Prediction of Response to Immune Modifying Therapy for Patients with Chronic Hepatitis B using Hepatitis B Surface Antigen Levels

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Colofon

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Prediction of Response to Immune Modifying Therapy for Patients with Chronic Hepatitis B using Hepatitis B Surface Antigen Levels

Immuunmodulatoire behandeling voor chronische hepatitis B: predictie van respons met behulp van hepatitis B surface antigeen concentraties

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Table of contents

General introduction		7
Chapter 1	A randomized trial of peginterferon alfa-2a with or without ribavirin for HBeAg-negative chronic hepatitis B	15
Chapter 2	Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir	33
Chapter 3	Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline	47
Chapter 4	Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B using HBsAg and HBV DNA levels	63
Chapter 5	Early on-treatment identification of hepatitis B e antigen-negative patients not responding to peginterferon alfa-2a	79
Chapter 6	Durable HBsAg decline in HBeAg-positive chronic hepatitis B patients treated with peginterferon alfa-2b: Relation to response and HBV genotype	93
Chapter 7	Long-term follow-up of HBeAg-negative patients treated with peginterferon alfa-2a: Progressive HBsAg decline in responders	107
Summary and discussion		123
Samenvatting en bespreking		133
Dankwoord		145
Bibliografie		149
Curriculum vitae		151
PhD portfolio		153

General introduction

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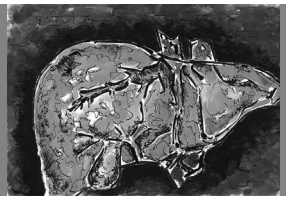

The hepatitis B virus (HBV) was discovered in 1967 with the identification of the Australia antigen in Aboriginals by Dr. Blumberg, who was awarded the 1976 Nobel Prize in Medicine for his work. The Australia antigen is nowadays known as the hepatitis B surface antigen (HBsAg). More than 4 decades later, chronic infection with HBV still is one of the most serious and prevalent infectious diseases worldwide. It is estimated that about one third of the world's population has evidence of past or current HBV infection.(1) Although safe and effective vaccines have been available for more than 2 decades, (2) there are still more than 350 million people worldwide who are chronically infected with HBV.(1) Progression of HBV-related liver disease to cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) is estimated to result in 0.5-1.2 million annual deaths.(3)

Phases of infection

Patients with chronic hepatitis B (CHB) may present in any one of four, not necessarily sequential, phases of infection.(4) During the immunotolerant phase, which is common among perinatally infected patients, the hepatitis B e antigen (HBeAg) is detectable and the serum HBV DNA level is high (>100,000 copies/mL or >20,000 IU/mL), while the serum alanine aminotransferase (ALT) concentration is within the normal range and liver histology shows minimal inflammation. In the immunoactive phase, the host immune response results in a decline in HBV DNA level, elevated ALT level and hepatic inflammation on liver biopsy. During this phase HBeAg loss and seroconversion to anti-HBe can occur. HBeAg seroconversion is often followed by the inactive carrier state characterized by a low serum HBV DNA level (<10,000 copies/mL or <2,000 IU/mL) and normalization of ALT. However, in a significant proportion of HBeAg-negative patients viral replication recurs or persists at higher levels, resulting in HBeAg-negative CHB. This phase of the infection develops through presence of viral strains harbouring mutations in the precore or basal core promoter region that reduce or abolish the expression of HBeAg.(5)

Patients eligible for antiviral treatment

There continues to be a debate as to when antiviral treatment should be initiated in patients with precirrhotic HBV-related liver disease.(6) The guidelines of the American Association for the Study of Liver Diseases (AASLD) advocate treatment for patients who remain HBeAg positive with serum HBV DNA levels >20,000 IU/mL in combination with persistently elevated ALT levels (>2 times the upper limit of normal (ULN) over a 3-6 month period).(7) The same HBV DNA and ALT criteria apply to HBeAg-negative patients, but it is emphasized that treatment should also be considered for these patients if the HBV DNA concentration is >2,000 IU/mL combined with a liver biopsy showing moderate to severe necroinflammation and/or fibrosis.(7) According to the

2009 HBV guidelines of the European Association for the Study of the Liver (EASL) antiviral therapy should be considered, irrespective of HBeAg status, if HBV DNA levels are >2,000 IU/mL, ALT levels are >1 x ULN and if a liver biopsy shows moderate to severe necroinflammation and/or fibrosis.(8)

Treatment options

Interferon (IFN-)alfa has been a mainstay in the treatment of chronic hepatitis B (CHB) since it was licensed for this indication in the early 1990s. IFN mainly acts through enhancement of the host immunological response against the virus, although it also exerts a limited direct antiviral effect.(9) Since 1998 considerable progress has been made in the treatment of CHB with the introduction of nucleos(t)ide analogues (NA), which directly inhibit the HBV polymerase and provide, at least on the short term, a well-tolerated and effective alternative to IFN therapy. IFN-based therapy also improved significantly by pegylation of IFN which allowed a more convenient once-weekly dosing interval, with treatment efficacy equal or superior to conventional IFN.(10) Currently, IFN (conventional or pegylated) and 5 NA (lamivudine, telbivudine, adefovir, entecavir and tenofovir), have been approved for the treatment of CHB in many parts of the world.

Treatment goals

Since complete eradication of HBV is rarely achieved due to persistence of covalently closed circular DNA (cccDNA) in host hepatocytes,(11) the main goal of antiviral treatment is to prevent progression to cirrhosis, hepatic decompensation and HCC.(12) Since these events develop only after decades of infection, clinical studies have focussed on various short-term, surrogate outcomes to assess treatment efficacy.(7, 12) The most widely used surrogate markers include suppression of HBV DNA levels and HBeAg loss with or without appearance of anti-HBe in HBeAg-positive patients (virologic response), normalization of serum ALT (biochemical response) and improvement of liver histology (histological response).(7)

Parameters used to assess treatment response differ between NA and PEG-IFN therapy because of their different modes of action. During NA therapy, maintained suppression of HBV DNA is the main goal given the risk of developing antiviral resistance associated with detectable HBV DNA levels.(13) The main goal of PEG-IFN therapy is to achieve immunological control over HBV resulting in a sustained response after a finite treatment course.

In HBeAg-positive patients treated with PEG-IFN, HBeAg seroconversion is frequently used as a primary endpoint since it is associated with increased survival and a reduced risk of developing HCC.(14-16) In contrast, suppression of serum HBV DNA below the detection limit of a sensitive polymerase chain reaction (PCR) assay is often applied for

NA therapy in HBeAg-positive CHB. In HBeAg-negative patients, suppression of HBV DNA levels in combination with normalization of ALT is considered the most important treatment goal. Whilst HBV DNA undetectability is pursued during NA treatment, an HBV DNA level <2,000 IU/mL (~10,000 copies/mL) is often used to define a sustained off-treatment response to PEG-IFN therapy.(8) Large population-based studies have shown that an HBV DNA concentration below this threshold is associated with a low risk of cirrhosis and HCC.(14, 17, 18) HBsAg seroclearance is the ultimate surrogate endpoint as it is associated with improved outcomes, provided that it occurs at a younger age and precedes the development of cirrhosis.(19)

Practice guidelines recommend both PEG-IFN and NA as initial treatment options.(7, 8) In order to select the optimal first-line drug, the advantages and limitations of each agent should be taken into account. NA therapy offers oral administration and newly registered NA are able to maintain suppression of viral replication in the majority of patients for prolonged periods of time.(20, 21) It is however highly uncertain whether oral antiviral therapy can be discontinued, thus necessitating indefinite therapy in many patients. Although NA are well tolerated, extended therapy still poses a risk for antiviral resistance and its long-term safety is currently unknown.(13) PEG-IFN therapy offers the advantage of higher sustained off-treatment response rates compared to NA due to its immunomodulatory effects.(22, 23) However, PEG-IFN should only be considered for patients who are likely to respond to therapy because of the costs and side-effects associated with this agent.(24)

The general aims of this thesis are (1) to optimize treatment strategies using PEG-IFN for CHB, (2) to explore the role of on-treatment HBsAg levels in the prediction of response to PEG-IFN therapy and (3) to evaluate the long-term durability of PEG-IFN induced responses.

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A randomized trial of peginterferon alfa-2a with or without ribavirin for HBeAg-negative chronic hepatitis B

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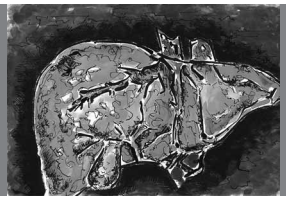


ABSTRACT

Objectives

Hepatitis B e antigen (HBeAg-)negative chronic hepatitis B patients are at high risk of treatment relapse following any antiviral therapy. Combining peginterferon alfa-2a with ribavirin might improve sustained response rates.

Methods

138 HBeAg-negative chronic hepatitis B patients were randomized to receive monotherapy (peginterferon alfa-2a 180 µg weekly plus placebo) or combination therapy (peginterferon alfa-2a weekly plus ribavirin 1000 or 1200 mg daily, depending on body weight) for 48 weeks. Post-treatment follow-up lasted 24 weeks. Analyses were based on the modified intention-to-treat population after exclusion of 5 patients.

Results

At the end of follow-up, 14 (20%) of 69 patients assigned to monotherapy and 10 (16%) of 64 assigned to combination therapy had a combined response (hepatitis B virus (HBV) DNA <10,000 copies/mL (<1,714 IU/mL) and a normal alanine aminotransferase level, p=0.49). At the end of treatment, more patients had a combined response (25 (36%) versus 26 (41%) in the monotherapy and combination therapy group, respectively, p=0.60), but subsequently relapsed during follow-up. Serum HBV DNA and hepatitis B surface antigen (HBsAg) levels decreased during treatment (mean change at week 48 compared to baseline -3.9 versus -2.6 log copies/mL, p<0.001 and –0.56 versus –0.34 log IU/mL, p=0.23, respectively). HBV DNA levels relapsed after treatment discontinuation, HBsAg remained at end-of-treatment levels. In general, combination therapy was well tolerated, although it was associated with a higher risk of anemia and neutropenia.

Conclusions

Treatment with peginterferon alfa-2a resulted in a limited sustained response rate in HBeAg-negative chronic hepatitis B patients. Addition of ribavirin did not improve response to therapy.

INTRODUCTION

Chronic infection with hepatitis B virus (HBV) is a serious global health problem affecting 350 to 400 million people worldwide. Progression of HBV-related liver disease to cirrhosis, liver failure and hepatocellular carcinoma (HCC) results in approximately 1 million annual deaths.(1) Hepatitis B e antigen (HBeAg-)negative chronic hepatitis B is a late phase of the infection, which develops spontaneously through mutations in the precore or basal core promoter region that reduce the expression of HBeAg.(2) This type of chronic hepatitis B is distributed worldwide with an increasing prevalence and represents the majority of cases in Europe.(3, 4) In general, HBeAg-negative patients have more advanced liver disease and spontaneous recovery rarely occurs resulting in a poor long-term prognosis.(2) Therefore, effective treatment strategies for this expanding patient population are of vital importance.

Despite the development of new nucleos(t)ide analogues in recent years, (peg-)interferon is still considered as one of the first-line treatment options because of the higher chance of achieving a sustained off-treatment response.(5-7) Although HBeAg-negative chronic hepatitis B responds well to continuous nucleos(t)ide analogue therapy,(8-11) responses are usually not durable when therapy is discontinued.(12-14) In early studies, conventional interferon therapy for a duration of 3 to 6 months resulted in biochemical and virological response in many patients at the end of treatment, but sustained response rates were disappointing.(15-17) Treatment with peginterferon alfa-2a for 48 weeks has been investigated in one large trial and led to a virological response (HBV DNA <20,000 copies/mL) in 43% of patients 24 weeks after discontinuation of therapy. Although the addition of lamivudine resulted in stronger viral suppression during the treatment period, sustained response rates were comparable to peginterferon alfa-2a alone.(18)

Ribavirin is a guanosine nucleoside analogue, which in contrast to many other nucleos(t)ide analogues not only inhibits viral replication by interference with viral messenger RNA, but also modulates the immune response by increasing interferon-γ production and modulating the Th1/Th2 balance.(19-22) In a small study HBeAg-negative non-responders to interferon were re-treated with a combination of standard interferon (5 million units three times a week) and ribavirin (1000 or 1200 mg daily) for 12 months. In this difficult-to-treat population a virological response (HBV DNA <400 copies/mL) was observed in 50% of patients after 12 months of follow-up, despite the low dose of interferon given.(23) Furthermore, a significant proportion of patients (11%) treated with peginterferon alfa-2a and ribavirin for dual chronic hepatitis B and C cleared hepatitis B surface antigen (HBsAg).(24) These findings suggest this combination regimen could be more effective than (peg-)interferon alone or its combination with other nucleos(t)ide analogues to achieve sustained off-treatment response in HBeAg-negative chronic hepatitis B. Our study was thus designed to investigate whether the

combination of peginterferon alfa-2a and ribavirin increases the rate of such response in HBeAg-negative disease.

PATIENTS AND METHODS

Patients

Chronic hepatitis B patients, aged 18 to 70 years, were enrolled after assessment of their eligibility at the trial coordinating center. Eligible patients had been positive for HBsAg for longer than 6 months; were negative for HBeAg and positive for anti-HBe using local enzyme immunoassays on 2 occasions within 2 months prior to randomization; had an HBV DNA level of more than 100,000 copies/mL (17,143 IU/mL); had had 2 episodes of raised serum levels of alanine aminotransferase (ALT >1.5 and ≤10 times the upper limit of the normal range) within the 2 months before randomization; and were requested to be using 2 forms of effective contraception until 6 months after treatment discontinuation. Exclusion criteria included: co-infection with hepatitis C, hepatitis D or human immunodeficiency virus (HIV); antiviral or immunosuppressive therapy within the previous 6 months; substance abuse during the preceding 2 years; other acquired or inherited causes of liver disease; coexisting serious medical or psychiatric illness; uncontrolled thyroid disease; inadequate blood counts (neutrophils ≤1.5x10^9/L; platelets ≤90x10^9/L or haemoglobin ≤11.5 g/dL for females and ≤12.5 g/dL for males); radiological or biochemical evidence of HCC; and advanced liver disease with a prothrombin time prolonged by at least 3 seconds, serum albumin concentration less than 35 g/L, bilirubin more than 1.46 mg/dL (25 µmol/L), or a history of ascites, variceal bleeding or hepatic encephalopathy.

Study design

This investigator-initiated, randomized, double-blind, controlled trial was carried out between June 2005 and February 2008 (ClinicalTrials.gov registration number: NCT00114361). A total of 25 European centers in 7 countries participated in this study. With the use of a central interactive voice response system (S-Clinica), randomization was done centrally by a computer-generated randomization list and stratified by country and by ALT level (≤2 or >2 times ULN) in blocks of 4 to one of the treatment arms. Eligible patients were randomly assigned in a one-to-one ratio to receive combination therapy with 180 µg peginterferon alfa-2a (Pegasys, F. Hoffmann-La Roche Ltd., Basel, Switzerland) weekly and ribavirin (Copegus, F. Hoffmann-La Roche Ltd., Basel, Switzerland) 1000 mg daily (body weight <75 kg) or 1200 mg daily (body weight ≥75 kg) or monotherapy with 180 µg/week peginterferon alfa-2a and placebo, which was similar in appearance to ribavirin. The 48-week treatment period was followed by

a 24-week observation period. Patients attended the outpatient clinic at least every 4 weeks for routine examinations and laboratory assessments. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice and it was formally approved by the ethics committee of each participating centre. All patients gave written informed consent.

Assessment of efficacy and safety

The predefined primary endpoint was the combined presence of an HBV DNA level below 10,000 copies/mL (1,714 IU/mL) and normalization of ALT at the end of follow-up (week 72). This HBV DNA level corresponds to that accepted by the recent European guidelines as definition of response to peginterferon therapy.(6) Patients were categorized as non-responder in case HBV DNA or ALT result at week 72 was missing. Secondary outcome measures included HBV DNA less than 10,000 and 400 copies/ml (69 IU/mL), normalization of ALT levels and clearance of HBsAg. Secondary endpoints were assessed both at the end of treatment (week 48) and at the end of follow-up (week 72).

Serum ALT was measured at the participating centers in accordance with standard procedures. To correct for heterogeneity of the different local assays and for differences in reference between males and females, ALT levels are expressed as values representing a ratio to the local upper limit of normal (ULN). Serum HBV DNA (Taqman HBV assay, Roche Diagnostics, lower limit of quantification: 35 copies/mL (6 IU/mL)) and serum HBsAg (ARCHITECT HBsAg assay, Abbott laboratories; range 0.05-250 IU/mL) were quantified with 12-week intervals at the central laboratory at the Erasmus MC, University Medical Center, Rotterdam. Confirmation of HBeAg (ETI-EBK PLUS, DiaSorin) and anti-HBe (ETI-AB-EBK PLUS, DiaSorin) status was retrospectively performed at the central laboratory, as predefined in the protocol. HBV genotype (INNO-LiPA assay; Innogenetics) was also assessed.

All patients underwent liver biopsy within one year before randomization. A second liver biopsy was performed at the end of follow-up (week 72). Improvement in liver histology was defined as either a decrease in necroinflammatory score of ≥2 points (range 0-18) or a decrease in fibrosis score of ≥1 point (range 0-6) according to the Ishak scoring system.(25) The biopsies were evaluated by one experienced pathologist (PZ) who was not aware of treatment arm or biopsy dates.

The safety analysis included clinical adverse events reported by the local investigators and safety laboratory data.

Statistical analysis

A sample size of 61 patients per treatment arm provided the study with a statistical power of 80% at the 0.05 level of significance, on the assumption of a combined

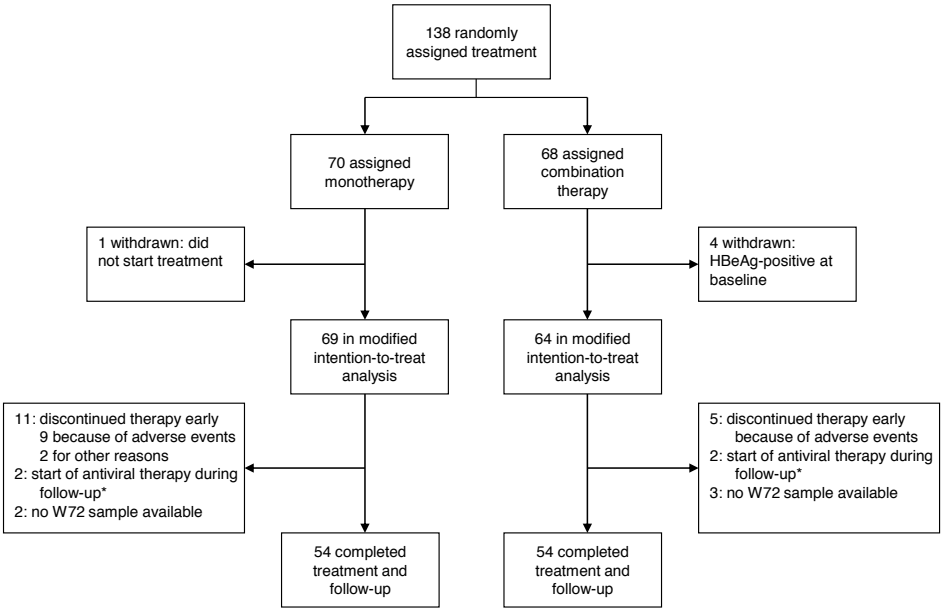


Figure 1: Trial profile. Flow of patients receiving monotherapy (peginterferon alfa-2a and placebo) and combination therapy (peginterferon alfa-2a and ribavirin) through the study.
*All non-responders.

response rate at week 72 of 30% for peginterferon alfa-2a monotherapy versus 55% for peginterferon alfa-2a and ribavirin combination therapy. During the trial, the sample size was increased to 69 patients per treatment arm to account for withdrawal.

Efficacy analyses were based on the modified intention-to-treat population, which includes all randomized patients who met the inclusion criteria and received at least one dose of study medication. Continuous variables are expressed as mean (standard deviation) or median (interquartile range), where appropriate. Serum HBV DNA and HBsAg levels were logarithmically transformed for analysis. Differences in treatment effect were assessed by the Chi-square test. Continuous variables were compared using t-tests. The relation between baseline characteristics and response to therapy was examined by logistic regression analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance. SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

Role of the funding source

The study was initiated by the Foundation for Liver Research (SLO), Rotterdam, the Netherlands, who was also the sponsor of the study. Financial support, study medication and drug supply were provided by F. Hoffmann-La Roche Ltd., Basel, Switzerland. No funding source had any role in the collection, management, analysis, or interpretation of the data, nor in writing of the report or the decision to submit the paper for publication.

The trial coordinating center in Rotterdam performed the data analysis, wrote the manuscript and had final responsibility for the decision to submit for publication.

RESULTS

Of the 226 patients who were screened, 138 patients underwent randomization. Most patients who did not meet the inclusion criteria had a low HBV DNA level (56%) or a low ALT level (27%). The final modified intention-to-treat analysis included 133 (96%) of the 138 randomized patients. One patient randomly assigned to receive monotherapy (peginterferon alfa-2a and placebo) and 4 patients assigned to combination therapy (peginterferon alfa-2a and ribavirin) were excluded because 1 patient did not receive any study medication and 4 patients were found to be positive for HBeAg at baseline (figure 1). In the final analysis 69 patients were treated with monotherapy and 64 patients with combination therapy. The two groups were well balanced regarding clinical and virological characteristics at baseline. HBV genotype D was predominant (table 1).

Table 1: Baseline characteristics

Characteristics	Peginterferon alfa-2a and placebo (n=69)	Peginterferon alfa-2a and ribavirin (n=64)
Demography		
Mean (SD) age, years	42 (10)	43 (11)
Male/Female	54 (78%)/15 (22%)	44 (69%)/20 (31%)
Race		
Caucasian	66 (96%)	61 (95%)
Other	3 (4%)	3 (5%)
Laboratory results		
Median (IQR) ALT*	2.4 (1.7-4.1)	2.0 (1.6-3.7)
Mean (SD) HBV DNA, log copies/mL	6.9 (1.2)	6.8 (1.3)
Mean (SD) HBsAg, log IU/mL	3.8 (0.6)	3.8 (0.6)
HBV Genotype		
A	7 (10%)	10 (16%)
D	58 (84%)	49 (77%)
Other/mixed	4 (6%)	5 (8%)
Histology		
Median (IQR) necroinflammation	5 (4-7)	5 (3-7)
Median (IQR) fibrosis	3 (1-3)	3 (1-3)
Cirrhosis†	2 (3%)	2 (3%)
History		
Previous interferon therapy‡	17 (25%)	7 (11%)
Previous lamivudine therapy§	16 (23%)	8 (13%)

*Multiples of upper limit of the normal range
†Ishak fibrosis score 5-6
‡At least 4 weeks of (peg-)interferon treatment
§At least 3 months of lamivudine treatment

Table 2: Rates of virological and biochemical response

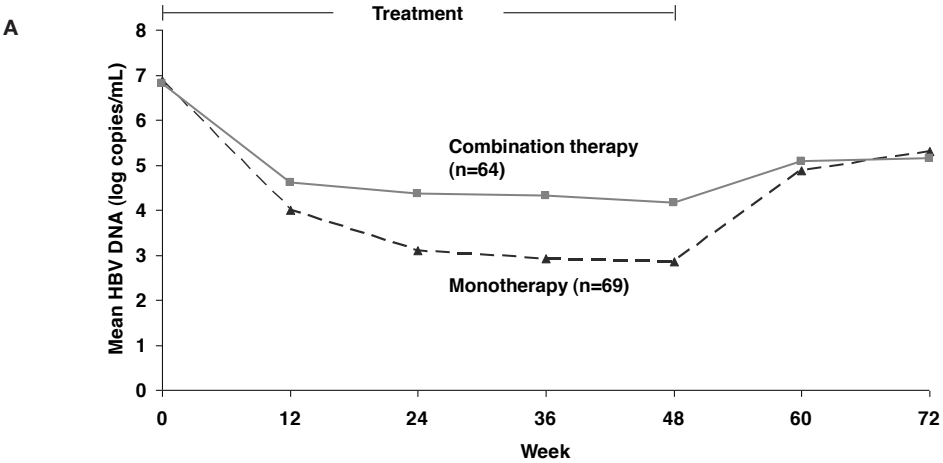
	End of treatment (Week 48)			End of follow-up (Week 72)		
	Peginterferon alfa-2a and placebo (n=69)	Peginterferon alfa-2a and ribavirin (n=64)	p	Peginterferon alfa-2a and placebo (n=69)	Peginterferon alfa-2a and ribavirin (n=64)	p
Virological response						
HBV DNA <10,000 copies/mL	46 (67%)	35 (55%)	0.16	14 (20%)	12 (19%)	0.82
HBV DNA <400 copies/mL	36 (52%)	18 (28%)	0.005	6 (9%)	4 (6%)	0.59
Mean (95% CI) change in HBV DNA (log copies/mL)	-3.9 (-3.5 to -4.4)	-2.6 (-2.1 to -3.2)	<0.001	-1.5 (-1.0 to -2.1)	-1.6 (-1.1 to -2.1)	0.90
Mean (95% CI) change in HBsAg (log IU/mL)	-0.56 (-0.30 to -0.81)	-0.34 (-0.09 to -0.59)	0.23	-0.57 (-0.29 to -0.85)	-0.44 (-0.18 to -0.69)	0.48
Biochemical response						
ALT normalization	28 (41%)	34 (53%)	0.15	28 (41%)	33 (52%)	0.20
Combined response						
ALT normalization and HBV DNA <10,000 copies/mL	25 (36%)	26 (41%)	0.60	14 (20%)	10 (16%)	0.49

At the end of treatment (week 48), there was no difference in the number of patients with a combined response (HBV DNA <10,000 copies/mL and a normal ALT level) between the monotherapy and combination therapy group (25 (36%) versus 26 (41%), p=0.60; table 2). After 24 weeks of follow-up (week 72), this response was sustained in a comparable proportion of patients in the two treatment arms (14 (20%) versus 10 (16%) patients, respectively, p=0.49; table 2).

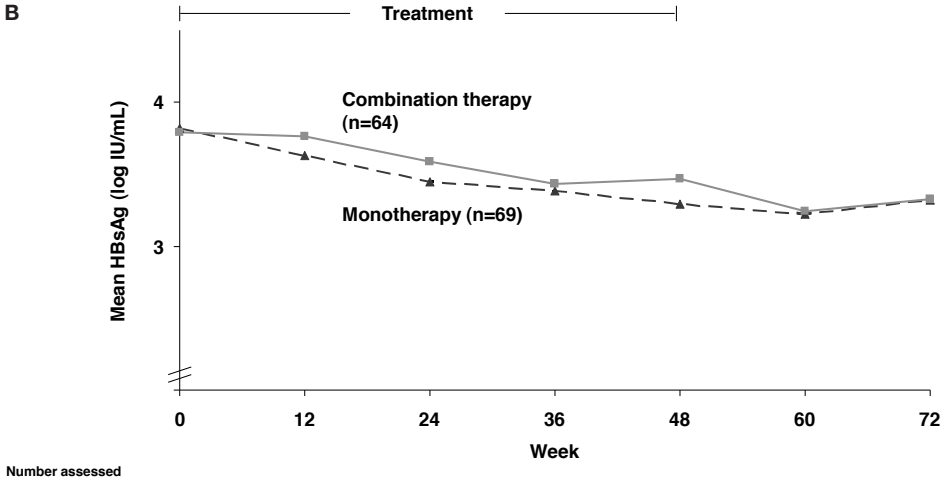
Peginterferon alfa-2a monotherapy induced greater on-treatment HBV DNA suppression compared to its combination with ribavirin (figure 2A; table 2). This was reflected in a higher number of patients with HBV DNA <400 copies/mL in the monotherapy group compared to the combination therapy group at the end of the 48-week treatment period (36 (52%) versus 18 (28%), p=0.005; table 2). In contrast, decline in HBV DNA concentration from baseline was similar for the two treatment regimens at week 72 (figure 2A; table 2).

One patient in the combination therapy group, a 24-year-old genotype D infected female, cleared HBsAg and developed anti-HBs. Loss of HBsAg did not occur in the monotherapy group. Mean HBsAg levels over time are displayed in figure 2B. Serum HBsAg consistently decreased during the 48-week treatment period, without significant differences between the two groups (mean change compared to baseline -0.56 versus -0.34 log IU/mL at week 48 in the monotherapy and combination therapy group, respectively, p=0.23; table2). HBsAg remained at end-of-treatment levels after treatment discontinuation (figure 2B; table 2).

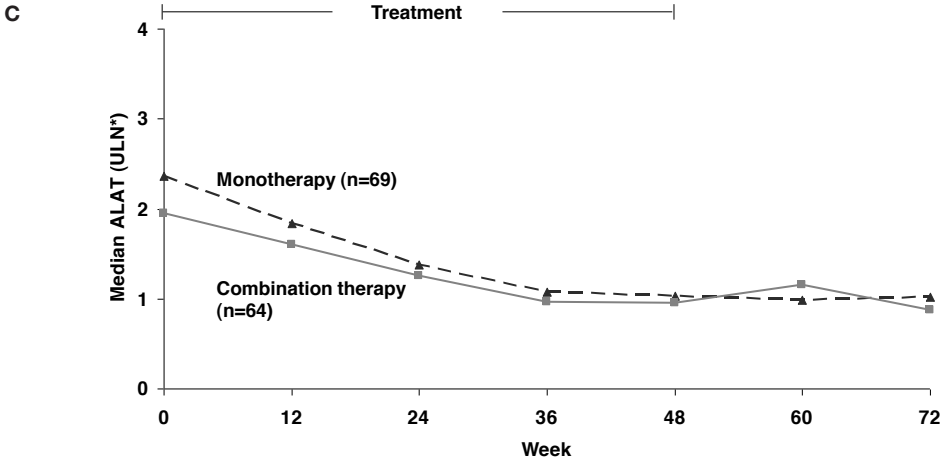
The rate of biochemical remission (normal ALT level) was slightly lower in patients receiving monotherapy compared to patients receiving combination therapy at week 48, but did not differ significantly between the groups (28 (41%) versus 34 (53%),



Number assessed							
Monotherapy	66	60	59	58	58	48	54
Combination therapy	64	60	60	53	59	55	55



Number assessed							
Monotherapy	69	60	59	58	57	48	54
Combination therapy	64	60	59	53	58	54	55



Number assessed							
Monotherapy	67	65	62	58	58	54	56
Combination therapy	64	61	60	58	59	57	58

Figure 2: Mean serum HBV DNA (panel A), mean serum HBsAg (panel B) and median serum ALT concentrations (panel C) during treatment and follow-up in patients receiving monotherapy (peginterferon alfa-2a and placebo) and combination therapy (peginterferon alfa-2a and ribavirin). *Multiples of upper limit of the normal range.

p=0.15; table 2). The number of patients with a biochemical response remained stable at week 72 (28 (41%) versus 33 (52%), p=0.20; table 2).

Paired liver biopsies of adequate quality were available for 36 patients in the monotherapy group and 39 patients in the combination therapy group. In 17 patients one or both

biopsies were judged to be of insufficient length or quality by the pathologist. Forty-one patients refused a second biopsy. By ranked assessment, a reduction in necroinflammation was observed in a significant proportion of patients in both patients treated with monotherapy and combination therapy (19 (53%) versus 19 (49%), p<0.001 compared to baseline for both groups). Necroinflammation scores worsened in few patients (3 (8%) versus 8 (21%), respectively). On average fibrosis scores did not change in both treatment groups. A decrease in fibrosis score was reported in a comparable number of patients receiving monotherapy and combination therapy (11 (31%) versus 10 (26%)). The fibrosis score deteriorated in 10 (28%) patients in the monotherapy group and 10 (26%) patients in the combination therapy group. Our results on the change of liver histology should be interpreted with caution, since the number of sustained responders tended to be higher in the group of patients for whom scored biopsies were available at baseline and week 72 (16 (21%) versus 8 (14%), p=0.26).

The majority of patients were infected with HBV genotype D (table 1). Twenty-three (21%) of the 107 genotype D infected patients had a combined response at week 72. None of the genotype A infected patients responded. Due to the low response rate, baseline characteristics predictive of a combined response at week 72 could not be identified using univariate logistic regression analyses. Variables analysed included HBV genotype, serum HBV DNA, HBsAg and ALT level and previous interferon or lamivudine therapy.

The most common adverse events are listed in table 3. Overall the number of patients with at least one reported adverse event was slightly higher in the combination therapy group than in the monotherapy group (58 (91%) versus 58 (84%)). Alopecia, anorexia and cough were reported more frequent in the patients treated with combination therapy, while myalgia occurred more often in patients receiving monotherapy. During the treatment period more patients in the combination therapy group than in the monotherapy group developed anemia and neutropenia (table 3). In both treatment groups there was a rapid increase in haemoglobin level and neutrophil counts after treatment discontinuation. Investigators reported 25 serious adverse events in 18 (14%) patients. Seven serious adverse events, 4 in the monotherapy and 3 in the combination therapy group, were probably related to therapy: anemia (3 cases), neutropenia, convulsion, rheumatoid arthritis and hypothyroidism.

Adverse events led to premature discontinuation of study medication in 9 (13%) of patients in the monotherapy group and 5 (8%) of patients in the combination therapy group (figure 1). In contrast to withdrawals, dose reduction or treatment interruption was necessary in more patients treated with combination therapy than with monotherapy (32 (50%) versus 20 (29%), p=0.013), mainly due to a reduction in haemoglobin level or neutrophil counts. Overall, the proportion of patients receiving at least 80% of peginterferon alfa-2a was comparable between the two groups (54 (78%) versus 48 (75%) patients receiving monotherapy and combination therapy, respectively).

Table 3: Adverse events

	Peginterferon alfa-2a and placebo (n=69)	Peginterferon alfa-2a and ribavirin (n=64)
	Number of patients (percent)	
Reported serious adverse event*	14 (20.3)	4 (6.3)
Reported adverse event (serious or non-serious)*	58 (84.1)	58 (90.6)
Fatigue	18 (26.1)	22 (34.4)
Pruritus	11 (15.9)	17 (26.6)
Influenza-like illness	16 (23.2)	11 (17.2)
Pyrexia	14 (20.3)	10 (15.6)
Myalgia†	17 (24.6)	7 (10.9)
Headache	10 (14.5)	12 (18.8)
Arthralgia	11 (15.9)	8 (12.5)
Cough†	5 (7.2)	13 (20.3)
Alopecia†	4 (5.8)	12 (18.8)
Nausea	7 (10.1)	9 (14.1)
Anorexia†	4 (5.8)	11 (17.2)
Asthenia	4 (5.8)	10 (15.6)
Hematological (laboratory data)		
Anemia (<10 g/dL)†	6 (8.7)	14 (21.9)
Neutropenia (<0.75x10^9/L)†	13 (18.8)	26 (40.6)
Neutropenia (<0.50x10^9/L)	3 (4.4)	6 (9.4)
Thrombocytopenia (<75x10^9/L)	3 (4.4)	3 (4.7)

*Values are based on all randomized patients who received at least 1 dose of study medication, patients may have had >1 (serious) adverse event
†p<0.05 (Chi-square test)

DISCUSSION

HBeAg-negative chronic hepatitis B patients are at high risk for disease relapse after discontinuation of any antiviral therapy. Peginterferon seems to be the only agent capable of inducing a true sustained off-treatment response in HBeAg-negative disease, but such a response is observed in a minority of HBeAg-negative patients.(18) However, the probability of HBsAg clearance, which is regarded as the best virological endpoint, increases over time in sustained virological responders.(26) Nucleos(t)ide analogues typically need to be continued indefinitely to maintain viral suppression, without a clearly defined endpoint for stopping treatment.(7) This approach increases the risk of antiviral resistance and the toxicity of continuous nucleos(t)ide analogue therapy on the long-term is unclear.

This study, which is only the second well-designed large randomized study on peginterferon in HBeAg-negative chronic hepatitis B, is important for several reasons. Firstly,

it shows that the proportion of patients with an off-treatment combined response, defined by HBV DNA <10,000 copies/mL and a normal ALT level at 24 weeks after treatment discontinuation, was not higher than 20%. Secondly, it shows that ribavirin did not add to the response of peginterferon alfa-2a in HBeAg-negative disease in any way. Thirdly, it shows that combination of peginterferon alfa-2a and ribavirin in HBV-infected patients was relatively well tolerated and that less patients had to discontinue therapy in comparison with HCV-infected patients.

The limited efficacy of peginterferon alfa-2a in this study population might be explained by the infecting HBV genotype. HBV genotype is increasingly recognized as a predictor of response to antiviral therapy in HBeAg-positive, and to a lesser extent HBeAg-negative chronic hepatitis B.(27-30) For example, HBeAg-positive patients infected with genotype A are more likely to respond to peginterferon than those with genotype D.(29) HBV genotype D was predominant in this study, for this reason a direct comparison between different genotypes was not possible. The response rate in genotype D patients (21%) was however slightly higher than the rate reported in a previous pivotal randomized trial with peginterferon in HBeAg-negative patients (16% of peginterferon alfa-2a monotherapy treated patients with HBV DNA <20,000 copies/mL and ALT normalization 24 weeks after treatment discontinuation).(18, 27) Overall the role of HBV genotype as predictor of response appears to be less well defined in HBeAg-negative disease, as compared to HBeAg-positive disease.(31)

With respect to the potential role of ribavirin in the treatment of chronic viral hepatitis, adding this nucleoside analogue to peginterferon in the treatment of chronic hepatitis C dramatically improved sustained response rates and has become the mainstay of therapy.(32) Although several effects of ribavirin have been proposed, including direct inhibition of HCV replication and immunomodulation, its major mechanism of action against HCV has not yet been elucidated.(33) In this study the combination of peginterferon alfa-2a and ribavirin was however not superior to peginterferon alfa-2a monotherapy in the treatment of HBeAg-negative chronic hepatitis B. Inversely, a significant lower number of patients in the combination therapy group had a profound HBV DNA decline (HBV DNA <400 copies/mL) at the end of treatment. The higher number of dose reductions in the combination therapy group is unlikely to explain the difference in HBV DNA decline since the number of patients receiving 80% of peginterferon alfa-2a was comparable between the two groups. The results of our study are in line with a previous randomized controlled trial from Taiwan, containing mainly patients infected with HBV genotype B and C, which did not show a benefit of combining ribavirin with conventional interferon in HBeAg-positive patients.(34) The addition of lamivudine to peginterferon alfa-2a did also not result in higher sustained response rates in HBeAg-negative chronic hepatitis B.(18) Different treatment regimens with stronger antiviral agents or extended treatment duration may improve treatment outcomes (35-37), the

latter approach may however be limited by the side-effects associated with interferon-based therapy. Continuation of peginterferon therapy in a reduced dose beyond one year may therefore provide another option, since several studies have suggested that the duration of interferon-based therapy, and not the actual interferon dose, is associated with sustained off-treatment response.(37, 38)

Neither baseline HBV DNA nor HBsAg levels predicted response to therapy in this study. In contrast, low baseline serum HBV DNA levels were significantly associated with a combined virological and biochemical response in one study involving peginterferon alfa-2a and/or lamivudine treated HBeAg-negative patients.(27) Serum HBV DNA and HBsAg kinetics clearly differed during the treatment phase. A marked and fast decrease in HBV DNA concentration was observed during therapy, whereas serum HBsAg levels decreased more gradually. HBV DNA levels relapsed after treatment discontinuation, but HBsAg remained at end-of-treatment levels. These findings suggest that the decline of serum HBsAg is more associated with clearance of infected hepatocytes than the decline of serum HBV DNA.

Regarding the tolerability and safety of therapy, the proportion of patients who prematurely discontinued treatment in the combination therapy group appeared to be somewhat lower than in HCV studies, although comparisons should be made with caution due to differences in study design and the different study population.(32, 39) Surprisingly, a high proportion of patients in the combination therapy group developed neutropenia relative to the monotherapy group. Otherwise there were no unexpected side-effects.

In conclusion peginterferon alfa-2a monotherapy for 48 weeks results in a limited sustained response rate in HBeAg-negative chronic hepatitis B patients. Combination therapy with peginterferon alfa-2a and ribavirin is not superior to peginterferon alfa-2a alone.

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APPENDIX

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Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir

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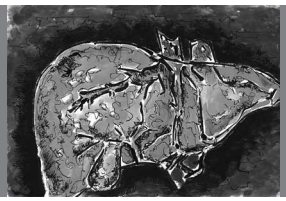

ABSTRACT

Background & Aims

We aimed to investigate serum hepatitis B surface antigen (HBsAg) levels in patients with chronic hepatitis B virus (HBV) infection during peginterferon (PEG-IFN) and entecavir (ETV) monotherapy.

Methods

HBsAg was quantified (Abbott ARCHITECT) at baseline and during antiviral therapy (weeks 12, 24, 36, 48) in hepatitis B e antigen (HBeAg-)positive patients treated with ETV (N=33) or PEG-IFN (N=61) and in HBeAg-negative patients treated with ETV (N=37) or PEG-IFN (N=69).

Results

Within the HBeAg-positive population, patients treated with PEG-IFN tended to have a steeper HBsAg decline than ETV-treated patients (mean decline 0.94 versus 0.38 log IU/mL at week 48, p=0.07 for comparison of the slope of HBsAg decline). The HBsAg decline was largest in those who became HBeAg negative, irrespective of treatment regimen. A decline of HBsAg was confined to ETV-treated patients with elevated baseline alanine aminotransferase (ALT) levels, whereas HBsAg decline was not associated with baseline ALT in patients treated with PEG-IFN. Within the HBeAg-negative population, PEG-IFN induced a significant HBsAg decline, while HBsAg did not decrease in ETV-treated patients (0.56 versus -0.10 log IU/mL, p<0.001). Both in HBeAg-positive and HBeAg-negative patients, decline of serum HBV DNA was larger in patients who received ETV compared with PEG-IFN.

Conclusions

In HBeAg-positive patients, decline of serum HBsAg is largely confined to patients who clear HBeAg, either induced by PEG-IFN or ETV. In HBeAg-negative patients, PEG-IFN therapy resulted in a significant reduction in HBsAg levels, whereas HBsAg did not decrease in ETV-treated patients.

INTRODUCTION

Chronic hepatitis B (CHB) can be controlled in most patients with the currently available treatment options, but complete eradication of the hepatitis B virus (HBV) is rarely achieved. HBV covalently closed circular DNA (cccDNA) plays a major role in viral persistence and its clearance is thought to be the limiting factor in eliminating infection (1). Previous studies demonstrated that both (pegylated) interferon (IFN) and nucleos(t)ide analogue (NA) therapy result in a reduction of intrahepatic cccDNA (2-4). In addition, intrahepatic cccDNA was shown to be a strong predictor of sustained off-treatment virological response (5). Serum hepatitis B surface antigen (HBsAg) levels are known to reflect cccDNA in the liver, and reduction of HBsAg levels correlates well with that of cccDNA (2-4).

HBsAg clearance from serum approximates clinical cure and is associated with improved survival (6). The kinetics of HBsAg decline have recently been described in patients treated with standard IFN, PEG-IFN, lamivudine (LAM), and adefovir (ADV) monotherapy (2, 7, 8). It was demonstrated that measurement of the serum HBsAg concentration during therapy may allow the identification of sustained responders to PEG-IFN more reliably than serum HBV DNA (9). However, the effect of potent NA such as entecavir (ETV) and tenofovir (TDF) on serum HBsAg levels is unknown. Furthermore, the efficacy of PEG-IFN in terms of HBsAg decline was only compared to inferior oral agents such as LAM and ADV.

The aim of our study was (1) to assess on-treatment serum HBsAg kinetics in hepatitis B e antigen (HBeAg-)positive and HBeAg-negative CHB patients treated with PEG-IFN or ETV monotherapy, (2) to compare the efficacy of PEG-IFN and ETV monotherapy in terms of HBsAg decline, and (3) to identify baseline factors associated with HBsAg decline after 48 weeks of antiviral therapy.

PATIENTS AND METHODS

Study population

We studied all consecutive HBV-monoinfected patients treated with ETV monotherapy for at least 48 weeks between January 2005 and May 2008 at the Erasmus MC University Medical Center Rotterdam. Patients treated with PEG-IFN monotherapy were derived from two randomized controlled trials (total treatment duration 48 and 52 weeks) (10, 11). The studies conformed to the ethical guidelines of the Declaration of Helsinki. Informed consent was obtained from all patients.

Laboratory tests

Patients attended the outpatient clinic at least every 12 weeks for routine examinations and laboratory assessments. Serum alanine aminotransferase (ALT) levels were measured using automated techniques and are expressed as values representing a ratio to the upper limit of the normal range (ULN). Determination of HBeAg and antibody against HBeAg (anti-HBe) status was performed using commercially available enzyme immunoassays. Serum HBsAg was quantified at baseline and during antiviral therapy (weeks 12, 24, 36 and 48) using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL). Serum HBV DNA levels were measured using commercial TaqMan polymerase chain reaction (PCR) assays (Roche Molecular Systems; lower limit of detection 70 copies/mL), except for the HBeAg-positive patients treated with PEG-IFN for whom an in-house-developed TaqMan PCR assay based on the EuroHep standard was used (lower limit of detection 400 copies/mL) (12). It has previously been demonstrated that there is an excellent correlation between these assays (13)

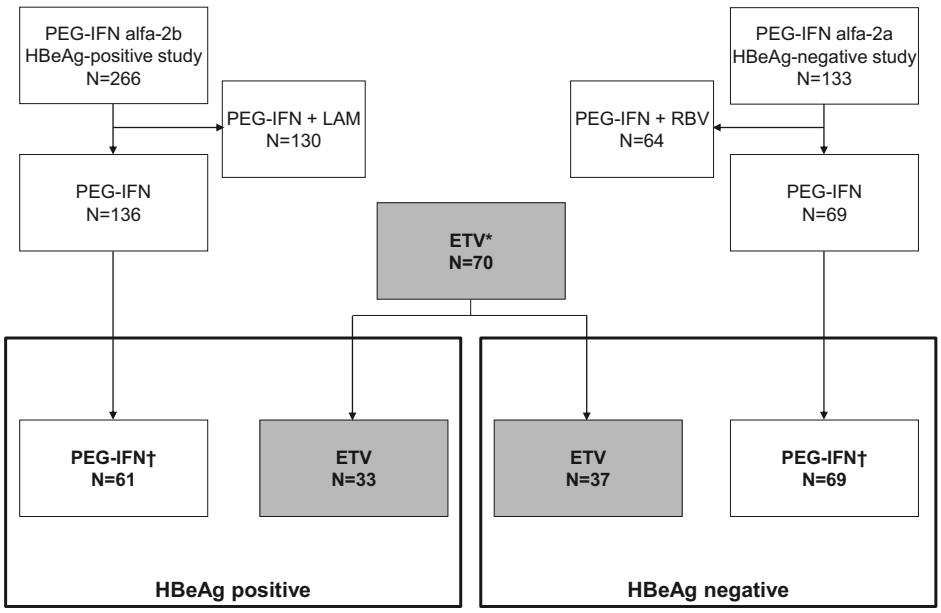

Figure 1: Study profile.
*All consecutive patients treated with ETV monotherapy for at least 48 weeks were included.
†Patients treated with PEG-IFN monotherapy were randomly selected from two randomized controlled trials (10,11), the PEG-IFN and ETV groups were group-matched according to their baseline HBV DNA level.
RBV: ribavirin.

Statistical analysis

Group-matching between the ETV and PEG-IFN groups was performed by their baseline HBV DNA concentration and aimed at a 2:1 ratio in order to increase power, as described by Pocock (14). Baseline HBV DNA was selected, because this factor was previously found to be associated with the degree of HBsAg decline during PEG-IFN therapy (7).

Serum HBsAg and HBV DNA levels were logarithmically transformed for analysis. Continuous variables are presented as mean (standard deviation) or median (interquartile range), where appropriate. The lower limit of detection of 400 copies/mL of the in-house PCR assay was applied to all HBV DNA results to allow comparison between the treatment groups. Continuous variables were compared using the t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square or Fisher's exact test. The association between baseline factors and the degree of HBsAg decline was assessed by linear regression analyses applying mixed modelling techniques with a random intercept and a random slope per subject, and with a covariance structure depending on the treatment regimen. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

Baseline characteristics

At total of 200 HBV-infected patients were included. The HBeAg-positive population consisted of 61 patients treated with PEG-IFN and 33 patients treated with ETV. The HBeAg-negative population consisted of 69 patients treated with PEG-IFN and 37 ETV-treated patients (Fig.1). Baseline characteristics are presented in Table 1. HBeAg-positive patients treated with PEG-IFN and ETV were comparable at baseline except for serum ALT level, distribution of HBV genotypes and prevalence of liver cirrhosis (Table 1). Within the HBeAg-negative population, the treatment groups were balanced except for ethnicity, distribution of HBV genotypes and prevalence of liver cirrhosis (Table 1).

HBeAg-positive patients tended to be younger (38 versus 41 years, $p=0.10$) and had higher baseline serum HBV DNA and HBsAg levels compared with HBeAg-negative patients (8.4 versus 6.8 log copies/mL for HBV DNA and 4.2 versus 3.8 log IU/mL for HBsAg, $p<0.001$ for both comparisons), while median ALT levels were similar (2.3 versus 2.5 ULN, $p=0.42$).

Table 1: Baseline characteristics

Characteristics	HBeAg positive		p	HBeAg negative		p
	PEG-IFN (N=61)	ETV (N=33)		PEG-IFN (N=69)	ETV (N=37)	
Mean (SD) age, years	38 (13)	38 (15)	0.93	42 (10)	40 (12)	0.59
Sex, male (%)	51 (84%)	27 (82%)	0.83	54 (78%)	28 (76%)	0.76
Race (%)			0.12			<0.001
Caucasian	44 (72%)	22 (67%)		66 (96%)	19 (51%)	
Asian	15 (25%)	6 (18%)		1 (1%)	9 (24%)	
Other	2 (3%)	5 (15%)		2 (3%)	9 (24%)	
Median (IQR) ALT*	3.2 (2.3-5.4)	1.5 (1.1-2.4)	<0.001	2.4 (1.7-4.1)	2.2 (1.5-3.3)	0.26
Mean (SD) HBV DNA, log copies/mL	8.6 (1.1)	8.0 (2.1)	0.09	6.9 (1.2)	6.7 (1.7)	0.67
Mean (SD) HBsAg, log IU/mL	4.2 (0.8)	4.3 (0.7)	0.51	3.8 (0.6)	3.7 (0.7)	0.41
HBV Genotype (%)			0.04			<0.001
A	29 (48%)	13 (39%)		7 (10%)	5 (14%)	
B	0	4 (12%)		0	3 (8%)	
C	11 (18%)	2 (6%)		1 (1%)	8 (22%)	
D	14 (23%)	10 (30%)		58 (84%)	20 (54%)	
Other/mixed	7 (12%)	4 (12%)		3 (4%)	1 (3%)	
Presence of cirrhosis (%)	9 (15%)	12 (36%)	0.02	2 (3%)	9 (24%)	0.002

*Multiples of upper limit of the normal range

Baseline serum HBsAg and HBV DNA levels showed a significant positive correlation in HBeAg-positive patients (R=0.54, p<0.001), while HBsAg and HBV DNA were not correlated in HBeAg-negative patients (R=0.09, p=0.36).

HBeAg-positive patients

Virological and biochemical response rates

Within the HBeAg-positive population, PEG-IFN therapy resulted in a higher rate of HBeAg clearance at week 48 compared to ETV (21 (34%) versus 3 (9%) patients, p=0.007; Table 2). HBsAg loss (HBsAg <0.05 IU/mL) occurred in 6 (10%) patients in the PEG-IFN group, but was not achieved in patients treated with ETV (p=0.09; Table 2). In contrast, the proportion of patients with HBV DNA <400 copies/mL at week 48 was higher in the ETV group (17 (52%) versus 10 (16%) patients in the ETV and PEG-IFN group, respectively; p<0.001; Table 2).

Table 2: Rates of virological and biochemical response

Response at week 48	HBeAg positive		p	HBeAg negative		p
	PEG-IFN (N=61)	ETV (N=33)		PEG-IFN (N=69)	ETV (N=37)	
HBV DNA <400 copies/mL	10 (16%)	17 (52%)	<0.001	36 (52%)	31 (84%)	0.001
HBeAg clearance	21 (34%)	3 (9%)	0.007	-	-	-
HBeAg seroconversion	16 (26%)	3 (9%)	0.05	-	-	-
HBsAg clearance	6 (10%)	0	0.09	0	0	-
HBsAg seroconversion	4 (7%)	0	0.29	0	0	-
ALT normalization	21 (34%)	16 (48%)	0.18	28 (41%)	27 (73%)	0.001

On-treatment HBsAg and HBV DNA decline

The decline of serum HBsAg during 48 weeks of monotherapy with PEG-IFN and ETV in HBeAg-positive patients is displayed in Figure 2A. HBsAg decreased significantly during PEG-IFN therapy (mean decline 0.94 log IU/mL at week 48, p<0.001) and to a lesser extent in ETV-treated patients (0.38 log IU/mL, p=0.07). The difference in HBsAg decline was not significant between these two groups (p=0.15). Figure 2B shows the decline of serum HBV DNA for the two treatment groups. HBV DNA levels decreased significantly during PEG-IFN and ETV therapy (p<0.001 compared to baseline in both groups). In contrast to HBsAg, suppression of HBV DNA was stronger in the ETV-treated patients (mean decline 4.5 versus 2.2 log copies/mL at week 48 in the ETV and PEG-IFN group, respectively; p<0.001).

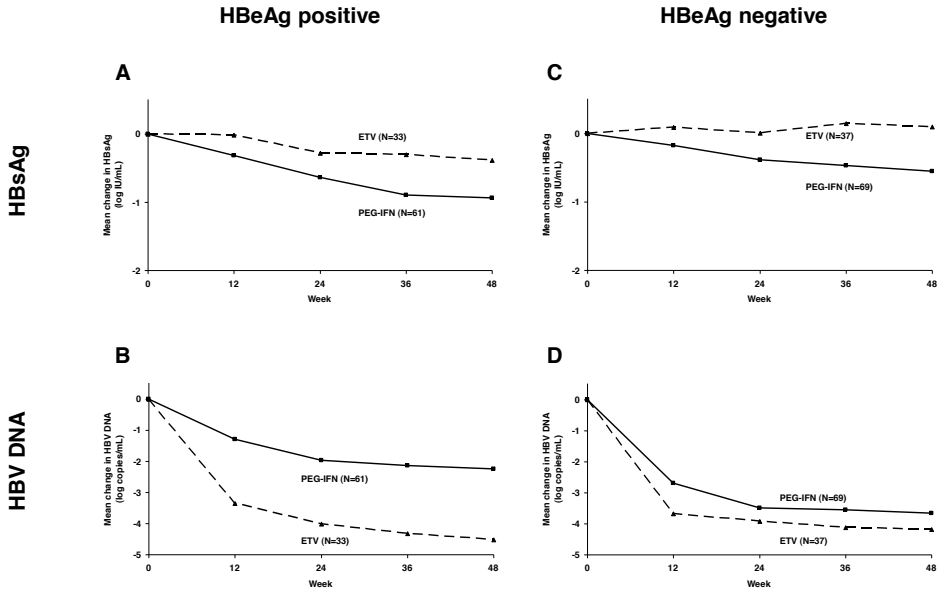

Figure 2: Mean change compared to baseline for (A) HBsAg and (B) HBV DNA levels in HBeAg-positive patients and for (C) HBsAg and (D) HBV DNA levels in HBeAg-negative patients treated with PEG-IFN and ETV.

Baseline factors associated with HBsAg decline

By univariate analysis, the baseline factors age, serum ALT level, serum HBV DNA level and HBV genotype (A versus non-A) were significantly associated with HBsAg decline. Patients treated with PEG-IFN tended to have a steeper HBsAg decline compared to patients treated with ETV (p=0.07). After correction of the model for each of these factors in multivariate analysis, a higher HBsAg decline in patients treated with PEG-IFN compared to ETV remained, yet this difference did not reach the level of significance (all p<0.17). Interestingly, HBsAg levels only decreased in ETV-treated patients with elevated ALT levels at baseline (mean decline -0.03 log IU/mL for ALT ≤2 ULN versus 1.03 for ALT >2 ULN at week 48, p=0.007), whereas HBsAg decline was comparable in both ALT categories in HBeAg-positive patients treated with PEG-IFN (Fig. 3). Although the initial decline of HBsAg was more pronounced in the PEG-IFN group with ALT >2 ULN and ETV group with ALT >2 ULN compared with the PEG-IFN group with ALT ≤2 ULN (mean decline 0.67 and 0.73 versus 0.42 log IU/mL at week 24, respectively), this difference was not significant, potentially due to the limited number of patients treated with PEG-IFN who had baseline ALT ≤2 ULN (p=0.61).

Association between HBsAg decline and HBeAg loss at week 48

The magnitude of HBsAg decline was larger in the 24 patients who lost HBeAg compared to patients who remained HBeAg positive after 48 weeks of therapy (mean

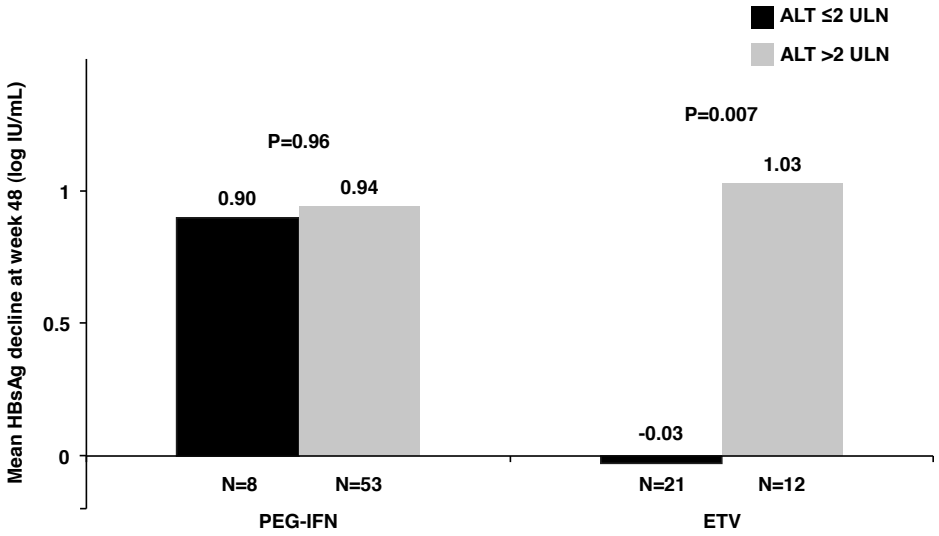

Figure 3: Degree of HBsAg decline after 48 weeks of PEG-IFN and ETV therapy for HBeAg-positive patients according to the categories of baseline ALT ≤2 ULN and >2 ULN. Within the HBeAg-negative population, decline of HBsAg was only related to treatment regimen and not to baseline ALT or any other baseline variable.

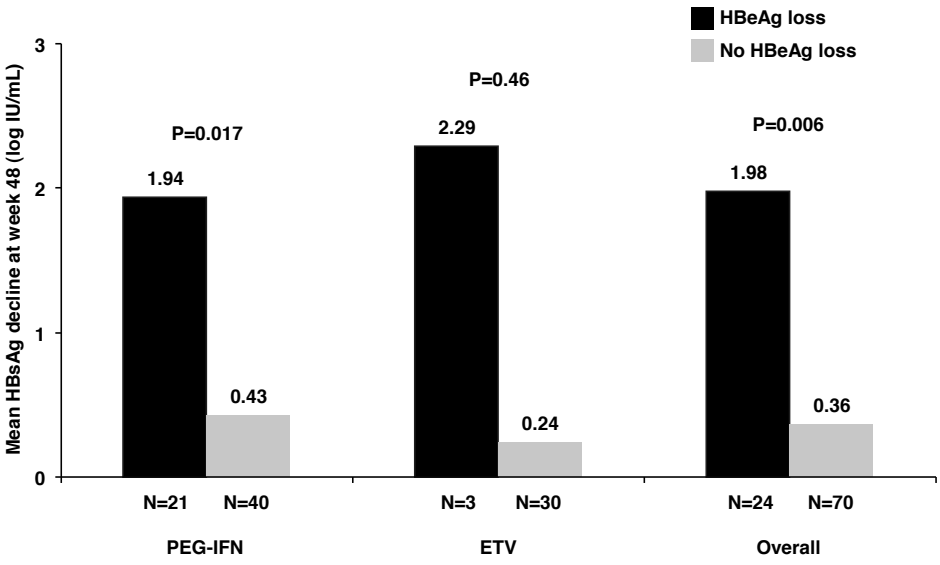

Figure 4: Degree of HBsAg decline at week 48 of PEG-IFN and ETV therapy for HBeAg-positive patients according to the achievement of HBeAg loss after 48 weeks of treatment.

decline 1.98 versus 0.36 log IU/mL at week 48, p=0.006; Fig. 4). Interestingly, the magnitude of HBsAg decline in the patients who achieved HBeAg loss was not associated with treatment regimen (1.94 and 2.29 log IU/mL for PEG-IFN and ETV, respectively, p=0.85; Fig. 4).
The decline of HBV DNA was also associated with HBeAg loss at week 48 (4.6 versus 2.6 log copies/mL for patients who were HBeAg negative at week 48 compared to patients who remained HBeAg positive, p<0.001), irrespective of treatment regimen (4.6 versus 4.9 log copies/mL for PEG-IFN and ETV, respectively, p=0.77).

HBeAg-negative patients

Virological and biochemical response rates

Within the HBeAg-negative population, the number of patients with HBV DNA <400 copies/mL at week 48 was higher in the ETV group as well (31 (84%) versus 36 (52%) patients, p=0.001; Table 2). Neither in the PEG-IFN nor in the ETV group HBsAg clearance was observed.

On-treatment HBsAg and HBV DNA decline

The decline of serum HBsAg in HBeAg-negative patients is shown in Figure 2C. Serum HBsAg consistently decreased in the PEG-IFN group (p<0.001 compared to baseline), while HBsAg levels did not change during ETV therapy (mean decline 0.56 versus -0.10 log IU/mL at week 48, p<0.001). Figure 2D shows the decline of serum HBV DNA for the PEG-IFN and ETV group. HBV DNA levels decreased significantly in both treatment groups (p<0.001 compared to baseline), but viral suppression tended to be stronger in the ETV group (mean decline 4.2 for ETV versus 3.7 log copies/mL for PEG-IFN at week 48, p=0.13).

Baseline factors associated with HBsAg decline

Multivariate analysis showed that the decline of HBsAg was only related to treatment regimen (p<0.001) and not to any other baseline variable.

DISCUSSION

This is the first detailed study comparing on-treatment serum HBsAg kinetics in patients with both HBeAg-positive and HBeAg-negative CHB receiving either PEG-IFN or ETV monotherapy. In HBeAg-positive patients, decline of HBsAg was significantly associated with HBeAg loss, and, subsequently, HBsAg decline tended to be higher in PEG-IFN treated subjects compared to ETV-treated subjects (p=0.07). Interestingly,

patients who achieved HBeAg loss during either PEG-IFN or ETV therapy demonstrated a similar reduction in HBsAg levels. In contrast, in HBeAg-negative patients, only treatment with PEG-IFN resulted in a significant HBsAg decline, whereas HBeAg-negative patients treated with ETV demonstrated no HBsAg reduction at all.

In HBeAg-positive patients, previous studies suggested that serum HBsAg levels gradually decrease during LAM and ADV therapy (2, 15). Our study indicates that a decline of HBsAg in HBeAg-positive CHB is primarily confined to ETV-treated patients with a baseline serum ALT >2 ULN, and can be attributed to a large extent to patients achieving HBeAg loss. This finding suggests that the presence of an active preexisting immune response against HBV is required to lower HBsAg levels for patients treated with ETV. In contrast to ETV, PEG-IFN is able to modulate immune reactivity itself (16), which is underlined by our observation that PEG-IFN therapy reduced serum HBsAg levels irrespective of baseline ALT level, yet was also mostly confined to patients who demonstrated HBeAg loss after one year of therapy. Even more interesting is that a similar decline of HBsAg was observed in patients who achieved HBeAg loss during either PEG-IFN or ETV therapy.

With regard to our findings in HBeAg-negative patients, it has recently been demonstrated by Brunetto et al. that serum HBsAg levels do not decrease during 48 weeks of LAM therapy (7). Despite the higher antiviral potency of ETV compared with LAM, ETV therapy for 48 weeks did also not result in a reduction of serum HBsAg in HBeAg-negative patients in our study. However, a study from Greece suggested that HBsAg levels decrease in HBeAg-negative patients during long-term LAM monotherapy, although at a significantly slower rate compared with IFN (8). Thus, a longer duration of NA therapy may be required to reduce HBsAg levels in HBeAg-negative CHB. The study from Brunetto et al. also demonstrated a significant on-treatment HBsAg decline of 0.71 log IU/mL in HBeAg-negative patients treated with PEG-IFN monotherapy for 48 weeks, which is somewhat higher compared with our study (0.56 log IU/mL) (7). This difference may be caused by the predominance of HBV genotypes B and C in their study population. HBV genotype D was predominant among the HBeAg-negative patients treated with PEG-IFN in our study, therefore a direct comparison between different genotypes was not possible. However, the degree of HBsAg decline induced by PEG-IFN appears to be less pronounced in genotype D compared with other genotypes (7).

Quantitative HBsAg in serum reflects the cccDNA concentration in the liver, which plays a major role in viral persistence (2-4). It has been shown that ADV monotherapy is able to decrease intrahepatic cccDNA. Yet, when ADV was combined with PEG-IFN, clearance of cccDNA was enhanced, and in contrast to ADV monotherapy, it also resulted in a strong reduction of HBs-antigen- and HBc-antigen-positive hepatocytes (3). Our study, in fact, confirms previous observations that immune modulation is of vital importance to completely eradicate HBV. First, PEG-IFN resulted in higher HBeAg

loss rates compared to ETV, and in significant HBsAg decline in both HBeAg-negative and HBeAg-positive patients. Second, although ETV demonstrated to be an potent inhibitor of viral replication, a significant decline of HBsAg was only observed in those patients with preexisting immune activity, reflected by high baseline ALT levels, and in those patients who achieved HBeAg loss after one year of therapy. Moreover, in HBeAg-negative patients, ETV therapy did not even result in HBsAg decline at all.

Our study is limited by the suboptimal study design, which lacked the possibility to randomize at baseline, and therefore led to somewhat different patient characteristics between the two treatment groups at baseline. The higher rate of HBeAg loss among HBeAg-positive patients treated with PEG-IFN compared with ETV may partially be caused by the higher baseline ALT level and the slightly unbalanced distribution of HBV genotypes. However, for the analysis of HBsAg decline in HBeAg-positive patients we corrected for confounders such as HBV genotype, HBV DNA and age using multivariate analysis, and performed a stratified analysis for baseline ALT. Cirrhosis was not associated with HBsAg decline. Furthermore, it should be noted that the predictors of HBsAg decline in our study are exactly the same as the predictors of HBeAg loss identified in previous studies, which only supports our finding that in HBeAg-positive patients reduction in HBsAg levels is largely confined to those patients achieving HBeAg loss during antiviral therapy (17-19). Within the HBeAg-negative population, the only independent factor that was associated with HBsAg decline was treatment regimen. No other baseline factors were related to HBsAg decline, and therefore have not influenced the study results.

In summary, in HBeAg-positive patients decline of serum HBsAg is largely confined to those patients that achieved HBeAg loss during antiviral therapy, either induced by PEG-IFN or ETV therapy. In HBeAg-negative patients PEG-IFN resulted in a significant reduction in HBsAg levels, whereas ETV resulted in no HBsAg decline at all. No other factors were related to HBsAg decline in HBeAg-negative patients.

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3

Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline

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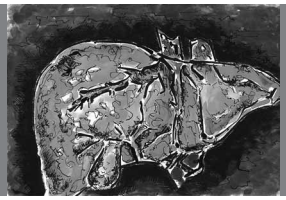


ABSTRACT

Serum hepatitis B surface antigen (HBsAg) levels may reflect the immunomodulatory efficacy of peginterferon (PEG-IFN). We investigated within a large randomised trial whether quantitative HBsAg levels predict response to PEG-IFN in HBeAg-positive chronic hepatitis B patients. Serum HBsAg was measured in samples taken at baseline, week 4, 8, 12, 24, 52 and 78 of 221 patients treated with PEG-IFN alfa-2b±lamivudine for 52 weeks. HBsAg decline was compared between treatment arms and between responders and non-responders. Response was defined as HBeAg loss with HBV DNA<10,000 copies/mL at 26 weeks post-treatment (week 78); 43 of 221 (19%) patients achieved a response. One year of PEG-IFN ± lamivudine resulted in a significant decline in serum HBsAg, which was sustained post-treatment (decline 0.9 log IU/mL at week 78, $P<0.001$). Patients treated with combination therapy experienced a more pronounced on-treatment decline, but relapsed subsequently. Responders experienced a significantly more pronounced decline in serum HBsAg compared to non-responders (decline at week 52: 3.3 versus 0.7 log IU/mL, $P<0.001$). Patients who achieved no decline at week 12 had a 97% probability of non-response through post-treatment follow-up and no chance of HBsAg loss. In a representative subset of 149 patients similar results were found for prediction through long-term (mean 3.0 years) follow-up.

Conclusion

PEG-IFN induces a significant decline in serum HBsAg in HBeAg-positive patients. Patients who experience no decline from baseline at week 12 have little chance of achieving a sustained response and no chance of HBsAg loss and should be advised to discontinue therapy with PEG-IFN.

INTRODUCTION

Chronic hepatitis B (CHB) is a major health problem, affecting more than 350 million people worldwide. Prolonged infection with the hepatitis B virus (HBV) may ultimately result in severe liver-related morbidity and mortality, and treatment of CHB is therefore indicated in patients with persistent liver inflammation.(1-4) The ideal outcome of treatment of CHB would be complete eradication of HBV, but this is only scarcely, if ever, achieved, for HBV covalently closed circular DNA (cccDNA) persists in host hepatocytes.(5) Therefore, the main goal of therapy is to halt the progression of liver inflammation to fibrosis, cirrhosis or hepatocellular carcinoma.(6, 7)

Current treatment options for CHB consist of nucleo(s)tide analogues (NA) and (pegylated) interferons (PEG-IFN). Antiviral treatment with NA aims at inhibiting viral polymerase activity,(8) and the most recently approved NA can effectively maintain suppression of HBV DNA levels for prolonged periods of time in the vast majority of patients.(9-11) Nevertheless, PEG-IFN remains an important first-line treatment option for CHB, especially in hepatitis B e antigen (HBeAg)-positive disease, since a long-term off-treatment sustained response can be achieved in about 25% of patients after a finite treatment course.(12-14) Response to IFN-based therapy in these patients is accompanied by high rates of hepatitis B surface antigen (HBsAg) seroconversion,(15) a reduced incidence of hepatocellular carcinoma and prolonged survival.(16, 17)

The development of a durable off-treatment response is attributed to the immunomodulatory effect of PEG-IFN,(18) which results in a decrease in intrahepatic cccDNA.(19) CccDNA levels at the end of therapy are indeed predictive of a sustained off-treatment response,(20) but since these can only be assessed invasively the clinical utility is limited. Recent studies report an excellent correlation between decline in intrahepatic cccDNA and serum HBsAg levels in HBeAg-positive patients.(5, 21) A decline in serum HBsAg levels may therefore reflect the efficacy of PEG-IFN in decreasing intrahepatic cccDNA and consequently predict a sustained off-treatment response.

The aims of our study were to investigate the effects of one year of PEG-IFN ± lamivudine (LAM) therapy on serum HBsAg levels in HBeAg-positive CHB patients, and to describe the relationship between on-treatment HBsAg decline and a sustained off-treatment response.

PATIENTS AND METHODS

Patients

In this study serum HBsAg levels were assessed in HBeAg-positive CHB patients who were previously enrolled in an investigator-initiated multicenter randomized controlled

trial and a subsequent long-term follow-up (LTFU) study.(12, 13) Patients were eligible for the initial study if they had been HBsAg positive for at least 6 months prior to randomization, were HBeAg positive on two occasions within 8 weeks prior to randomization, had elevated serum alanine aminotransferase (ALT) levels of 2 - 10 times the upper limit of normal (ULN), and had a serum HBV DNA concentration above 1.0 x 10^5 copies/mL. Key exclusion criteria were: antiviral therapy within 6 months prior to randomization, presence of viral co-infections, pre-existing cytopenia or decompensated liver disease. Treatment comprised of PEG-IFN alfa-2b 100 µg weekly (PegIntron, Schering-Plough, Kenilworth, NJ, USA) in combination with placebo or LAM (Zeffix, GlaxoSmithKline, Greenford, UK) 100 mg daily for 52 weeks. To limit the probability of early treatment discontinuation, the dose of PEG-IFN was reduced to 50 µg per week after 32 weeks of treatment. Patients attended the outpatient clinic at least every 4 weeks for routine examinations and laboratory assessments during both the treatment and the post-treatment follow-up phase of the initial study. For the LTFU study, patients were re-evaluated at one additional visit at the local participating center. The mean duration of follow-up was 3 years.(12)

Inclusion criteria for the present analysis were completion of the 26-week follow-up phase of the main study and availability of a baseline serum sample for HBsAg quantification. Of the 266 patients in the initial study, 221 fulfilled these criteria. Of these patients, 149 participated in the associated LTFU study.(12) The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written informed consent according to standards of the local ethics committees.

Laboratory measurements

Serum HBsAg was quantified in samples taken at baseline, during the treatment period (weeks 4, 8, 12, 24 and 52) and during follow-up (week 78) using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05 - 250 IU/mL).(22) HBV DNA quantification for the initial study was performed with 4-week intervals using an in-house developed TaqMan polymerase chain reaction (PCR) assay (lower limit of quantification 400 copies/mL) based on the EuroHep standard.(23) For the LTFU study, HBV DNA was measured with the Cobas TaqMan HBV assay (Roche Molecular Systems, Branchburg, NJ, USA), with a dynamic range of quantification of 174 - 6.4 x 10^8 copies/mL (30 - 1.1 x 10^8 IU/mL). It has previously been demonstrated that there is an excellent correlation between the two assays.(12) HBeAg was assessed using EIA (AxSYM, Abbott, Abbott Park, IL, USA) or ELISA (DiaSorin SpA, Saluggia, Italy). ALT was measured locally in accordance with standard procedures and is presented as multiples of the upper limit of normal (ULN). HBV genotype was assessed using the INNO-LiPA assay (Innogenetics).

Statistical analysis

For the current study a composite endpoint of HBeAg loss and HBV DNA level <10,000 copies/mL was chosen for definition of response.(24) Patients who were retreated after the initial study were considered non-responders at LTFU. Associations between variables were tested using Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents when appropriate. The differences in HBsAg decline between treatment arms and (non-)responders were analysed using repeated measurement models with an unstructured covariance allowing heterogeneity across compared groups. Discrimination, or the ability of HBsAg concentration and decline at various time-points to distinguish patients who will develop a response from those who will not, was quantified by the area under the receiver-operating characteristic curve (AUC). Our aim was to use on-treatment HBsAg levels to identify a stopping rule that would enable a clinician to discontinue patients who had a very low chance of response as early as possible, while maintaining >90% of responders on treatment. The optimal cut-off in HBsAg decline was identified using a grid-search of possible cut-off points at weeks 4, 8, 12, and 24. For each cut-off point the chi-square test was calculated together with the sensitivity and the negative predictive value (NPV). The highest chi-square identified the optimal cut-off point.(25) SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used to perform statistical analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

Patient characteristics

The characteristics of the 221 patients are shown in table 1 according to assigned treatment regimen. Patients were comparable across both groups with regard to age, race, HBV genotype distribution, baseline prevalence of cirrhosis and ALT and HBV DNA levels. Overall, 43 (19%) patients had a response at week 78, and these patients were distributed equally across the two study arms. Baseline mean serum HBsAg was 4.4 log IU/mL in both treatment groups. Serum HBsAg was positively correlated with HBV DNA (r = 0.66, $P<0.01$) and inversely correlated with age (r = -0.16, $P = 0.02$) but did not correlate with ALT. Variation was observed in pre-treatment HBsAg levels between genotypes, with the highest baseline levels in genotypes A and D (mean 4.5 log IU/mL for both) and lower levels in genotypes B (mean 4.3 log IU/mL) and C (mean 3.8 log IU/mL) ($P<0.001$ for genotype C versus other genotypes with Bonferroni correction).

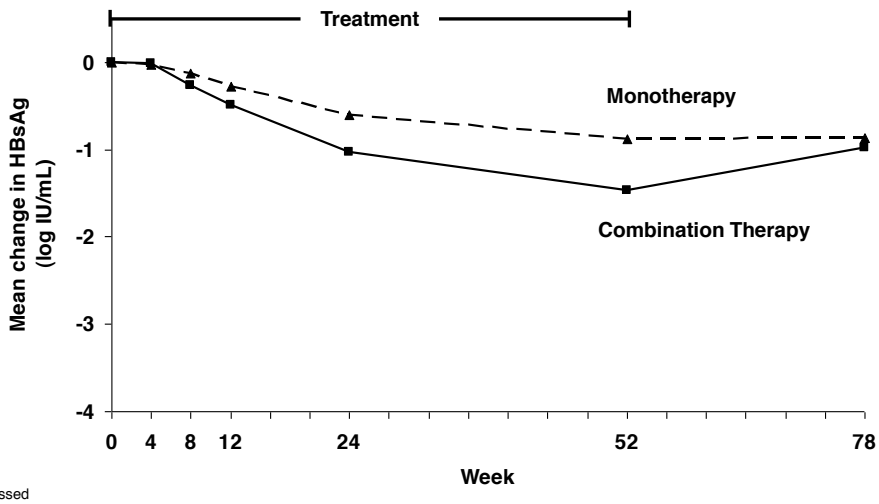
Table 1: Patient characteristics according to treatment regimen

Characteristics	PEG-IFN and placebo (n=111)	PEG-IFN and lamivudine (n=110)	P
Demography			
Mean (SD) age, years	34 (13)	33 (12)	0.52
Male	90 (81%)	83 (75%)	0.33
BMI	24.2 (3.4)	25.1 (4.5)	0.10
Race			0.31
Caucasian	80 (72%)	80 (73%)	
Asian	25 (23%)	19 (17%)	
Other	6 (5%)	11 (10%)	
Laboratory results			
Mean (SD) ALT*	4.3 (3.0)	4.2 (3.0)	0.75
Mean (SD) HBV DNA, log copies/mL	9.1 (0.8)	9.1 (1.0)	0.87
Mean (SD) HBsAg, log IU/mL	4.4 (0.6)	4.4 (0.7)	0.74
HBV Genotype			0.52
A	40 (36%)	34 (31%)	
B	10 (9.0%)	10 (9.1%)	
C	18 (16%)	14 (13%)	
D	41 (37%)	46 (42%)	
Other/mixed	2 (1.8%)	6 (5.5%)	
Response at week 78			
Response#	20 (18%)	23 (21%)	0.61
HBV DNA <400 copies/mL	9 (8.1%)	13 (12%)	0.38
HBeAg loss	43 (39%)	41 (37%)	0.89
HBsAg loss	8 (7.3%)	11 (10%)	0.48

*Multiples of upper limit of the normal range
#HBeAg loss and HBV DNA <10,000 copies/mL

On-treatment HBsAg decline according to treatment regimen

Overall, HBsAg levels decreased significantly through 52 weeks of therapy (mean decline 1.2 log IU/mL, $P<0.001$), and the decrease was sustained after 26 weeks of follow-up (mean decline compared to baseline 0.9 log IU/mL, $P<0.001$). Patterns of HBsAg decline for both treatment groups are depicted in figure 1. Declines were similar in both treatment arms at weeks 4, 8 and 12, but slightly more pronounced in the combination (PEG-IFN + LAM) compared to the monotherapy group (PEG-IFN + placebo) at week 24 (mean decline 1.0 log IU/mL versus 0.6 log IU/mL, $P = 0.04$) and at week 52 (mean decline 1.46 and 0.87 log IU/mL for combination therapy and monotherapy, respectively, $P = 0.04$). This difference was not sustained through post-treatment follow-up (mean decline of 0.98 and 0.86 log IU/mL for combination and monotherapy at week 78, respectively, $P = 0.63$). Considering the equal response rates and HBsAg levels at week 78 in the two treatment groups, we analysed the relationship between HBsAg decline and treatment response in all 221 patients.



No assessed							
Monotherapy	111	105	106	103	103	105	110
Combination therapy	110	103	104	99	106	104	110

Figure 1: Mean change in serum HBsAg from baseline in patients treated with monotherapy (PEG-IFN + placebo) and combination therapy (PEG-IFN + LAM).

HBsAg decline according to treatment response at week 78

Baseline mean HBsAg levels were comparable in the 43 patients who achieved a response at week 78 and those who did not; 4.4 versus 4.3 log IU/mL in non-responders and responders, respectively ($P = 0.19$). Mean HBsAg declines from baseline for responders and non-responders at week 78 are shown in figure 2. Non-responders showed a modest decline through 52 weeks of therapy (0.69 log IU/mL, $P<0.001$), and relapsed during follow-up (decline from baseline at week 78 was 0.35 log IU/mL, $P<0.001$ compared to week 52). Mean decline from baseline in responders was 3.3 log IU/mL at week 52 and 3.4 at week 78 ($P<0.001$ for both when compared to baseline). Responders thus showed a more vigorous decline in HBsAg starting at week 4, and this difference increased through 52 weeks of therapy and was sustained during post-treatment follow-up ($P<0.005$ for week 4 and $P\leq0.001$ for all other time-points compared to non-responders).

Prediction of response

Since HBsAg decline patterns differed depending on treatment response, we investigated the discriminatory capabilities of HBsAg decline at weeks 4, 8, 12 and 24 for predicting response. Using ROC analysis, areas under the curve (AUC) were 0.70, 0.76, 0.75 and 0.78 for decline at week 4, 8, 12 and 24, respectively, for predicting response at week 78. We also investigated the discriminatory values of absolute HBsAg levels (in log IU/mL) and HBV DNA decline, but these proved inferior to HBsAg declines.

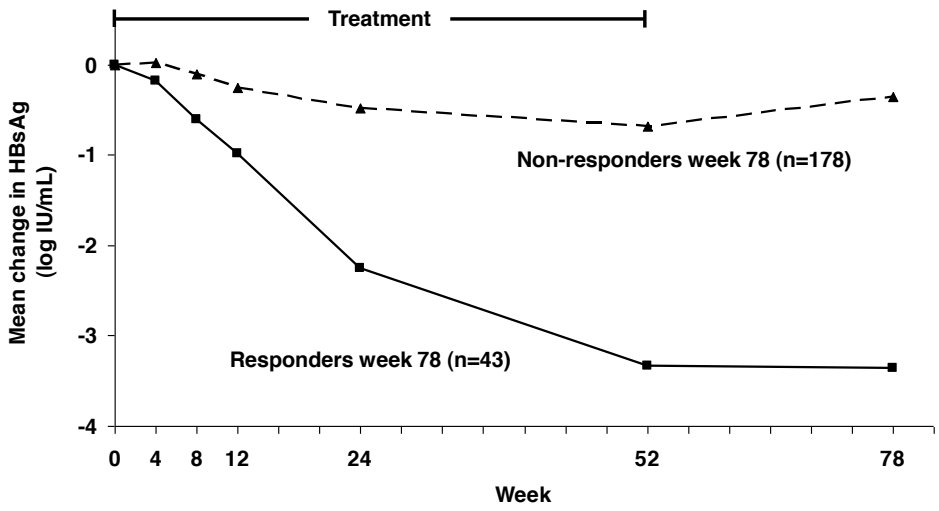


Figure 2: Mean change in serum HBsAg from baseline in patients who achieved a response (HBeAg loss and HBV DNA <10,000 copies/mL) at week 78 and those who did not.

Next, we proceeded to investigate the optimal cut-off point, according to our preset criteria, in HBsAg decline at week 4, 8, 12 and 24 for prediction of response. A cut-off of any decline in serum HBsAg level from baseline (i.e. the HBsAg level on-treatment was lower than the level measured at baseline: $\log(HBsAg_{on\text{-}treatment})-\log(HBsAg_{baseline}) < 0$) proved superior. Subsequently, prediction of response at weeks 12 and 24 was superior to weeks 4 and 8 since it allowed for more patients to be stopped, while maintaining >90% of responders on-treatment (Figure 3). In addition, week 12 was superior to week 24 because it allowed for earlier discontinuation of therapy, while maintaining high predictive values for both response and HBsAg loss (Table 2).
At week 12, 69% of patients achieved a decline in HBsAg when compared to baseline. Of the 31% who did not only 3% achieved a response at week 78. Consequently, the negative predictive value (NPV) of the presence of any decline in HBsAg at week 12 is 97% for prediction of response at week 78. Comparable NPVs were found for prediction of response at week 24 (Table 2, figure 4). Of those patients that developed a decline at week 12, 25% achieved a response at week 78, and 12% achieved HBsAg loss.

Prediction of response through LTFU

Of the 149 patients with LTFU data available, 36 (24%) had a response at LTFU. Similar decline patterns were observed for responders and non-responders at LTFU when compared to (non-)responders at week 78; responders showed a steeper on-treatment decline. Declines were 0.53 log IU/mL versus 2.76 log IU/mL at week 52,

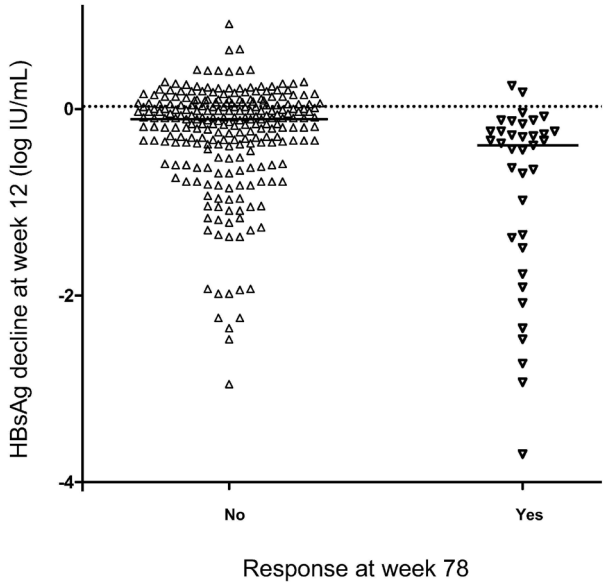


Figure 3: Individual HBsAg declines at week 12, stratified by response at week 78. All but two patients who achieved a response at week 78 experienced a decline in HBsAg from baseline to week 12. Lines represent median decline.

Table 2: Positive and negative predictive values for any HBsAg decline at week 12 and 24 for prediction of response and HBsAg loss at week 78

		Response week 78*				HBsAg loss week 78			
		No	Yes	PPV	NPV	No	Yes	PPV	NPV
Any decline week 12	Yes	104	35	25%	-	122	17	12%	-
	No	61	2	-	97%	63	0	-	100%
Any decline week 24	Yes	122	36	23%	-	140	18	11%	-
	No	47	4	-	92%	51	0	-	100%

*Response is defined as HBeAg loss and HBV DNA <10,000 copies/mL
PPV, positive predictive value
NPV negative predictive value

for (non-)responders, respectively (P = 0.007 for weeks 4 and 8, $P \leq 0.002$ for all other time-points), and the difference was sustained post-treatment. Furthermore, of the patients who did not achieve a decline through 12 weeks of therapy, only 5% achieved a sustained response through LTFU and none lost HBsAg (table 3).

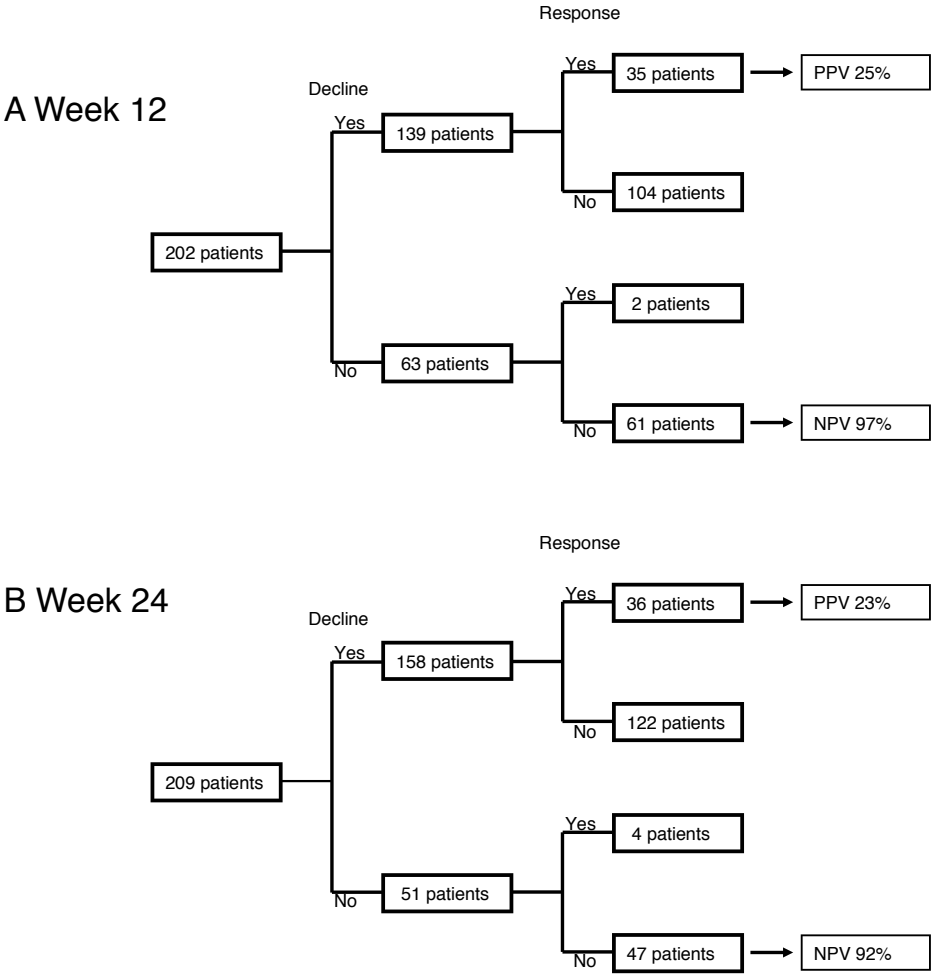


Figure 4: Flowcharts for any decline in serum HBsAg levels from baseline at weeks 12 (A) or 24 (B) in relation to sustained off-treatment response at week 78.

Table 3: Positive and negative predictive values for any HBsAg decline at week 12 and 24 for prediction of response and HBsAg loss at LTFU

		Response LTFU *				HBsAg loss LTFU			
		No	Yes	PPV	NPV	No	Yes	PPV	NPV
Any decline week 12	Yes	65	29	31%	-	80	14	15%	-
	No	42	2	-	95%	44	0	-	100%
Any decline week 24	Yes	76	30	28%	-	90	16	15%	-
	No	32	3	-	91%	35	0	-	100%

*Response is defined as HBeAg loss and HBV DNA <10,000 copies/mL
PPV, positive predictive value
NPV negative predictive value

DISCUSSION

We report the first large study on serum HBsAg decline during PEG-IFN treatment for HBeAg-positive CHB in relation to a sustained off-treatment response. One year of therapy with PEG-IFN significantly reduced serum HBsAg levels, and the decrease was sustained through post-treatment follow-up. HBsAg decline was significantly more pronounced in patients who achieved a response (HBeAg loss and HBV DNA <10,000 copies/mL). Furthermore, we found that reliable prediction of non-response to PEG-IFN is possible as early as week 12 of therapy, based on the absence of a decline in serum HBsAg. Patients who do not experience a decline in serum HBsAg from baseline to week 12, comprising 31% of our study population, have a minimal chance of achieving a sustained off-treatment response. Our results can help clinicians in their decision of whether to continue PEG-IFN therapy based on an individual patient's probability of non-response.

PEG-IFN can induce an off-treatment sustained response in a substantial proportion of HBeAg-positive CHB patients,(12-15) but its clinical use is compromised by the frequent occurrence of side-effects(26) and the uncertainty as to whether a patient will actually benefit from this therapy. Reliable prediction of non-response at baseline or during the first weeks of therapy is therefore essential to optimal utilization of this agent. Recently, a baseline prediction model has been published, based upon data from the two largest studies involving PEG-IFN in HBeAg-positive chronic hepatitis B.(24) The model enables the clinician to predict response (HBeAg loss and HBV DNA < 2,000 IU/mL (~10,000 copies/mL)) of HBeAg-positive patients to PEG-IFN, based on readily available data, such as HBV genotype, HBV DNA and ALT levels, age and sex. While the model provides considerable support when considering a patient for PEG-IFN therapy, substantial uncertainty remains as to whether an individual patient will respond to a one year course of PEG-IFN. On-treatment monitoring of viral replication using HBV DNA, HBeAg and HBsAg levels may aid decision-making and frequent HBV DNA monitoring is therefore recommended in treatment guidelines.(3) However, modeling of HBV DNA kinetics during PEG-IFN therapy has shown only limited clinical utility,(27, 28) and reliable prediction of non-response is only possible at week 24 of therapy (negative predictive value (NPV) 86%).(29)

Recent technical advances have allowed for the quantitative assessment of HBsAg in serum. HBsAg is secreted from the hepatocyte during viral replication as part of the HBV nucleocapsid, or as part of noninfectious viral particles.(30) Several studies have reported that serum HBsAg levels correlate with intrahepatic cccDNA levels in HBeAg-positive patients.(21, 31) On-treatment HBsAg decline may therefore reflect the efficacy of PEG-IFN in decreasing intrahepatic cccDNA and consequently predict a sustained response.(21, 31) This hypothesis was first tested in HBeAg-negative patients, and it

was found that patients with low HBsAg levels at the end of treatment had the highest probability of achieving a sustained off-treatment response.(32) Furthermore, another study showed that patients who did not achieve a 0.5 log decline in serum HBsAg from baseline to week 12 of therapy had only 10% probability of achieving a response (NPV 90%).(33)

Our observations in HBeAg-positive patients corroborate these results on the excellent predictive capabilities of on-treatment HBsAg decline. In our study population, patients who did not achieve a decline in serum HBsAg concentration from baseline to week 12 of therapy had only 3% chance of achieving a sustained off-treatment response. The resulting NPV of 97% is superior to that achieved using HBV DNA and comparable to HBeAg monitoring.(29) Furthermore, our findings indicate that prediction of non-response to PEG-IFN is possible as early as week 12, as opposed to week 24 when using serum HBV DNA or HBeAg levels(29) and that prediction of non-response using HBsAg decline can accurately indentify those patients with a low probability of sustained response through 3 years of post-treatment follow-up. Furthermore, if our on-treatment stopping-rule was applied combined with the baseline prediction model,(24) the AUC increased from 0.75 for the stopping-rule alone to 0.79 for the combination, showing that application of both two models to guide therapy decisions may be beneficial.

Other studies have reported that HBsAg levels of <1500 IU/mL at week 12 or week 24 of therapy were highly predictive of sustained HBeAg seroconversion 6 months post-treatment.(34) We found comparable positive predictive values (PPVs) for HBsAg levels <1500 IU/mL at week 12 for response at LTFU (PPV: 55%) and for loss of HBsAg at LTFU (PPV: 35%). Prediction did not improve at week 24, with PPVs of 53% for response at LTFU, and 41% for HBsAg loss at LTFU. Anyhow, these results have limited clinical significance, since even patients with HBsAg levels >1500 IU/mL at either of these time-points have a considerable probability of response. If one were to discontinue therapy all patients with HBsAg >1500 IU/mL at week 24, one would miss out on 48% of patients with a response at LTFU in our study population.

A possible caveat of our study is that we pooled data from the two treatment arms for the formulation of our stopping-rule. Patients who received combination therapy experienced a somewhat larger decline from week 24 to week 52. To account for this, we validated our stopping-rule in both treatment groups, and found that it performed equally well in both populations. Sensitivity analysis confirmed that a cut-off of any decline was superior in both groups. Additionally, our LTFU population comprised only a subgroup of the total study group (149 out of 221). However, it was previously shown that the LTFU group was representative of the entire study cohort,(12) and we confirmed these findings (data not shown). Also, the cut-off of any decline performed well in both groups (tables 2 and 3). Furthermore, one could argue that we should have

chosen a different definition of response. In this study, we defined response as off-treatment sustained HBeAg loss combined with HBV DNA <10,000 copies/mL (~2000 IU/mL), since HBeAg loss 6 months post-treatment has been reported to be highly durable(12) and since patients with low HBV DNA levels are less likely to develop HBV related liver complications or require antiviral therapy according to recent guidelines. (3, 35-37) Moreover, this end-point is in line with other recently published papers on response to PEG-IFN in HBeAg-positive CHB,(24) and the high negative predictive values were maintained if HBeAg seroconversion combined with HBV DNA <10,000 copies/mL was applied as end-point.

Concluding, a one year course of PEG-IFN results in a significant decline in serum HBsAg in HBeAg-positive CHB patients. The decline is considerably more pronounced in patients who achieve a response (HBeAg loss and HBV DNA <10,000 copies/mL) when compared to non-responders. Patients who do not experience a decline in HBsAg levels through 12 weeks of therapy have a low chance of achieving a sustained off-treatment response (<5%) and no chance of HBsAg loss, and should therefore be considered for treatment discontinuation.

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Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B using HBsAg and HBV DNA levels

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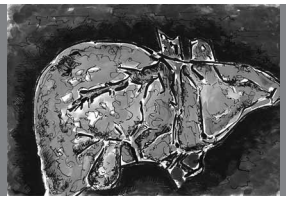

ABSTRACT

Peginterferon alfa-2a results in a sustained response (SR) in a minority of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients. This study investigated the role of early on-treatment serum hepatitis B surface antigen (HBsAg) levels in the prediction of SR in HBeAg-negative patients receiving peginterferon alfa-2a. HBsAg (Abbott ARCHITECT) was quantified at baseline, during treatment (weeks 4, 8, 12, 24, 36 and 48) and follow-up (weeks 60 and 72) in the sera from 107 patients who participated in an international multicenter trial (peginterferon alfa-2a, n=53 versus peginterferon alfa-2a and ribavirin, n=54). Overall, 24 (22%) patients achieved SR (serum hepatitis B virus (HBV DNA) <10,000 copies/mL and normal alanine aminotransferase level at week 72). Baseline characteristics were comparable between sustained responders and non-responders. From week 8 onwards, serum HBsAg levels markedly decreased in sustained responders, whereas only a modest decline was observed in non-responders. However, HBsAg declines alone were of limited value in the prediction of SR (area under the receiver-operating characteristic curve (AUC) 0.59, 0.56 and 0.69 at weeks 4, 8 and 12, respectively). Combining HBsAg and HBV DNA declines allowed the best prediction of SR (AUC 0.74 at week 12). None of the 20 patients (20% of the study population) in whom a decrease in serum HBsAg level was absent and HBV DNA declined less than 2 log copies/mL exhibited a SR (NPV 100%).

Conclusion

At week 12 of peginterferon alfa-2a treatment for HBeAg-negative CHB a solid stopping rule was established using a combination of declines in serum HBV DNA and HBsAg level from baseline. Quantitative serum HBsAg in combination with HBV DNA enables on-treatment adjustment of peginterferon therapy in HBeAg-negative CHB.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 350 to 400 million people worldwide and is responsible for 1 million deaths every year.(1) Hepatitis B e antigen (HBeAg-) negative chronic hepatitis B (CHB) represents a late phase in the course of the infection, which is recognized worldwide with an increasing prevalence.(2) Therapeutic intervention is often indicated for HBeAg-negative patients, because spontaneous remission rarely occurs and patients have more advanced liver disease in comparison with HBeAg-positive patients.(3)

In the last decade great strides have been made in the treatment of CHB, but the management of the HBeAg-negative type remains difficult. Nucleos(t)ide analogues are able to maintain suppression of viral replication in the majority of HBeAg-negative patients and are well tolerated,(4, 5) but it is highly uncertain whether oral antiviral therapy can be discontinued.(6-8) In contrast to nucleos(t)ide analogues, one year of peginterferon therapy can result in an off-treatment sustained response (SR) in HBeAg-negative patients.(9, 10) However, treatment with peginterferon is often complicated by the occurrence of side effects and a minority of patients with HBeAg-negative disease achieve SR. It is therefore a major challenge to identify patients who are likely to benefit from peginterferon therapy as early as possible during the treatment course.

HBV DNA quantification is widely used as a marker of viral replication to assess response to nucleos(t)ide analogues, but prediction of response to peginterferon by means of serum HBV DNA levels is difficult.(11, 12) Advances in technology have enabled the development of a quantitative assay for hepatitis B surface antigen (HBsAg). The serum concentration of HBsAg appears to reflect the amount of covalently closed circular DNA (cccDNA) in the liver, which acts as a template for the transcription of viral genes.(13, 14) Recently, several studies have suggested that serum HBsAg levels may be indicative of the likelihood of response to interferon-based therapy.(15-17) The aim of this study was to clarify the role of early on-treatment quantitative serum HBsAg in the prediction of SR in HBeAg-negative CHB patients treated with peginterferon alfa-2a.

PATIENTS AND METHODS

Patients

HBsAg levels were measured in sera from a total of 107 of 133 HBeAg-negative chronic hepatitis B patients who participated in an investigator-initiated, multicenter, randomized, double-blind, controlled trial.(9) Patients were randomly assigned in a one-to-one ratio to receive 180 µg peginterferon alfa-2a weekly and ribavirin 1000 mg (body weight <75 kg) or 1200 mg daily (body weight ≥75 kg) or peginterferon alfa-2a

180 µg weekly and placebo daily. Duration of therapy was 48 weeks, followed by a 24-week observation period. Patients attended the outpatient clinic every 4 weeks. Results at the end of treatment (week 48) and at the end of follow-up (week 72) have been reported previously.(9) Patients who were treated according to the protocol and completed the follow-up phase were selected for the present study.

Eligible patients for the original study had been positive for HBsAg for more than 6 months; were HBeAg negative and anti-HBe positive on 2 occasions within 2 months before randomization; had had 2 episodes of elevated serum alanine aminotransferase (ALT) levels (>1.5 but ≤10 times the upper limit of normal (ULN) of the normal range) within 2 months prior to randomization and had a serum HBV DNA level >100,000 copies/mL (17,143 IU/mL). Exclusion criteria were: antiviral or immunosuppressive therapy within the previous 6 months; co-infection with hepatitis C, hepatitis D or human immunodeficiency virus (HIV); other acquired or inherited causes of liver disease; pre-existing cytopenia or decompensated liver disease. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written, informed consent.

Laboratory measurements

Serum HBsAg was quantified in samples taken at baseline, during the treatment period (weeks 4, 8, 12, 24, 36, 48) and during follow-up (weeks 60 and 72) using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL).(18) Serum HBV DNA was measured at the same time points using the Taqman polymerase chain reaction assay (Taqman HBV assay, Roche Diagnostics, lower limit of quantification: 35 copies/mL (6 IU/mL)). Transaminases were measured locally at the time of sampling in accordance with standard procedures. HBV genotype was assessed using the INNO-LiPA assay (Innogenetics).

Liver histology

A liver biopsy was performed in all patients within one year before randomization. Necroinflammation grade (range 0-18) and fibrosis stage (range 0-6) were assessed using the Ishak scoring system.(19)

Statistical analysis

Sustained response (SR), the predefined primary endpoint in the original study, was defined according to the EASL guidelines as the combined presence of serum HBV DNA level below 10,000 copies/mL (1,714 IU/mL) and normalization of ALT at the end of follow-up (week 72).(20) The association between baseline factors and SR was assessed by univariate logistic regression analyses. Predictive values of early on-treatment serum HBsAg, as well as HBV DNA and ALT levels (weeks 4, 8, and 12)

were explored applying logistic regression analysis techniques. Discrimination, which is the ability to distinguish patients who will develop SR from those who will not, was quantified by the area under the receiver-operating characteristic curve (AUC). The best model-fit was assessed comparing the AUC and the Akaike's Information Criteria (AIC). Hereafter the optimal cut-off values for serum HBsAg and HBV DNA levels during treatment were established with the use of explanatory plots and the maximum chi-square approach to find a clinically useful rule for (dis)continuation of therapy. (21) SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used to perform statistical analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

Sustained response rate

Twenty-four (22%) of 107 patients developed SR. The number of sustained responders was comparable between the peginterferon alfa-2a monotherapy and the peginterferon alfa-2a and ribavirin combination therapy group (14 (26%) of 53 versus 10 (19%) of 54 patients, respectively, p=0.33). The two treatment groups were therefore pooled for further analysis. Among the 24 sustained responders, one patient cleared HBsAg from serum and developed anti-HBs.

Baseline characteristics

Baseline characteristics of the 107 patients are shown in table 1. The mean pretreatment serum HBsAg level was 3.8 log IU/mL (range 1.1-5.0 log IU/mL) and the mean serum HBV DNA level was 6.8 log copies/mL (range 4.3-9.5 log copies/mL), both were stable during the screening period. There was no significant correlation between serum HBsAg and other factors at baseline including serum HBV DNA and ALT, HBV genotype, age, gender, body mass index (BMI) or liver histology. Baseline characteristics were comparable for patients with and without SR, including age, gender, HBV genotype, serum ALT, HBV DNA and HBsAg levels and liver necroinflammatory and fibrosis scores (Table 1).

Serum HBsAg and HBV DNA levels during treatment and follow-up

Overall, the mean serum HBsAg concentration decreased significantly after 48 weeks of therapy (mean change compared to baseline -0.47 log IU/mL, $p<0.001$). HBsAg remained at end-of-treatment levels during post-treatment follow-up (mean change at week 72 compared to baseline -0.52 log IU/mL, $p<0.001$). Serum HBV DNA declined significantly during the treatment period as well (mean change at week 48 compared

Table 1: Baseline characteristics according to SR

Characteristics	All patients (n=107)	SR + (n=24)	SR – (n=83)	*P* value
Mean (SD) age, years	42 (10)	41 (11)	42 (10)	0.59
Male (%)	77 (72.0)	16 (66.7)	61 (73.5)	0.51
Ethnicity (%)				0.73
Caucasian	102 (95.3)	23 (95.8)	79 (95.2)	
Other	5 (4.7)	1 (4.2)	4 (4.8)	
HBV genotype (%)				0.13
A	15 (14.0)	0	15 (18.1)	
D	85 (79.4)	23 (95.8)	62 (74.7)	
Other/mixed	7 (6.5)	1 (4.2)	6 (7.2)	
Median (IQR) ALT*	2.3 (1.6-4.1)	2.0 (1.7-3.9)	2.3 (1.6-4.1)	0.82
Mean (SD) HBV DNA, log copies/mL	6.8 (1.2)	6.9 (1.2)	6.7 (1.2)	0.52
Mean (SD) HBsAg, log IU/mL	3.8 (0.5)	3.8 (0.4)	3.8 (0.6)	0.80
Median (IQR) liver necroinflammation	5 (4-7)	5 (4-6)	5 (4-7)	0.52
Median (IQR) liver fibrosis	3 (1-3)	2 (1-3)	3 (1-3)	0.57
Cirrhosis† (%)	3 (2.8)	0	3 (3.6)	1.0

*Multiples of upper limit of the normal range
†Ishak fibrosis score 5-6

to baseline -3.29 log copies/mL p<0.001). In contrast to HBsAg levels, HBV DNA levels relapsed after treatment discontinuation (mean change at week 72 compared to baseline -1.55 log copies/mL, p=0.004).

A weak positive correlation was present between serum HBsAg and HBV DNA levels when all available samples were considered (R=0.35, p<0.001). From baseline until week 12, serum HBsAg and HBV DNA were not correlated (R<0.15, p>0.11). However, the correlation became stronger at the end of the treatment phase (week 48; R=0.36, p<0.001) and further increased at the end of follow-up (week 72; R=0.53, p<0.001).

Serum HBsAg and HBV DNA levels according to response

Mean HBsAg declines from baseline for sustained responders and non-responders are shown in figure 1A. During the first 8 weeks of therapy mean serum HBsAg levels remained stable in both patient groups (Fig. 1A). From week 8 onwards however, HBsAg levels markedly decreased among the 24 patients who developed SR, whereas only a modest decrease in HBsAg level was observed in patients who failed to achieve SR (p<0.05 for comparison of HBsAg declines between patients with and without SR at all time points from week 8 with correction for multiple testing).

Mean HBV DNA declines from baseline for patients with and without SR are displayed in figure 1B. A significant reduction in serum HBV DNA level was observed at week 4, in contrast to the later on-treatment decline in serum HBsAg level. Although the magnitude of on-treatment HBV DNA decline was larger in patients who eventually developed SR (p<0.01 for comparison of HBV DNA declines between patients with

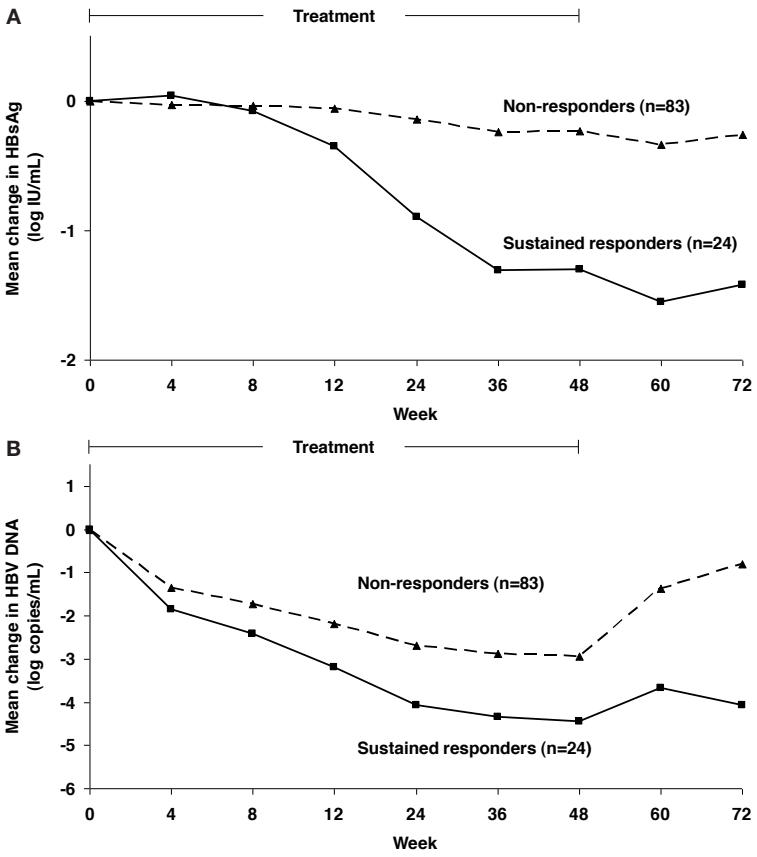

Figure 1: Mean change compared to baseline for HBsAg (A) and HBV DNA (B) levels in patients who achieved SR and those who did not.

and without SR at all time points with correction for multiple testing), HBV DNA also decreased substantially in patients who did not achieve SR (Fig. 1B).
Serum ALT levels behaved similarly in sustained responders and non-responders during the treatment period and were not predictive of SR.

Prediction of sustained response

The relationship between serum HBsAg and HBV DNA levels and subsequent achievement of SR was assessed at weeks 4, 8 and 12 of therapy. The performance of HBsAg and HBV DNA declines from baseline on SR was superior to absolute values. The AUC for declines in HBsAg and HBV DNA level is shown in figure 2. The reductions in HBsAg level at weeks 4 and 8 were not associated with SR using logistic regression analysis. HBsAg decline at week 12 was significantly associated with SR, but the overall discrimination remained unsatisfactory (AUC 0.59, 0.56 and 0.69 at weeks 4, 8 and 12, respectively).

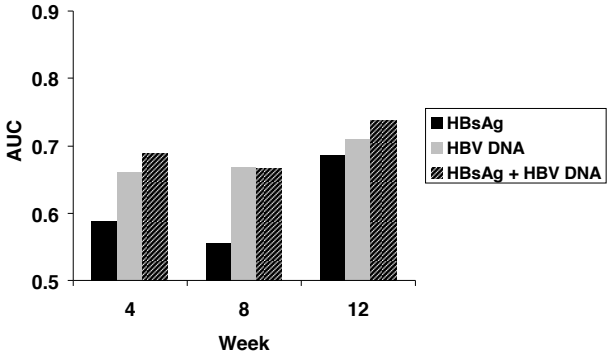

Figure 2: AUC for HBsAg decline from baseline, HBV DNA decline from baseline and a combination of these 2 markers for the prediction of SR.

In contrast to HBsAg declines, HBV DNA declines were associated with SR as early as week 4 of treatment. HBV DNA declines performed better with regard to the prediction of SR than HBsAg declines at weeks 4, 8 and 12 (Fig. 2). The best model-fit however, based on the AUC and AIC, was achieved through a combination of HBsAg and HBV DNA declines (AUC 0.74 at week 12). The performance of the model at week 24 did not improve significantly compared to week 12 (p=0.37). Treatment regimen was not associated with SR when added to the logistic regression models (p≥0.35 for all time points).

Treatment algorithm

To find a clinically useful guiding rule, optimal cut-off values for a combination of HBsAg and HBV DNA decline at week 12 were established. We aimed to identify a stopping rule which enables discontinuation of therapy in patients who have a very low chance of SR, while maintaining more than 95% of sustained responders on treatment. Serum samples to measure HBsAg and HBV DNA decline at week 12 were available for 102 patients. Figure 3 illustrates the chance of SR within 4 patient groups defined according to the presence of HBsAg decline and/or HBV DNA decline ≥2 log copies/mL at week 12. None of the patients in whom a decline in serum HBsAg level was absent and HBV DNA decreased less than 2 log copies/mL (20% of the study population) exhibited a SR (NPV 100%). In contrast, patients in whom both these virological declines were achieved had the highest probability of SR (39%), which is almost double the overall response rate of 22%. Rates of SR were intermediate in patients with either a ≥2 log copies/mL decline in HBV DNA (24%) or a decline in HBsAg concentration only (25%). Separate analyses for the two treatment regimens (peginterferon alfa-2a with or without ribavirin) resulted in identical cut-off values for HBsAg and HBV DNA decline at week 12.

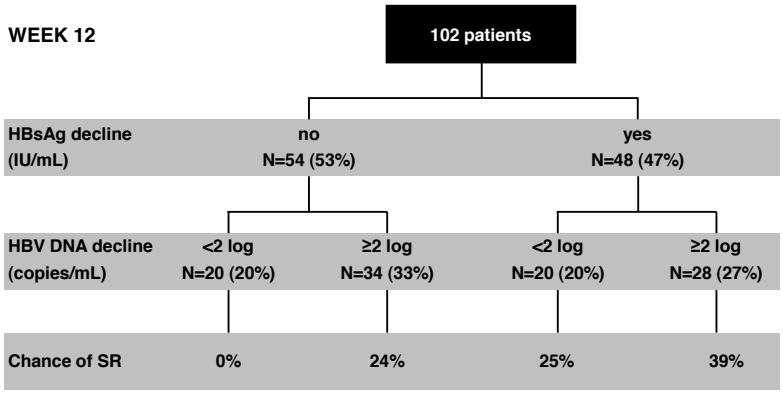

Figure 3: Algorithm showing chances of SR based on (1) HBsAg decline and (2) HBV DNA decline ≥2 log copies/mL at week 12 compared to baseline.

DISCUSSION

HBeAg-negative CHB represents a difficult-to-treat population at high risk for liver-related complications.(3) All of the major practice guidelines recommend both peginterferon and nucleos(t)ide analogues as initial treatment options(20, 22, 23), but the optimal choice for individual patients remains controversial. Due to the higher chance of disease relapse after treatment discontinuation peginterferon is relatively less often prescribed to HBeAg-negative as compared to HBeAg-positive patients. A treatment course with peginterferon should however be considered for HBeAg-negative patients with a high likelihood of response, because a finite treatment course can lead to an off-treatment SR. Otherwise prolonged or indefinite treatment with a nucleos(t)ide analogue is likely. Unfortunately, baseline predictors of response to peginterferon are poorly defined in comparison with HBeAg-positive disease.(24, 25) One study reported that baseline serum HBV DNA and ALT levels, patient age and gender, and infecting HBV genotype were significantly associated with response to peginterferon alfa-2a with or without lamivudine therapy,(26) but this was not confirmed in our patient population. Recent studies on peginterferon in HBeAg-negative patients have focussed on the identification of markers allowing on-treatment prediction of response.(15-17)

We found that accurate prediction of SR to peginterferon for HBeAg-negative disease in an early treatment phase is not possible based on serum HBsAg levels alone. However, combining on-treatment declines in serum HBsAg and HBV DNA concentration resulted in a solid stopping rule. At week 12, the absence of a decline in HBsAg level combined with less than 2 log copies/mL decrease in HBV DNA level identified a substantial proportion of the total study population (20%) in which therapy could be discontinued without losing sustained responders. In contrast, patients in whom

Table 2: Recommendations for continuation of peginterferon alfa-2a therapy for HBeAg-negative CHB at week 12

Week 12 versus baseline		Chance of SR	Recommendation to continue
HBsAg decline	HBV DNA decline ≥2 log copies/mL		
-	-	Absent	-
-	+	Intermediate	+
+	-	Intermediate	+
+	+	High	++

both declines were present had the highest probability of SR (39%). This patient group should be encouraged to complete the 48-week treatment phase because they are the most likely group to benefit from therapy. Table 2 provides recommendations for (dis)continuation of therapy for patient groups based on the chance of developing SR. Obviously, the final decision to (dis)continue therapy is at the discretion of the treating physician, taking into account other factors like drug tolerability as well. Another important finding is that a guiding rule before 12 weeks of therapy could not be established because discrimination of serum HBsAg and HBV DNA levels during the first 8 weeks of treatment did not prove sufficient. Also, the decision to discontinue therapy should not be postponed, because the prediction of SR did not improve significantly at week 24 compared to week 12.

The kinetics of serum HBsAg and HBV DNA levels clearly differed during the treatment phase. HBV DNA decreased throughout the entire treatment period, while a later decline was observed in serum HBsAg levels. HBsAg and HBV DNA levels were not correlated at baseline and early during the treatment phase, further underlining the additional value of HBsAg levels in the prediction of SR. The added information that is provided by quantitative assessment of serum HBsAg may be explained by the dual antiviral and immunomodulatory mode of action of peginterferon. The on-treatment reduction in serum HBV DNA primarily reflects the direct antiviral effect of peginterferon. In contrast, the decline in serum HBsAg may be a marker of its immunomodulatory effects resulting in gradual clearance of infected hepatocytes from the liver through the induction of cytotoxic T-cell activity.(27) In line with these findings, it has been demonstrated that reductions in serum HBsAg mirror the decline in intrahepatic cccDNA.(13, 14)

Recently high predictive values for on-treatment HBsAg declines at weeks 12 and 24 on sustained virological response (HBV DNA <70 copies/mL) were reported in a cohort of 48 patients treated with peginterferon alfa-2a for 48 weeks.(17) This finding was not confirmed in our larger study population, which was derived from a randomized controlled trial. This discrepancy may be generated by the substantial difference in response rates between the two studies. In the study by Moucari et al., 25 percent of patients developed a sustained virological response. (17) This response rate is substantially higher than in any peginterferon study for

HBeAg-negative patients, suggesting that a selection bias may have affected the results of this retrospective study.

In our study SR had previously been defined as the combined presence of a serum HBV DNA level <10,000 copies/mL and a normal ALT level at 6 months after treatment discontinuation. One could argue that the HBV DNA threshold should have been set at a lower level. Indeed, off-treatment undetectability of serum HBV DNA by a sensitive PCR assay is a major virological endpoint and strongly associated with HBsAg clearance from serum in the years afterwards.(28) However, these preferred treatment endpoints occur infrequently in HBeAg-negative patients treated with peginterferon. In fact, another important goal of therapy for HBeAg-negative CHB is the induction of the HBsAg inactive carrier phase. Our endpoint of a serum HBV DNA level <10,000 copies/mL combined with a normal ALT level appears to differentiate reliably between inactive carriers and HBeAg-negative chronic hepatitis B patients.(29) In addition, large population studies have shown that HBsAg-positive patients with an HBV DNA concentration below this level of viral replication have a reduced risk of progression to cirrhosis and hepatocellular carcinoma.(30-32) Furthermore, this HBV DNA threshold and the duration of follow-up correspond with the definition of response to peginterferon therapy according to the recent European guidelines and the pivotal studies on peginterferon in chronic hepatitis B, respectively.(10, 20, 33)

The large majority of our patients were of Caucasian origin and infected with HBV genotypes A and D. Responsiveness to interferon-based therapy appears to be lower in genotype D compared to other genotypes, which may explain the limited efficacy of peginterferon in our study population.(9, 10, 26, 34) A recent retrospective analysis of 264 HBeAg-negative patients treated with peginterferon alfa-2a alone or in combination with lamivudine reported that pretreatment HBsAg levels varied according to genotype. The highest concentrations were found in patients infected with genotypes A and D. Although serum HBsAg levels decreased during the treatment phase in all genotypes, HBsAg decline was least pronounced in genotype D.(35) Therefore, our data on HBsAg decline need to be confirmed in genotypes B and C.

In summary, the current study shows that a combination of early quantitative serum HBsAg and HBV DNA levels allows the best selection of patients with HBeAg-negative CHB who will not respond to a 48-week course of peginterferon alfa-2a therapy. Discontinuation of peginterferon therapy and a switch to alternative treatment appears to be indicated in patients without a decline in HBsAg level combined with less than 2 log copies/mL decline in HBV DNA level at week 12.

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5

Early on-treatment identification of hepatitis B e antigen-negative patients not responding to peginterferon alfa-2a

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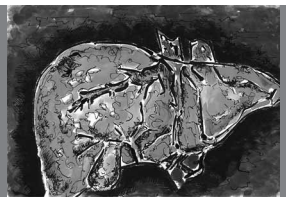

ABSTRACT

Background

It was recently demonstrated that none of the hepatitis B e antigen (HBeAg-)negative patients who did not experience any serum hepatitis B surface antigen (HBsAg) decline and had <2 log hepatitis B virus (HBV) DNA decline at week 12 of a 48-week peginterferon alfa-2a (PEG-IFN) treatment course achieved a sustained response (SR). We aimed to validate this stopping rule in two independent trials.

Methods

HBeAg-negative patients who received 48/96 weeks of PEG-IFN in the phase III registration trial (N=85) and PegBeLiver study (N=75) were stratified according to the presence of any HBsAg decline and/or ≥2 log HBV DNA decline at week 12. For the current study, SR was defined as HBV DNA <2,000 IU/mL and normal alanine aminotransferase 24 weeks after treatment.

Results

The original PARC trial included 102 patients (HBV genotype A/D/other: 14/81/7), of whom 25 (25%) had a SR. The validation dataset consisted of 160 patients (genotype A/B/C/D/other: 10/18/34/91/7), 57 (36%) achieved a SR. The stopping rule performed well across the two studies (p=0.001 with correction for study) and its high negative predictive value [NPV] was confirmed across HBV genotypes A-D (95%). Also among the 34 patients treated for 96 weeks, none of the 7 (21%) without HBsAg decline and with <2 log HBV DNA decline at week 12 achieved a SR (NPV 100%).

Conclusions

We confirmed in two large trials that a combination of HBsAg and HBV DNA levels at week 12 identifies HBeAg-negative patients with no or a very low chance of SR to either 48 or 96 weeks of PEG-IFN therapy.

INTRODUCTION

The management of hepatitis B e antigen (HBeAg-)negative chronic hepatitis B (CHB) remains problematic because this disorder is characterized by high rates of virologic relapse following any antiviral therapy.(1) Although newly registered nucleos(t)ide analogues (NA), such as entecavir and tenofovir, are able to maintain suppression of hepatitis B virus (HBV) replication for up to 5 years with a low risk of resistance and a favourable safety profile, (2, 3) it is currently unclear when NA therapy can be stopped. (4-6) Compliance with ongoing treatment is therefore required in the vast majority of patients and this approach may still be associated with a considerable risk for resistance and toxicity on the long term.

In parallel with NA, current guidelines consider peginterferon (PEG-IFN) therapy as a first-line treatment option because this agent results in the highest rate of sustained off-treatment response (SR) after a 1-year course of therapy.(7-9) Nevertheless, a minority of HBeAg-negative patients receive PEG-IFN as initial treatment, which may be explained by its modest rate of SR in this population, its side effects and need for subcutaneous administration.(10) Moreover, current guidelines do not provide specific recommendations as to which HBeAg-negative patients should be treated with PEG-IFN since clinically useful baseline predictors of SR are lacking.(11) For this reason, there has been much interest in on-treatment quantified serum hepatitis B surface antigen (HBsAg) as a possible marker of the immunomodulatory effects of PEG-IFN in HBeAg-negative disease.(12-14) We recently reported that monitoring of both HBsAg and HBV DNA levels may provide the best predictive value and a solid stopping rule was established: none of the patients who did not experience a decrease in serum HBsAg and who had less than 2 log HBV DNA decline at week 12 of PEG-IFN alfa-2a treatment achieved a SR to a 48-week treatment course.(15) Discontinuation of PEG-IFN therapy would thus prevent the side effects and costs associated with unnecessary treatment in these patients, attributing to more efficient utilization of this agent in clinical practice.

The purpose of the present study was (1) to validate this stopping rule in the two other large trials performed to date investigating the efficacy of PEG-IFN alfa-2a in HBeAg-negative disease and (2) to investigate how this rule performs in HBeAg-negative patients treated with PEG-IFN alfa-2a for 96 weeks.

PATIENTS AND METHODS

Patients

The stopping rule was based on a total of 102 HBeAg-negative patients treated with PEG-IFN alfa-2a (± ribavirin) for 48 weeks who completed the post treatment follow-up phase of 24 weeks and had serum HBsAg and HBV DNA levels available at baseline and at week 12.(15) External validation of this rule was performed in 85 patients treated with PEG-IFN alfa-2a monotherapy for 48 weeks in the phase III registration trial,(16) and 75 patients treated with PEG-IFN alfa-2a monotherapy for 48 or 96 weeks in the PegBeLiver study.(17) In accordance with the initial PARC trial, the selection criteria for inclusion in the present study were completion of 24 weeks of post treatment follow-up and availability of HBsAg and HBV DNA measurements at baseline and at week 12. PEG-IFN alfa-2a was administered in a dose of 180 µg/week, the dose was reduced to 135 µg after week 48 in PegBeLiver participants treated for 96 weeks.(17) Patients treated with PEG-IFN and lamivudine combination therapy in the phase III trial were not eligible for the present study because they experienced a stronger degree of HBV DNA suppression during the treatment period compared with PEG-IFN monotherapy.(16)
The inclusion and exclusion criteria were described in detail previously and were similar across the three trials.(16-18) In summary, adult patients were eligible if they had been HBsAg positive for at least 6 months, were HBeAg negative and anti-HBe positive, had elevated serum alanine aminotransferase (ALT) levels between 1 and 10 times the upper limit of the normal range (ULN) and had a serum HBV-DNA level exceeding 100,000 copies/mL (≈20,000 IU/mL). Key exclusion criteria included antiviral therapy within 6 months prior to randomization, viral co-infections (hepatitis C virus, hepatitis delta virus, or human immunodeficiency virus) and decompensated liver disease.(16-18)

Laboratory measurements

Serum HBsAg and HBV DNA levels were measured in samples obtained at baseline and every 12 weeks during the treatment period using the ARCHITECT HBsAg assay (Abbott laboratories) and commercial polymerase chain reaction (PCR) assays (Roche Diagnostics), respectively. Serum ALT was measured locally in accordance with standard procedures.

Statistical analysis

Continuous variables are expressed as means ± standard deviation (SD). Continuous baseline variables were compared between the 3 trials by one-way analysis of variance (ANOVA), categorical variables using the Chi-square or Fisher's exact test. For the present study, SR was defined as the combined presence of serum HBV DNA below

2,000 IU/mL and normal ALT after 24 weeks of post treatment follow-up. This definition is accepted by the most recent guidelines for the management of CHB as an appropriate marker of response to PEG-IFN therapy in HBeAg-negative patients.(7) Patients were categorized into 4 groups according to the presence of any HBsAg decline and/or ≥2 log HBV DNA decline at week 12 of therapy and the rates of SR were subsequently compared. The performance of the stopping rule was assessed across the 3 trials using the Chi-square test. All statistical tests were two-sided and were evaluated at the 0.05 level of significance. SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics

Baseline characteristics for the three trials are shown in table 1. Patient demographic and virologic characteristics were significantly different across the three groups. While

Table 1: Baseline characteristics of the study patients

Characteristic	PARC N=102	Phase III N=85	PegBeLiver N=75	*P*
Treatment duration (%)				-
48 weeks	102 (100.0)	85 (100.0)	41 (54.7)	
96 weeks	0	0	34 (45.3)	
Mean (SD) age, years	41 (10)	41 (10)	44 (10)	0.17
Gender (%)				0.24
Male	74 (72.5)	70 (82.4)	55 (73.3)	
Female	28 (27.5)	15 (17.6)	20 (26.7)	
Ethnicity (%)				<0.001
Caucasian	97 (95.1)	30 (35.3)	75 (100.0)	
Asian	3 (2.9)	52 (61.2)	0	
Other	2 (2.0)	3 (3.5)	0	
HBV genotype (%)				<0.001
A	14 (13.7)	8 (9.4)	2 (2.7)	
B	0	18 (21.2)	0	
C	2 (2.0)	34 (40.0)	0	
D	81 (79.4)	21 (24.7)	70 (93.3)	
Other/mixed	5 (4.9)	4 (4.7)	3 (4.0)	
Mean (SD) ALT*	3.2 (2.5)	3.1 (2.8)	3.2 (2.8)	0.98
Mean (SD) HBV DNA, log IU/mL	6.0 (1.2)	6.7 (1.9)	6.2 (1.4)	0.005
Mean (SD) HBsAg, log IU/mL	3.8 (0.6)	3.4 (0.6)	3.7 (0.4)	<0.001
Cirrhosis† (%)	3 (2.9)	7 (8.2)	5 (6.7)	0.26

*Multiples of upper limit of the normal range

†Ishak fibrosis score 5-6 or METAVIR fibrosis score 4

most patients (61%) participating in the phase III trial were Asian, the vast majority (>95%) of patients in the PARC and PegBeLiver study were Caucasian (p<0.001). Consequently, most phase III patients were infected with HBV genotype B or C, whereas genotype D was predominant in the PARC en PegBeLiver trial (p<0.001). Baseline serum HBsAg concentrations were higher for patients included in the PARC and PegBeLiver study compared with the phase III trial (3.8±0.6 and 3.7±0.4 versus 3.4±0.6 log IU/mL, respectively; p<0.001). In contrast, baseline serum HBV DNA levels were higher for the phase III trial compared with the PARC and PegBeLiver study (6.7±1.9 versus 6.0±1.2 and 6.2±1.4 log IU/mL, respectively; p=0.005). The three groups were comparable at baseline regarding age, gender distribution, serum ALT level and prevalence of cirrhosis (table 1).

Serum HBsAg and HBV DNA levels in sustained responders and nonresponders

Twenty-five (25%) of 102 patients participating in the PARC study and 32 (38%) of 85 patients in the phase III trial achieved a SR. Overall, a SR was achieved in 25 (33%) of 75 PegBeLiver participants: 11 (27%) of 41 patients treated for 48 weeks and 14 (41%) of 34 patients treated for 96 weeks.

In the initial PARC study, sustained responders experienced a stronger decline of serum HBsAg during the treatment course compared with those without SR (figure 1A). The mean decline of HBsAg from baseline at week 48 was 1.26±1.43 log in sustained responders compared with 0.24±0.59 log in nonresponders (p=0.002). The same pattern was observed in the two other trials (figure 1B-D). Mean HBsAg declines at week 48 for sustained responders versus nonresponders were 1.15±1.37 versus 0.38±0.66, 0.64±1.26 versus 0.17± 0.37 and 1.04±1.11 versus 0.05±0.44 log in the phase III trial, the 48-week arm and the 96-week arm of the PegBeLiver study, respectively (all p<0.05).

Figure 2A shows that the degree of serum HBV DNA decline throughout the treatment course in the PARC trial was stronger in patients who had a SR, although HBV DNA levels also decreased considerably in those without a SR. The same observation was made in the phase III trial and 96-week arm of the PegBeLiver study (figure 2B and 2D). Sustained responders in the 48-week arm of the PegBeLiver study also showed a stronger HBV DNA decline during the first 12 weeks of therapy compared with nonresponders. In this group, however, HBV DNA declines at a later stage of the treatment course were comparable between patients with and without a SR (figure 2C).

Validation of stopping rule

Figure 3 shows the chances of SR according to the presence of any HBsAg decline and/or more than 2 log HBV DNA decline at week 12 of therapy in the original PARC

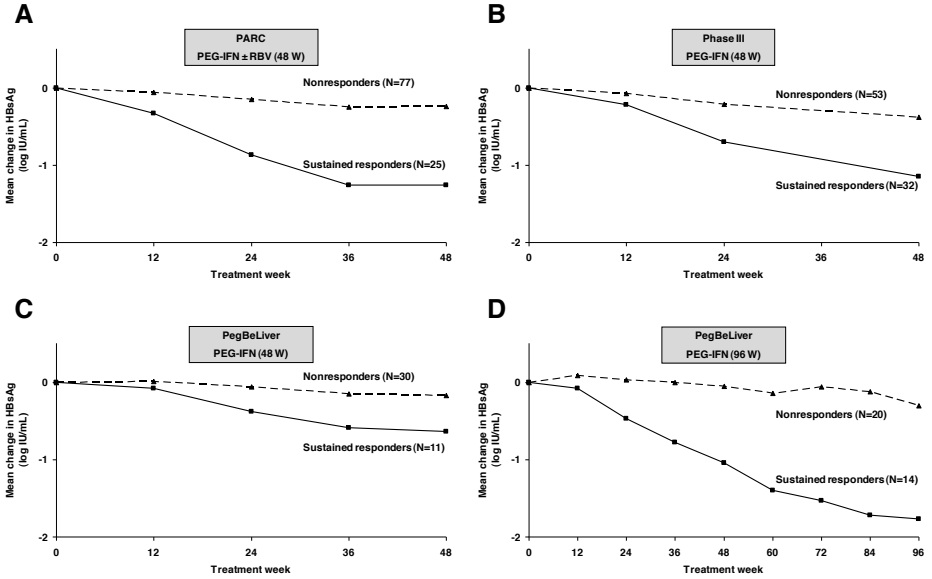

Figure 1: Mean change in HBsAg compared to baseline according to the achievement of a sustained response within the PARC trial (A), phase III trial (B) and the 48-week (C) and 96-week (D) arm of the PegBeLiver trial.
RBV, ribavirin.

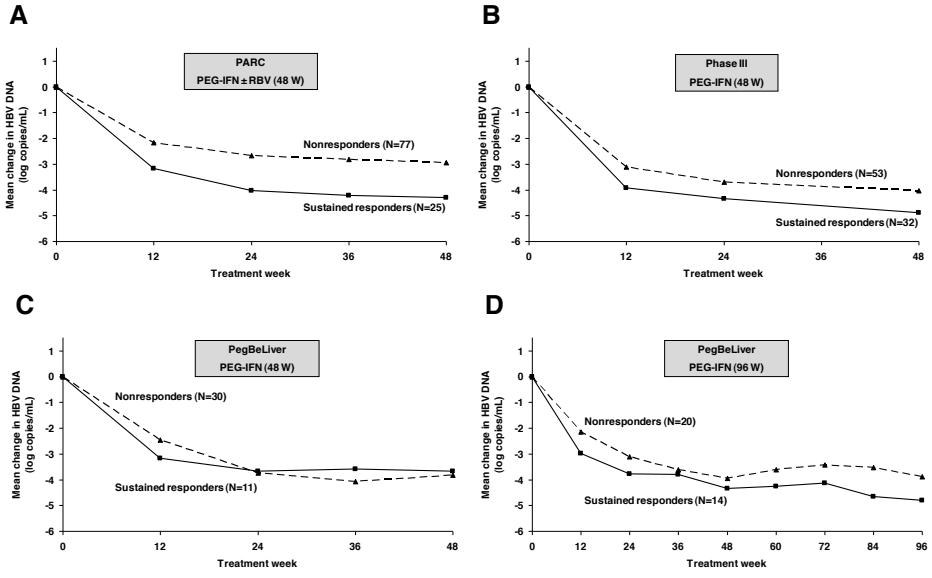

Figure 2: Mean change in HBV DNA compared to baseline according to the achievement of a sustained response within the PARC trial (A), phase III trial (B) and the 48-week (C) and 96-week (D) arm of the PegBeLiver trial.
RBV, ribavirin.

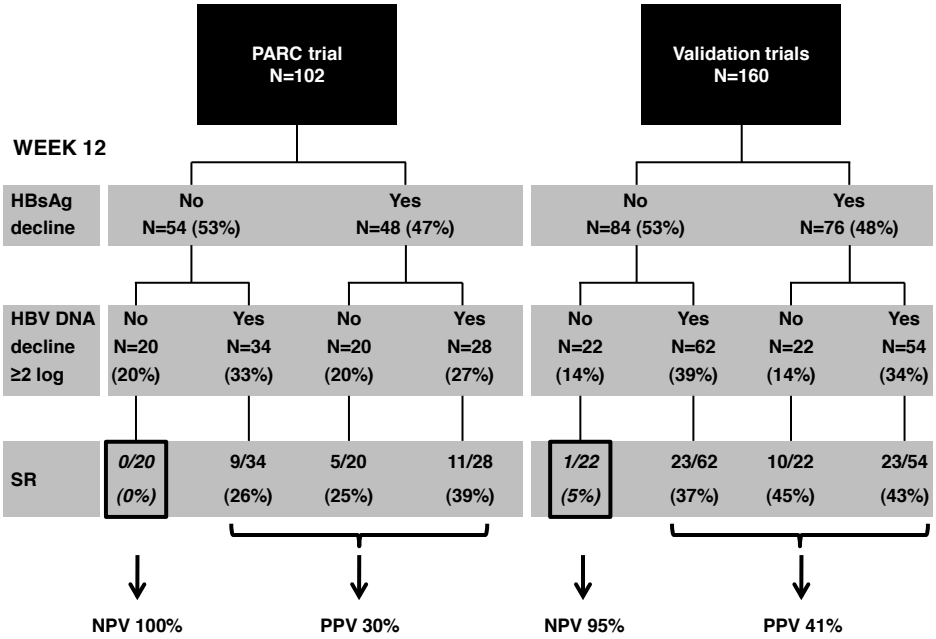

Figure 3: Flowchart showing chances of sustained response (SR) within the PARC trial and the validation trials (phase III and PegBeLiver), based on the presence of (1) any HBsAg decline and (2) HBV DNA decline ≥2 log at week 12 compared to baseline.
NPV, negative predictive value.
PPV, positive predictive value.

trial and the validation trials (phase III and PegBeLiver). None of the PARC participants without a decrease in HBsAg and with less than 2 log HBV DNA decline at week 12 achieved a SR (negative predictive value [NPV] 100%). Despite the different baseline characteristics, the stopping rule performed well across the trials (p=0.001 with correction for trial): only 5% of the patients in the validation trials without a decrease in HBsAg and with less than 2 log HBV DNA decline at week 12 had a SR (NPV 95%; figure 3).

Nevertheless, 20% of patients would be allowed to discontinue therapy at week 12 in the PARC study, while keeping all sustained responders on treatment, compared with 14% in the validation trials (figure 3). Therefore, analyses were repeated for patients infected with HBV genotype D only, which was the infecting genotype in 79% of PARC participants (table 1). The performance of the stopping rule for genotype D was similar in the PARC trial and the validation dataset. Patients without a decrease in HBsAg and with less than 2 log HBV DNA decline at week 12 (19% of the study population) had no chance of achieving a SR (NPV 100%).

Table 2: Chance of response assessed at 24 and 48 weeks after 96 weeks of PEG-IFN therapy in the PegBeLiver trial based on the presence of either any HBsAg decline or ≥2 log HBV DNA decline or both at week 12.

	PegBeLiver trial - 96 weeks N=34			PegBeLiver trial - 96 weeks N=33	
HBsAg decline and/ or ≥2 log HBV DNA decline at week 12	No 7 (21%)	Yes 27 (79%)	**HBsAg decline and/ or ≥2 log HBV DNA decline at week 12**	No 6 (18%)	Yes 27 (82%)
Response* 24 weeks after treatment	0/7 (0%)	14/27 (52%)	**Response* 48 weeks after treatment**	0/6 0%	12/27 (44%)
Predictive values	NPV 100%	PPV 52%	**Predictive values**	NPV 100%	PPV 44%

*HBV DNA <2,000 IU/mL and normal ALT
NPV, negative predictive value
PPV, positive predictive value

Performance of stopping rule in patients treated with PEG-IFN for 96 weeks

Thirty-four patients, of whom 32 (94%) harbored HBV genotype D, were treated with PEG-IFN for 96 weeks in the PegBeLiver study (table 1). Interestingly, the stopping rule performed equally well in this patient population (table 2). None of the 7 patients (21%) without a decrease in HBsAg and with less than 2 log HBV DNA decline at week 12 achieved a SR.

Follow-up data at 48 weeks after treatment were available in 33 patients treated for 96 weeks. Three patients, having HBV DNA <2,000 IU/mL and normal ALT at 24 weeks after treatment, experienced a relapse during extended follow-up. Furthermore, one patient without HBV DNA <2,000 IU/mL and normal ALT at 24 weeks after treatment, developed such a response at 48 weeks after treatment. Importantly, similar results were found for the performance of the stopping rule in relation to outcome at 48 weeks after treatment (table 2).

DISCUSSION

We recently reported a stopping rule recommending discontinuation of PEG-IFN therapy in HBeAg-negative patients who fail to achieve any decrease of HBsAg and >2 log HBV DNA decline at week 12 of a 48-week treatment course.(15) Clinical prediction rules usually demonstrate diminished performance in another patient population, because they are optimally modelled to the original data set. However, it was confirmed in two independent large trials investigating the efficacy of PEG-IFN for HBeAg-negative CHB that this rule reliably identifies those patients with no or a very low chance of achieving a SR early during the treatment course. The high NPV of the

stopping rule was confirmed across all genotypes. Furthermore, the rule performed equally well in patients receiving 96 weeks of PEG-IFN therapy.

PEG-IFN therapy for HBeAg-negative CHB is more likely to result in a SR compared with NA.(4-6, 16, 18) Moreover, rates of HBsAg clearance have been shown to increase over time in sustained responders to PEG-IFN therapy,(19) while this endpoint is scarcely achieved in HBeAg-negative patients treated with NA.(20) However, clinical application of this agent is compromised by its side effects and modest response rates. Furthermore, clinically useful baseline factors associated with an increased likelihood of response to IFN-based therapy are not readily available for HBeAg-negative disease.(11) The ability of the stopping rule to identify patients who do not have any chance of achieving a SR early during the PEG-IFN treatment course is therefore of high clinical relevance for the use of PEG-IFN in clinical practice. Moreover, the rule can be applied without risking discontinuation of therapy in potential responders to treatment.

An important finding of the current study is that the stopping rule can be used in patients infected with all four major HBV genotypes (A-D), while maintaining its excellent NPV. The rule was based on a cohort which mainly consisted of Caucasian patients infected with HBV genotype D.(15) A mixture of Asian and Caucasian patients participated in the validation trials, hence all major HBV genotypes were included.(16, 17) It has previously been demonstrated that baseline HBsAg levels and the degree of HBsAg decline during PEG-IFN therapy for HBeAg-negative CHB vary according to the infecting HBV genotype.(12, 21) Data from the PEG-IFN alfa-2a phase III registration trial showed that HBeAg-negative patients infected with HBV genotype D had the highest HBsAg levels at baseline, but also experienced a lower degree of on-treatment HBsAg decline compared with genotypes A-C.(12) These differences in HBsAg kinetics are likely to contribute to the higher proportion of patients infected with HBV genotype D in whom therapy could be stopped at week 12 of therapy compared with those infected with non-D genotypes.

An important disadvantage of PEG-IFN therapy for patients with HBeAg-negative CHB is the considerable risk of relapse following 48 weeks of treatment.(16, 18) Older studies investigating the efficacy of conventional IFN suggested that the risk of relapse may be reduced by prolonging the treatment duration.(22) The benefits of extending IFN-based therapy beyond one year were recently confirmed in the PegBeLiver study.(17) A sustained off-treatment response to PEG-IFN alfa-2a, defined as HBV DNA ≤2,000 IU/mL at 1 year after treatment discontinuation, was achieved in 31% of genotype D infected HBeAg-negative patients treated for 2 years compared with 10% of those treated for 1 year (p=0.01).(17) Although the stopping rule was based on a cohort of patients treated for 48 weeks, it performed as least as good in patients receiving 96 weeks of therapy: treatment discontinuation would be possible in 21% of patients at week 12 of

therapy without losing sustained responders. Of note, the majority of patients with a response to PEG-IFN at the end of treatment who subsequently experience a relapse do so within the first year after treatment discontinuation.(19) An important observation was therefore that similar results were found for the performance of the stopping rule in relation to outcome assessed at 48 weeks after treatment cessation.

A caveat of the current study was the fact that we only included patients who had completed 24 weeks of post treatment follow-up. The response rates were therefore higher compared to those reported in the original studies using intention-to-treat analyses.(16-18) Consequently, the positive predictive value (PPV) provided in the current manuscript may be higher than that achieved in clinical practice, since patients may drop out after week 12 of PEG-IFN therapy due to side effects or for other reasons. However, our clinical prediction rule did not aim to identify responders and it is very unlikely that the selection criteria influenced the NPV, which is the most important characteristic of a stopping rule.

In conclusion, we confirmed in two large trials that a combination of HBsAg and HBV DNA levels at week 12 identifies patients with HBeAg-negative CHB who have no or a very low chance of achieving a SR to 48 weeks of PEG-IFN therapy. The stopping rule can be used in patients infected with all four major HBV genotypes (A-D), and performed at least as good in patients treated for 96 weeks.

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6

Durable HBsAg decline in HBeAg-positive chronic hepatitis B patients treated with peginterferon alfa-2b: Relation to response and HBV genotype

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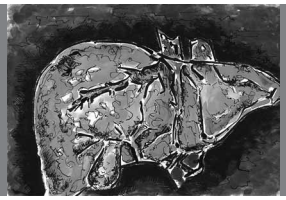


ABSTRACT

Background

On-treatment decline of serum Hepatitis B surface Antigen (HBsAg) may reflect the immunomodulatory effect of peginterferon (PEG-IFN) for HBeAg-positive chronic hepatitis B (CHB). We compared HBsAg decline across HBV genotypes between combined responders (HBeAg loss and HBV DNA <10,000 copies/mL at week 78), HBeAg responders (HBeAg loss with HBV DNA >10,000 copies/mL) and nonresponders.

Methods

HBsAg was measured at baseline, on-treatment and 6 months post-treatment in 221 HBeAg-positive CHB patients treated with PEG-IFN±lamivudine for 52 weeks, and in a representative subgroup of 142 patients at long-term follow-up (LTFU; mean follow-up 3.0 years).

Results

On-treatment HBsAg decline significantly varied according to HBV genotype (A and B more than C and D, $p<0.001$). On-treatment HBsAg decline also differed between patients with a combined response (n=43) and those without (n=178, 3.34 versus 0.69 log IU/mL decline at week 52; $p<0.001$). Among patients without a combined response, no difference was observed between HBeAg responders (n=41) versus nonresponders (n=137). HBsAg decline was sustained in combined responders and progressed to 3.75 log IU/mL at LTFU. Patients with a combined response achieved pronounced HBsAg declines, irrespective of HBV genotype, and those who achieved HBsAg levels <1,000 IU/mL at week 78 had a high probability of a sustained response and HBsAg clearance through LTFU.

Conclusions

On-treatment HBsAg decline during PEG-IFN therapy for HBeAg-positive CHB depends upon HBV genotype. Patients with a combined response to PEG-IFN achieve a pronounced HBsAg decline, irrespective of HBV genotype, which is sustained through 3 years of off-treatment follow-up.

INTRODUCTION

Chronic hepatitis B (CHB) is an important global health problem, with over 350 million people being chronically infected.(1) Prolonged liver inflammation due to infection with the hepatitis B virus (HBV) may progress to cirrhosis, liver failure and hepatocellular carcinoma (HCC).(1, 2) Hepatitis B e Antigen (HBeAg)-positive CHB is generally regarded as the earliest phase of infection in what is essentially a four phase disease continuum.(2) Current treatment guidelines recommend both pegylated interferon (PEG-IFN) and nucleos(t)ide analogues (NA) for the treatment of HBeAg-positive patients.(2, 3) A one year course of PEG-IFN results in an off-treatment sustained response, defined as HBeAg loss and HBV DNA <10,000 copies/mL at 6 months post-treatment, in around 25 percent of patients.(4, 5) Response to IFN-based therapy has been reported to be associated with a lower incidence of HCC and prolonged survival.(6-8)

Covalently closed circular DNA (cccDNA) is the main replication template of HBV (9) and low cccDNA levels following antiviral therapy have been shown to be predictive of a sustained response.(10) Intrahepatic cccDNA can only be assessed invasively, but it has recently been demonstrated that serum levels of hepatitis B surface antigen (HBsAg) reflect intrahepatic cccDNA levels in HBeAg-positive CHB patients and may consequently predict a sustained response.(11-13)

PEG-IFN induces a strong decline in serum HBsAg levels in both HBeAg-positive and HBeAg-negative patients.(14-18) Patients who achieve a sustained response to PEG-IFN exhibit a steeper HBsAg decline compared with non-responders. A recent study among HBeAg-negative patients suggested that the degree of HBsAg decline may be influenced by the infecting HBV genotype as well.(19) Given these findings, a durable suppression of HBsAg may reflect immunological control over the virus. The long-term sustainability of PEG-IFN induced HBsAg decline is however currently unknown. The aims of our study were therefore to investigate (1) which factors are associated with HBsAg decline induced by PEG-IFN for HBeAg-positive CHB and (2) whether HBsAg decline is durable through long-term follow-up.

PATIENTS AND METHODS

Patients

Serum HBsAg levels were assessed in 221 HBeAg-positive CHB patients who were previously enrolled in an investigator-initiated multicenter randomized controlled trial and a subsequent long-term follow-up (LTFU) study.(4, 20) The in- and exclusion criteria for this study have been described elsewhere. In short, patients were eligible if they had been HBsAg positive for at least 6 months prior to randomization, were HBeAg

positive twice within 8 weeks prior to randomization, had elevated serum alanine aminotransferase (ALT) levels of 2 - 10 times the upper limit of normal, and had a serum HBV DNA level above 1.0 x 10^5 copies/mL. Exclusion criteria were antiviral therapy within 6 months prior to randomization, viral co-infections, pre-existing cytopenia and/or decompensated liver disease. Patients were treated with PEG-IFN alfa-2b 100 µg weekly (PegIntron, Merck, Whitehouse Station, NJ, USA) in combination with a placebo or lamivudine (LAM) 100 mg (Zeffix, GlaxoSmithKline, Greenford, UK) daily for 52 weeks. The PEG-IFN dose was reduced to 50 µg per week after 32 weeks of therapy to limit the probability of early treatment discontinuation. Patients attended the outpatient clinic every 4 weeks for routine examinations and laboratory assessments during both the treatment and the post-treatment follow-up phase of the study. For the LTFU study, patients were re-evaluated at one additional visit at the local participating center. The mean duration of follow-up was 3.0 years.(20)

Inclusion criteria for the present study were: completion of the 26-week follow-up phase of the main study and availability of a baseline serum sample for HBsAg quantification. Of the 266 patients in the initial study, 221 fulfilled these criteria. Of these patients, 142 participated in the associated LTFU study and had LTFU samples available for HBsAg quantification.(20)

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written informed consent according to standards of the local ethics committees.

Laboratory measurements

Serum HBsAg was quantified in samples taken at baseline, during the treatment period (weeks 4, 8, 12, 24 and 52), during follow-up (week 78) and at LTFU using the ARCHITECT HBsAg assay (Abbott laboratories, Abbott Park, IL; range 0.05 - 250 IU/mL). HBV DNA quantification was performed using an in-house developed TaqMan polymerase chain reaction (PCR) assay (lower limit of quantification 400 copies/mL) based on the EuroHep standard.(21) For the LTFU study, HBV DNA was measured with the Cobas TaqMan HBV assay (Roche Molecular Systems, Branchburg, NJ, USA), with a dynamic range of quantification of 174 - 6.4 x 10^8 copies/mL (30 - 1.1 x 10^8 IU/mL). There is an excellent correlation between the two assays.(20) ALT was measured locally in accordance with standard procedures and is presented as multiples of the upper limit of normal (ULN). HBV genotype was assessed using the INNO-LiPA assay (Innogenetics, Gent, Belgium).

Statistical analysis.

Response to treatment was assessed at week 78 in all patients. A composite endpoint of HBeAg loss and HBV DNA level <10,000 copies/mL was chosen for definition of

combined response,(5) and patients who achieved HBeAg loss but failed to achieve HBV DNA <10,000 copies/mL were considered HBeAg responders. All others were non-responders. Associations between variables were tested using Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents when appropriate. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used to perform statistical analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

Patient characteristics

A total of 221 patients were included in this study. The mean age of the total study population was 34 years at the start of therapy, patients were predominantly male (78%), and of Caucasian origin (72%). HBV genotypes A and D were most prevalent in the study cohort (34% and 39%), followed by C (15%) and B (9%). Mean baseline ALT levels were 4.2 times ULN, HBV DNA levels were 9.1 log copies/mL and HBsAg levels were 4.4 log IU/mL (Table 1). Of the 221 patients, 43 (19%) achieved a combined response (HBeAg negativity and HBV DNA <10,000 copies/mL at week 78). An additional 41 patients were HBeAg responders (HBeAg loss with HBV DNA levels >10,000 copies/mL), and 19 patients (9%) lost HBsAg.

A total of 142 patients had serum available at LTFU (mean follow-up 3.0 years). The characteristics of the main study population and the LTFU cohort were fully comparable with regard to baseline HBV DNA, ALT and HBsAg levels, and HBV genotype distribution (table 1). Follow-up duration was similar in responders and non-responders. Importantly, only one patient with a combined response at week 78 experienced HBeAg relapse, and 54% of combined responders at week 78 was HBsAg negative at LTFU.

HBsAg decline on-treatment

One year of treatment with PEG-IFN resulted in a mean decline in serum HBsAg levels of 1.17 log IU/mL. This decline was sustained through 6 months of post-treatment follow-up: the mean decline from baseline was 0.92 log IU/mL at week 78. At baseline, only age ($p<0.01$) and HBV genotype ($p<0.01$) were related to HBsAg decline at week 78 by univariate analysis. Combination therapy, ALT, log HBV DNA, sex and race were all not associated with HBsAg decline at week 78. By multivariate analysis, HBV genotype, age and log HBV DNA level at baseline were related to HBsAg decline at week 78.

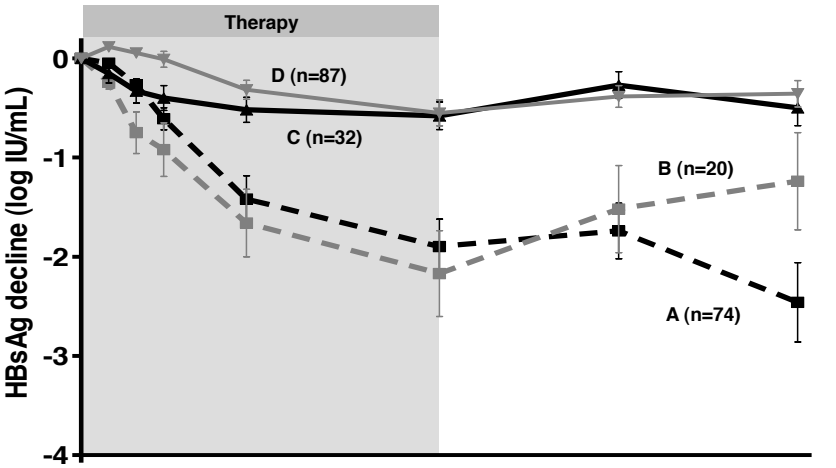
Table 1: Patient characteristics

Characteristics	Main study population (n=221)	LTFU study population (n=142)	P
Demography			
Mean (SD) age, years	34 (12)	34 (12)	1.0
Male	173 (78%)	115 (81%)	0.60
BMI	25 (4.0)	25 (4.2)	0.66
Monotherapy	111 (50%)	75 (53%)	0.63
Race			0.83
Caucasian	160 (72%)	99 (70%)	
Asian	44 (20%)	30 (21%)	
Other	17 (8%)	13 (9%)	
Laboratory results			
Mean (SD) ALT*	4.2 (3.0)	4.6 (3.4)	0.31
Mean (SD) HBV DNA, log copies/mL	9.1 (0.89)	9.1 (0.80)	1.0
Mean (SD) HBsAg, log IU/mL	4.4 (0.64)	4.3 (0.69)	0.26
HBV Genotype			0.78
A	74 (34%)	41 (29%)	
B	20 (9%)	12 (9%)	
C	32 (15%)	27 (19%)	
D	87 (39%)	56 (39%)	
Other/mixed	8 (4%)	6 (4%)	
Response at week 78			
Combined response#	43 (19%)	24 (17%)	0.58
HBeAg loss	84 (38%)	49 (35%)	0.51
HBsAg loss	19 (9%)	10 (7%)	0.69

*Multiples of upper limit of the normal range
#HBeAg loss and HBV DNA <10,000 copies/mL

HBsAg decline according to HBV genotypes

Baseline HBsAg levels were significantly different in genotypes A through D. Mean HBsAg levels were 4.53 log IU/mL in patients with genotype A, 4.33 in genotype B, 3.79 in genotype C, and 4.51 in genotype D ($p<0.001$ for genotype C versus other genotypes). Furthermore, considerably different HBsAg decline patterns were observed in the respective genotypes. While patients with genotype D experienced a slight increase during the first 12 weeks of therapy, patients with genotypes A and B showed a strong initial decline (figure 1). At the end of treatment, patients infected with genotypes A and B had a significantly more pronounced HBsAg decline (mean 1.90 and 2.17 log IU/mL, respectively), when compared to patients harbouring genotypes C or D (0.59 and 0.55, respectively; $p<0.001$ for A and B versus C and D). Through post-treatment LTFU, considerable relapse was observed in patients with genotype B (decline at LTFU 1.24 log IU/mL), whereas HBsAg levels in patients with genotype A continued to decrease (decline at LTFU 2.46 log IU/mL). HBsAg levels remained stable through LTFU in patients infected with genotypes C and D.

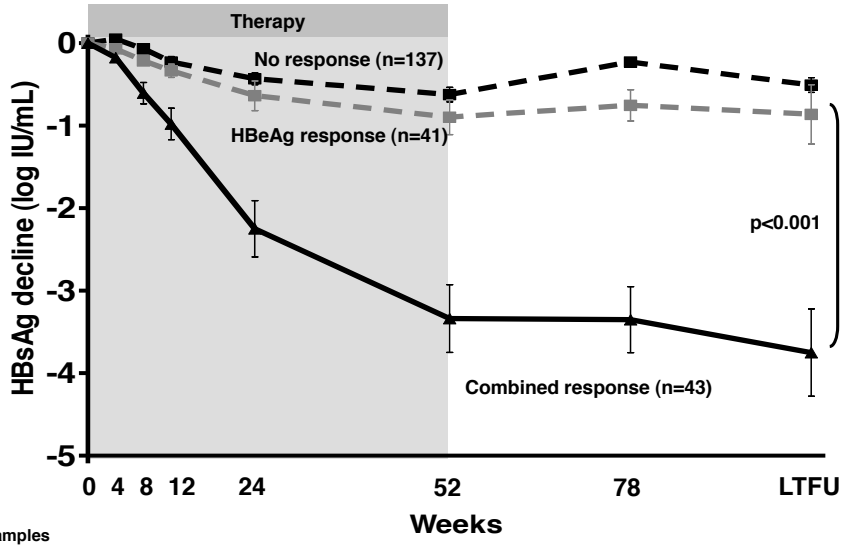


Available samples					
A	74	65	68	73	41
B	20	19	20	20	12
C	32	32	32	32	27
D	87	86	81	87	56

Figure 1: HBsAg decline in all patients according to HBV genotype. Differences between patients with genotype A or B versus C or D were statistically significant from week 24. Error bars represent the standard error of the mean.

HBsAg decline according to treatment response

Pre-treatment HBsAg levels were comparable in patients who achieved a combined response and in those who did not; 4.29 versus 4.43 log IU/mL (p=0.19). However, on-treatment HBsAg kinetics clearly differed between patients with a combined response and those without. Combined responders exhibited a decline of 3.34 log IU/mL, compared to 0.69 in all other patients (p<0.001, figure 2). Within the group who failed to achieve a combined response, no significant difference was observed between patients who achieved a HBeAg response (n=41) versus those who remained HBeAg positive (n=137); mean declines were 0.90 and 0.62 log IU/mL at week 52, respectively (p=0.17). The HBsAg decline induced by PEG-IFN was sustained in patients with a combined response, and declined further through LTFU to 3.75 log IU/mL (p=0.27 versus end of treatment). In those who were HBeAg positive at week 78, mean HBsAg decline at LTFU was 0.51 log IU/mL, (figure 2, p<0.001 compared to combined responders), while the HBeAg responders achieved a decline of 0.86 log IU/mL at LTFU (p=0.35 versus those with positive HBeAg).



Available samples					
No response	137	129	132	137	93
HBeAg response	41	40	39	41	25
Combined Response	43	40	38	42	24

Figure 2: HBsAg decline in patients with a combined response (HBeAg loss and HBV DNA <10,000 copies/mL) versus patients with an HBeAg response (HBeAg loss but HBV DNA >10,000 copies/mL) or no response. Response was assessed at week 78. Differences between patients with a combined response versus those with no response or HBeAg response were significant from week 8 of treatment. Error bars represent the standard error of the mean.

HBsAg decline according to treatment response and HBV genotype

Of the 43 combined responders, 42 were infected with genotype A through D (28 (65%) genotype A, 5 (12%) genotype B, 3 (7%) C and 6 (14%) D). Of the 41 HBeAg responders, 9 (22%) were infected with genotype A, 5 (12%) with B, 5 (12%) with C and 18 (44%) with D. Similar to the overall population, combined responders infected with genotypes A through D achieved more HBsAg decline than did patients with the same genotype who were HBeAg responders or who remained HBeAg positive (figure 3A-D). Combined responders experienced marked declines in HBsAg levels from baseline to end of treatment (genotype A 3.81, genotype B 2.98, genotype C 1.47 and genotype D 2.68 log IU/ml; p=0.59). At LTFU, declines were 4.18, 2.38, 1.87 and 3.09 log IU/mL in combined responders with genotypes A, B, C and D, respectively (p=0.56).

In contrast, on-treatment decline in the HBeAg responders (patients who cleared HBeAg but did not achieve HBV DNA <10,000 copies/mL) significantly varied according to HBV genotype; patients with genotype A and B achieved declines of 2.02 and 1.56 log IU/mL at week 52, compared to 0.50 and 0.43 log IU/mL in patients with C and D (P=0.02 for A and B versus C and D). At LTFU, declines were 3.33, 0.92, 0.09 and 0.22 in HBeAg responders with genotypes A through D, respectively (p=0.03).

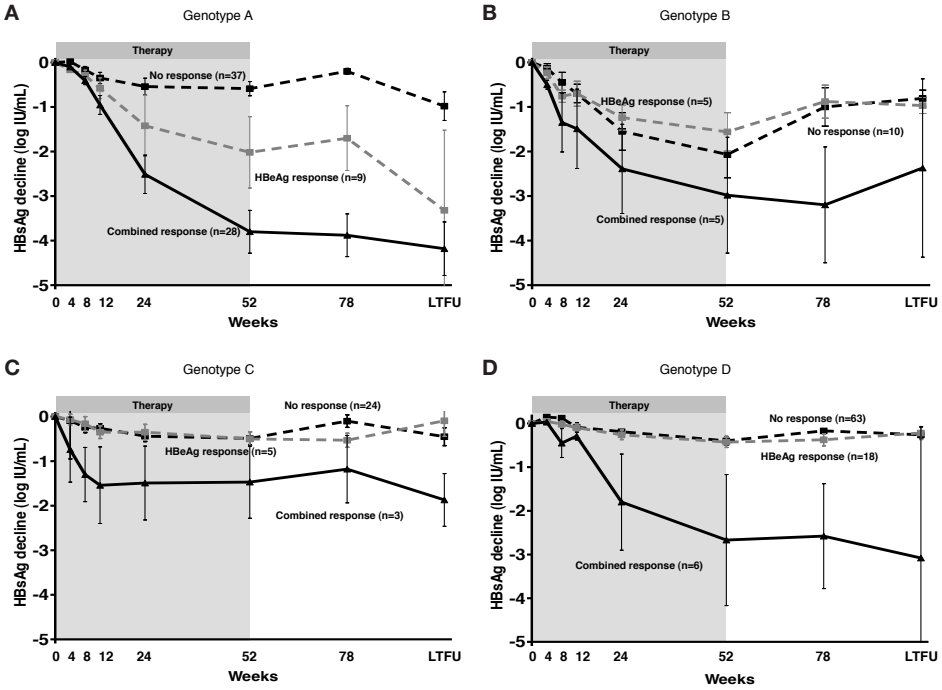


Figure 3: HBsAg decline in patients with a combined response (HBeAg loss and HBV DNA <10,000 copies/mL at week 78) versus patients with an HBeAg response (HBeAg loss but HBV DNA >10,000 copies/mL at week 78) or no response for genotypes A (A), B (B), C (C) and D (D). Error bars represent the standard error of the mean.

Relationship between HBsAg levels at week 78 and response at LTFU

Thirty-three of 142 (23%) patients with HBsAg data at LTFU achieved a combined response at LTFU. Analysis was limited to the 141 with available HBsAg levels at week 78. Probabilities of achieving a combined response at LTFU in relation to HBsAg levels and decline at week 78 are shown in table 2, both for the overall population (n=141) and for patients with a combined response at week 78 with available LTFU data (n=23). Importantly, no patient without a decline at week 78 achieved HBsAg clearance by LTFU, whereas patients with an HBsAg level <1,000 IU/mL at week 78 (n=28) had a probability of HBsAg clearance of 46% (13/28). Only 2 of 113 (2%) patients with HBsAg ≥1,000 IU/mL achieved HBsAg clearance at LTFU. Among patients with a combined response, those with HBsAg <1,000 IU/mL achieved HBsAg negativity at LTFU in 73% (11/15), compared to 25% (2/8) in those with HBsAg ≥1,000 IU/mL.

Table 2. HBsAg level and decline at week 78 and response at LTFU

		All patients* (n=141)				Combined response 78# (n=23)			
Combined response LTFU		No	Yes	NPV	PPV	No	Yes	NPV	PPV
HBsAg level week 78	<1,000	11	17	-	61%	0	15	- 100%	
	≥1,000	98	15	87%	-	4	4	50%	-
Any decline week 78	Yes	71	31	-	30%	3	19	-	86%
	No	38	1	97%	-	1	0	100%	-

*All patients with HBsAg levels at week 78 and at LTFU
#Patients with a combined response at week 78. Combined response was defined as HBeAg negativity with HBV DNA <10,000 copies/mL.
NPV, negative predictive value
PPV, positive predictive value

DISCUSSION

In this large study we show that on-treatment HBsAg decline is a sensitive marker for response to PEG-IFN, showing a steep decline in patients who achieved a combined response (defined as HBeAg loss and HBV DNA <10,000 copies/mL), irrespective of HBV genotype. A very limited decline was observed in patients who remained HBeAg positive or those who cleared HBeAg but progressed to active HBeAg-negative CHB, and patients without an HBsAg decline at week 78 have a very limited probability of response at LTFU. Furthermore, HBsAg decline was sustained in patients with a combined response through 3 years of follow-up, reflecting a long-term sustained suppression after therapy discontinuation.

The HBV replication pathway that produces HBsAg is separate from the pathway that produces HBV DNA. HBsAg is transcribed and subsequently translated from the HBV envelope gene, and produced as small, medium or large HBsAg proteins.(22, 23) Synthesized HBsAg proteins may then be incorporated into mature HBV nucleocapsids, and subsequently secreted from the hepatocyte. However, HBsAg production far exceeds that required for the production of HBV virions and HBsAg is therefore also secreted in the form of non-infectious particles. Commercially available HBsAg quantification assays probably detect all forms of HBsAg,(24) but the clinical relevance of the different HBsAg forms is so far unclear.

HBsAg levels in the sera of patients with CHB depend on the phase of infection. Patients classified as being in the immune tolerant and immune clearance phases of the disease have the highest HBsAg levels, whereas HBsAg is lowest in inactive carriers.(24-26) Furthermore, patients classified as inactive carriers who experienced a subsequent HBV DNA increase (reactivation) had higher HBsAg levels compared to those who did not.(25) A post-treatment sustained reduction in HBsAg levels achieved

with PEG-IFN therapy may therefore signify an immunological response, resulting in transition to the inactive carrier state.(26) In our study, one year of PEG-IFN therapy induced a pronounced decline in serum HBsAg levels, particularly in patients who achieved HBeAg loss and HBV DNA <10,000 copies/mL at week 78. Importantly, the decline achieved in these combined responders was durable through LTFU. This implies not only long-term sustained disease remission with a very low probability of relapse,(25) but also reflects the high probability of subsequent HBsAg loss as described previously in HBeAg-positive responders to (PEG-)IFN.(20, 27) Furthermore, we found that patients who achieved a combined response (HBeAg clearance with HBV DNA <10,000 copies/mL at week 78) with concomitant HBsAg levels <1,000 IU/mL did not experience relapse during off-treatment follow-up and were very likely to clear HBsAg. Conversely, patients who failed to achieve an HBsAg decline from baseline by week 78 had little chance of combined response, and no chance of HBsAg loss, suggesting the necessity of retreatment with other agents.

The current study also shows that baseline HBsAg levels and on-treatment HBsAg decline in HBeAg-positive patients are dependent upon HBV genotype. These findings are in line with a report on HBeAg-negative subjects,(19) and may reflect a difference in transcription efficacy between respective genotypes.(28) However, the differences observed in HBsAg decline according to genotype may also be a reflection of the variance in the efficacy of PEG-IFN across the genotypes. Among HBeAg responders, those with genotypes C and D experienced only a limited reduction in HBsAg levels, and these patients were reported to have a high probability of HBeAg seroreversion and persistently detectable HBV DNA through LTFU.(20, 29) Conversely, HBeAg responders infected with genotype A experienced a pronounced decline in HBsAg levels, and also had the highest probability of losing HBsAg through LTFU.(20) Interestingly, we found detectable precore and/or core promoter mutants by INNO-LiPA line-probe assay in all but one of the HBeAg responders with available serum, irrespective of HBV genotype (n=29, data not shown). This suggests that the differences in HBsAg decline across genotypes are not due to the presence or absence of these mutants.

In contrast to the genotype specific differences among HBeAg responders, all combined responders experienced pronounced HBsAg declines. This shows that, irrespective of HBV genotype, a combined response is associated with a sustained reduction in HBsAg levels and a high probability of HBsAg loss through long-term follow-up.(27) Taken together, these observations corroborate recent data highlighting the influence of HBV genotype on response to PEG-IFN, and show that a combined response of HBeAg loss and HBV DNA <10,000 copies/mL is the most appropriate marker for response to PEG-IFN, especially in patients with non-A genotypes.(5, 20)

Recent studies among HBeAg-positive (18) and HBeAg-negative (14, 30) patients treated with PEG-IFN have shown that HBsAg levels during therapy may be used to

predict response to treatment. However, the current study now shows that HBsAg decline during PEG-IFN treatment for HBeAg-positive CHB depends upon HBV genotype as well, and the differences in HBsAg decline suggest that genotype specific thresholds may be required when using HBsAg to guide PEG-IFN based therapy.

A possible caveat of our study is that we pooled data from the two treatment arms of the original trial. Combination therapy of PEG-IFN and LAM is known to cause a slightly steeper on-treatment HBsAg decline. However, this effect of LAM was the same regardless of treatment response or HBV genotype, and was not sustained post-treatment. Furthermore, we found no difference in any of the outcomes when we analyzed the treatment arms separately. Response rates were similar in patients treated with PEG-IFN monotherapy versus the combination with LAM (18 versus 21%, p=0.61), as were HBsAg decline at week 78 (0.86 versus 0.98 log IU/mL, p=0.63) and at LTFU (0.93 versus 1.32, p=0.22). A subgroup of non-responders was retreated after the initial study. Retreatment did not affect HBsAg kinetics during LTFU.

In conclusion, HBeAg-positive CHB patients who achieve a combined response (HBeAg loss and HBV DNA <10,000 copies/ml) to PEG-IFN therapy achieve a pronounced decline in HBsAg levels, irrespective of HBV genotype. In contrast, patients who clear HBeAg but have HBV DNA levels >10,000 copies/mL experience a limited decline, showing that HBeAg loss alone may be a suboptimal marker for response to PEG-IFN in patients with non-A genotypes. The HBsAg decline achieved in patients with a combined response is sustained through 3 years of post-treatment follow-up reflecting a durable response with a low chance of relapse and a high probability of subsequent HBsAg loss.

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7

Long-term follow-up of HBeAg-negative patients treated with peginterferon alfa-2a: Progressive HBsAg decline in responders

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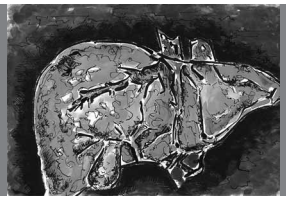
Submitted.



ABSTRACT

Background

The aim of this study was to evaluate the long-term response to peginterferon alfa-2a (PEG-IFN) in patients with hepatitis B e antigen (HBeAg-)negative chronic hepatitis B.

Methods

All patients enrolled in the PARC study who completed the treatment phase were eligible for this long-term follow-up (LTFU) study. Patients received PEG-IFN alfa-2a (180 µg weekly) ± ribavirin (1000-1200 mg daily) for 48 weeks and had at least one additional LTFU visit after the initial follow-up period of 24 weeks (mean duration 2.1 ± 0.2 years). Re-treated patients were considered nonresponders.

Results

Of 117 patients who completed the treatment phase, 79 (68%) were included in this LTFU study. Among 19 patients with a combined response at 24 weeks after treatment (initial responders; hepatitis B virus DNA <10,000 copies/mL (<1,714 IU/mL) and normal alanine aminotransferase), 12 (63%) sustained this response through LTFU. Three additional patients had such a response at LTFU, resulting in a total of 15 (19%) combined responders at LTFU. A profound decrease of serum HBsAg levels was observed in initial responders, resulting in HBsAg clearance in 26% (6% of all LTFU participants).

Conclusions

About one third of HBeAg-negative patients with a response to PEG-IFN at 24 weeks after treatment subsequently relapsed during 2 years of follow-up. Despite the limited overall efficacy of PEG-IFN, patients responding to PEG-IFN treatment experienced a strong serum HBsAg decline resulting in a high rate of HBsAg clearance, which indicates the need for predictors of response to PEG-IFN in HBeAg-negative disease.

INTRODUCTION

Hepatitis B e antigen (HBeAg-)negative chronic hepatitis B (CHB) has become the predominant type of hepatitis B virus (HBV) infection in many geographical areas.(1) HBeAg-negative CHB typically arises at a later stage of the disease due to replication of naturally occurring HBV variants harbouring mutations in the precore and/or basal core promoter regions.(2) The prognosis of HBeAg-negative patients is poor if left untreated and spontaneous disease remission rarely occurs.(3)

Treatment for HBeAg-negative CHB may be given using two approaches.(4) On the one hand, long-lasting maintained HBV DNA suppression without antiviral resistance is achievable in a vast majority of patients by the last generation nucleos(t)ide analogues (entecavir and tenofovir).(5, 6) However, responses induced by nucleos(t)ide analogues (NA), at least when administered for relatively short periods, are usually not sustained in the absence of therapy.(7) On the other hand, peginterferon (PEG-IFN) therapy results in relatively weak viral suppression, but sustained off-treatment responses are achieved in a selected group of HBeAg-negative patients due to its immunomodulatory properties.(8, 9) Nevertheless, responses to PEG-IFN have usually been assessed at 6 months after treatment discontinuation and data on the long-term durability of response are limited.

An advantage of PEG-IFN compared with NA in HBeAg-negative disease is the increased rate of hepatitis B surface antigen (HBsAg) clearance. In line with this finding, recent studies reported that PEG-IFN therapy results in a significant decline of HBsAg levels in HBeAg-negative patients.(10, 11) In contrast, a reduction of HBsAg levels was not observed in patients treated with lamivudine or entecavir.(10, 12) Whether the decline of HBsAg induced by PEG-IFN is sustained during long-term follow-up is currently unknown.

This study examines the long-term response to PEG-IFN alfa-2a and evaluates the long-term sustainability of PEG-IFN induced HBsAg decline in patients with HBeAg-negative CHB.

PATIENTS AND METHODS

Study population

Initial study

The initial PARC study involved 133 patients (25 centers in 7 European countries), who were randomly assigned to receive PEG-IFN alfa-2a 180 µg/week (Pegasys, F. Hoffmann-La Roche Ltd., Basel, Switzerland) in combination with placebo (N=69) or

PEG-IFN alfa-2a with ribavirin 1000-1200 mg/day (Copegus, F. Hoffmann-La Roche Ltd., Basel, Switzerland; N=64) for 48 weeks.(9) The primary endpoint was the combined presence of serum HBV DNA <10,000 copies/mL (1,714 IU/mL) and alanine aminotransferase (ALT) within the normal range at 24 weeks after treatment (week 72).(9) Patients who had HBV DNA <10,000 copies/mL and normal ALT at week 72 were classified as initial responders for the current long-term follow-up (LTFU) study.
For the initial study, eligible patients had been HBsAg positive for longer than 6 months; were HBeAg negative and anti-HBe positive on 2 occasions within 2 months prior to randomization; had a serum HBV DNA level >100,000 copies/mL (17,143 IU/mL) and had had 2 episodes of elevated ALT (>1.5 but ≤10 times the upper limit of normal (ULN) of the normal range) within 2 months prior to randomization. Exclusion criteria included: antiviral or immunosuppressive therapy within the preceding 6 months; co-infection with hepatitis C, hepatitis D or human immunodeficiency virus (HIV); other acquired or inherited causes of liver disease; pre-existing cytopenia or decompensated liver disease.(9)

Long-term follow-up study

All patients included in the initial study who completed the treatment phase were eligible for this LTFU study. For the LTFU study, patients had at least one additional visit after completing the initial follow-up phase of 24 weeks. If the patient had received re-treatment during the follow-up period, a serum sample and local data before re-treatment were obtained. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Informed consent was obtained from each patient included in the study.

Assessment of efficacy

In accordance with the initial study, the primary endpoint at LTFU was the combined presence of HBV DNA <10,000 copies/mL and normal ALT. Secondary endpoints included HBV DNA <10,000 and <400 copies/mL (69 IU/mL), normal ALT as well as the occurrence of HBsAg loss. Re-treated patients were considered nonresponders for all efficacy analyses.
Serum ALT levels were measured locally according to standard procedures. Serum HBV DNA (Taqman HBV assay, Roche Diagnostics, Basel, Switzerland, lower limit of quantification: 35 copies/mL (6 IU/mL)), HBsAg (range 0.05-250 IU/mL) and anti-HBs antibodies (ARCHITECT assay, Abbott laboratories, Abbott Park, IL) were measured centrally at the Erasmus MC University Medical Center, Rotterdam.

Statistical analysis

Continuous variables are expressed as means ± standard deviation (SD) or medians with interquartile range (IQR), where appropriate. HBV DNA and HBsAg concentrations were logarithmically transformed for analysis. Continuous variables were compared between groups using the t-test, one-way analysis of variance (ANOVA), or the Mann-Whitney test, categorical variables using the Chi-square or Fisher's exact test. The association between baseline factors and combined response (HBV DNA <10,000 copies/mL and normal ALT) at LTFU was assessed by univariate regression analyses. The performance of HBsAg levels to discriminate between initial responders who sustained their combined response through LTFU and those who did not was evaluated by the area under the receiver-operating characteristic (ROC) curve. All statistical tests were two-sided and were evaluated at the 0.05 level of significance. SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients

A total of 117 (88%) out of 133 patients completed the 48-week treatment phase of the initial study, of whom 79 (68%) were included in the LTFU study. The mean interval between the end of treatment (week 48) and the LTFU visit was 2.1±0.2 years. As shown in the LTFU study profile (figure 1), most of the 38 patients who were not enrolled in the LTFU study did not participate because the local center did not take part in this study (N=15; 39%) or because of loss to follow-up (N=10; 26%). None of the patients died during the LTFU study. All baseline characteristics of LTFU participants (N=79) and patients who were not included in the LTFU study (N=54) were comparable except for serum HBsAg level (3.9±0.4 versus 3.7±0.8 log IU/mL, respectively; p=0.049).

Baseline characteristics and response at 24 weeks after treatment

Baseline characteristics and response rates at 24 weeks after treatment (week 72) from patients participating in the initial study and in the LTFU study are shown in table 1. The two groups were similar regarding clinical and virological characteristics at baseline. Sixty-one (77%) of the LTFU participants were infected with HBV genotype D. Thirty-seven (47%) patients included in the LTFU study received PEG-IFN monotherapy. In the initial study, the number of patients with a combined response at week 72 (HBV DNA <10,000 copies/mL and normal ALT) was comparable in the PEG-IFN monotherapy and PEG-IFN and ribavirin combination therapy group (14 of 69 (20%) versus 10 of 64 (16%) patients, p=0.49).(9) These patients were classified as initial responders. The proportion of initial responders did also not differ significantly between the treatment

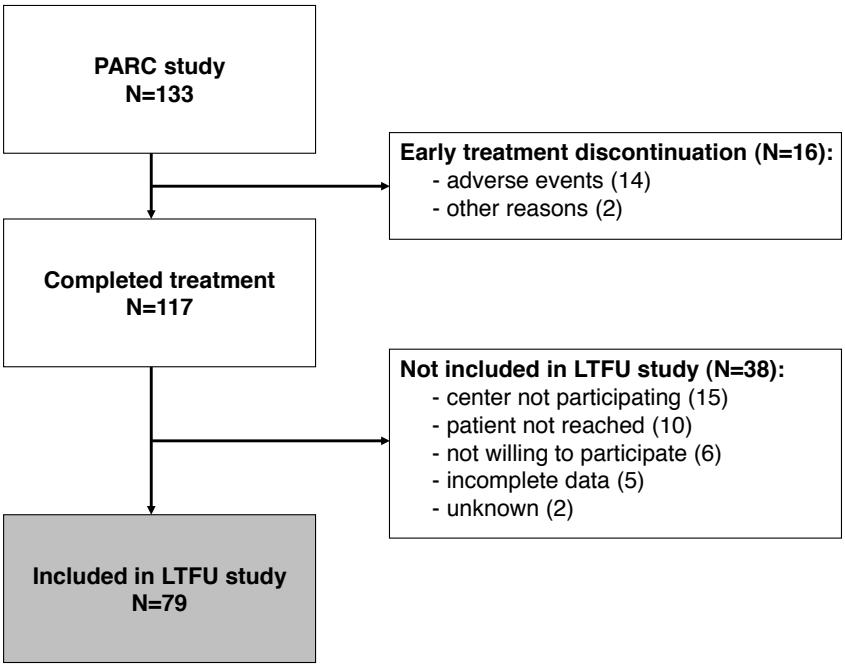


Figure 1: Study profile showing the inclusion of patients from the initial PARC study in the LTFU study and the reasons for not participating of those patients not included in the LTFU study.

groups in the subset of patients included in the LTFU study (11 of 37 (30%) versus 8 of 42 (19%) patients treated with monotherapy and combination therapy, respectively; p=0.27). Further analyses are therefore presented for both treatment groups combined.

Virological and biochemical response rates at LTFU

Among 79 patients enrolled in the LTFU study, nucleos(t)ide analogue therapy was initiated in 41 (52%) patients after week 48. Initial responders were less likely to receive re-treatment compared with initial nonresponders (2 (11%) of 19 versus 39 (65%) of 60 patients, p<0.001). Re-treated patients were considered as nonresponders for all efficacy endpoints.

Virological and biochemical response rates for the LTFU study population at week 72 and at LTFU are shown in figure 2. Among the 19 initial responders, 12 (63%) also had a combined response (HBV DNA <10,000 copies/mL and normal ALT) at LTFU (figure 3). Three additional patients developed such a response during LTFU, resulting in a total of 15 (19%) patients with a combined response at LTFU (figure 2). All patients with a combined response at LTFU were infected with HBV genotype D (15 (25%) of 61 patients with a combined response for D versus none of 18 for non-D, p=0.02). Other baseline characteristics, including serum HBV DNA, HBsAg and ALT level, were not associated with a combined response at LTFU.

Table 1: Baseline characteristics and response at 24 weeks after treatment of patients from the initial study and the LTFU study

	Initial study (N=133)	LTFU study (N=79)
Baseline		
Peginterferon monotherapy	69 (52%)	37 (47%)
Mean (SD) age, years	42 (11)	41 (10)
Male	98 (74%)	56 (71%)
Race		
Caucasian	127 (95%)	74 (94%)
Other	6 (5%)	5 (6%)
Median (IQR) ALT*	2.1 (1.6-4.0)	2.1 (1.6-4.1)
Mean (SD) HBV DNA, log copies/mL	6.8 (1.2)	7.0 (1.2)
Mean (SD) HBsAg, log IU/mL	3.8 (0.6)	3.9 (0.4)
HBV Genotype		
A	17 (13%)	9 (11%)
D	107 (80%)	61 (77%)
Other/mixed	9 (7%)	9 (11%)
Median (IQR) necroinflammation	5 (4-7)	5 (4-7)
Median (IQR) fibrosis	3 (1-3)	3 (1-3)
Previous interferon therapy†	24 (18%)	10 (13%)
Previous lamivudine therapy‡	24 (18%)	10 (13%)
Response 24 weeks after treatment		
Combined response§	24 (18%)	19 (24%)
HBV DNA <10,000 copies/mL	26 (20%)	20 (25%)
HBV DNA <400 copies/mL	10 (8%)	8 (10%)
Normal ALT	61 (46%)	42 (53%)

*Multiples of upper limit of the normal range
†At least 4 weeks of (peg-)interferon treatment
‡At least 3 months of lamivudine treatment
§HBV DNA <10,000 copies/mL and normal ALT

Nine (11%) patients had HBV DNA levels <400 copies/mL at LTFU, all were initial responders. HBsAg was negative in 5 (6%) patients at LTFU, which was accompanied by the appearance of anti-HBs in 3 cases. HBsAg loss was exclusively observed among initial responders and HBV DNA was <400 copies/mL in all patients who achieved HBsAg negativity.

Sustainability of serum HBsAg decline during LTFU

Levels of serum HBsAg at LTFU were available in 35 of 38 patients who had not been re-treated after week 48. The degree of HBsAg decline was larger in patients who had a combined response at LTFU compared to those without (mean HBsAg decline 2.6±2.3 log IU/mL compared with 0.3±0.4 log IU/mL, p=0.003). Of note, the mean HBsAg decline was similar in re-treated patients at the time new antiviral therapy was initiated (0.3±0.5 log IU/mL) and untreated patients without a combined response at LTFU.

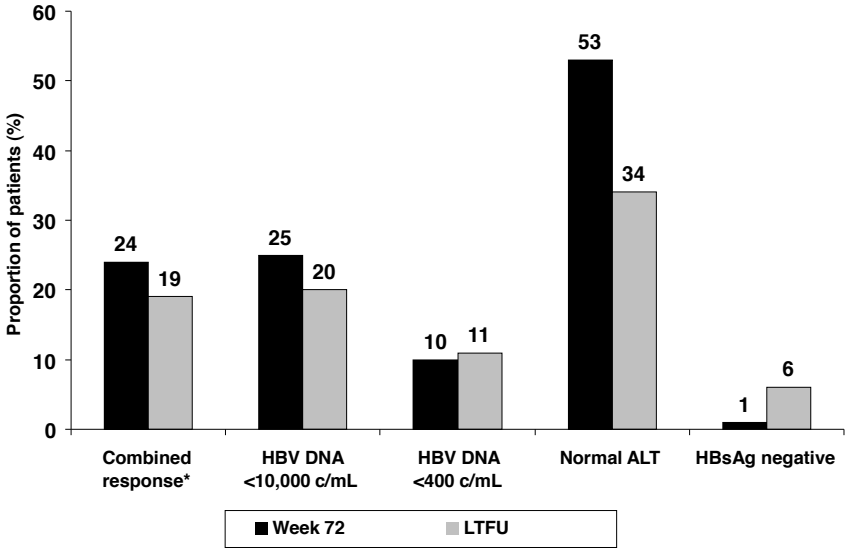


Figure 2: Response rates at 24 weeks after treatment (week 72) and at LTFU (mean 2.1±0.2 years after treatment) of 79 patients enrolled in the LTFU study. *HBV DNA <10,000 copies/mL and normal ALT.

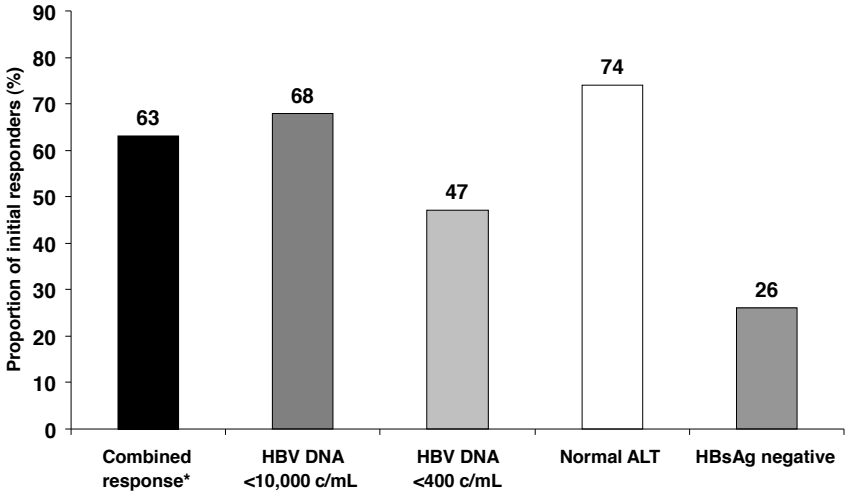


Figure 3: Response at LTFU of 19 initial responders (HBV DNA <10,000 copies/mL and normal ALT at 24 weeks after treatment). This figure displays the percentage of initial responders who had a *combined response (HBV DNA <10,000 copies/mL and normal ALT), HBV DNA <10,000 and <400 copies/mL, normal ALT and negative HBsAg at LTFU.

Next, we studied HBsAg decline over time in the 79 LTFU participants according to the pattern of response. Patients were categorized into 4 groups based on the presence of a combined response at week 72 and/or at LTFU. Figure 4 shows that the degree of

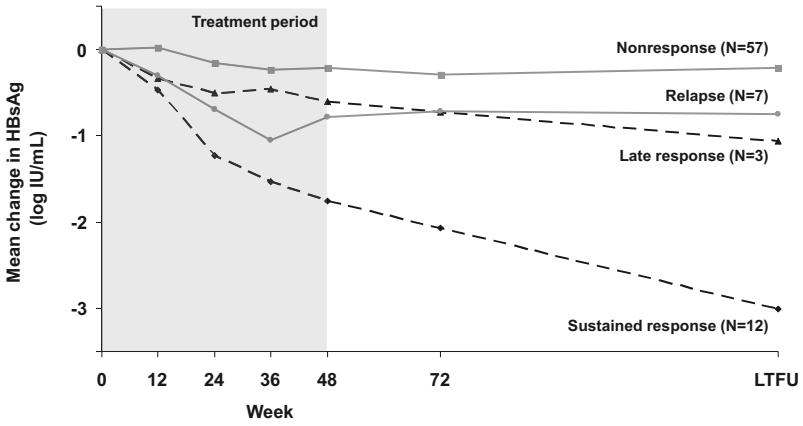


Figure 4: HBsAg kinetics according to pattern of response. Mean change in serum HBsAg level compared to baseline in patients who did not have a combined response (HBV DNA <10,000 copies/mL and normal ALT) at 24 weeks after treatment (week 72) and at LTFU (nonresponse), those who experienced a relapse (combined response at week 72, but not at LTFU), those who had a late response (combined response at LTFU, but not at week 72) and sustained responders (combined response both at week 72 and LTFU). p<0.001 by ANOVA for comparison between the groups.

HBsAg decline significantly varied according to the pattern of response (p<0.001 by ANOVA). Patients who did not achieve a combined response at both occasions hardly experienced a decrease of HBsAg (nonresponse, N=57; mean decline at LTFU 0.2±0.4 log IU/mL, p=0.03 compared to baseline). In patients who experienced a relapse (N=7; initial responders without a combined response at LTFU) and in patients with a late response (N=3; combined response at LTFU, but not at week 72) a considerable degree of HBsAg decline was observed (0.8±0.2 and 1.1±0.6 log IU/mL, respectively; p=0.008 and p=0.09 compared to baseline). The 12 sustained responders, defined as initial responders who sustained their combined response during LTFU, experienced the most profound decrease of HBsAg (3.0±2.4 log IU/mL, p=0.002 compared to baseline).

Prediction of relapse in initial responders

Given the higher degree of HBsAg decline in initial responders who also had a combined response at LTFU compared with those who relapsed, we assessed by ROC analysis whether the HBsAg concentration at week 72 could be useful for the prediction of relapse in the 19 initial responders. The mean decline of HBsAg at week 72 was larger among sustained responders compared with those who relapsed (2.1±1.9 versus 0.7±0.5 log IU/mL, p=0.03). The area under the ROC curve for serum HBsAg to predict relapse was comparable for absolute values and declines compared to baseline (0.77 versus 0.74). We aimed to identify a cut-off point for week 72 HBsAg to allow reliable identification of initial responders with a low risk of relapse. All 9 initial responders who

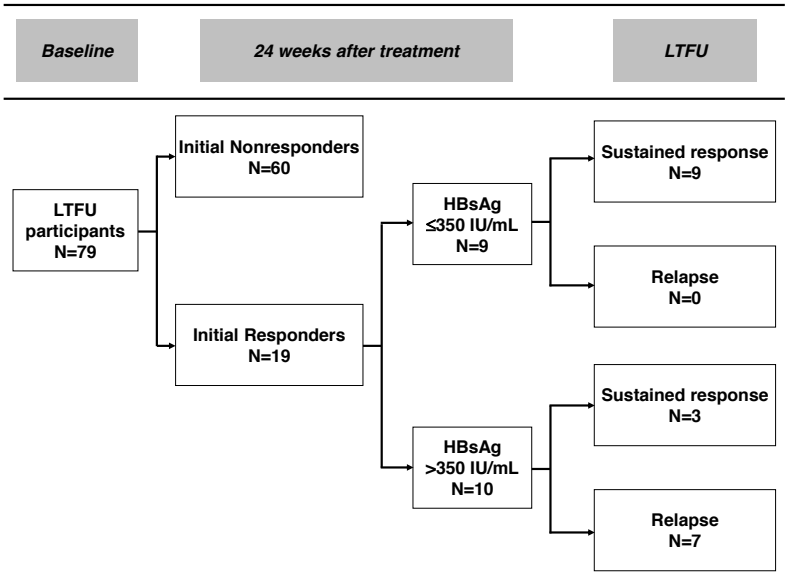


Figure 5: Algorithm showing the chances of sustained response and relapse for initial responders (HBV DNA <10,000 copies/mL and normal ALT at 24 weeks after treatment) according to serum HBsAg at 24 weeks after treatment (week 72).

had serum HBsAg ≤350 IU/mL at week 72 also had a combined response at LTFU. In contrast, relapse occurred in 7 out of 10 initial responders with HBsAg >350 IU/mL (figure 5). The level of HBV DNA at week 72 was similar in sustained responders and those who relapsed (2.7±1.1 versus 2.9±0.7 log copies/mL, p=0.68).

DISCUSSION

Current guidelines support the use of PEG-IFN for the initial treatment of patients with HBeAg-negative CHB because a sustained off-treatment response is achievable in a selected group of patients.(13, 14) However, data on the long-term durability of response to PEG-IFN are still scarce. Our LTFU study shows that 19% of HBeAg-negative patients treated with PEG-IFN alfa-2a for 48 weeks had a combined response (HBV DNA <10,000 copies/mL and normal ALT) at more than 2 years after treatment discontinuation. This definition of response is acknowledged by the most recent guidelines as an appropriate marker of response to PEG-IFN therapy.(13) Because fluctuations in HBV DNA and ALT levels are associated with HBeAg-negative disease, we also explored the sustainability of response through LTFU in initial responders (HBV DNA <10,000 copies/mL and normal ALT at 24 weeks after treatment). Relapse occurred in approximately one third of the initial responders, so 15% of the total study population

had a combined response documented at 24 weeks after treatment and at LTFU. A high degree of serum HBsAg decline was observed in initial responders resulting in a substantial rate of HBsAg clearance (26%) at LTFU.

This is only the second study investigating the long-term effects of PEG-IFN in HBeAg-negative CHB. Another recent study showed that HBV DNA levels were <10,000 copies/mL in 28% of HBeAg-negative patients both at 2 and 3 years after treatment with PEG-IFN ±lamivudine,(15) compared with 20% of patients having HBV DNA <10,000 copies/mL at 2 years after treatment in our study. This difference in response rates may be accounted for by the demographic constitution of the populations studied, with the majority being of Asian origin in the first study versus the predominance of Caucasian patients infected with HBV genotype D in our study.(15) Nevertheless, the rate of HBsAg negativity reported by Marcellin et al. increased to 6% at 2 years after treatment, which is similar to that reported here, and HBsAg loss was observed across all HBV genotypes with the highest probability in genotype A patients (20%).(15) Interestingly, we found that none of the genotype A patients experienced HBsAg loss, although the number of patients infected with non-D genotypes was limited in our study, which precludes a direct comparison between the different genotypes.

Our study is the first to describe serum HBsAg levels in HBeAg-negative patients treated with PEG-IFN up to 2 years after treatment discontinuation. Patients with a combined response at LTFU showed a marked HBsAg decline compared with baseline, in contrast to those without a response. Moreover, the strongest decrease of HBsAg was observed in patients with a combined response both at 24 weeks after treatment (week 72) and at LTFU. However, patients who experienced a relapse between week 72 and LTFU also showed a considerable degree of HBsAg decline. Although the number of patients was limited, an interesting finding of our study is that all initial responders who had serum HBsAg ≤350 IU/mL at week 72 sustained their combined response through LTFU, while relapse occurred in 70% of initial responders with HBsAg >350 IU/mL. These findings suggest that careful monitoring and prompt initiation of antiviral therapy is required for this group of patients. The considerable on-treatment HBsAg decline in patients who experienced a relapse suggests that these patients may benefit from prolongation of PEG-IFN therapy. Prolongation of treatment with conventional IFN significantly reduced the risk of relapse in HBeAg-negative patients.(16) A recent Italian study confirmed that this approach also applies to PEG-IFN with a sustained off-treatment response (HBV DNA ≤2000 IU/mL at 1 year after treatment discontinuation) in 31% of genotype D infected HBeAg-negative patients treated for 2 years compared with 10% of those treated for 1 year (p=0.01).(17) Further studies are warranted to determine whether HBsAg quantification can be helpful in determining the duration of PEG-IFN treatment in individual patients with HBeAg-negative CHB.

PEG-IFN therapy resulted in a limited response rate in our HBeAg-negative population. Nevertheless, a progressive decrease of HBsAg levels was observed among patients achieving a response resulting in an increased rate of HBsAg clearance. These results emphasize the need for predictors of response to PEG-IFN in HBeAg-negative CHB. We previously reported that baseline factors were not predictive of a combined response assessed at 24 weeks after treatment and we confirmed this for response assessed at LTFU.(9) Overall, baseline predictors of response remain less well-defined in HBeAg-negative compared with HBeAg-positive disease.(18, 19) Several studies have therefore investigated the use of on-treatment markers in predicting response to PEG-IFN in HBeAg-negative patients.(11, 20) We recently found that none of the patients in whom a decrease in serum HBsAg level was absent and HBV DNA declined less than 2 log copies/mL achieved a combined response at 24 weeks after treatment (negative predictive value 100%), and should be advised to discontinue therapy.(21) Importantly, none of the LTFU participants without HBsAg decline and with <2 log copies/mL HBV DNA decline at week 12 of PEG-IFN therapy had a combined response at LTFU (data not shown). This stopping rule thus reliably identifies nonresponders at an early stage of treatment, but needs to be validated in patients with other HBV genotypes and in patients treated with PEG-IFN beyond 48 weeks.

A caveat of the current LTFU study was that a subgroup of the patients participating in the initial study was included. However, the LTFU group was representative of the initial study population with regard to baseline characteristics. Furthermore, a considerable proportion of patients dropped out during LTFU because antiviral therapy was initiated. These patients were considered as nonresponders for all efficacy analyses.

In conclusion, about one third of HBeAg-negative patients with a combined response (HBV DNA <10,000 copies/mL and normal ALT) to PEG-IFN at 24 weeks after treatment subsequently experienced a relapse during 2 years of follow-up, resulting in a combined response rate of 19% at LTFU. Despite the limited overall efficacy of PEG-IFN, a high degree of serum HBsAg decline was observed in patients responding to PEG-IFN treatment resulting in a substantial rate of HBsAg clearance. These results further emphasize the need for predictors of response to PEG-IFN in patients with HBeAg-negative disease.

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APPENDIX

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Summary and discussion

Derived from:

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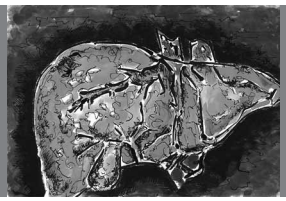
(2) Rijckborst V, Janssen HL. The Role of Interferon in Hepatitis B Therapy. Curr Hepat Rep. 2010 Nov;9(4):231-238.



In the last decade great strides have been made in the treatment of chronic hepatitis B (CHB) with the introduction of nucleos(t)ide analogues (NA) and pegylated forms of interferon (PEG-IFN). The increasing number of treatment options has added to the complexity of antiviral therapy for CHB, leading to the development of multiple international practice guidelines.(1-3) All of these guidelines support both PEG-IFN and NA as first-line treatment options, but the optimal choice for individual patients remains controversial.

Peginterferon therapy

IFN has been used for the treatment of CHB for almost three decades. Modification of IFN through the attachment of a polyethyleneglycol (PEG) molecule improved its pharmacokinetic and pharmacodynamic properties. PEG-IFN is administered once-weekly by subcutaneous injection, which results in a relatively continuous drug exposure during the dosing interval. Two types of PEG-IFN have been developed: PEG-IFN alfa-2a and PEG-IFN alfa-2b.(4) PEG-IFN alfa-2a proved to be at least as effective as conventional IFN in hepatitis B e antigen (HBeAg-)positive patients, with a comparable tolerability profile.(5)

HBeAg-positive patients

The efficacy of PEG-IFN alfa-2a and PEG-IFN alfa-2b for HBeAg-positive CHB has been evaluated in 2 pivotal trials.(6, 7) PEG-IFN alfa-2a monotherapy during 1 year resulted in HBeAg seroconversion in 27% of patients, whereas this was 22% in patients treated with PEG-IFN alfa-2b. Six months after treatment discontinuation, HBeAg seroconversion rates were 32% and 29%, respectively. Sustained off-treatment viral suppression (serum hepatitis B virus (HBV) DNA <400 copies/mL) was achieved in 7-14% of patients. In both studies, PEG-IFN monotherapy was compared with PEG-IFN and lamivudine combination therapy. Although combination therapy was associated with more potent viral suppression during the treatment phase, sustained response rates were comparable with monotherapy.(6, 7) At 6 months after discontinuation of PEG-IFN therapy, hepatitis B surface antigen (HBsAg) seroconversion occurred in 3-5% of HBeAg-positive patients.

HBeAg-negative patients

In HBeAg-negative patients, the phase III registration study showed that PEG-IFN alfa-2a monotherapy for 1 year resulted in a sustained response (HBV DNA <20,000 copies/mL and normal alanine aminotransferase (ALT) at 6 months after treatment) in 36% of patients.(8) Serum HBV DNA was <400 copies/mL in 19% of patients and 4% had lost HBsAg at 6 months after treatment. In parallel with HBeAg-positive disease, addition of lamivudine to PEG-IFN was beneficial in terms of on-treatment viral suppression, but

did not result in higher sustained response rates.(8) In **chapter 1**, the efficacy of PEG-IFN alfa-2a monotherapy and PEG-IFN alfa-2a and ribavirin combination therapy for patients with HBeAg-negative CHB is compared. This study, which is only the second large randomized study on PEG-IFN in HBeAg-negative CHB, shows that the proportion of patients with a sustained response, defined by HBV DNA <10,000 copies/mL and a normal ALT level at 24 weeks after treatment discontinuation, was not higher than 20%. Furthermore, ribavirin did not improve the response to PEG-IFN in any way. The limited efficacy of PEG-IFN might be explained by the predominance of HBV genotype D in this study, which appears to be a "difficult-to-treat" genotype.(9, 10) These findings indicate that pretreatment or early on-treatment selection of patients with a high probability of achieving a response is essential for a successful application of PEG-IFN in clinical practice. Furthermore, although combining PEG-IFN and NA theoretically offers advantages compared to monotherapy, the addition of lamivudine, adefovir and ribavirin to PEG-IFN therapy did not result in higher sustained response rates.(8, 11) New trials on a combination of PEG-IFN with potent NA (entecavir and tenofovir) are therefore needed. In addition, studies exploring different ways of combining PEG-IFN with NA are indicated since most studies started and ended combination therapy at the same time.

When to use peginterferon therapy?

Both treatment modalities (NA and PEG-IFN) have substantial advantages and limitations.(12) PEG-IFN therapy should always be considered for HBeAg-positive CHB, because a sustained response is achieved in approximately one third of patients.(6, 7) Furthermore, HBsAg loss, which approximates clinical cure, is more likely to be achieved during PEG-IFN therapy compared with NA. However, the clinical use of PEG-IFN is compromised by its side-effects and costs.(13) Selection of patients with the highest probability of achieving a response is therefore essential. This is even more critical in HBeAg-negative disease, where a sustained response is achieved in not more than 20% of patients. Patients should ideally be selected for PEG-IFN therapy based on their individual probability of response. In case of a low baseline probability of response to PEG-IFN, it should be advised to initiate NA therapy.

Prediction of response to peginterferon

HBeAg-positive patients

In recent years, many reports demonstrated the importance of HBV genotypes for IFN-based therapy.(10, 14) HBV has been classified into 8 genotypes (A-H) defined by divergence of more than 8% in the entire genome.(15) The main HBV genotypes (A-D) have a distinct distribution throughout the world, with a predominance of A and D in

Europe and North America and B and C in Asia. The association between HBV genotypes and responsiveness to IFN was confirmed in a study that pooled the data from the 2 large trials investigating the efficacy of PEG-IFN in HBeAg-positive CHB.(16) A multivariable prediction model was constructed which allowed for individual prediction of a sustained response to PEG-IFN (HBeAg clearance and HBV DNA <10,000 copies/mL at 6 months after treatment) based on HBV genotype, age, sex, previous IFN therapy, serum ALT and HBV DNA levels.(16) Nonetheless, considerable uncertainty remains on the individual level and additional predictors of response may augment the baseline prediction model. In parallel with HBV DNA, quantitative assays for HBeAg and HBsAg have become available.(17) Fried et al. reported that patients with lower baseline HBeAg levels have a higher probability of sustained off-treatment HBeAg seroconversion.(18) HBsAg, another HBV marker, is secreted from the hepatocyte during viral replication as part of the HBV nucleocapsid, or as part of noninfectious viral particles.(17) Serum HBsAg levels correlate with the amount of intrahepatic covalently closed circular (ccc)DNA, at least in HBeAg-positive patients.(19) However, baseline serum HBsAg levels were not associated with a sustained response to PEG-IFN for HBeAg-positive CHB.(20)

Even in the face of careful selection of patients for PEG-IFN, a considerable number of patients fail to achieve a response. On-treatment predictors of response to PEG-IFN may guide clinicians in their decision of whether to continue PEG-IFN or switch to NA therapy in specific patients. In **chapter 2**, on-treatment kinetics of serum HBsAg in patients receiving either PEG-IFN or entecavir monotherapy, are studied. In HBeAg-positive patients, decline of HBsAg was significantly associated with HBeAg loss and, subsequently, HBsAg decline tended to be higher in subjects treated with PEG-IFN compared to entecavir-treated subjects. Interestingly, patients who achieved HBeAg loss during either PEG-IFN or entecavir therapy demonstrated a similar reduction in HBsAg levels. In contrast, in HBeAg-negative patients, only treatment with PEG-IFN resulted in a significant HBsAg decline, whereas HBeAg-negative patients treated with entecavir demonstrated no HBsAg reduction at all. A decline of serum HBsAg may therefore, more than HBV DNA decline, reflect the immunomodulatory effects of antiviral therapy. Previous studies indeed showed that the usefulness of HBV DNA levels in prediction of response during the first months of PEG-IFN therapy is limited. Different patterns of decline were observed during PEG-IFN alfa-2b therapy and even patients with a late or post-treatment decline of HBV DNA had a considerable chance of achieving HBeAg loss after 26 weeks of posttreatment follow-up.(21) High HBeAg levels after 24 weeks of PEG-IFN alfa-2a therapy had a somewhat greater negative predictive value upon HBeAg seroconversion in comparison with HBV DNA (96% versus 86%).(18) Recent studies have explored the role of on-treatment HBsAg levels for prediction of response to PEG-IFN in HBeAg-positive CHB. A preliminary report showed that

PEG-IFN alfa-2a treated patients with HBsAg levels <1,500 IU/mL at week 12 had 51% chance of sustained off-treatment HBeAg seroconversion compared with only 16% of those with HBsAg levels >20,000 IU/mL.(20) Unfortunately, a considerable proportion of responders would be lost if one would discontinue therapy in patients with HBsAg levels >20,000 IU/mL. In **chapter 3**, the predictive value upon a sustained response (HBeAg loss and HBV DNA <10,000 copies/mL at 26 weeks after treatment) of HBsAg levels during PEG-IFN alfa-2b therapy in HBeAg-positive patients is assessed. HBsAg decline was significantly more pronounced in patients who achieved a sustained response and those patients who did not experience any HBsAg decline from baseline at week 12 of the treatment course had only 3% chance (negative predictive value (NPV) 97%) of such a response and no chance of HBsAg loss. These patients should therefore be advised to discontinue PEG-IFN therapy, which would prevent the side-effects and costs associated with unnecessary treatment.

HBeAg-negative patients

Currently, data on baseline predictors of response to PEG-IFN in HBeAg-negative CHB are limited. A post-hoc analysis of HBeAg-negative patients participating in the registration trial of PEG-IFN alfa-2a found that younger age, female gender, higher baseline ALT and lower baseline HBV DNA levels were associated with a higher probability of achieving a sustained response (HBV DNA levels <20,000 copies/mL combined with normal ALT at 6 months after treatment).(9) Unfortunately, the authors did not provide tools for easy clinical application of their findings and calculation of the probability of response for individual patients is therefore cumbersome.

In parallel with HBeAg-positive disease, on-treatment prediction of response to PEG-IFN using HBV DNA levels is difficult in HBeAg-negative patients.(22) Recent studies have focussed on the predictive value of on-treatment HBsAg levels, the only viral marker that remains detectable in HBeAg-negative patients with suppressed HBV DNA levels. Low HBsAg levels at the end of 1 year of PEG-IFN treatment were associated with the highest probability of achieving a sustained response, suggesting that HBsAg decline during therapy may allow discrimination between responders and non-responders.(23) Indeed, high predictive values for on-treatment HBsAg declines at weeks 12 and 24 on sustained response (HBV DNA <70 copies/mL at 24 weeks after treatment) were reported in a cohort of 48 patients treated with PEG-IFN alfa-2a for 1 year: only 10% of patients who did not achieve a 0.5 log decline in serum HBsAg from baseline to week 12 of therapy achieved a response (NPV 90%).(24) In **chapter 4**, the role of early on-treatment HBsAg levels in the prediction of response in HBeAg-negative patients receiving PEG-IFN alfa-2a is investigated. The serum decline of HBsAg at week 12 alone was of limited value in prediction of sustained response (HBV DNA <10,000 copies/mL and normal ALT 24 weeks after treatment). Nevertheless, it was possible to

establish a solid stopping rule by combining early on-treatment declines of HBsAg and HBV DNA. None of the patients (20% of the study population) without HBsAg decline combined with <2 log HBV DNA decline achieved a sustained response (NPV 100%). In **chapter 5** the performance of this stopping rule is validated in the two other large trials performed to date investigating the efficacy of PEG-IFN alfa-2a for HBeAg-negative CHB.(8, 25) Clinical prediction rules usually demonstrate diminished performance in another patient population, because they are optimally modelled to the original data set. However, it was confirmed that this rule reliably identifies those patients with no or a very low chance of achieving a sustained response early during the treatment course. The high NPV of the stopping rule was confirmed across all HBV genotypes, although therapy could be stopped at week 12 of therapy in a higher proportion of patients infected with HBV genotype D compared with those infected with non-D genotypes. Furthermore, the rule performed equally well in patients receiving 96 weeks of PEG-IFN therapy, which is an important finding given the lower risk of relapse after treatment discontinuation compared with 48 weeks of treatment.(25)

Long-term durability of PEG-IFN induced responses

Long-term follow-up (mean duration 3 years) results of 172 HBeAg-positive patients treated with PEG-IFN alfa-2b ± lamivudine showed that among initial responders (defined as HBeAg negativity 26 weeks after treatment) HBeAg clearance was sustained in 81% of cases and the rate of HBsAg loss increased to 30%. Overall, HBsAg loss was achieved in 11% of patients at the last visit.(26) In **chapter 6**, the durability of PEG-IFN induced HBsAg decline through long-term follow-up is investigated. Irrespective of HBV genotype, patients with a combined response (HBeAg loss and HBV DNA <10,000 copies/mL at 26 weeks after treatment) demonstrated an increasing degree of HBsAg decline through 3 years of follow-up, reflecting a durable response with a low chance of relapse and a high probability of subsequent HBsAg loss.

Results of long-term follow-up studies have shown that the off-treatment sustainability of response to IFN-based therapy is lower in patients with HBeAg-negative compared with HBeAg-positive CHB.(27) In **chapter 7**, long-term effects of PEG-IFN in HBeAg-negative CHB are described. Nineteen percent of HBeAg-negative patients treated with PEG-IFN alfa-2a for 48 weeks had a combined response (HBV DNA <10,000 copies/mL and normal ALT) at more than 2 years after treatment discontinuation. We also explored the sustainability of response through long-term follow-up in initial responders (HBV DNA <10,000 copies/mL and normal ALT at 24 weeks after treatment), because of the fluctuations in HBV DNA and ALT levels characterizing HBeAg-negative disease. Relapse occurred in approximately one third of the initial responders, so 15% of the total study population had a combined response documented at 24 weeks after treatment and at long-term follow-up. A high degree of serum HBsAg decline was

observed in initial responders resulting in a substantial rate of HBsAg clearance (26%) at long-term follow-up.

CONCLUSIONS

The treatment of CHB has greatly improved with the introduction of potent NA with a high barrier to resistance and PEG-IFN. The advantages and limitations of both treatment options should be considered when a patient has an indication to initiate antiviral therapy. NA can be prescribed to all adult CHB patients in whom treatment is indicated and offer easy daily oral dosing, are well tolerated and NA with a high barrier to resistance can maintain suppression of viral replication for prolonged periods of time. However, a sustained off-treatment response is unlikely in the majority of patients. A sustained response is more likely to be achieved in a subgroup of patients with a finite course of PEG-IFN therapy. Furthermore, sustained responders to PEG-IFN experience a strong degree of serum HBsAg decline resulting in a higher rate of HBsAg clearance. Both PEG-IFN and NA can be given as first-line treatment option for CHB. However, PEG-IFN should only be considered for patients with a high chance of response because of the considerable side effects associated with this agent. Predictors of response at baseline, such as HBV genotype, ALT and HBV DNA levels, aid in selecting patients for PEG-IFN therapy, especially in HBeAg-positive disease. Furthermore, on-treatment viral markers, in particular quantitative HBV DNA and HBsAg, allow the identification of patients who are unlikely to benefit from PEG-IFN early during the treatment course, thereby avoiding unnecessary treatment. Patients who are not eligible for PEG-IFN or who have a low probability of response to PEG-IFN based on baseline or on-treatment factors should be advised to initiate or switch to NA therapy.

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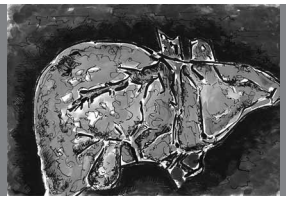
Samenvatting en bespreking



De behandeling van chronische hepatitis B is de afgelopen 10 jaar sterk verbeterd met de introductie van nucleos(t)ide analogen (NA) en gepegyleerd interferon (PEG-IFN). Met de beschikbaarheid van een toenemend aantal antivirale middelen is de behandeling van chronische hepatitis B complexer geworden, wat heeft geleid tot de ontwikkeling van meerdere internationale richtlijnen.(1-3) Deze richtlijnen ondersteunen het gebruik van zowel PEG-IFN als NA als initiële therapie, maar er bestaat controverse over de optimale keuze voor een individuele patiënt.

Peginterferon therapie

IFN wordt al bijna drie decennia gebruikt voor de behandeling van chronische hepatitis B. Het toevoegen van polyethyleenglycol (PEG) aan IFN heeft geleid tot verbeterde farmacokinetische en farmacodynamische eigenschappen. PEG-IFN wordt wekelijks toegediend door middel van een subcutane injectie. Dit resulteert in een redelijk gelijkmatige IFN spiegel gedurende dit interval. Er zijn twee soorten PEG-IFN ontwikkeld: PEG-IFN alfa-2a and PEG-IFN alfa-2b.(4) PEG-IFN alfa-2a bleek minstens even effectief als conventioneel IFN voor hepatitis B e antigeen (HBeAg-)positieve patiënten, met vergelijkbare bijwerkingen.(5)

HBeAg-positieve patiënten

De effectiviteit van PEG-IFN alfa-2a en PEG-IFN alfa-2b voor HBeAg-positieve chronische hepatitis B is onderzocht in 2 grote studies.(6, 7) PEG-IFN alfa-2a monotherapie gedurende 1 jaar resulteerde in HBeAg seroconversie (verlies van HBeAg met ontwikkeling van anti-HBe) bij 27% van de patiënten, in vergelijking met 22% van de patiënten behandeld met PEG-IFN alfa-2b. Deze percentages liepen op naar respectievelijk 32% en 29% op 6 maanden na het staken van de behandeling. Een duurzame onderdrukking van de virale replicatie (serum hepatitis B virus (HBV) DNA <400 kopieën/ml) werd bereikt in 7-14% van de patiënten na het staken van de behandeling. In beide studies werd PEG-IFN monotherapie vergeleken met PEG-IFN en lamivudine combinatie therapie. Ondanks een sterkere onderdrukking van de virale replicatie gedurende de behandeling met combinatie therapie was het aantal patiënten met een blijvende respons vergelijkbaar in beide groepen.(6, 7) Zes maanden na het staken van de behandeling had hepatitis B surface antigeen (HBsAg) seroconversie (verlies van HBsAg met ontwikkeling van anti-HBs) plaats gevonden bij 3-5% van de HBeAg-positieve patiënten.

HBeAg-negatieve patiënten

PEG-IFN alfa-2a monotherapie gedurende 1 jaar resulteerde in een blijvende respons (HBV DNA <20.000 kopieën/ml en een normaal serum alanine aminotransferase (ALAT) op 6 maanden na de behandeling) bij 36% van de patiënten.(8) Het HBV DNA was

onderdrukt (<400 kopieën/ml) bij 19% en HBsAg was negatief bij 4% van de patiënten op 6 maanden na de behandeling. Net als bij HBeAg-positieve patiënten resulteerde de toevoeging van lamivudine aan PEG-IFN niet in een hoger percentage patiënten met een blijvende respons.(8) In **hoofdstuk 1** wordt de effectiviteit van PEG-IFN alfa-2a monotherapie en PEG-IFN alfa-2a en ribavirine combinatie therapie voor patiënten met HBeAg-negatieve chronische hepatitis B vergeleken. Deze studie, de tweede grote gerandomiseerde studie naar de effectiviteit van PEG-IFN in deze patiëntengroep, toont aan dat het percentage patiënten met een blijvende respons (HBV DNA <10.000 kopieën/ml en een normaal ALAT op 24 weken na de behandeling) niet hoger was dan 20%. Het toevoegen van ribavirine aan PEG-IFN had geen gunstige effecten. De beperkte effectiviteit van PEG-IFN kan mogelijk worden verklaard door de hoge prevalentie van HBV genotype D in deze studie, hetgeen een lastig te behandelen genotype lijkt te zijn.(9, 10) Deze bevindingen benadrukken dat het selecteren van patiënten met een hoge kans op een blijvende respons, voor aanvang van de behandeling of in het begin van de behandeling, essentieel is voor een effectieve toepassing van PEG-IFN in de praktijk. Overigens resulteerde het toevoegen van zowel lamivudine, adefovir als ribavirine aan PEG-IFN niet in een hoger percentage patiënten met een blijvende respons.(8, 11) Nieuwe studies naar combinaties van PEG-IFN en potente NA (entecavir and tenofovir) zijn daarom gewenst. Verder is onderzoek naar andere manieren van het combineren van PEG-IFN en NA geïndiceerd, omdat in de meeste studies combinatie therapie tegelijkertijd werd gestart en beëindigd.

Peginterferon of NA behandeling?

Beide behandelvormen (NA en PEG-IFN) hebben voor- en nadelen.(12) PEG-IFN zou altijd overwogen moeten worden voor de behandeling van HBeAg-positieve chronische hepatitis B, omdat ongeveer een derde van de patiënten een blijvende respons behaalt. (6, 7) Ook is de kans op HBsAg verlies, hetgeen vrijwel gelijk staat aan genezing, hoger gedurende PEG-IFN therapie dan gedurende behandeling met NA. Behandeling met PEG-IFN kent echter aanzienlijke bijwerkingen.(13) Het selecteren van patiënten met een hoge kans op een blijvende respons is daarom essentieel. Dit geldt in nog sterkere mate voor patiënten met HBeAg-negatieve chronische hepatitis B, die in niet meer dan 20% van de gevallen een blijvende respons ontwikkelen. Idealiter zou de selectie van patiënten voor PEG-IFN therapie plaats moeten vinden op individuele basis. Als er een lage kans is op een blijvende respons na PEG-IFN behandeling dient de voorkeur gegeven te worden aan NA behandeling.

Voorspellers van respons op peginterferon

HBeAg-positieve patiënten

In de afgelopen jaren hebben vele studies het belang van het HBV genotype als voorspeller van een blijvende respons op IFN behandeling aangetoond.(10, 14) HBV is onderverdeeld in 8 genotypes (A-H).(15) De belangrijkste genotypes (A-D) kennen een specifieke verdeling over de wereld, waarbij A en D met name in Europa en Noord Amerika voorkomen, en B en C in Azië. De associatie tussen HBV genotypes en respons op IFN werd bevestigd in een studie waarbij de data van de 2 grote trials van PEG-IFN voor HBeAg-positieve chronische hepatitis B werden samengevoegd. (16) In deze studie werd een model opgesteld waarmee het mogelijk is de kans op een blijvende respons (HBeAg verlies en HBV DNA <10.000 kopieën/ml) op PEG-IFN voor individuele patiënten te berekenen met behulp van het HBV genotype, leeftijd, geslacht, eventueel eerdere IFN behandeling(en), ALAT en HBV DNA concentraties.(16) Toch rest er een aanzienlijke onzekerheid of een individuele patiënt daadwerkelijk gaat responderen en extra voorspellers zouden het model kunnen verbeteren. Naast het HBV DNA, kunnen nu ook de concentraties van het HBeAg en HBsAg in serum worden gemeten.(17) Fried et al. toonden aan dat patiënten met een lage serum HBeAg concentratie voor aanvang van PEG-IFN behandeling een hogere kans hadden op het bereiken van HBeAg seroconversie.(18) HBsAg, een andere virale marker, wordt door de hepatocyt uitgescheiden als onderdeel van het complete virus, of als niet-infectieus deeltje.(17) De HBsAg concentratie in het serum is gecorreleerd met de hoeveelheid covalently closed circular (ccc)DNA in de hepatocyt, in elk geval in HBeAg-positieve patiënten.(19) Serum HBsAg concentraties waren echter niet geassocieerd met een blijvende respons op PEG-IFN in HBeAg-positieve patiënten.(20)

Zelfs wanneer patiënten zorgvuldig worden geselecteerd voor een behandeling met PEG-IFN behaalt een aanzienlijk gedeelte van hen geen blijvende respons. Voorspellers van respons op PEG-IFN gedurende de behandelfase zouden dus behulpzaam kunnen zijn bij de beslissing om de behandeling te continueren, of om over te gaan op NA therapie. In **hoofdstuk 2** wordt de kinetiek van serum HBsAg concentraties bij HBeAg-positieve en HBeAg-negatieve patiënten onderzocht, die worden behandeld met PEG-IFN of entecavir. In HBeAg-positieve patiënten was de daling in HBsAg concentraties sterk geassocieerd met het optreden van HBeAg verlies tijdens de behandeling. Aangezien behandeling met PEG-IFN vaker leidt tot HBeAg verlies resulteerde het in vergelijking met entecavir dan ook in een grotere daling van het HBsAg. Indien er echter HBeAg verlies optrad, was er geen verschil te zien in de mate van HBsAg daling tussen deze twee behandelingen. Bij patiënten met HBeAg-negatieve chronische hepatitis B leidde alleen PEG-IFN behandeling tot een daling van HBsAg concentraties. Een daling van de HBsAg concentratie lijkt daarom, meer dan een daling van het

HBV DNA, de immuunmodulatoire effecten van antivirale therapie te reflecteren. De voorspellende waarde van HBV DNA concentraties tijdens PEG-IFN behandeling is inderdaad beperkt. Tijdens behandeling met PEG-IFN alfa-2b werden verschillende patronen van daling waargenomen en zelfs patiënten die zeer laat een HBV DNA daling door maakten hadden een aanzienlijke kans op het bereiken van HBeAg verlies op 26 weken na het staken van de behandeling.(21) Hoge HBeAg concentraties na 24 weken PEG-IFN alfa-2a therapie hadden een iets grotere negatief voorspellende waarde in vergelijking met HBV DNA (96% versus 86%).(18) In recente studies is de voorspellende waarde van HBsAg concentraties gedurende behandeling met PEG-IFN voor HBeAg-positieve patiënten onderzocht. Patiënten behandeld met PEG-IFN alfa-2a met een HBsAg concentratie <1.500 IU/ml op week 12 hadden 51% kans op een HBeAg seroconversie, vergeleken met slechts 16% van de patiënten met HBsAg concentraties >20.000 IU/ml.(20) Wanneer de behandeling zou worden gestaakt in patiënten met HBsAg concentraties >20.000 IU/ml op week 12 zou dit echter ook gelden voor een aanzienlijk deel van de patiënten die bij het voltooien van de behandeling wel een blijvende respons zouden behalen. In **hoofdstuk 3** wordt de voorspellende waarde van HBsAg concentraties gedurende PEG-IFN alfa-2b therapie voor HBeAg-positieve chronische hepatitis B voor een blijvende respons (HBeAg verlies en HBV DNA <10.000 kopieën/ml) bestudeerd. De mate van daling in HBsAg concentraties was sterker in patiënten die uiteindelijk een blijvende respons zouden ontwikkelen. Patiënten waarbij geen HBsAg daling optrad op week 12 hadden slechts 3% kans (negatief voorspellende waarde 97%) op een blijvende respons en geen kans op HBsAg verlies. Deze patiënten zou daarom moeten worden geadviseerd de behandeling met PEG-IFN te staken, waarmee de bijwerkingen en kosten van onnodige behandeling voorkomen kunnen worden.

HBeAg-negatieve patiënten

Momenteel zijn er bij aanvang van een PEG-IFN behandeling bij HBeAg-negatieve chronische hepatitis B weinig voorspellende factoren bekend voor een blijvende respons. Een post hoc analyse van HBeAg-negatieve patiënten behandeld in de PEG-IFN alfa-2a registratie studie toonde aan dat lagere leeftijd, vrouwelijk geslacht, hoger ALAT en lager HBV DNA geassocieerd waren met een hogere kans op een blijvende respons (HBV DNA <20.000 kopieën/ml gecombineerd met een normaal ALAT op 6 maanden na de behandeling).(9) Helaas werd door de auteurs geen praktisch hulpmiddel opgesteld en het berekenen van de kans op een blijvende respons voor individuele patiënten is dus zeer lastig.

Net als bij HBeAg-positieve chronische hepatitis B is het voorspellen van een blijvende respons aan de hand van HBV DNA concentraties tijdens PEG-IFN behandeling moeilijk in HBeAg-negatieve patiënten.(22) Recente onderzoeken hebben zich daarom gericht

op de voorspellende waarde van HBsAg concentraties tijdens de behandeling, de enige virale marker die aanwezig blijft in het serum van HBeAg-negatieve patiënten met een onderdrukt HBV DNA. Lage HBsAg concentraties na 1 jaar PEG-IFN behandeling waren geassocieerd met een hoge kans op een blijvende respons. Dit suggereert dat de mate van daling in HBsAg concentraties onderscheid kan maken tussen patiënten die wel of geen blijvende respons zullen bereiken.(23) De hoge voorspellende waarde van HBsAg dalingen op 12 en 24 weken van de behandeling werd bevestigd in een cohort van 48 patiënten behandeld met PEG-IFN alfa-2a gedurende 1 jaar: slechts 10% van de patiënten die geen 0,5 log HBsAg daling bereikten op week 12 van de behandeling ontwikkelde een blijvende respons (negatief voorspellende waarde 90%).(24) In **hoofdstuk 4** wordt de voorspellende waarde van HBsAg concentraties in een vroege fase van de behandeling met PEG-IFN alfa-2a bij HBeAg-negatieve patiënten onderzocht. De daling van de HBsAg concentratie op week 12 had een beperkte predictieve waarde voor een blijvende respons (HBV DNA <10.000 kopieën/ml en normaal ALAT op 24 weken na de behandeling). Het was echter wel mogelijk om een betrouwbare stopregel te creëren met behulp van een combinatie van de daling van HBsAg en HBV DNA concentraties. Er bestond geen kans op een blijvende respons in de patiënten (20% van de gehele populatie) bij wie na 12 weken behandeling geen daling van de HBsAg concentratie en <2 log HBV-DNA daling aantoonbaar was (negatief voorspellende waarde 100%). In **hoofdstuk 5** vindt validatie van deze stopregel plaats in de twee andere grote studies naar PEG-IFN alfa-2a in HBeAg-negatieve patiënten.(8, 25) Klinische beslisregels werken in het algemeen minder goed in een andere patiëntengroep, omdat ze zijn gebaseerd op de originele patiëntenpopulatie. Er werd echter bevestigd dat deze regel in een vroege fase van de behandeling op betrouwbare wijze patiënten identificeert met geen of een zeer lage kans op een blijvende respons. De hoge negatief voorspellende waarde werd bevestigd voor alle HBV genotypes, alhoewel therapie gestaakt zou kunnen worden in een hoger percentage van de patiënten met genotype D in vergelijking met andere genotypes. Bovendien presteerde de regel even goed bij patiënten die gedurende 96 weken werden behandeld met PEG-IFN. Dit is een belangrijk gegeven, omdat er na deze behandelduur een lager risico bestaat op reactivatie van chronische hepatitis B in vergelijking met 48 weken.(25)

Duurzaamheid van respons op peginterferon

Een follow-up studie in 172 patiënten met HBeAg-positieve chronische hepatitis B toonde aan dat onder patiënten die HBeAg negatief waren op 26 weken na de behandeling met PEG-IFN alfa-2b ± lamivudine (initiële responders) na gemiddeld 3 jaar 81% nog steeds HBeAg negatief was. Verder was het percentage HBsAg verlies 30% onder initiële responders. In de gehele groep behandelde patiënten was het percentage HBsAg verlies 11%.(26) In **hoofdstuk 6** wordt de duurzaamheid van door PEG-IFN

geïnduceerde daling van HBsAg concentraties op de lange termijn beschreven. Onafhankelijk van het HBV genotype hadden alle patiënten met een gecombineerde respons (HBeAg verlies en HBV DNA <10.000 kopieën/ml op 26 weken na de behandeling) een progressieve daling van de HBsAg concentratie in de 3 jaar na de behandeling. Deze bevinding weerspiegelt een duurzame respons met een lage kans op terugkeer van de ziekte activiteit en daarbij een hoge kans op toekomstig verlies van HBsAg.
Verschillende studies hebben aangetoond dat de duurzaamheid van een respons op IFN lager is bij HBeAg-negatieve dan HBeAg-positieve patiënten.(27) In **hoofdstuk 7** worden de lange termijn resultaten van PEG-IFN voor HBeAg-negatieve chronische hepatitis B bestudeerd. Van de HBeAg-negatieve patiënten behandeld met PEG-IFN alfa-2a gedurende 48 weken had 19% een gecombineerde respons (HBV DNA <10.000 kopieën/ml en een normaal ALAT) op meer dan 2 jaar na het staken van de behandeling. Vanwege het fluctuerende karakter van HBV DNA en ALAT concentraties die kenmerkend zijn voor HBeAg-negatieve chronisch hepatitis B, onderzochten we tevens de duurzaamheid van respons in initiële responders (HBV DNA <10.000 kopieën/ml en een normaal ALAT op 24 weken na de behandeling). Ongeveer een derde van de initiële responders vertoonde een terugval, dus 15% van de gehele onderzochte populatie had een gecombineerde respons op zowel 24 weken als 2 jaar na de behandeling. In de groep initiële responders was een sterke mate van HBsAg daling aantoonbaar, wat resulteerde in een aanzienlijk percentage van HBsAg verlies (26%) op 2 jaar na de behandeling.

CONCLUSIE

De behandeling van chronische hepatitis B is sterk verbeterd met de introductie van potente NA met een hoge genetisch barrière tegen resistentie en PEG-IFN. De voor- en nadelen van beide behandelopties zijn van belang indien een patiënt in aanmerking komt voor behandeling. NA kunnen worden voorgeschreven aan alle volwassen patiënten met een behandelindicatie. Ze worden oraal ingenomen, goed verdragen en de NA met een hoge genetisch barrière tegen resistentie kunnen langdurig de virale replicatie onderdrukken. Patiënten dienen echter langdurig te worden behandeld, aangezien een blijvende respons na het staken van de behandeling slechts bij een beperkt aantal patiënten kan worden bereikt. Er bestaat een hogere kans op een blijvende respons na het staken van een PEG-IFN behandeling in een subgroep patiënten. Bovendien treedt er een sterke daling van de serum HBsAg concentratie op in patiënten met een blijvende respons op PEG-IFN, wat resulteert in een hogere kans op HBsAg verlies.
Zowel PEG-IFN als NA kunnen als initiële therapie worden toegepast voor chronische hepatitis B. Gezien de aanzienlijke bijwerkingen van PEG-IFN moet dit middel echter

alleen worden overwogen voor patiënten met een hoge kans op een blijvende respons. Voorspellers van een blijvende respons, zoals het HBV genotype, serum ALAT en HBV DNA concentraties, zijn behulpzaam bij het selecteren van patiënten voor PEG-IFN therapie, vooral bij HBeAg-positieve chronische hepatitis B. Verder kunnen virale markers gedurende de behandeling, met name serum HBV DNA en HBsAg concentraties, de identificatie van patiënten met een zeer lage kans op een blijvende respons op PEG-IFN mogelijk maken. Daardoor kan onnodige behandeling worden voorkomen. Aan patiënten met een contra-indicatie voor het gebruik van PEG-IFN of met een lage kans op een blijvende respons, gebaseerd op voorspellende factoren bij aanvang of tijdens de behandeling, zou moeten worden geadviseerd te starten met behandeling met NA.

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De hepatitis B groep heeft altijd een grote bijdrage geleverd aan het onderzoeksplezier. Ten eerste wil ik mijn voorganger Martijn bedanken voor al het werk dat al was gedaan voor de PARC studie. Daarnaast wil ik de directe collega’s binnen de hepatitis B groep bedanken: Erik, Roeland, Milan en Jurriën. Erik, bedankt voor je hulp gedurende de eerste maanden en je tips in Las Vegas. Roeland, ik zal altijd tijd vrij maken voor een gezamenlijk ontbijtje met Koffietijd op de achtergrond. Milan, ik ken niemand die zo kan goochelen met een database als jij. Bedankt voor de super samenwerking die geresulteerd heeft in enkele mooie publicaties. Wat een mooie congressen hebben we gehad (Halloween feesten, overstroomde hotelkamers…), ik wens jullie beiden

veel succes met het afronden van jullie onderzoeken. Tot slot Jurriën: ook al zijn wij inderdaad twee totaal verschillende personen, uiteindelijk blijk ook jij te eindigen met een stationwagon voor je deur. Ik had vooraf niet kunnen bedenken waar een impulsieve beslissing tijdens een scherp etentje toe bij Chili Time (afgeblust met de nodige alcoholische consumpties) toe kon leiden. Enorm bedankt voor je hulp gedurende de laatste maanden, na augustus mag je altijd bij mij aankloppen ;-).

Dan mijn statistische steun Bettina, bedankt voor de perfecte samenwerking die heeft geresulteerd in onze ViBe rule. Na jouw hulp aan vele promovendi vond ik het super om een (kleine) bijdrage te mogen leveren aan jouw eigen promotie. Het hoogtepunt was toch wel de rondleiding door “jouw” Kopenhagen, die spetterend eindigde in de Karriere Bar.

Een multicenter onderzoek is echt teamwerk. Een speciaal woord van dank gaat daarom uit naar de leden van het lab, met name Hanneke, Jan, Angela, Martine en ook Andrea, die onder andere zorg droegen voor een snelle verwerking van de vele honderden samples. Sandra, Thijs, Martin en Suzan van de afdeling virologie: bedankt voor de vele assays. Daarnaast wil ik Pieter Zondervan, patholoog, bedanken voor het grote aantal espresso’s (en vergelijkbare aantal biopten). Een betrouwbare database is goud waard. Met name Elke, maar ook Irene en Wanda van het clinical research bureau waren onmisbaar bij het data managen voor onder andere de PARC studie. Dankzij Heleen, Cokki, Anneke, Lucille en Melek was de onderzoekspoli altijd tot in de puntjes geregeld, jullie speciale aandacht voor de studiepatiënten is essentieel. Een welkome afwisseling met het onderzoekswerk was altijd de hepatitis B poli op dinsdagmiddag. Wilma, Nermin, Lakshmie, Ronald, Ellen, en alle anderen: bedankt voor het zoeken van alle statussen. Gelukkig is dit met het digitale dossier nu verleden tijd.

De meeste tijd van het promotieonderzoek bracht ik door op de beruchte dakpoli. De eerste dag bleek dat ik mijn kamer zou gaan delen met Robert. Jij zou ook op 1 november beginnen, maar was alvast één dag eerder gekomen om je in te lezen (lees: om het beste bureau op te eisen). Drie jaar lang hebben wij met elkaar door gebracht op enkele vierkante meters, waarbij we vaak de vorderingen van ons onderzoek (of het uitblijven daarvan) door namen, maar vooral de nieuwe collectie racefietsen bestudeerden onder het genot van vele koppen koffie. Veel succes met het afronden van je promotie, je vooropleiding in Delft en in je nieuwe huis. Ook mijn andere kamergenoten, Moniek, Esmay en later Edith, wil ik bedanken voor de leuke tijd. De groep collega-onderzoekers, zowel op het dak als op de flex, heeft ook zeker meegedragen aan het werkplezier: Paul(us), Femme, kleine Vincent, Ad, Nicoline, Jilling, Aafke, Jildou, Leonie, Daphne, Jerome, Lieke, Edith, Desirée, Margot, Geert, Judith, Lisette, Aria,

Edmee, Caroline, Renate, Susanne en Pauline, de wisselende groep arts-assistenten en alle anderen: bedankt voor alle gezelligheid in de koffiepauzes, op skireis, borrels en congressen.

Dan Marion en Margriet, het magische secretaresseduo van de hepatologie. Marion, vanaf het moment dat mijn eerste manuscript, na zes pogingen door mijzelf, door jou eindelijk succesvol werd gesubmit begon mijn onderzoek pas echt te lopen. Zonder alle hulp in de laatste fase van mijn promotie was dit boekje er zeker nog niet geweest.

Sinds november 2010 ben ik werkzaam binnen de afdeling interne geneeskunde van het Ikazia ziekenhuis. Ik wil de club arts-assistenten en de maatschap interne bedanken voor de plezierige werksfeer en samenwerking. Graag wil ik hier nog Peter van Wijngaarden uit mijn periode in het Amphia ziekenhuis te Breda noemen. Mijn eerste publicatie is dankzij jou tot stand gekomen, net als mijn interesse in leverziekten. Bedankt voor je advies om een promotietraject in te gaan.

Ik had mijn boekje niet af kunnen maken zonder ontspanning met mijn vrienden, die ik de laatste tijd lang niet zo veel heb gezien als ik zou willen. Zeeuwse (Bastiaan, Elwin, Frank, Wolfert en Martin) en Rotterdamse (Bas-Peter, Elwin, Jur en Werner) boyz, op naar de volgende weekendjes en feesten! Ook mijn “oude” tennismaatjes Dimp, Vip en Kwek en de A-selectie: bedankt voor alle afleiding.

Daarnaast wil ik Joke en Henri enorm bedanken. Het is super hoe wij altijd een beroep op jullie kunnen doen, waarna jullie zonder mopperen binnen een uur bij ons op de stoep staan. Bovendien zou de “tuin“ er zonder jullie heel anders uit zien. Jullie zijn een fantastische opa en oma voor Jasmijn. Rob en Sasja, dank voor de gezellige middagen en etentjes. Ik wens jullie alle geluk in jullie nieuwe huis.

Dan mijn paranimfen. Wolfert, van mijn vrienden ben jij degene die ik het beste (en langste) ken. Jij trok na de middelbare school naar Tilburg en ik naar Rotterdam. Na je studie nam je ons oude appartement in Rotterdam over en hebben we genoten van menig maaltje Knaks. Helaas besloot je te verhuizen naar Zeeland, maar dat is denk ik een goede keuze geweest en inmiddels woon je al weer even met Marinka in Middelburg. Gelukkig zien we elkaar nog regelmatig, ik vind het geweldig dat jij mijn paranimf wil zijn. Daniël, kleine broertjes worden groot. Ik heb er veel respect voor hoe jij na je eerste studie probleemloos nog even je master haalde in de sociologie. We kunnen gelukkig vaak “spontaan” bij jou en Thea op bezoek gaan in jullie penthouse in Rotterdam. Bedankt voor je interesse in mijn onderzoek en je steun als paranimf.

Dit dankwoord is niet volledig zonder mijn lieve vader en moeder. Jullie hebben mij altijd gestimuleerd om door te zetten en alles voor mij mogelijk gemaakt. Ik vind het fantastisch hoe jullie wekelijks op Jasmijn passen, dat is onbetaalbaar. Zonder jullie steun zou het allemaal zo veel lastiger zijn. Papa, het is super dat jij de omslag hebt geïllustreerd, het eindresultaat mag er zeker zijn. Ook mijn opa en oma's wil ik bedanken, ik ben heel blij dat jullie er bij kunnen zijn.

Tot slot kom ik dan toch bij jou, lieve Anke, hoewel je meerdere malen hebt gevreesd niet opgenomen te worden in het dankwoord ;-). Je hebt de afgelopen maanden gezorgd dat ik in alle rust dit onderzoek kon afronden, zonder jou was het me niet gelukt. Ik beloof je dat ik onze projecten weer op zal pakken na vandaag. Ik vind dat je een geweldige huisarts bent en kan me geen betere moeder bedenken voor ons toppertje Jasmijn. Op de toekomst samen!

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HBeAg-positive chronic hepatitis B patients treated with peginterferon alfa-2b: Relation to response and HBV genotype. Antiviral Therapy 2011. Accepted for publication.

Curriculum vitae

De auteur van dit proefschrift werd geboren op 21 februari 1982 te Vlissingen. In 2000 behaalde hij zijn gymnasiumdiploma aan de Stedelijke Scholengemeenschap Nehalennia te Middelburg, om vervolgens te starten met de opleiding Geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraalexamen werd behaald in 2004 en het artsexamen in 2006. Van januari tot november 2007 werkte hij als arts niet in opleiding tot specialist (ANIOS) Interne Geneeskunde in het Amphia ziekenhuis te Breda (opleider: dr. C. van Guldener). Hierna begon hij aan zijn promotieonderzoek op de afdeling Maag-, Darm-, en Leverziekten van het Erasmus MC te Rotterdam (afdelingshoofd: prof. dr. E.J. Kuipers) onder supervisie van prof. dr. H.L.A. Janssen. Per november 2010 is hij gestart met de opleiding tot Maag-Darm-Leverarts (opleider: dr. R.A. de Man). De vooropleiding Interne Geneeskunde volgt hij gedurende twee jaar in het Ikazia Ziekenhuis te Rotterdam (opleider: dr. A. Dees, per maart 2011 dr. A.A.M. Zandbergen). Hij woont samen met Anke Peelen en hun dochter Jasmijn in Berkel en Rodenrijs.

PhD portfolio

Erasmus MC
Universitair Medisch Centrum Rotterdam

Summary of PhD training and teaching

Name PhD student: Vincent Rijckborst
Erasmus MC Department: Gastroenterology and Hepatology

PhD period: 2007-2011
Promotor: Prof. Dr. H.L.A. Janssen

1. PhD training

	Year	Workload
General courses		
Classical methods of data analysis. Netherlands Institute for Health Sciences, Rotterdam, the Netherlands.	2008	104 hours
Presentations and workshops		
48 weeks of peginterferon alfa-2a alone or in combination with ribavirin for HBeAg-negative chronic hepatitis B: Addition of ribavirin does not increase response rates. Annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2008	18 hours
Early reduction of serum HBsAg levels in HBeAg-negative chronic hepatitis B patients achieving sustained virological response after peginterferon alfa-2a ± ribavirin treatment. Annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2008	12 hours
Clinical decisions in viral hepatitis. Case reports. Dutch Liver Week, Soestduinen, the Netherlands.	2009	2 hours
Combinatiebehandeling voor HBV is niet meer noodzakelijk met de huidige antivirale middelen. Tweede Lagerhuisdebat Hepatitis B en C, Amsterdam, the Netherlands.	2009	4 hours
On-treatment prediction of sustained response in HBeAg-negative chronic hepatitis B patients treated with pegylated interferon alfa-2a. Annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2009	18 hours
Mutations in the precore and basal core promoter regions do not influence responsiveness to pegylated interferon alfa-2a treatment for HBeAg-negative chronic hepatitis B. Annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2009	12 hours
Prediction of response to peginterferon and nucleos(t)ide analogue treatment for chronic hepatitis B: The usefulness of HBsAg and HBV DNA levels. 7th Post-AASLD symposium, Rotterdam, the Netherlands.	2009	18 hours
Hepatitis B/C in vogelvlucht: Behandelingsmogelijkheden. Landelijke Hepatitisweek, Amersfoort, the Netherlands.	2010	6 hours

Early prediction of sustained response to peginterferon alfa-2a in HBeAg-negative patients: The role of on-treatment HBsAg and HBV DNA levels. 45th Annual Meeting of the European Association for the Study of the Liver (EASL), abstract 8, Vienna, Austria.	2010	36 hours
Behandelingsmogelijkheden HBV. Medisch Wetenschappelijke Dag 2010, Leiden, the Netherlands.	2010	6 hours
On-treatment predictors of response: HBsAg measurements. Preceptorship Program, Rotterdam, the Netherlands.	2010	6 hours
Interest of markers for the management of viral hepatitis. 1st European Young Hepatologist Workshop, Bendor, France.	2010	12 hours
Serum HBsAg levels decrease through long-term follow-up in HBeAg-negative patients achieving a sustained response to peginterferon alfa-2a. Annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2010	18 hours

Poster presentations

48 weeks of peginterferon alfa-2a alone or in combination with ribavirin for HBeAg-negative chronic hepatitis B: Addition of ribavirin does not increase response rates. 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 991, San Francisco, CA, United States of America.	2008	32 hours
Early reduction of serum HBsAg levels in HBeAg-negative chronic hepatitis B patients achieving sustained virological response after peginterferon alfa-2a ± ribavirin treatment. 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 986, San Francisco, CA, United States of America.	2008	24 hours
On-treatment prediction of sustained response in HBeAg-negative chronic hepatitis B patients treated with pegylated interferon alfa-2a. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 492, Boston, MA, United States of America.	2009	32 hours
Mutations in the precore and basal core promoter regions do not influence responsiveness to pegylated interferon alfa-2a treatment for HBeAg-negative chronic hepatitis B. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 497, Boston, MA, United States of America.	2009	24 hours
Serum HBsAg levels decrease through long-term follow-up in HBeAg-negative patients achieving a sustained response to peginterferon alfa-2a. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 486, Boston, MA, United States of America.	2010	32 hours
Early on-treatment HBsAg and HBV DNA levels identify HBeAg-negative patients not responding to 48 or 96 weeks of peginterferon alfa-2a therapy. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), abstract 479, Boston, MA, United States of America.	2010	32 hours

International conferences

43rd Annual Meeting of the European Association for the Study of the Liver (EASL). Milan, Italy.	2008	28 hours
The Liver Meeting 2008, 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA, United States of America.	2008	28 hours
44th Annual Meeting of the European Association for the Study of the Liver (EASL). Copenhagen, Denmark.	2009	28 hours

The Liver Meeting 2009, 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2009	28 hours
45th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria.	2010	28 hours
The Liver Meeting 2010, 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2010	28 hours

Attended seminars and workshops

5th Post-AASLD symposium. Rotterdam, the Netherlands.	2007	2 hours
Hepatitis Masterclass. Utrecht, the Netherlands	2008	18 hours
Studiemiddag HIV en hepatitis B: Maakt de dokter het verschil? Rotterdam, the Netherlands.	2008	2 hours
Eerste Lagerhuisdebat Hepatitis B en C. Amsterdam, the Netherlands.	2008	2 hours
Tweede Lagerhuisdebat Hepatitis B en C. Amsterdam, the Netherlands.	2009	2 hours
De 24-uur van De Vanenburg. Putten, the Netherlands.	2009	6 hours
7th Post-AASLD symposium. Rotterdam, the Netherlands.	2009	2 hours
1st European Young Hepatologist Workshop. Bendor, France.	2010	12 hours
Derde Lagerhuisdebat Hepatitis B en C. Utrecht, the Netherlands.	2010	2 hours

2. Teaching

	Year	Workload
Lecturing		
Hepatitis B. 3rd year Erasmus MC medical students participating in a 4-week Gastroenterology and Hepatology training program. Rotterdam, the Netherlands.	2010	8 hours
Hepatitis B. Education gastroenterologists in training. Rotterdam, the Netherlands.	2010	4 hours
Diagnosis and treatment of chronic hepatitis B. 3rd year Erasmus MC medical students participating in a Gastroenterology and Hepatology training program. Rotterdam, the Netherlands.	2010	4 hours