copies/ml at week 48 in 26% of PEG-IFN treated patients and in none of the placebo group (p<.001) (see table 2). In genotypes C and D, this difference was not significant.

	PEG-IFN				**Placebo**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
HBV-DNA <1000c/ml	26%	22%	9%	2%	0%	0%	0%	0%
ALT<40 U/L	41%	45%	18%	33%	5%	33%	20%	38%

Table 2: End of treatment results (both HBV-DNA level below 1000 copies/ml and ALT below 40 U/L) in the two treatment groups.

ALT kinetics

The mean ALT at baseline was comparable in both groups, 167±128 U/L in the PEG-IFN α-2b group and 148 ± 154 U/L in the placebo group (p=0.54). Mean reductions in ALT levels at week 48 of treatment were 95 IU/L (SD 139) in the PEG-IFN α-2b group and 42 IU/L (SD 130) in the placebo group (p<0.001) (see Figure 2 for per genotype ALT decline). Patients in the PEG-IFN treated group with genotype A achieved significantly more ALT decline compared to the placebo group (p=0.01) (table 2, figure 2). In genotype B, C and D there was no significant difference. In all genotypes, there was a pronounced overlap in ALT levels.

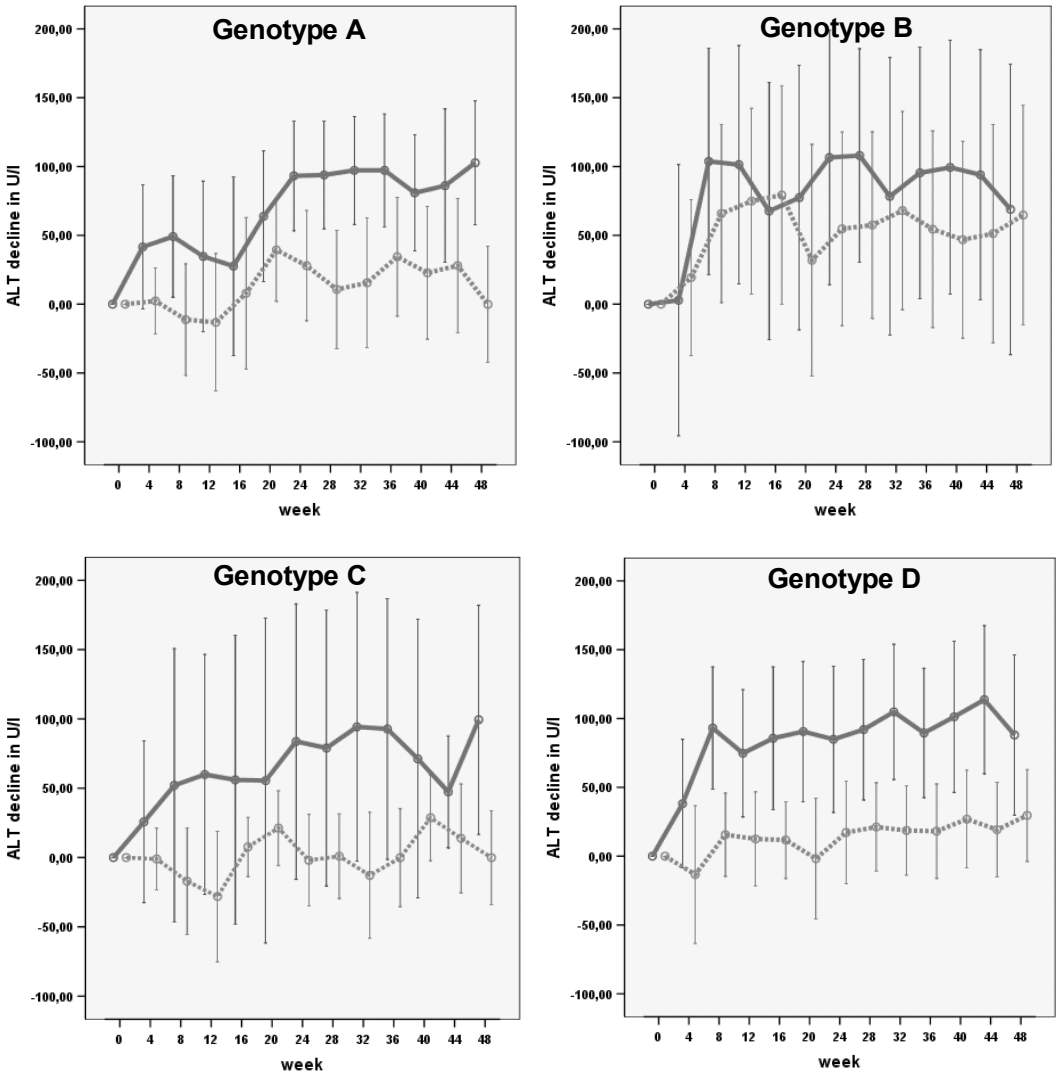


Figure 2: ALT decline from baseline during treatment in the PEG-IFN group (solid line) and the placebo group (dashed line) per genotype.

<u>The occurrence of a significant viral decline (SVD)</u>

A significant viral decline decline (SVD) was defined as a more than 1 log copies/ml viral decline on two occasions (see methods section). SVD's occurred in 42% of patients in the PEG-IFN treated group compared to 13% in the placebo group ($p<0.01$). Most SVD's occurred at week 4 of treatment in the PEG-IFN group, with a second peak at week 20 (Figure 3).

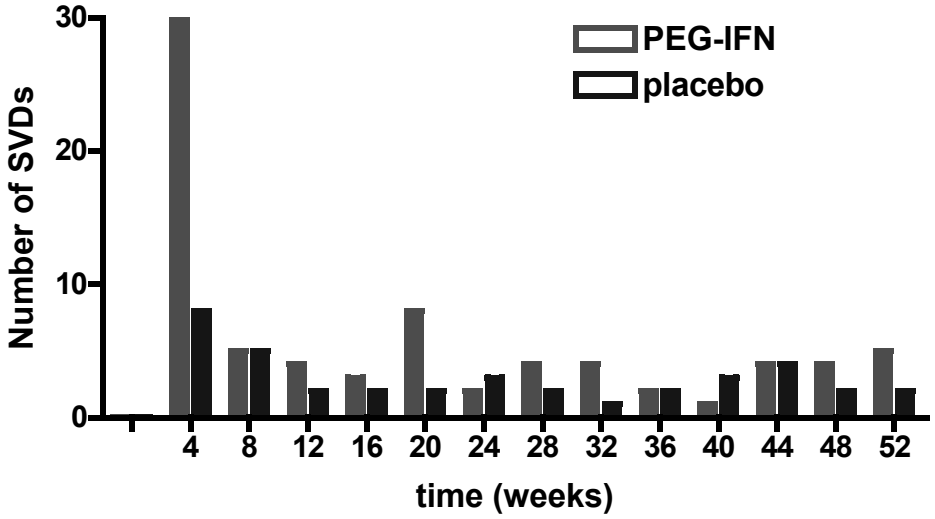


Figure 3: Number of SVD's over time in both treatment arm.

In the placebo group there is a stable amount of SVD's over time however with significant more SVD's at week 4 after the start of placebo treatment compared to the other timepoints.

Discussion

In this study, we analyzed HBV DNA viral kinetics during PEG-IFN α-2b treatment in HBeAg-positive chronic hepatitis B and compared viral decline with a control group of placebo treated patients. Even patients in the placebo group exhibited almost 1 $\log_{10}$ copies/mL decline in viral load at the end of treatment, in PEG-IFN α-2b treated patients a decline of 2.3 $\log_{10}$ copies/mL was observed. Patients with genotype A and B treated with PEG-IFN had the most pronounced decline in viral load. With mixed procedure analysis, the effect of PEG-IFN treatment on viral decline was estimated and compared to placebo. Significant more viral decline was observed, especially in genotype A.

In the PEG-IFN group there was significantly more ALT decline compared to the placebo group. There were however considerable fluctuations in ALT levels in the placebo group, especially in genotypes A and B. It is known that genotype A and B exhibit a favourable natural disease course[2] and this is probably the explanation for this observation.

Significant viral declines (SVD's) were more frequently observed in PEG-IFN treated patients especially in the first week after start of treatment. There was however a second peak in the number of SVD's during PEG-IFN treatment at week 20 week. This is in line with our previous observation that patients with a delayed HBV DNA decline between week 4 and 32 had the highest chance of response (both HBeAg and HBsAg loss).[8]

Although this study is one of the first to analyse the effects of PEG-IFN treatment in chronic hepatitis B compared to placebo, it has some limitations. The two groups of patients used in this study are not completely comparable. Baseline HBV-DNA levels where significantly higher in the PEG-IFN treated group of this study but despite these higher HBV-DNA levels, which is associated with a less favourable outcome of PEG-IFN treatment[2], we observed most decline in viral load and ALT in genotype A. If there were no differences in baseline viral load, the effects of PEG-IFN treatment would probably be better. Furthermore, there were significant differences in the patient's race between the two groups. Therefore we investigated the HBV DNA genotypes separate in most analysis to correct for these differences. Race and genotypes seem to be important with respect to treatment outcome, especially in HBeAg-positive hepatitis B, therefore genotype testing is recommended in future studies.

In conclusion, PEG-IFN α-2b is able to induce a pronounced viral load and ALT decline, especially in genotype A. Despite the favourable natural disease course in genotype A patients, they are the best candidates for PEG-IFN treatment compared to placebo.

References

1. Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13 Suppl 1:S47-9.
2. 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 Suppl 1:S3-25.
3. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337(24):1733-45.
4. 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(10):1001-10.
5. 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.
6. 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(17):1256-63.
7. 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(26):2682-95.
8. ter Borg MJ, van Zonneveld M, Zeuzem S, et al. Patterns of viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B: Relation to treatment response. Hepatology 2006;44(3):721-7.
9. 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.
10. Neumann AU. Hepatitis B viral kinetics: a dynamic puzzle still to be resolved. Hepatology 2005;42(2):249-54.
11. 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(8):2897-901.

CHAPTER 6

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

Erik H.C.J. Buster [1], Martijn J. ter Borg [1], Johannes R. Vingerling [2], Harry L.A. Janssen [1]

1. Departments of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands.
2. Ophthalmology Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands.

J Hepatol. 2006 Jul;45(1):160-1

Summary

Ophthalmologic side-effects can occur during interferon (IFN) therapy for viral hepatitis. In a recent study, retinopathy was found on fundoscopic examination in 24% of patients after IFN therapy, none of whom experienced ophthalmologic symptoms.

The observed incidence of retinopathy in our study (4%) was significantly lower compared to that in other studies. This may be explained by multiple factors: (1) we performed fundoscopic examination relative early during treatment (median 14 weeks); (2) in our study mean age was profoundly lower; (3) hepatitis C patients may be, irrespective of age and other risk factors, more at risk to develop retinopathy than hepatitis B patients.

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.

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 & methods

As part of a global randomized controlled trial we performed a 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. 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).

Results

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.

Clinical characteristics at baseline (n=28)		
Age		
Mean age	34 ± 4.3 years (range 18 – 60 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	$1.6x10^9$ copies/ml (range $2.0x10^7$ – $1.4x10^{10}$ copies/ml)	
Risk factors for developing 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 (p = 0.18) *
Retinopathy	0/28 (0%)	1/28 (4%)

* Mean corrected visual acuity during peginterferon treatment compared to baseline testing by paired *t* test.

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

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 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, Delplace MP, Bacq Y. Ophthalmologic side effects during alpha-interferon therapy for viral hepatitis. J Hepatol. 2006 Jan;44(1): 56-61.
2. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HbeAg-positive chronic hepatitis B: a randomised trial. Lancet. 2005 Jan 8-14;365(9454):123-9.
3. Tu KL, Bowyer J, Schofield K, Harding S. Severe interferon associated retinopathy. Br J Ophthalmol. 2003 Feb;87(2):247-8.
4. Hayasaka S, Nagaki Y, Matsumoto M, Sato S. Interferon associated retinopathy. Br J Ophthalmol. 1998 Mar;82(3):323-5.
5. Schulman JA, Liang C, Kooragayala LM, King J. Posterior segment complications in patients with hepatitis C treated with interferon and ribavirin. Ophthalmology. 2003 Feb;110(2):437-42.
6. Abe T, Nakajima A, Satoh N, Koizumi T, Sakuragi S, Ono T, 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, Gordon SR. Ocular manifestations of hepatitis C virus infection. Curr Opin Ophthalmol. 2002 Dec;13(6):423-7.

CHAPTER 7

Exacerbation of chronic hepatitis B infection after delivery

M.J. ter Borg, W.F. Leemans, R.A. de Man, H.L.A. Janssen.

Department of Gastroenterology and Hepatology
Erasmus MC, University Medical Center Rotterdam
Rotterdam, The Netherlands.

Journal of Viral Hepatitis 2008; 15(1):37-41

Summary

During pregnancy several alterations in the immune status allow mothers to tolerate the genetically different fetal tissues. We investigated the evolution of liver disease during and after pregnancy in chronic hepatitis B patients. Between 1998 and 2006 there were 38 pregnancies in 31 chronic HBsAg-positive women at our liver unit. Twenty-four subjects (63%) were HBeAg-positive, 14 (37%) HBeAg-negative. In 13 pregnancies (34%), lamivudine therapy was started during the last trimester of pregnancy to lower HBV DNA levels in order to reduce the risk of vertical transmission.

A significant increase in liver disease activity after pregnancy, defined as a 3 times increase in ALT within 6 months after delivery, occurred in 17 out of 38 patients (45%). In those treated with lamivudine during the last trimester of pregnancy, this occurred in even 8/13 patients (62%). Prediction during pregnancy of these exacerbations was not possible using HBV DNA, ALT level, HBeAg status or any other characteristic. The median maximal ALT of these exacerbations was 4.0 x ULN and none led to decompensated liver disease.

In conclusion, a significant increase in liver inflammation occurs often after pregnancy. This may be due to a reactivation of the immune system after delivery. Based on our data we recommend to monitor closely and if necessary treat women with chronic HBV shortly after delivery.

Introduction

Chronic hepatitis B virus (HBV) infection is an important health issue and one of the most prevalent viral diseases in human. Approximately 400 million people are chronically infected with HBV worldwide.[1,2] HBV is a non-cytopathic virus and the associated hepatic inflammation is mainly mediated by the host's immune response. In patients with chronic HBV infection an inadequate immune response of the host plays an important role in the development of chronicity.[3]

During pregnancy there is extensive contact between fetal and maternal tissues. Between these two tissues the placenta, composed of fetal and maternal tissue, acts both as a barrier and a zone for nutritional exchange. There are several mechanisms to prevent rejection by the maternal immune system.[4] After pregnancy, these adaptations disappear and the immune system fully restores its function. These alterations in the immune system during pregnancy could influence liver disease activity and thereby may alter the need for therapy after delivery.

We therefore analyzed the influence of pregnancy on liver disease activity in both HBeAg-positive and HBeAg-negative chronic hepatitis B patients. Furthermore we investigated the role of lamivudine treatment in the last trimester of pregnancy on liver disease activity in this patient category.

Material and methods

Patients

In this retrospective cohort study we analyzed all pregnancies in women chronically infected with HBV between 1998 and 2006 at our liver unit. Data were compiled from patient files. Patients with an acute hepatitis B infection during pregnancy and those who were treated at the time of conception were excluded from the study. During pregnancy, patients attended the outpatient clinic at regular time intervals for routine examination and laboratory tests. In patients with a viral load ≥1.2 x 10^9 copies/ml at the end of the second trimester of pregnancy, lamivudine therapy was started in the last trimester and stopped immediately after delivery.[5]

Biochemical and virological assessments

The extent of liver inflammation was determined by measuring serum alanine

aminotransferase (ALT) levels. HBV DNA levels were measured using an in-house developed TaqMan real-time PCR assay (dynamic range $4x10^2$-10^{10} copies/ml).[6] The Eurohep HBV DNA standard was used for validation of HBV DNA levels.[7] Hepatitis B 'e' antigen (HBeAg), hepatitis B 's' antigen (HBsAg), antibodies to HBeAg (anti-HBe) and antibodies to HBsAg (anti-HBs) were measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, Il, USA).

Definition of a significant increase in liver disease activity

A significant increase in liver disease activity after pregnancy was defined as a 3 times increase in ALT within 6 months after delivery compared to the lowest ALT value during pregnancy.[8,9]

Statistical analysis:

Comparisons between groups were done using the chi-square test or Fisher's exact test for categorical variables, and the Mann-Whitney U test for continuous variables. Dependent variables were tested using the Wilcoxon Signed Rank test. All data were analyzed using SPSS (version 14.0.1 SPSS Inc., Chicago, IL). All tests for significance and resulting P values were two-sided, with a level of significance of 0.05.

Results

Patient characteristics

Between 1998 and 2006 there were 38 pregnancies in 31 women with a chronic HBV infection. During pregnancy, 24 mothers (63%) were HBeAg-positive and 14 (37%) HBeAg-negative (Table 1). Seventeen subjects (45%) were in the immunotolerant, four (11%) in the immune-active, nine (23%) in the immune-control and eight (21%) in the immune-escape phase before pregnancy.[10] The median age at time of delivery was 25.6 years (range, 18.2-40.5). In 13 pregnancies (34%) with high HBV DNA levels during pregnancy, lamivudine therapy was started in the last trimester of pregnancy to lower HBV DNA levels and thereby reducing the risk of vertical transmission.[5] Lamivudine was stopped immediately after delivery in all those pregnancies. In this group, more patients tended to be HBeAg-positive and significantly fewer patients were in the immune-control phase of infection (Table 1).

	All pregnancies (n=38)	Without lamivudine (n=25)	With lamivudine (n=13)	*p*
Median age (years, range)	25.6 (18.2-40.5)	26.0 (18.2-40.5)	24.3 (18.9-35.4)	*0.21*
Phase of infection				*0.02*
- immunotolerant	17 (45%)	7 (28%)	10 (77%)	
- immuno-active	4 (11%)	3 (12%)	1 (8%)	
- immune-control	9 (23%)	9 (36%)	2 (15%)	
- immune-escape	8 (21%)	6 (24%)	0 (0%)	
HBeAg positive	24 (63%)	13 (52%)	11 (85%)	*0.08*
Median HBV DNA level (log_{10} copies/ml)	7.75 (3.00-9.90)	5.19 (3.00-9.29)	8.82 (7.32-9.90)	*0.07*
Median ALT x ULN (range)	0.83 (0.53-3.97)	0.77 (0.53-3.97)	0.90 (0.53-1.63)	*0.86*
Previous LAM treatment	11 (29%)	7 (28%)	4 (31%)	*1.00*
Previous IFN treatment	5 (13%)	4 (16%)	1 (8%)	*0.64*

ALT = alanine aminotransferase; IFN = interferon; LAM = lamivudine; ULN = upper limit of normal.

Table 1: Baseline characteristics in all pregnancies (n=38) and in the subgroups with (n=25) and without (n=13) lamivudine therapy in the last trimester of pregnancy.

Influence of pregnancy on HBV DNA

There was an overall increase in median HBV DNA level from 7.8 log_{10} copies/ml before to 8.2 log_{10} copies/ml during pregnancy (p=0.06), despite lamivudine therapy in 13 patients (Figure 1). After pregnancy, there was a decline towards 6.8 log_{10} copies/ml (p=0.01).

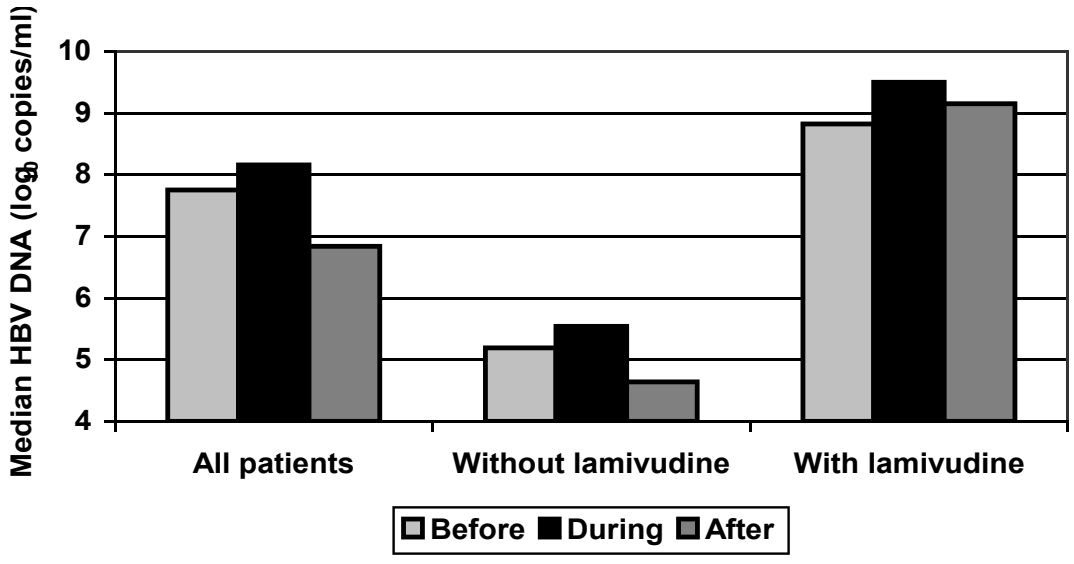


Figure 1: Median HBV DNA levels before, during and after pregnancy for all pregnancies (n=38), for those without lamivudine (n=25) and for those with lamivudine (n=13) given only in the last trimester of pregnancy.

Among patients without lamivudine treatment (n=25), median HBV DNA level was 5.2 $\log_{10}$ copies/ml before pregnancy and increased during pregnancy to 5.5 $\log_{10}$ copies/ml (p=0.48) (Figure 1). After pregnancy there was a decline to 4.6 $\log_{10}$ copies/ml (during vs. after pregnancy; p=0.05). In 5 pregnancies, HBV DNA was below the lower limit of detection of the real-time PCR assay.

Among the 13 out of 38 patients (34%) that were treated with lamivudine in the last trimester of pregnancy, median HBV DNA level was 8.8 $\log_{10}$ copies/ml prior to pregnancy (Table 1). Because lamivudine treatment was only given in patients with a high viral load, median HBV DNA levels during pregnancy were higher in patients treated with lamivudine compared to those not treated with lamivudine in the last trimester of pregnancy (9.5 vs. 5.5 $\log_{10}$ copies/ml, respectively; p<0.001). After pregnancy, the median HBV DNA level was 9.2 $\log_{10}$ copies/ml in this patient group treated with lamivudine.

Influence of pregnancy on ALT

Before pregnancy, median ALT was 0.8 x ULN (range, 0.5-4.0) and decreased during pregnancy to 0.5 x ULN (range, 0.3-3.3) (p=0.001). After pregnancy there was an increase to 1.6 x ULN (range, 0.4-13.2) (p=0.001) (Figure 2). A significant increase in liver disease activity was observed in 17 out of 38 pregnancies (45%) at a median of 56 days (range, 5-129) after delivery. The maximum ALT level of these exacerbations was 4.0 x ULN (median; range, 1.8-13.2). In those without an exacerbation, ALT returned to 0.8 x ULN (range, 0.4-2.1). None of the increases in liver disease activity led to an increase in bilirubin, development of ascites or variceal bleeding.

In the subgroup of patients not treated with lamivudine, a significant increase in liver disease activity occurred in 9 out of 25 pregnancies (36%). The maximum ALT level of these exacerbations was 3.3 x ULN (median; range, 2.1-13.2) (Figure 2). In those without an exacerbation, ALT returned to 0.8 x ULN (range, 0.4-2.1).

In the 13 patients treated with lamivudine, median ALT before pregnancy was 0.9 x ULN (range, 0.5-1.6). Eight out of 13 (62%) exhibited a significant increase in liver disease activity. The maximum ALT level of the exacerbation was 4.3 x ULN (median; range, 1.8-8.7). In those without an exacerbation, ALT returned to 1.0 x ULN (range, 0.5-1.9).

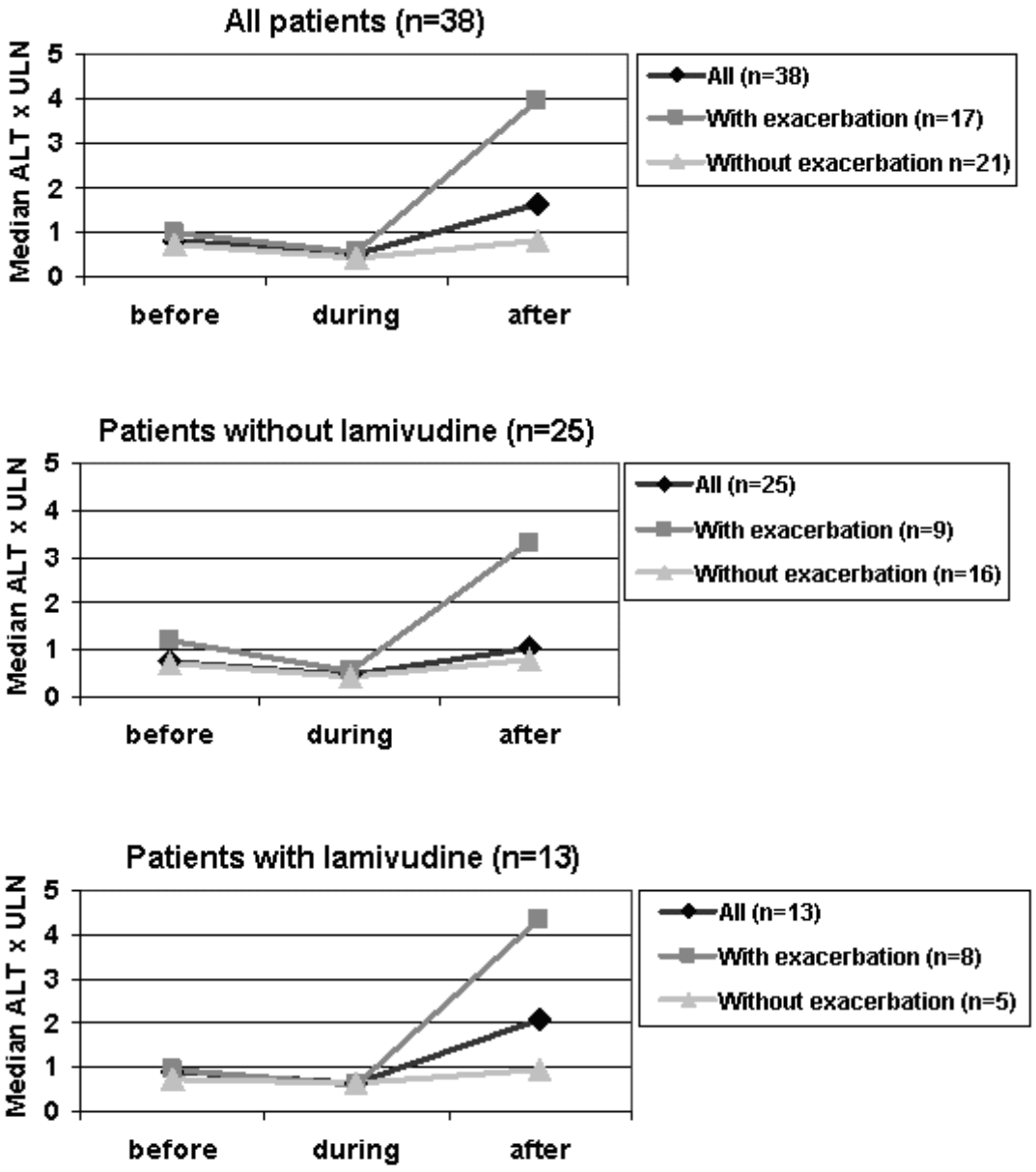


Figure 2: Median ALT levels before, during and after pregnancy for all pregnancies, for those without and for those with lamivudine treatment in the last trimester of pregnancy.

Prediction of exacerbations

Median viral load before and during pregnancy were 7.8 and 8.2 $\log_{10}$ copies/ml in patients with an significant increase in liver disease activity compared to 6.6 and 8.3 $\log_{10}$

copies/ml in those without (p=0.64 and 0.46, respectively) (Table 2). After pregnancy, median viral load was 7.7 $\log_{10}$ copies/ml in patients with an exacerbation and 5.5 $\log_{10}$ copies/ml in those without (p=0.13). Median baseline ALT tended to be higher in patients with (0.7 x ULN) compared to patients without an exacerbation (1.0 x ULN) (p=0.11). A significant increase in liver disease activity occurred in 11 out of 24 (46%) HBeAg positive and in 6 out of 14 (43%) HBeAg negative patients; therefore, HBeAg status did not influence the occurrence of these exacerbations (p=0.86).

	Without exacerbation (n=21)	**With exacerbation (n=17)**	***p***
Median age (years, range)	25.9 (18.9-40.5)	24.7 (18.2-37.6)	*0.40*
Phase of infection			*0.27*
- immunotolerant	8 (38%)	9 (53%)	
- immuno-active	3 (14%)	1 (6%)	
- immune-control	7 (34%)	2 (12%)	
- immune-escape	3 (14%)	5 (29%)	
HBeAg positive	13 (62%)	11 (65%)	*0.86*
Median HBV DNA level ($\log_{10}$ copies/ml)	6.62 (3.00-9.90)	7.75 (3.00-9.63)	*0.64*
Median ALT x ULN (range)	0.73 (0.53-1.63)	0.98 (0.67-3.97)	*0.11*
Previous LAM treatment	6 (29%)	5 (29%)	*0.96*
Previous IFN treatment	2 (10%)	3 (18%)	*0.64*

ALT = alanine aminotransferase; IFN = interferon; LAM = lamivudine; ULN = upper limit of normal.

Table 2: Baseline characteristics in all pregnancies with (n=17) and without (n=21) a post-pregnancy increase in liver disease activity.

Discussion

In this cohort of 38 pregnancies in women with a chronic hepatitis B infection, a significant increase in liver disease activity was observed in 45% of cases after delivery. In those not treated with lamivudine, an exacerbation was observed in 36% versus 62% in patients treated with lamivudine in the last trimester to reduce vertical transmission.

During pregnancy, there are numerous alterations in the maternal immune system to prevent rejection of the fetus. Several factors are produced by both the fetal trophoblast and the maternal uterine tissue like CD95L (Fas ligand), indoleamine 2,3-dioxygenase (IDO), the Crry protein and leukemia inhibitory factor (LIF).[11-14] There is also a shift in the T_H1 – T_H2 balance towards a T_H2 response with increased amounts of regulatory T

cells.[13,15] Regulatory T cells also seem to play a role in chronic hepatitis B infection, contributing to an inadequate immune response against the virus.[16] A further increase in the amount of regulatory T cells could explain the tolerance against the hepatitis B virus during pregnancy with the observed rise in viral load and decline in ALT levels. All these changes in the immune status recover after delivery and the immune system fully restores its function. We hypothesize that this reactivation of the immune system is responsible for the high amount of patients with a post-pregnancy increase in ALT. Additional research is however necessary to confirm this hypothesis.

Many studies have been performed in pregnant women infected with HBV concerning the prevention of vertical transmission, both with lamivudine treatment as well as with hepatitis B immunoglobulin's during the last trimester of pregnancy.[5, 17-19] In China, many studies have been performed on hepatitis B immunoglobulin's and this treatment is becoming a standard regimen in this country. The results on the effectiveness of immunoglobulin's are conflicting and so far this treatment has not been adopted by other countries.[17,18] Postnatal immunization hepatitis B immunization of the child is an effective treatment to prevent vertical transmission of the hepatitis B virus (http://www.cdc.gov/nip/ACIP/). Maternal chronic HBV infection also seems to influence pregnancy outcome with more maternal morbidity in HBsAg positive women compared to HBsAg negative controls.[20]

Nevertheless, little information is available on the outcome of the hepatitis B infection of the new mother. The percentage of patients with a significant increase in liver disease activity in our cohort of patients (45%) in the first 6 months after pregnancy is high compared to the expected yearly risk of a hepatic flare of 27% in HBeAg-positive and 10% in HBeAg-negative patients.[21] The percentage of exacerbations after pregnancy was even higher in patients treated with lamivudine during the last trimester of pregnancy. These patients are more likely to have an exacerbation because it is a selection of patients with a high viral load during pregnancy. Furthermore these patients may exhibit an exacerbation after withdrawal of lamivudine treatment. These withdrawal flares usually occur in approximately 17-25% of patients after lamivudine discontinuation.[8,22] A previous study on the effects of chronic hepatitis B virus infection in pregnant women on HBV DNA levels showed a significant increase in ALT 6 weeks after pregnancy, however, the increase in ALT in this study was recorded at two fixed time points 6 weeks and 1 year post partum.[23]

It was not possible to predict the occurrence of these exacerbations using viral load or

HBeAg-status. Only baseline ALT levels tended to be higher in patients exhibiting an exacerbation, this difference was however small and cannot be used in the clinic to predict flares. HBV DNA during pregnancy did also not differ between patients with or without an increase in liver disease activity. We therefore recommend to monitor chronic hepatitis B patients after delivery for a significant increase in liver disease activity. If lamivudine is given in the last trimester of pregnancy, it can be considered to continue therapy after delivery. However, none of the increases in liver disease activity led to hepatic decompensation and the patient population is relatively young. Therapy with PEG-interferon or nucleos(t)ide analogues conferring less resistance than lamivudine should therefore be considered as a first line therapy outside the setting of pregnancy.

There are some limitations of the present study. First, the design was retrospective; we searched our electronic patient files for all patients with both a pregnancy and hepatitis B infection thereby inducing a possible bias. Second, there is no general accepted definition of a significant increase in liver disease activity, we therefore used a 3 times increase in ALT levels, which was previously used as a definition of a hepatic flare.[8,9] Finally, the amount of samples that was available during and after pregnancy was limited. In future research, frequent testing of both transaminases and viral load is recommended in particular during the period after birth.

In conclusion, a significant increase in liver disease activity is often observed after pregnancy, even in the absence of lamivudine treatment. This may be due to a reactivation of the immune system after delivery. Based on our data we recommend to monitor closely for these exacerbations and if necessary treat women with chronic HBV in the months after delivery.

References

1. Kane M. Global programme for control of hepatitis B infection. Vaccine. 1995;13 Suppl 1:S47-9.
2. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997 Dec 11;337(24):1733-45.
3. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol. 1995;13:29-60.
4. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat Immunol. 2006 Mar;7(3):241-6.
5. van Zonneveld M, van Nunen AB, Niesters HG, de Man RA, Schalm SW, Janssen HL. Lamivudine treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. J Viral Hepat. 2003 Jul;10(4):294-7.
6. 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(8):2897-901.
7. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol. 1999 Jan;37(1):68-73.
8. 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(3):635-9.
9. Flink HJ, Sprengers D, Hansen BE, van Zonneveld M, de Man RA, Schalm SW, 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 Nov; 54(11):1604-9.
10. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, et al. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol. 2003;39 Suppl 1:S3-25.
11. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998 Aug 21;281(5380):1191-3.
12. Hunt JS, Vassmer D, Ferguson TA, Miller L. Fas ligand is positioned in mouse

uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. J Immunol. 1997 May 1;158(9):4122-8.

13. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today. 1993 Jul;14(7):353-6.
14. Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator crry in fetomaternal tolerance. Science. 2000 Jan 21;287(5452):498-501.
15. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004 Mar;5(3):266-71.
16. Stoop JN, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG, et al. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology. 2005 Apr;41(4):771-8.
17. Yuan J, Lin J, Xu A, Li H, Hu B, Chen J, et al. Antepartum immunoprophylaxis of three doses of hepatitis B immunoglobulin is not effective: a single-centre randomized study. J Viral Hepat. 2006 Sep;13(9):597-604.
18. Xu Q, Xiao L, Lu XB, Zhang YX, Cai X. A randomized controlled clinical trial: interruption of intrauterine transmission of hepatitis B virus infection with HBIG. World J Gastroenterol. 2006 Jun 7;12(21):3434-7.
19. Su GG, Pan KH, Zhao NF, Fang SH, Yang DH, Zhou Y. Efficacy and safety of lamivudine treatment for chronic hepatitis B in pregnancy. World J Gastroenterol. 2004 Mar 15;10(6):910-2.
20. Tse KY, Ho LF, Lao T. The impact of maternal HBsAg carrier status on pregnancy outcomes: a case-control study. J Hepatol. 2005 Nov;43(5):771-5.
21. Liaw YF, Tai DI, Chu CM, Pao CC, Chen TJ. Acute exacerbation in chronic type B hepatitis: comparison between HBeAg and antibody-positive patients. Hepatology. 1987 Jan-Feb;7(1):20-3.
22. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999;341(17):1256-63.
23. Soderstrom A, Norkrans G, Lindh M. Hepatitis B virus DNA during pregnancy and post partum: aspects on vertical transmission. Scandinavian journal of infectious

diseases. 2003;35(11-12):814-9.

CHAPTER 8

The effects of α-galactosylceramide on chronic hepatitis B infection in a randomized placebo controlled phase I/II trial

Martijn J. ter Borg[1*], Andrea M. Woltman[1*], Rekha S. Binda, Dave Sprengers[1], B. Mary E. von Blomberg[2], Rik J. Scheper[2], Kunihiko Hayashi[3], Nobusuke Nishi[3], André Boonstra, Renate van der Molen[1], Harry L.A. Janssen[1].

1. Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands.
2. Department of Pathology,Vrije Universiteit Medical Center, Amsterdam, The Netherlands.
3. Pharmaceutical division, Kirin Brewery Co. Ltd., Tokyo, Japan
*Equal contribution

Submitted

Summary

The glycosphingolipid alpha-galactosylceramide (a-GalCer) has been shown to stimulate invariant natural killer T (NKT) cells and is able to induce powerful antiviral immune responses. The aim of the present dose-escalating randomized placebo-controlled phase I/II trial was to investigate the antiviral activity and safety of a-GalCer as a novel class of treatment for chronic hepatitis B patients.

Twenty-seven patients (HBeAg positive or negative, HBV DNA $>10^5$ copies/ml) were randomly assigned to a dose of 0.1 mg/kg (n=8), 1 mg/kg (n=6) or 10 mg/kg (n=6) a-GalCer or placebo (n=7).

Almost all patients responded to a-GalCer by a rapid and strong decline in NKT cell numbers. Especially patients with relatively high circulating NKT cell levels showed signs of immune activation, including enhanced levels of circulating activated NK cells, elevated serum TNF-a and IL-6 levels and development of fever. However, this immune activation did not result in pronounced antiviral activity.

Three patients demonstrated a pronounced but transient decline in HBV DNA in the first week of treatment. Only one patient treated with 1 mg/kg a-GalCer had a sustained drop in HBV DNA at the end of follow-up. No clear effect on ALT was observed upon a-GalCer treatment. Four patients discontinued therapy because of an episode of fever shortly after drug administration. Otherwise no significant side effects were observed.

a-GalCer used as monotherapy for chronic hepatitis B infection at the doses (0.1-10 mg/kg) used in this trial resulted in a strong decline of NKT cells but had no clear effect on HBV DNA and ALT levels. a-GalCer was poorly tolerated, and is unlikely to provide an alternative as monotherapy to the current treatment of pegylated interferon-a and nucleos(t)ide analogs.

Introduction

Chronic hepatitis B remains a major health problem. Worldwide 2 billion people show evidence of infection with hepatitis B virus (HBV) and it chronically affects around 400 million people.[1] Approximately 15-40% of these will develop serious complications such as liver cirrhosis and hepatocellular carcinoma.[2] Pegylated interferons have shown to induce a sustained response in about 35-45% of patients.[3-5] Because of the importance of immune control over the hepatitis B virus, immunomodulatory drugs are of special interest in the treatment of chronic hepatitis B infection.

The glycosphingolipid a-galactosyleramide (a-GalCer) has been shown to induce potent antiviral as well as antitumor immune responses.[6-8] a-GalCer, originally derived from marine sponge, activates invariant natural killer T (iNKT) cells, which recognize a-GalCer in the context of the MHC-like molecule CD1d.[9] iNKT cells constitute a distinct lymphocyte subpopulation characterized by expression of both NK receptors and a restricted T cell receptor repertoire, which in humans consists of a Va24 chain preferentially paired to Vb11.[10] Upon activation these cells rapidly secrete large amounts of both Th-1 and Th-2 type cytokines, which subsequently enhance innate as well as adaptive immune cells.[11-13] Besides their pivotal role in anti-tumor and anti-viral immune responses, NKT cells have also been implicated in several other antimicrobial immune responses, as well as in autoimmunity and allergy.

Approximately 20-30% of intrahepatic lymphocytes consist of NKT cells.[14] Interestingly, NKT cells activated by a-GalCer have been shown to inhibit hepatitis B virus replication in HBV transgenic mice.[15] This antiviral effect of a-GalCer is associated with a rapid induction of IFN-γ and IFN-α/β in the liver even before a significant number of inflammatory cells are recruited to the liver, suggesting that intrahepatic NK and NKT cells are involved in this process.[15,16] In addition, it has been suggested that a-GalCer also exerts direct anti-viral activity against HBV.[17]

KRN7000 is a synthetic a-GalCer that has been most frequently used in experimental mouse studies, but also in some human oncology trials and in hepatitis C.[18-22] Also in the human setting, a-GalCer administration has been shown to induce immune activation in individuals, which depended on pretreatment circulating iNKT cell numbers.[19] The aim of the present study was to investigate the safety, tolerability and the antiviral effect of a-

GalCer for the treatment of patients with chronic hepatitis B infection.

Methods

Patients

Male and female patients aged 18 to 70 years with either HBeAg-positive or -negative chronic hepatitis B infection were enrolled. All patients had an HBV DNA level above 10^5 copies/ml at screening and two alanine transaminase (ALT) values of >1.2 times the upper limit of normal (xULN) within 8 weeks before initiation of treatment. The ULN for ALT was 40 U/L for males and 30 U/L for females. A liver biopsy obtained within 3 years prior to screening, consistent with chronic hepatitis B infection and without cirrhosis, was required.
Exclusion criteria were the evidence of decompensated liver disease, as indicated by bilirubin >20 mmol/L, serum albumin <35 g/L, prothrombin time prolonged by >3 seconds, history of bleeding esophageal varices, ascites or hepatic encephalopathy; ALT level >10 xULN; pregnancy or the inability to practice adequate contraception; clinically significant or major illnesses; history of autoimmune disease; systemic interferon-a treatment, systemic antiviral agents or another investigational drug within 3 months prior to enrollment in the study; immune suppressive treatment; pre-existing severe cytopenia (i.e. hemoglobin <7 mmol/L, white blood cell count $<3.0*10^9$/L, lymphocytes $<0.5*10^9$/L or platelets $<100*10^9$/L); evidence of hepatocellular carcinoma as indicated by alpha fetoprotein >50 ng/ml and/or ultrasound demonstrating a mass suggestive of liver cancer; other acquired or inherited causes of liver disease.
The study was approved by the ethics committees at our hospital according to the Declaration of Helsinki, and all patients gave written informed consent before enrollment.

Study design

This phase I/II dose-escalation trial was performed in a randomized, double-blind, placebo-controlled manner. Patients with chronic hepatitis B who met the inclusion criteria were assigned to receive three dosages of a-GalCer (KRN7000 ((2*S*,3*S*,4*R*)-1-*O*-(a-D-galactopyranosyl)-*N*-hexacosanoyl-2-amino-1,3,4-octadecanetriol), Kirin Pharma Co., Ltd., Gunma, Japan) or placebo intravenously, with intervals of 4 weeks. Patients were enrolled into 3 dose escalating groups with 11 patients in the first group (8 verum; 3 placebo) and 8 patients in the second and third group (both 6 verum; 2 placebo) (Figure 1). After

enrollment, patients were randomized to receive either a-GalCer or placebo. The dosage of a-GalCer was 0.1 mg/kg body weight in the first, 1 mg/kg in the second and 10 mg/kg in the third group. After completion of 8 weeks of treatment, with injections at 0, 4 and 8 weeks, patients were monitored without further therapy for an additional 16 weeks.
Dose escalation to the next cohort was decided after evaluation by a safety review board of all the safety data collected on all the patients who had completed 3 weeks after the first injection in the preceding dose cohort. The safety review board consisted of three experienced hepatologists who were not involved in the study.

Study objectives

The primary objective of the study was to evaluate the safety and tolerability of the 3 ascending doses of a-GalCer. The secondary objective was to evaluate the effectiveness of a-GalCer, immunological responses, reduction of HBV DNA and ALT normalization.

Biochemical and virological assessments

The extent of liver inflammation was determined by measuring serum alanine aminotransferase (ALT) levels. HBV DNA levels were measured using an in-house developed TaqMan real-time PCR assay (dynamic range 400-10^{10} copies/ml).[23] The Eurohep HBV DNA standard was used for validation of HBV DNA levels.[24] Hepatitis B 'e' antigen (HBeAg), hepatitis B 's' antigen (HBsAg), antibodies to 'e' antigen (anti-HBe) and antibodies to 's' antigen (anti-HBs) were measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, Il).

Immunology testing

Lymphocyte numbers were determined by adding fixed volumes of FlowCount™ fluorospheres (Beckman-Coulter, Miami, USA) to the leukocytes after erythrocyte lysis (BD Biosciences, San Jose, USA) just before flow cytometric evaluation. FACS analysis was performed using monoclonal antibodies against CD3 (SK7), CD4 (SK3), CD8a (SK1), CD45 (2D1), CD69 (L78), and the isotype controls mouse IgG1 (X40) and IgG2a (X39)(all purchased from BD Biosciences), Vb11 (C21) and Va24 (C15), CD8b (2ST8.5H7)(all from Immunotech, Marseille, France), and CD56 (MOC-1; IQ products, Groningen, The Netherlands) before and 2 and 7 days after each injection as well as at the end of

treatment (EOT; day 84) and at the end of follow up (EFU; day 168). For staining with a-GalCer or vehicle loaded CD1d-tetramers (Gemini Science Inc., San Diego, CA), 150 ml of whole blood was incubated with the tetramers for 10 min at 37°C, followed by staining for CD3 and Va24 and erythrocyte lysis. Whole blood analysis of myeloid and plasmacytoid dendritic cell (DC) numbers was performed, as described previously,[25] before and 7 days after each injection as well as at EOT and EFU. Flow cytometric analysis was performed on a FACS Calibur using CELL Quest software (BD Biosciences).

Serum levels of IFNg, TNFa, IL-6, IL-1b, IL-10, IL-5 and GM-CSF were measured by flow cytometry using the CBA Human Soluble Protein Flex Set system (BD Biosciences) and ELISA (IFNg and TNFa, R&D Systems, Abingdon, UK) before and 4 hours and 2 days after injection. IL-12 production by polyI:C and IFNg stimulated myeloid DC was examined as described before.[25]

Statistical analyses

Because of the explorative character of the study, power analysis was not considered. Patients were randomized by the Clinical Research Bureau of the Erasmus MC University Medical Center Rotterdam using a computer generated randomization list. All analyses were performed on an intention-to-treat basis. For analysis purposes, the patients treated with placebo therapy from the different dose levels will be considered as one treatment group. Paired and unpaired Student T tests, Wilcoxon matched pairs test, repeated measures ANOVA and Pearson's correlation coefficient were used where appropriate. P-values of <0.05 were considered statistically significant.

Results

Patients

A total of 30 patients were screened between August 2003 and January 2006 at the Erasmus MC University Medical Center Rotterdam. Twenty-seven patients met the criteria for enrollment into the study. In total, 8 patients were allocated to a dose of 0.1 mg/kg body weight (dose level 1), 6 were allocated to 1 mg/kg body weight (dose level 2), 6 were allocated to 10 mg/kg body weight (dose level 3) and 7 to placebo (Fig. 1). One patient in the highest dosage group withdrew his informed consent before study medication was administered.

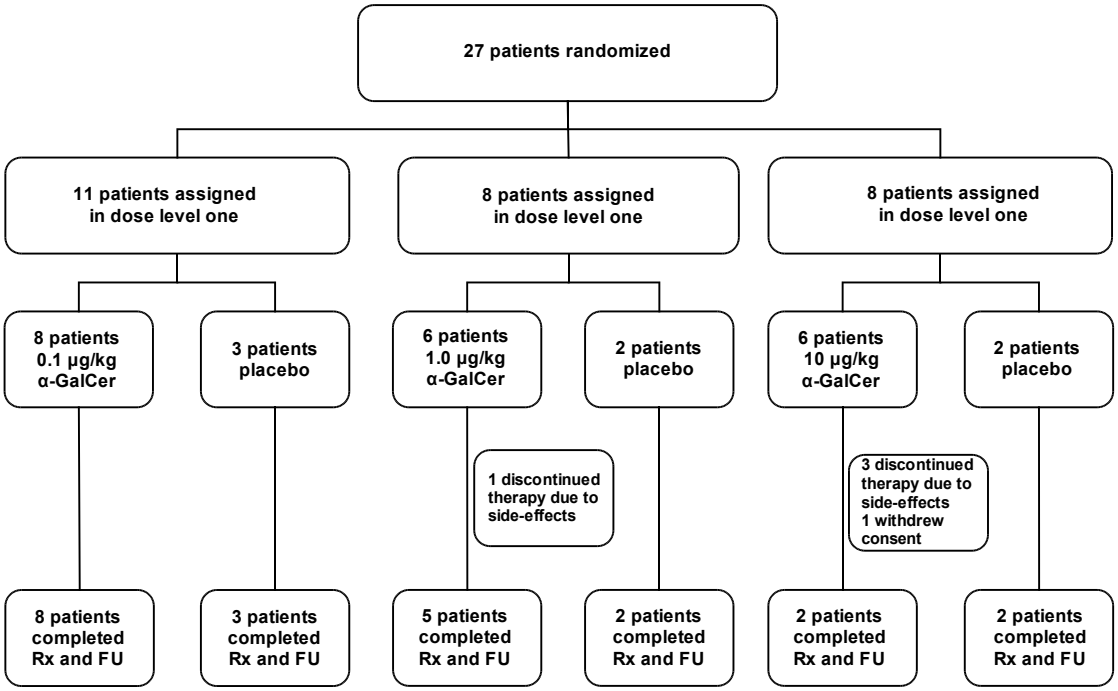


Figure 1: Trial profile. *Rx = treatment; FU = follow-up*

The median age at inclusion was 35 years (range, 21-58). At baseline the median ALT level was 103.5 IU/L (range 35-356) and the median HBV DNA level 8.1 $\log_{10}$ copies/mL (= 7.4 $\log_{10}$ IU/mL) (range 5.0-9.5 $\log_{10}$ copies/mL). Further demographics and baseline characteristics for the different dose levels are given in table 1. There were no significant differences between the patient characteristics of different treatment groups prior to therapy.

	0.1 μg/kg (n=8)	1 μg/kg (n=6)	10 μg/kg (n=6)	Placebo (n=7)
Male gender (%)	6/8 (75%)	3/6 (50%)	3/6 (50%)	6/7 (86%)
Age, years*	36.0 (24.3-57.6)	44.8 (23.8-50.9)	25.8 (20.7-49.7)	27.2 (23.8-44.0)
Weight, kg*	77 (74-111)	77 (42-87)	61 (43-85)	71 (61-81)
HBeAg positive (%)	4/8 (50%)	3/6 (50%)	6/6 (100%)	6/7 (86%)
Genotype				
A	5	2	2	2
B	0	1	1	2
C	0	1	1	0
D	3	1	1	3
E	0	1	1	0
Baseline ALT (IU/L)*	104.5 (84-356)	103 (67-262)	108 (59-212)	99 (35-229)
Baseline HBV DNA (log_{10} copies/mL)*	8.5 (5.4-9.5)	6.3 (5.0-8.9)	8.0 (7.0-8.3)	8.4 (6.4-9.4)

Table 1: Baseline characteristics.

NKT cell numbers decline after a-GalCer administration

At baseline, the number of circulating NKT cells, defined as $CD3^+V\alpha24^+V\beta11^+$ cells, did not significantly differ between the different groups (Fig. 2). The first administration of a-GalCer induced a rapid decrease in circulating NKT cells in all dose levels (day 0 vs day 2: dose level 1, p=0.0156); level 2, p=0.0313; level 3: p=0.1250), which was followed by a recovery of NKT cell numbers (Fig. 2BC). Although less pronounced, this decline in NKT cell numbers was also observed after the second and third administration of a-GalCer (Fig. 2B).

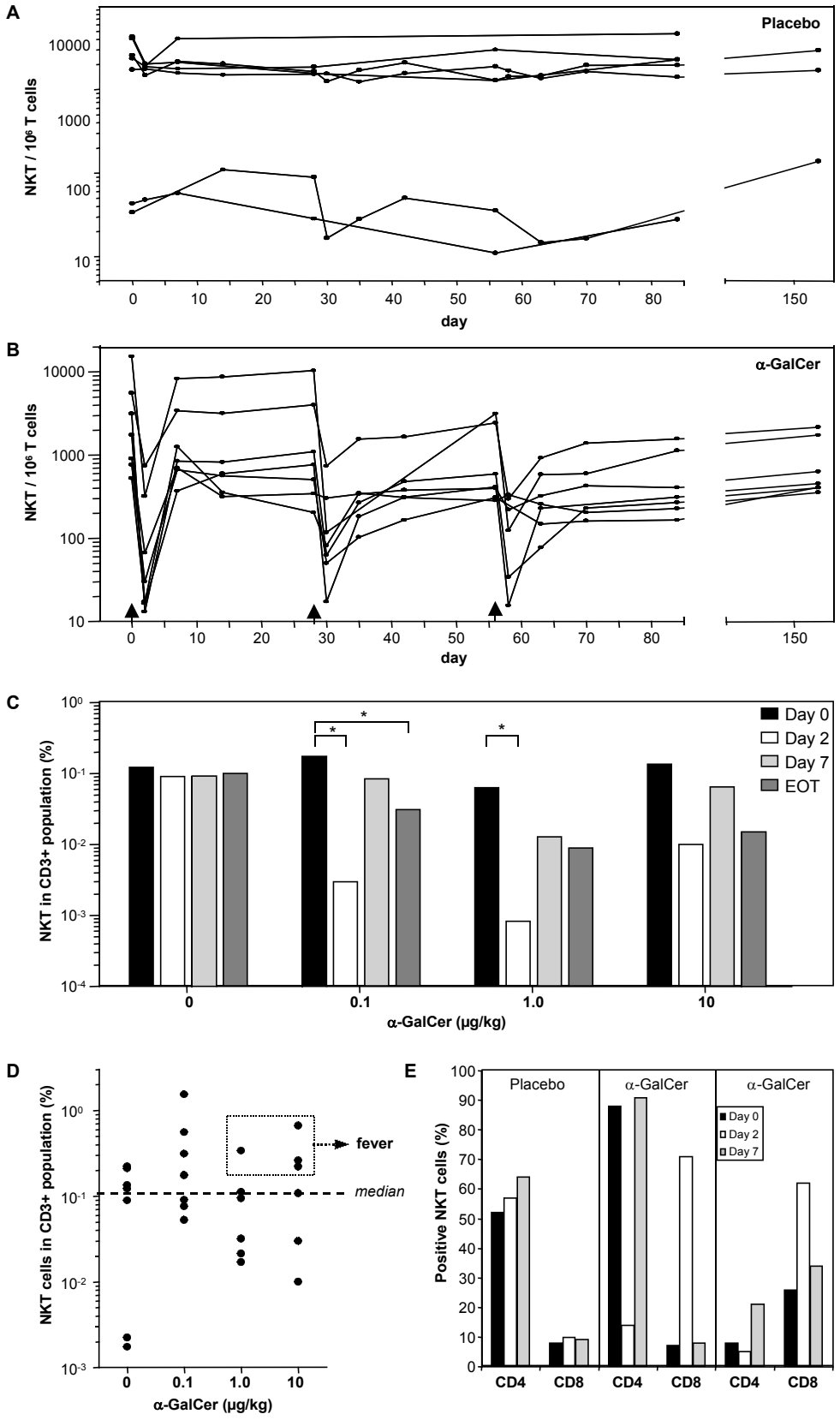

A
Placebo
NKT / 10⁶ T cells
10000
1000
100
10
0
10
20
30
40
50
60
70
80
150
day
B
α-GalCer
NKT / 10⁶ T cells
10000
1000
100
10
0
10
20
30
40
50
60
70
80
150
day
C
NKT in CD3+ population (%)
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
Day 0
Day 2
Day 7
EOT
*
*
*
0
0.1
1.0
10
α-GalCer (µg/kg)
D
NKT cells in CD3+ population (%)
10⁰
10⁻¹
10⁻²
10⁻³
fever
median
0
0.1
1.0
10
α-GalCer (µg/kg)
E
Positive NKT cells (%)
100
90
80
70
60
50
40
30
20
10
0
Placebo
α-GalCer
α-GalCer
Day 0
Day 2
Day 7
CD4
CD8
CD4
CD8
CD4
CD8

Figure 2: NKT cell numbers decline upon a-GalCer treatment.

A/B. *Peripheral blood $CD3^+Va24^+Vb11^+$ NKT cell numbers within $CD3^+$ T cell population in placebo (A) and a-GalCer-treated (B) patients. a-GalCer (0.1 µg/kg) was administered at day 0, 28 and 56 (arrows).*

C. *Median NKT cell numbers within CD3+ T cell population in placebo and a-GalCer-treated patients at day 0, 2, 7 and at the end of treatment (EOT). *p<0.05, Wilcoxon matched pairs test.*

D. *Pre-treatment NKT cell numbers within CD3+ T cell population in the different treatment groups. Dashed line represents the median of pre-treatment NKT cell number calculated from the patients included.*

E. *NKT cell subset analysis was performed at day 0, 2 and 7. Three representative patients with high NKT cell levels at baseline are shown.*

Furthermore, albeit not significantly different, the number of NKT cells was still decreased at the end of treatment (day 84) and approached baseline levels at the end of follow up (day 168). The NKT cell numbers in patients receiving placebo did not significantly differ during the study period (Fig. 2AC). Similar findings were observed with a-GalCer CD1d-tetramer staining, which was evaluated in 201 blood samples (data not shown).
Of note, all patients exhibiting high baseline NKT cell levels that received ³ 1 µg/kg a-GalCer developed fever and severe rigors 1 hour to 2 days after drug administration (see safety, Fig. 2D). NKT cell subset analysis in these patients revealed that after the first administration of a-GalCer the proportion of $CD4^+$ NKT cells decreased and the proportion of $CD8^+$ NKT cells increased, whereas in placebo treated patients with high baseline NKT cell levels the proportion of NKT cell subsets did not differ.

Analysis of circulating NK cells, T cells and DC

To evaluate a-GalCer induced indirect immune activation, peripheral blood NK cells, T cells and DC were analyzed. The analysis of circulating NK cells revealed that a-GalCer treatment significantly changed the number of NK cells 2 days post-injection (Fig. 3).

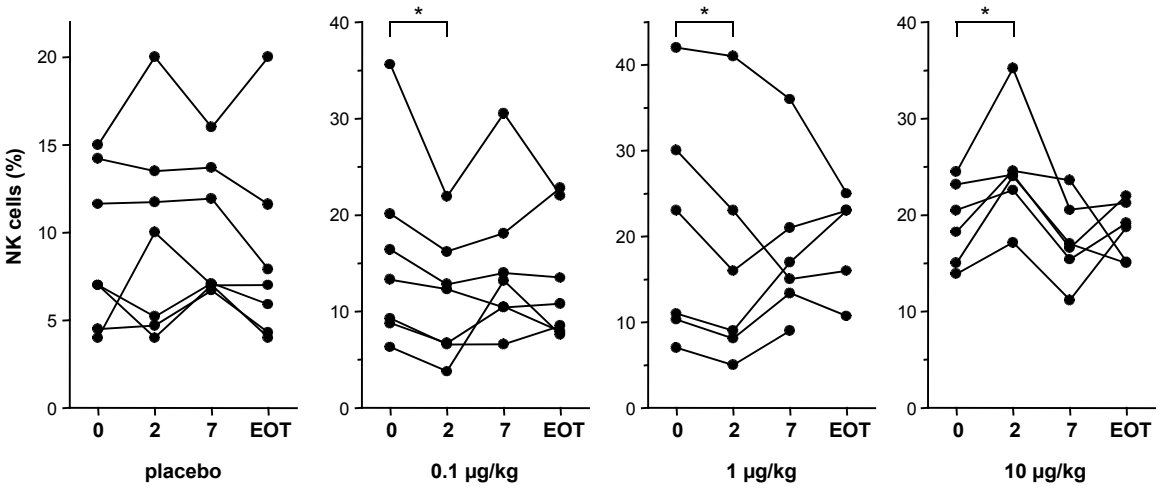


Figure 3: NK cell numbers change upon a-GalCer treatment. *Peripheral blood CD3$^-$ CD56$^+$ NK cell numbers present in the lymphocyte population in placebo and a-GalCer-treated patients at day 0, 2 and 7. *p<0.05, Wilcoxon matched pairs test.*

Surprisingly, in patients receiving 0.1 or 1.0 µg/kg a-GalCer, NK cell numbers significantly decreased (p=0.02 and p=0.03), whereas the highest dosage induced an increase in NK cells (p=0.03). Activated NK cells, as defined by CD69$^+$ cells, were observed in all treated patient groups, but the most pronounced increase in CD69 expressing NK cells was observed in patients with high NKT cell numbers at baseline (%CD69$^+$ NK cells: t=0, median 0.6, range 0.1-4.1, versus t=2, median 2.9, range 0.0-11.5; p=0.05). Significant differences in circulating T cells and DC were not observed. Of note, the only patient with a sustained drop in HBV DNA levels during treatment demonstrated an increased number of circulating myeloid DC at the end of treatment (t=0: 0.32%, t=84: 0.74%). Moreover, these myeloid DC displayed an increased capacity to produce IL-12 (t=0: 60 pg/ml, t=84: 1478 pg/ml), which is in line with our previous findings demonstrating that viral load reduction increases the number of circulating myeloid DC as well as their function.[25]

Serum cytokine levels

Serum cytokine levels were determined at baseline, 4 hr and 2 days after each a-GalCer administration. Cytokine levels remained undetectable in the patient group with low NKT cell numbers. However, in 5 of 9 patients with high NKT cell levels, a transient increase in

TNFa was observed. The patient exhibiting the highest TNFa level (35 pg/ml) experienced severe fever shortly after a-GalCer administration (see Safety). In addition, the patients exhibiting a period of fever shortly after a-GalCer administration demonstrated an increase in IL-6 from 2 ± 3 pg/mL at baseline to 719 ± 906 pg/mL 4 hours after drug administration that returned to baseline levels at day 2. No detectable levels of IFNg, IL-1b, IL-10, IL-5 and GM-CSF were observed in serum of those patients.

Virological and biochemical response

No significant decreases in HBV DNA following the first administration of a-GalCer were observed in any of the three dosages groups (Fig. 4). Four patients did show a more than 0.5 $\log_{10}$ copies/mL drop in HBV DNA levels following the first administration (1 mg/kg n=2, 10 mg/kg n=2) with a median decline in the first week of 1.09 $\log_{10}$ copies/mL (range 0.54 – 2.96; n=4) from baseline.

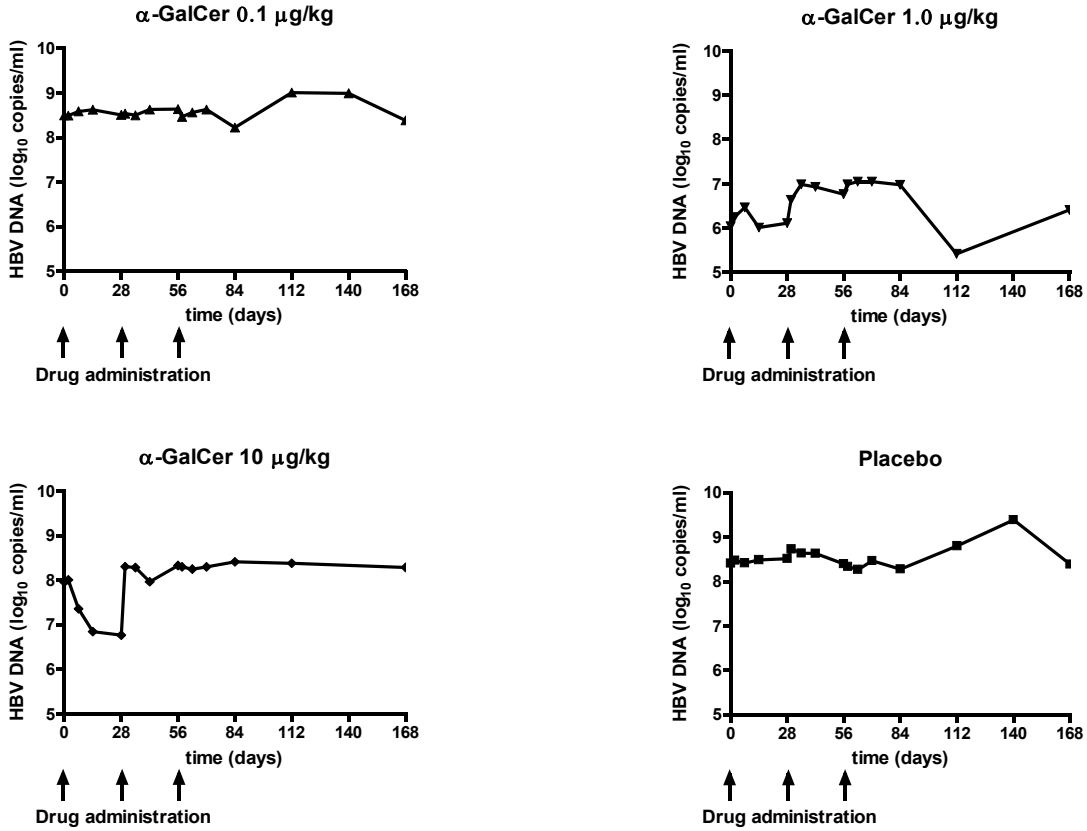


Figure 4: Median HBV DNA ($\log_{10}$ copies/ml) in the three different treatment arms and the placebo group.

For the whole group during follow-up of 168 days, also no statistically significant changes in HBV DNA level were observed among the different dose levels (Fig. 4). One patient in dose level 2, who displayed a low circulating NKT cell number and experienced a viral load drop of 0.54 $\log_{10}$ copies/mL one week after the first injection, had a sustained drop in HBV DNA level of 4.02 $\log_{10}$ copies/mL at the end of follow-up. The other three patients with a drop in HBV DNA level after the first administration, of whom 1 patient displayed low and 2 patients displayed high baseline NKT cell numbers, had no decline after the second and third injection of a-GalCer and HBV DNA levels returned to baseline. HBeAg-seroconversion was not observed in any of the a-GalCer treated patients. One HBeAg-positive patient in the placebo group had an HBeAg-seroconversion and a drop in HBV DNA of 4.48 $\log_{10}$ copies/mL at the end of follow-up. There were also no clear and significant differences in the ALT values over time in the 3 different dose levels of a-GalCer treated patients compared to placebo (Fig. 5).

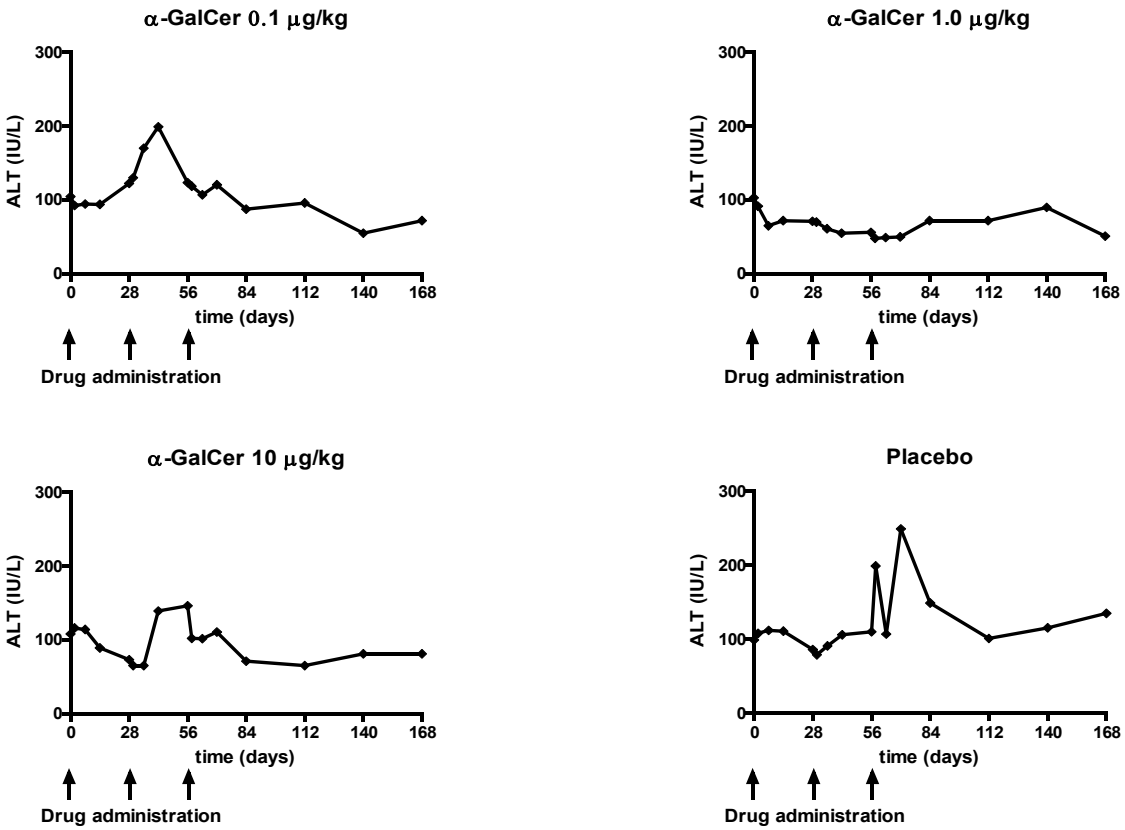


Figure 5: Median ALT (IU/L) in the three different treatment arms and the placebo group.

Safety

A total of 122 adverse events were reported in this study. The most frequent reported adverse events were flu like symptoms: fever (78%) and headache (63%). Other frequently reported side effects were abdominal pain (37%), and nausea and vomiting (30%) (Table 2). Two patients had an ALT flare above 10 times the ULN, both not resulting in hepatic decompensation. Four patients discontinued therapy prematurely due to an episode of fever and severe rigors 1 hour to 2 days after drug administration; one patient in the 1 mg/kg dose level, the other three in the 10 mg/kg dose level group. All these episodes of fever resolved within 1 week after onset.

Category	Adverse event	All patients (n=27)	0,1 μg/kg (n=8)	1 μg/kg (n=6)	10 μg/kg (n=6)	Placebo (n=7)
Flu-like symptoms	Fatigue	4	1	2	1	0
	Headache	17	5	5	7	0
	Fever	21	9	4	5	3
	Myalgia	2	0	1	1	0
Gastro-intestinal	Nausea/vomiting	8	0	2	6	0
	Abdominal pain	10	3	2	3	2
	Diarrhea	2	0	1	0	1
	Constipation	4	0	2	0	2
	ALT >10 xULN	2	2	0	0	0
Respiratory	Hay fever	2	0	1	1	0
	Cough	2	0	0	0	2
CNS	Dizziness	3	1	0	2	0
	Sleeping disorder	3	1	1	0	1
Cardiovascular	Hypertension	2	1	1	0	0
	Hypotension	2	0	1	1	0
	Chest pain	3	0	2	0	1
Other		35	13	6	4	12
Total number of adverse events		122	36	31	31	24

Table 2: Most common adverse events, defined as those occurring in at least 2 patients in any dosing group.

Discussion

This phase I/II randomized, double-blind, placebo-controlled trial provides unique data on the antiviral activity and safety of the immune-modulating glycosphingolipid a-GalCer for the treatment of chronic hepatitis B infection. In this trial, three administrations of a-GalCer with a 28-day interval were given in chronic hepatitis B patients in three different dose levels. Although almost all patients responded to a-GalCer as shown by a rapid and strong decline in NKT cell numbers, proper immune activation with a clear and sustained antiviral activity was however not observed.

As expected from literature, iNKT cells rapidly disappeared from the circulation upon a-GalCer administration. This decline in circulating iNKT cell numbers was observed after each a-GalCer injection but also rapidly returned close to pretreatment values. Whether circulating NKT cells die or migrate to the liver, as described previously,[26,27] remains to be determined. In contrast to the study of Veldt *et al.*[22] in which chronic hepatitis C patients showed only a moderate decrease in circulating iNKT cell numbers upon a-GalCer treatment, the current study on chronic hepatitis B patients showed a profound decrease in circulating iNKT cells upon similar doses of a-GalCer. Therefore, the effect of a-GalCer treatment on the immune system in the current study surpasses the effects observed in a-GalCer-treated hepatitis C patients.

The initial number of the circulating iNKT cells in chronic hepatitis B patients appears to be comparable to the number present in healthy controls and chronic hepatitis C patients.[19,22,28] The reduction of circulating iNKT cells in chronic hepatitis B patients was most pronounced after the first injection of a-GalCer and seemed to decline over time. This may suggest that the potential antiviral effect of a-GalCer in chronic hepatitis B patients diminishes over time, which may be related to the decreasing iNKT cell pool during treatment. In line with previous studies, we observed the strongest immune activating effects on patients with high NKT cell levels.[19,22] We defined the iNKT-high group by using the median number of circulating iNKT cells in patients (1100 $CD3^+V\alpha24^+V\beta11^+/10^6$ $CD3^+$ cells). This NKT-high group showed stronger immune activation upon a-GalCer administration than the other patients as demonstrated by stronger iNKT cell decline, transient rises in serum TNF-a and IL-6 levels and increased circulating activated NK cells. None of the immunological parameters tested showed clear a-GalCer dose-dependent effects. However, some dose-response effects were observed in relation to circulating NK

cell numbers and the development of fever. Patients with high level of circulating iNKT cells developed fever, as reported before,[19] but only when treated with at least 1 μg/kg a-GalCer. The specific, but transient, increase in the proportion of $CD8^+$ NKT cells in these patients may reflect a shift towards a more pronounced Th-1 phenotype,[29] but also in these patients IFNg serum levels remained undetectable.

Although the biological activity of a-GalCer in chronic hepatitis B patients seemed to be superior compared to chronic hepatitis C patients, a-GalCer did not significantly affect HBV DNA or ALT levels. Four patients treated with a-GalCer had a pronounced decline in HBV DNA in the first week of treatment, but this decline was not sustained and also not observed after the second and third administration of a-GalCer. One patient in dose level two had a sustained drop in HBV DNA at the end of follow-up (4 $\log_{10}$ copies/mL reduction). In HBV transgenic mice a-GalCer was able to inhibit HBV replication by directly activating NKT cells and by consequent activation of NK cells to secrete antiviral cytokines, such as IFN-g and IFN-a/b in the liver.[15] Although we were not able to study intrahepatic NK cell activation, patients with high circulating NKT cell levels did show enhanced levels of activated circulating NK cells upon a-GalCer injection, which is in line with previous reports.[19,30] This enhanced activation status of NK cells did not result in significant increases in serum IFNg levels and/or HBV-DNA decline. We did notice signs of immune activation as determined by increased serum IL-6 level in the patients exhibiting an episode of fever and severe rigors. This suggests that a-GalCer is able to induce immune activation in the liver since IL-6 is one of the primary inducers of the acute-phase response in liver.

Four a-GalCer treated patients discontinued therapy early due to an episode of fever short after drug administration. All these episodes resolved spontaneously. These side effects limit further development of treatment with a-GalCer in chronic hepatitis B patients. Furthermore, there is no clear and consistent effect of a-GalCer on the HBV DNA and ALT levels. This can be due to the relatively low NKT cell levels in humans compared to mice, where a-GalCer was able to inhibit HBV replication.[15] Serious side effects were not observed in a trial with a-GalCer treatment in patients chronically infected with hepatitis C.[22] This suggests that there is a different NKT-cell response in chronic hepatitis B patients.

A higher dosage of a-GalCer might be more effective, but the a-GalCer dosage is probably

limited by its side effects. Alternatively, administration of a-GalCer bound to dendritic cells has been shown to be much more potent than a-GalCer itself and seems to be well tolerated.[31-33] Nevertheless, previous studies on a-GalCer in humans did show immune responses with the relatively low levels of a-GalCer used in this trial.[19]

In conclusion, a-GalCer resulted in a strong decline of circulating NKT cells but had no effect on HBV DNA and ALT levels and was poorly tolerated in chronic hepatitis B infected patients when used as monotherapy at the doses 0.1-10 mg/kg. It is unlikely that a-GalCer will provide an alternative, at least as monotherapy, to the current treatment options.

Acknowledgements

We thank H.J. Bontkes (Cancer Center Amsterdam, VUMC Institute for Cancer and Immunology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands) for her contribution the NKT cell analyses.

References

1. Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13 Suppl 1:S47-49.

2. Bosch FX, Ribes J, Cleries R, Diaz M. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005;9:191-211, v.

3. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-2695.

4. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, 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;351:1206-1217.

5. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, 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. Johnson TR, Hong S, Van Kaer L, Koezuka Y, Graham BS. NK T cells contribute to expansion of CD8(+) T cells and amplification of antiviral immune responses to respiratory syncytial virus. J Virol 2002;76:4294-4303.

7. Exley MA, Bigley NJ, Cheng O, Tahir SM, Smiley ST, Carter QL, et al. CD1d-reactive T-cell activation leads to amelioration of disease caused by diabetogenic encephalomyocarditis virus. J Leukoc Biol 2001;69:713-718.

8. van Dommelen SL, Tabarias HA, Smyth MJ, Degli-Esposti MA. Activation of natural killer (NK) T cells during murine cytomegalovirus infection enhances the antiviral response mediated by NK cells. J Virol 2003;77:1877-1884.

9. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, et al. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science 1997;278:1626-1629.

10. Exley M, Garcia J, Balk SP, Porcelli S. Requirements for CD1d recognition by human invariant Valpha24+ CD4-CD8- T cells. J Exp Med 1997;186:109-120.

11. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H. The regulatory role of

Valpha14 NKT cells in innate and acquired immune response. Annu Rev Immunol 2003;21:483-513.

12. Taniguchi M, Seino K, Nakayama T. The NKT cell system: bridging innate and acquired immunity. Nat Immunol 2003;4:1164-1165.
13. Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ, Baxter AG. NKT cells: facts, functions and fallacies. Immunol Today 2000;21:573-583.
14. Ohteki T, MacDonald HR. Major histocompatibility complex class I related molecules control the development of CD4+8- and CD4-8- subsets of natural killer 1.1+ T cell receptor-alpha/beta+ cells in the liver of mice. J Exp Med 1994;180:699-704.
15. Kakimi K, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication in vivo. J Exp Med 2000;192:921-930.
16. Kakimi K, Lane TE, Chisari FV, Guidotti LG. Cutting edge: Inhibition of hepatitis B virus replication by activated NK T cells does not require inflammatory cell recruitment to the liver. J Immunol 2001;167:6701-6705.
17. Mehta AS, Gu B, Conyers B, Ouzounov S, Wang L, Moriarty RM, et al. alpha-Galactosylceramide and novel synthetic glycolipids directly induce the innate host defense pathway and have direct activity against hepatitis B and C viruses. Antimicrob Agents Chemother 2004;48:2085-2090.
18. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Sato H, et al. Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated Valpha14 NKT cells. Proc Natl Acad Sci U S A 1998;95:5690-5693.
19. Giaccone G, Punt CJ, Ando Y, Ruijter R, Nishi N, Peters M, et al. A phase I study of the natural killer T-cell ligand alpha-galactosylceramide (KRN7000) in patients with solid tumors. Clin Cancer Res 2002;8:3702-3709.
20. Nakagawa R, Motoki K, Ueno H, Iijima R, Nakamura H, Kobayashi E, et al. Treatment of hepatic metastasis of the colon26 adenocarcinoma with an alpha-galactosylceramide, KRN7000. Cancer Res 1998;58:1202-1207.
21. Kobayashi E, Motoki K, Uchida T, Fukushima H, Koezuka Y. KRN7000, a novel immunomodulator, and its antitumor activities; Oncol Res 1995;7:529-534.

22. Veldt BJ, van der Vliet HJ, von Blomberg BM, van Vlierberghe H, Gerken G, Nishi N, et al. Randomized placebo controlled phase I/II trial of alpha-galactosylceramide for the treatment of chronic hepatitis C. J Hepatol 2007;47:356-365.

23. 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.

24. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol 1999;37:68-73.

25. van der Molen RG, Sprengers D, Biesta PJ, Kusters JG, Janssen HLA. Favorable effect of adefovir on the number and functionality of myeloid dendritic cells of patients with chronic HBV. Hepatology 2006;44:907-914.

26. Osman Y, Kawamura T, Naito T, Takeda K, Van Kaer L, Okumura K, et al. Activation of hepatic NKT cells and subsequent liver injury following administration of alpha-galactosylceramide. Eur J Immunol 2000;30:1919-1928.

27. Nakagawa R, Nagafune I, Tazunoki Y, Ehara H, Tomura H, Iijima R, et al. Mechanisms of the antimetastatic effect in the liver and of the hepatocyte injury induced by alpha-galactosylceramide in mice. J Immunol 2001;166:6578-6584.

28. van der Vliet HJ, Molling JW, von Blomberg BM, Kolgen W, Stam AG, de Gruijl TD, et al. Circulating Valpha24+Vbeta11+ NKT cell numbers and dendritic cell CD1d expression in hepatitis C virus infected patients. Clin Immunol 2005;114:183-189.

29. Gadola SD, Dulphy N, Salio M, Cerundolo V. Valpha24-JalphaQ-independent, CD1d-restricted recognition of alpha-galactosylceramide by human CD4(+) and CD8alphabeta(+) T lymphocytes. J Immunol 2002;168:5514-5520.

30. Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y, et al. Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J Immunol 1999;163:4647-4650.

31. Fujii S, Shimizu K, Kronenberg M, Steinman RM. Prolonged IFN-gamma-producing NKT response induced with alpha-galactosylceramide-loaded DCs. Nat Immunol 2002;3:867-874.

32. Toura I, Kawano T, Akutsu Y, Nakayama T, Ochiai T, Taniguchi M. Cutting edge: inhibition of experimental tumor metastasis by dendritic cells pulsed with alpha-galactosylceramide. J Immunol 1999;163:2387-2391.

33. Ishikawa A, Motohashi S, Ishikawa E, Fuchida H, Higashino K, Otsuji M, et al. A phase I study of alpha-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer. Clin Cancer Res 2005;11:1910-1917.

CHAPTER 9

Summary, Samenvatting, Dankwoord, Curriculum vitae and Bibliography

Martijn ter Borg

SUMMARY

Chronic hepatitis B is a global health problem. Therefore, treatment of chronic hepatitis B remains an important clinical objective despite the introduction of effective hepatitis B vaccines for more than two decades. Estimates are that chronic hepatitis B currently affects about 400 million people, particularly in developing countries. Chronic hepatitis B is responsible for between 500.000 and 1.2 million deaths annually from cirrhosis and hepatocellular carcinoma. It is one of the world's most common infectious diseases and among the world's leading causes of death.

There are two types of chronic hepatitis B, which differ by the hepatitis B 'e' antigen (HBeAg) or anti-HBe status. HBeAg-positive chronic hepatitis B is a well defined disease which can be classified into three stages: the immunotolerant phase, immunoactive phase and the inactive carrier state. There is a subgroup of patients where HBeAg seroconversion occurs but in whom persistence or recurrence of biochemical and histological activity and high serum HBV DNA levels are found. These patients constitute the group of patients with HBeAg-negative chronic hepatitis B. HBeAg-negative chronic hepatitis B was first described and characterized in the early 80's in patients of the Mediterranean area. Nowadays, in the Mediterranean area, over 80% of chronic hepatitis B are related to HBeAg-negative variants.

Interferon-alpha has been the mainstay of therapy for both HBeAg-positive and -negative hepatitis B since the early 90's. Although hampered by side effects, interferon or pegylated interferon probably still offers the highest chance to achieve off-treatment sustained response for a large group of patients with chronic hepatitis B. Pegylated interferon-alpha can be administered once weekly and therapy for 1 year results in 35-45% sustained responses for HBeAg-positive and HBeAg-negative chronic hepatitis B. To further increase response, optimization of treatment schedules is necessary. Recent studies indicate that combination therapy with pegylated interferon and lamivudine does not improve the end of follow-up response. Combination with newer nucleos(t)ide analogs and different treatment regimens may lead to higher sustained response rates.

In chapter 2, we described different patterns of decline in viral load during treatment with PEG-IFN with or without lamivudine and its relation to response at the end of treatment. All patients treated with the combination of PEG-IFN and lamivudine exhibited a uniform biphasic viral decline pattern without marked differences in viral load between those who

lost HBeAg at the end of follow-up and those without HBeAg-loss. However, different viral decline patterns were observed in those treated with PEG-IFN monotherapy where those patients with a significant decline in viral load between week 4 and 32 had the highest chance of response. Furthermore we tried to find a stopping rule to identify non-responders early during treatment. However, since there was a considerable response, even in patients with a late or post-treatment decline pattern, prediction of response based on viral decline during the first months of therapy was difficult and only possible in patients with genotype A.

In chapter 3, we found that there was only a marginal decline in viral load during PEG-IFN monotherapy treatment with a 0.45 $\log_{10}$ copies/ml decline after four weeks of treatment. The peak IFN level was reached one day after PEG-IFN administration. At the end of the week, the PEG-IFN α-2b concentration returned to undetectable levels in the majority of patients. There was a relation between the IFN levels measured during weekly administration and the viral load: viral load rebounded near to baseline levels at the end of the dosing period in most patients treated with PEG-IFN monotherapy. Despite this minimal viral load decline during the first weeks of treatment, PEG-IFN α-2b was able to induce a sustained response in a considerable number of patients and further pronounced that immunomodulatory effects rather then direct antiviral activities of PEG-IFN-2b may explain its beneficial effect.

In chapter 4, we analyzed the relation between ALT and HBV DNA during PEG-IFN treatment and compared different patterns of on-treatment viral load decline described in chapter 2 with the occurrence of ALT flares. A total of 31 patients (25%) exhibited an ALT flare during treatment or follow-up of which 8 were a host induced flare associated with a favorable treatment outcome. Seventy-five percent of these host induced flares occurred in patients with a viral load decline during week 4 and 32 of treatment (i.e. the delayed decline pattern). Furthermore, the changes in viral load and ALT level were strongly interrelated both during treatment and follow-up, especially after the first four weeks of treatment.

In chapter 5, we compared the effects of PEG-IFN on HBV DNA and ALT decline with placebo treatment. We found that PEG-IFN treatment is able to reduce HBV DNA in a considerable amount of patients compared to placebo and despite the favourable natural course of genotype A, most viral load and ALT decline was observed in those with

genotype A compared to placebo.

In chapter 6, we investigated the ophthalmologic side effects during PEG-IFN treatment which include retinal hemorrhage, cotton wool spots, micro aneurysms, optic disc hyperemia and macular edema. The incidence of retinopathy during IFN-treatment varies between 18% and 86% in literature. In our study, the observed incidence was only 4%. This low incidence was possibly caused by the fact that we performed fundoscopic examination relative early during treatment, the mean age was lower in our study compared to literature and our study was performed in hepatitis B patient only. Based on the transient character of PEG-IFN-related retinopathy, we recommend to ophthlamologic examination only those with an increased risk for developing retinopathy or in patients with known pre-existing retinopathy.

In chapter 7, we investigated the effect of pregnancy on liver disease activity in female patients chronically infected with hepatitis B. Some of these patients were treated with lamivudine during the last trimester of pregnancy to reduce vertical transmission of the hepatitis B virus. We found that there was a significant increase in liver disease activity in a large proportion of patients in the first 6 months after pregnancy. Treatment with lamivudine did increase further this percentage. On the other hand, none of these exacerbations of liver disease led to decompensated liver disease. Based on the high incidence of increase in liver disease activity after pregnancy, we recommended to monitor women infected with hepatitis B closely after delivery.

Finally, we describe in chapter 8 the results of a dose-escalating randomized placebo-controlled phase I/II trial with the glycosphingolipid alpha-galactosylceramide (a-GalCer) as a novel class of treatment for chronic hepatitis B patients. a-GalCer has been shown to have an immunomodulatory effect by stimulation of natural killer T (NKT) cells and is able to induce a powerful antiviral immune response via the production of inflammatory cytokines. Almost all patients responded to a-GalCer by a rapid and strong decline in NKT cell numbers. Especially patients with relatively high circulating NKT cell levels showed signs of immune activation, including enhanced levels of circulating activated NK cells, elevated serum TNF-a and IL-6 levels and development of fever. However, as a monotherapy in chronic hepatitis B patients, a-GalCer was poorly tolerated, and was unlikely to provide an alternative as monotherapy to the current treatment of PEG-IFN and nucleos(t)ide analogs.

SAMENVATTIING

Chronische hepatitis B is wereldwijd een groot gezondheidsprobleem. Daarom blijft de behandeling van chronische hepatitis B belangrijk, alhoewel er al ongeveer 20 jaar een effectief vaccin bestaat. Naar schatting zijn er in de wereld 400 miljoen mensen chronisch geïnfecteerd met het hepatitis B virus, met name in ontwikkelingslanden. Verder sterven er jaarlijks tussen de 500.000 en 1.2 miljoen mensen aan de gevolgen van een hepatitis B infectie, meestal ten gevolge van levercirrose of leverkanker. Hepatitis B is dan ook een veel voorkomende infectieziekte en wereldwijd een belangrijke doodsoorzaak.

Er zijn twee typen chronische hepatitis B infectie die verschillen in de aan- dan wel afwezigheid van het hepatitis B 'e' antigeen (HBeAg) en de antistoffen hiertegen (anti-HBe). HBeAg-positieve chronische hepatitis B is een goed gedefinieerde ziekte die zich in drie fasen kan bevinden: de immuuntolerante fase, de immuunactieve fase of het inactieve dragerschap. Naast de welomschreven HBeAg-positieve chronische hepatitis B is er een subgroep van patiënten met verlies van het HBeAg en de vorming van anti-HBe bij wie er wel sprake is van ontsteking in de lever en een hoge virale load in het bloed. Bij deze patiënten is er sprake van een HBeAg-negatieve chronische hepatitis B. HBeAg-negatieve chronische hepatitis B werd in de jaren '80 van de vorige eeuw voor het eerst beschreven bij patiënten in het Middellandse zeegebied. Momenteel heeft ongeveer 80% van de patiënten in het Middellandse zeegebied deze HBeAg-negatieve vorm.

Interferon-alfa is sinds de jaren '90 van de vorige eeuw een belangrijke behandelingsmodaliteit voor zowel HBeAg-positieve als HBeAg-negatieve chronische hepatitis B. Ondanks de bijwerkingen die gepaard gaan met de behandeling met interferon is het nog steeds zo dat interferon de grootste kans geeft op een blijvende respons zonder dat andere therapie nodig is. Gepegyleerd interferon-alfa heeft als voordeel dat het maar 1 keer per week toegediend hoeft te worden en het geeft een blijvende respons in ongeveer 35-45% van de patiënten, zowel in de HBeAg-negatieve als de HBeAg-positieve vorm. Om dit responspercentage te kunnen verbeteren is optimalisatie van het behandelschema noodzakelijk. Studies tonen aan dat het toevoegen van lamivudine aan de behandeling met interferon bij chronische hepatitis B geen meerwaarde heeft. Combinaties met nieuwere nucleos(t)ide analogen moeten veelal nog worden onderzocht.

In hoofdstuk 2 beschreven we verschillende patronen van daling van de virale load tijdens de behandeling met PEG-interferon met of zonder lamivudine en de relatie met de respons

aan het einde van de behandeling. Alle patiënten die werden behandeld met de combinatietherapie hadden een uniforme bifasische daling van de virale load zonder duidelijke verschillen in de virale load daling tussen patiënten die uiteindelijk wel c.q. niet respondeerden aan het einde van de follow-up. In de patiëntengroep die alleen met PEG-interferon werd behandeld werden wel verschillende patronen van virale load daling waargenomen en de patiënten met een significante daling in de virale load tussen week 4 en 32 hadden de grootste kans op respons. Het was niet goed mogelijk om tijdens de behandeling te voorspellen wie er uiteindelijk zullen reageren omdat er veel patiënten zijn die pas heel laat tijdens de behandeling een virale load daling laten zien. Alleen in patiënten met genotype A was het enigszins mogelijk om tijdens de behandeling respons te voorspellen.

In hoofdstuk 3 lieten we zien dat er maar een marginale daling in de virale load van 0.45 $\log_{10}$ kopieën/mL optreedt na 4 weken behandeling met PEG-interferon. De hoogste interferon spiegel in het bloed wordt bereikt 1 dag na toediening. Aan het einde van de week is in de meeste patiënten geen PEG-interferon meer detecteerbaar. Er was een relatie tussen de gemeten interferon waarden en de virale load: de virale load steeg aan het eind van de week weer richting de uitgangswaarde in patiënten die behandeld werden met PEG-interferon monotherapie. Ondanks deze minimale daling van de virale load in de eerste maand van de behandeling, is er met PEG-interferon toch een blijvende respons te bereiken in een aanzienlijk deel van de patiënten. Dit benadrukt dat de immuunmodulerende eigenschappen van PEG-interferon van groter belang zijn dan de directe anti-virale activiteit.

In hoofdstuk 4 hebben we de relatie tussen ALT (een maat voor de ontsteking van de lever) en de virale load tijdens PEG-interferon bestudeerd. Verder onderzochten we de relatie tussen de patronen van virale load daling zoals beschreven in hoofdstuk 2 met het optreden van ALT flares. In totaal hadden 31 patiënten (25%) een ALT flare tijdens de behandeling of follow-up periode. In totaal hadden 8 patiënten een zogenaamde 'host-induced flare' die geassocieerd is met een gunstig beloop. Vijfenzeventig procent van deze 'host-induced flares' trad op in patiënten met een virale load daling tussen week 4 en 32 van de behandeling. Tenslotte was er een sterke relatie tussen de virale load en de ALT waarde tijdens de behandeling en de follow-up periode, vooral na de eerste maand van de behandeling.

In hoofdstuk 5, hebben we de effecten van de behandeling van PEG-IFN op het HBV DNA en ALAT vergeleken met patiënten die werden behandeld met placebo therapie. PEG-IFN is in staat om bij veel patiënten een daling te geven in zowel HBV DNA als ALAT, ook als dit vergeleken wordt met placebobehandeling. En alhoewel patiënten met genotype A ook zonder behandeling al een gunstig beloop van een hepatitis B infectie hebben, blijkt juist bij hen PEG-IFN behandeling zinvol vergeleken met placebo.

In hoofdstuk 6 hebben we de oogheelkundige bijwerkingen onderzocht van PEG-interferon behandeling. Deze bijwerkingen bestaan onder andere uit: retinabloedingen, 'cotton wool spots', micro-aneurysmata, opticus hyperaemie en macula oedeem. De incidentie van retinopathie tijdens interferonbehandeling varieert in de literatuur tussen de 18% en 86%. In onze studie was de geobserveerde incidentie slechts 4%. Deze lage incidentie kan worden verklaard door het feit dat het fundoscopie-onderzoek relatief vroeg tijdens de behandeling is uitgevoerd, de leeftijd van de patiënten relatief laag was in onze studie en verder was onze studie alleen uitgevoerd in hepatitis B patiënten. Mede gebaseerd op het tijdelijke karakter van de interferon gerelateerde retinopathie, bevelen wij bij hepatitis B patiënten die behandeld worden met PEG-interferon aan dat er alleen oogheelkundig onderzoek noodzakelijk is in patiënten met een verhoogd risico op oogafwijkingen en in patiënten die al een oogafwijking hebben.

In hoofdstuk 7 hebben we het effect van een zwangerschap op de activiteit van leverziekte activiteit onderzocht in vrouwelijke patiënten die chronisch geïnfecteerd waren met het hepatitis B virus. Een deel van deze patiënten is tijdens het laatste trimester van de zwangerschap behandeld met lamivudine om de verticale transmissie van het hepatitis B virus te verkleinen. We vonden een duidelijke stijging van de leverziekteactiviteit in het grootste deel van de patiënten in de eerste 6 maanden na de bevalling. Behandeling met lamivudine tijdens de zwangerschap deed dit alleen maar verder toenemen. Aan de andere kant waren er geen patiënten waarbij de stijging van de leverziekteactiviteit leidde tot een gedecompenseerde leverziekte. Gebaseerd op de hoge incidentie van toename van leverziekteactiviteit na de bevalling adviseren we om patiënten met een chronische hepatitis B infectie na de behandeling strak te vervolgen.

Tenslotte beschreven we in hoofdstuk 8 de resultaten van een gerandomiseerde fase I/II studie met alfa-galactosylceramide als een nieuwe behandelmethode voor patiënten met een chronische hepatitis B. Alfa-galactosylceramide heeft immuunmodulerende

eigenschappen doordat het 'natural killer T-cellen' stimuleert en in staat is om een antivirale immuunrepons teweeg te brengen via de vorming van inflammatoire cytokinen. Bijna alle patiënten lieten en snelle en sterke daling zien in het aantal NKT cellen. Deze immuunrespons, gemeten aan de hand van toename van NK cellen, stijging van TNF-alfa en IL-6 waarden, was het meest uitgesproken in patiënten met relatief veel circulerende NKT cellen. Een duidelijke daling van de virale load werd niet gezien. Concluderend was alfa-galactosylceramide als monotherapie bij chronische hepatitis B niet in staat om een daling van de hoeveelheid virus te bewerkstelligen. Tenslotte werd het ook slecht verdragen en het zal dan ook niet gebruikt kunnen worden als monotherapie voor de behandeling van chronische hepatitis B.

DANKWOORD

En dan eindelijk, het dankwoord....

Ten eerste wil ik natuurlijk mijn promotor, Harry Janssen, bedanken voor alle begeleiding gedurende dit hele promotieonderzoek. Je bent enorm gedreven in het doen van wetenschappelijk onderzoek en weet elke keer weer de juiste onderwerpen te vinden en een proefschrift in de juiste richting bij te sturen.

De collega's van andere afdelingen van het Erasmus MC die betrokken waren bij de verschillende onderzoeken waarbij met name de afdeling virologie niet onbenoemd mag blijven. Bart Haagmans, van harte bedankt voor al je hulp en bijdragen.

Dan is er natuurlijk Bettina Hansen, de statistische steun en toeverlaat van de hele afdeling. Zonder jouw SPSS en andere expertise was het afronden van dit boekje nooit gelukt.

Mijn voorgangers die hebben gewerkt aan de HBV9901 studie. Monika van Zonneveld en Hajo Flink, bedankt voor het leggen van de basis van dit voorlopig derde proefschrift met artikelen over deze groots opgezette studie!

Solko Schalm wil ik ook danken voor al zijn enthousiasme. Gelukkig heb ik je nog een laatste stuk op de afdeling Hepatologie en later ook via Liverdoc meegemaakt. Jouw drive en kennis zijn ongeëvenaard.

Mijn opleiders in de maag-darm-leverziekten, Ernst Kuipers en Rob de Man, bedankt voor de samenwerking en vooral jullie vertrouwen in mijn opleiding tot MDL-arts.

Secretaresses van de sectie Hepatologie: Margriet & Marion heel erg bedankt voor jullie hulp. Jullie zijn echt het middelpunt van de afdeling.

Het 'clinical research bureau' onder aanvoering van Elke. Maar natuurlijk ook Wanda,

Gaalda, Claudia. Zonder jullie enorme inzet in het tot stand komen van klinische studies en de strakke begeleiding erna, is wetenschappelijk onderzoek doen bijna onmogelijk.

Collega-onderzoekers en mensen van de dakpoli: Sarwa, Jilling, Geert, Erik, Lieke, Edith, Hajo, Joyce, Sanna, Bart, Rob, Claudia, Marianne, Manon en alle anderen. Hartelijk dank voor alle inbreng en natuurlijk vooral alle leuke uren die we hebben doorgebracht tijdens congressen of in Rotterdam in `Dizzy´ of een ander café.

Natuurlijk mijn kamergenoten op de dakpoli: Wim, Madeleen, Jurrien, Jolanda en Henk. Jullie hebben mijn ochtendhumeur altijd groots gedragen. Veel koffie hebben we voor elkaar gezet en ook menig idee is er zo na boven gekomen.

Mijn huidige opleider Interne Geneeskunde, Martin Gerding en alle andere collega's in het Deventer Ziekenhuis, bedankt voor het bieden van een leuke nieuwe werkomgeving en jullie belangstelling voor mijn onderzoek.

Zonder de researchverpleegkundigen Cokki, Anneke, Puck, Mirjam en vooral Heleen was er van dit proefschrift niets terechtgekomen. Bedankt voor al jullie vaak spontane en flexibele hulp!

Een klinisch onderzoek wordt pas echt een succes als er ook veel basaal (lees: lab) onderzoek bij betrokken is. Daarom wil ik vooral ook alle labmedewerkers bedanken: Andrea, André, Renate, Jeroen, Marjolein, Rekha en Paula, heel erg bedankt voor al jullie inzet en hulp!

Natuurlijk ook alle andere collega's van de afdeling maag-, darm- en leverziekten van het Erasmus MC bedankt voor de plezierige samenwerking en tot ziens in de toekomst. Ik hoop jullie snel weer te zien bij de bekende leverlunch.

De vaste poli-assistentes van de hepatitis B poli Nermin, Minou en Ester en alle anderen. Bedankt voor al jullie begeleiding op de polikliniek, onderzoek draait om patiënten en zonder alle patiënten die hebben deelgenomen aan de verschillende studies die zijn

beschreven was dit proefschrift niet mogelijk geweest.

Verder gaat mijn dank uit naar mijn ouders, die mij grotendeels hebben gevormd zoals ik nu ben. Helaas kan Lieneke de afronding van mijn promotie niet meer meemaken. Piet, heel erg bedankt voor alle steun!

Ook de rest van de familie, Pieter & Bhartie, Kees & Gerda, Wendy & Frank. Bedankt voor alle steun in de afgelopen periode!

En tenslotte gaat natuurlijk mijn dank uit naar Marjolein. Elke keer vond je het goed als ik weer eens achter de computer kroop. En je bent zelfs naar Deventer verhuisd voor mij! Zonder jou was het me niet gelukt.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 6 januari 1979 te Rotterdam. Na het behalen van zijn V.W.O. diploma aan het Comenius College te Capelle aan den IJssel in 1997 begon hij met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraal werd behaald in 2001 en het arts examen in november 2003. Van december 2003 tot juli 2004 was hij werkzaam als assistent niet in opleiding tot specialist (ANIOS) Interne Geneeskunde in het IJsselland ziekenhuis te Capelle aan den IJssel (opleider: dr. H.R.A. Fischer). Hierna werkte hij als arts-onderzoeker op de afdeling maag-, darm- en leverziekten van het Erasmus MC te Rotterdam (hoofd: prof.dr. E.J. Kuipers) onder supervisie van prof.dr. H.L.A. Janssen aan het onderzoek beschreven in dit proefschrift. Vanaf augustus 2007 is hij werkzaam als assistent in opleiding tot specialist (AIOS) op de afdeling Interne Geneeskunde (opleider: dr. M.N. Gerding) en per februari 2009 op de afdeling maag-, darm- en leverziekten (opleider: dr. F. ter Borg) van het Deventer Ziekenhuis te Deventer voor zijn opleiding tot maag-, darm- en leverarts via het Erasmus MC (opleider: dr. R.A. de Man).

BIBLIOGRAPHY

Ter Borg MJ, Hansen BE, Bigot G, Haagmans BL, Janssen HLA. *ALT and viral load decline during PEG-IFN alpha-2b treatment for HBeAg-positive chronic hepatitis B.* Journal of Clinical Virology. 2008 Jun;42(2):160-4 .

Ter Borg MJ, Leemans WF, De Man RA, Janssen HLA. *Exacerbation of chronic hepatitis B infection after delivery*. Journal of Viral Hepatitis. 2008 Jan;15(1):37-41.

Ter Borg MJ, Hansen BE, Van 't Veen A, Herrmann E, Zeuzem S, Cakaloglu Y, Karayalcin S, Flisiak R, De Man RA, Schalm SW, Janssen HLA, Haagmans BL. *Modelling of early viral kinetics and pegylated interferon-alpha-2b pharmacokinetics in HBeAg-positive chronic hepatitis B*. Antiviral Therapy. Antiviral Therapy 2007; 12(8):1285-94.

Leemans WF, Ter Borg MJ, de Man RA. *Success and failure of nucleoside and nucleotide analogues in chronic hepatitis B*. Aliment Pharmacol Ther 2007: 26 (Suppl 2), 171–182.

Ter Borg MJ, Janssen HLA. *Chronische hepatitis B: een update*. Tijdschrift voor Infectieziekten 2007;2(3):92-98.

Buster EH, Ter Borg MJ, Janssen HL. *Pegylated interferon alpha for chronic hepatitis B – alone or in combination with lamivudine*. Hot Topics in Viral Hepatitis nr. 4 – 2007: 21-27.

Ter Borg MJ, van Zonneveld M, Zeuzem S, Senturk H, Akarca US, Simon C, Hansen BE, Haagmans BL, De Man RA, Schalm SW, Janssen HL. *Patterns of viral decline during PEG-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B: Relation to treatment response*. Hepatology 2006;44(3): 721-7.

Ter Borg MJ, Buster EH, Janssen HL. *Interferon and Pegylated Interferon in Chronic Hepatitis B* in *Viral hepatitis B and C*, Maria Buti & Rafael Esteban (editors). Barcelona: Grup 3, 2006: 32-45.

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):160-1.

Ter Borg MJ, Janssen HL. *Pegylated interferon-alpha2b and lamivudine in hepatitis B e antigen-positive chronic hepatitis B*. Ann Intern Med 2005;143:391.

Ter Borg MJ, Janssen HL. *Interferon-alpha for chronic hepatitis B* in Hepatitis B, Maria Buti & Rafael Esteban (editors). Barcelona: Permanyer Publications, ISBN 84-934506-6-9, 2005: 59-68.

Cavelaars M, Tulen JH, van Bemmel JH, Ter Borg MJ, Mulder PG, van den Meiracker AH. *Determinants of ambulatory blood pressure response to physical activity*. J Hypertens 2002;20:2009-2015.