Original article

Baseline hepatitis B surface antigen (HBsAg) as predictor of sustained HBsAg loss in chronic hepatitis B patients treated with pegylated interferon-α2a and adefovir

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Background: In this study, we aimed to identify baseline predictors of response in chronic hepatitis B patients treated with a combination of pegylated interferon (PEG-IFN)-α2a and adefovir.

Methods: We treated 92 chronic hepatitis B patients (44 hepatitis B e antigen [HBeAg]-positive and 48 HBeAg-negative) with HBV DNA>100,000 copies/ml (>17,182 IU/ml) with PEG-IFN and adefovir for 48 weeks and followed them up for 2 years. Baseline markers for HBeAg loss, combined response (HBeAg negativity, HBV DNA levels ≤2,000 IU/ml and alanine aminotransferase [ALT] normalization) and hepatitis B surface antigen (HBsAg) loss were evaluated.

Results: Two years after the end of treatment, rates of HBeAg loss and HBsAg loss in HBeAg-positive patients were 18/44 (41%) and 5/44 (11%), respectively. In HBeAg-negative patients, rates of combined response and HBsAg loss were 12/48 (25%) and 8/48 (17%), respectively. HBeAg-negative patients with HBsAg loss had lower baseline HBsAg levels than those without HBsAg loss (mean HBsAg 2.35 versus 3.55 $\log_{10}$ IU/ml; $P<0.001$). They also had lower HBV DNA levels and were more often (PEG-)IFN experienced. Baseline HBsAg was the only independent predictor of HBsAg loss (OR 0.02; $P=0.01$).

Conclusions: With combination therapy of PEG-IFN and adefovir for 48 weeks, a high rate of HBsAg loss was observed in both HBeAg-positive (11%) and HBeAg-negative (17%) patients 2 years after treatment ended. In HBeAg-negative patients, a low baseline HBsAg level was a strong predictor for HBsAg loss.

Introduction

Worldwide, approximately 400 million people have chronic HBV infection. Such chronic infection increases the risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC). Approximately 25% of people who acquire chronic HBV infection early in life will develop cirrhosis or HCC during their lifetime [1].

Treatment of chronic hepatitis B has improved over the decades, and can be divided into direct antiviral or immunomodulatory therapy. The nucleoside/nucleotide analogues (NUCs), lamivudine, adefovir, entecavir, telbivudine and tenofovir, currently registered for such treatment, inhibit HBV DNA synthesis and viral replication. They are well-tolerated and have few side effects. However, they require prolonged, probably lifelong, use because reactivation is common if treatment is stopped [2–4].

Pegylated interferon (PEG-IFN)-α2a is mainly immunomodulatory, although it also has limited

direct antiviral effects. Unfortunately, treatment with PEG-IFN entails significant side effects. Therefore, treatment is usually limited to 1 year. More importantly, most patients do not benefit from PEG-IFN treatment [2,3,5].

Given the different target sites of PEG-IFN and NUCs, a potential way to improve therapeutic efficacy is by combining them. Large randomized trials investigated whether combination therapy might have an additive effect and, thereby, achieve more effective sustained viral suppression. However, such studies showed no beneficial effect of combining PEG-IFN with lamivudine or ribavirin compared with PEG-IFN alone in both HBeAg-positive and -negative patients [2,3,5–7]. The combination of PEG-IFN with other more potent NUCs remains of interest, but has not so far been reported in prospective studies.

Considering the low efficacy and significant side effects of PEG-IFN-based therapy, there is a need to establish predictors of response (or non-response) to allow selection of patients likely to benefit from treatment. Several pre-treatment markers have been found to predict combined response, defined as sustained viral suppression (HBV DNA<2,000 IU/ml) with alanine aminotransferase (ALT) normalization, in both HBeAg-positive and -negative patients treated with PEG-IFN. In HBeAg-positive patients, HBV genotype, lower baseline HBV DNA level ($\leq 2\times10^8$ IU/ml), high ALT level, female sex, older age and absence of previous IFN therapy were predictors of response [8]. In HBeAg-negative patients, besides high ALT and low HBV DNA levels, younger age, female sex and a lower Ishak fibrosis score were found to be baseline markers of combined response [9,10]. For HBsAg seroconversion in HBeAg-positive patients, genotype A and B, and HBeAg seroconversion before week 32 of treatment were favourable markers [11–13].

In this prospective study, we treated 92 chronic HBV patients (44 HBeAg-positive and 48 HBeAg-negative) with a combination of PEG-IFN and adefovir for 48 weeks. In this report, we present the results of up to 2 years of treatment-free follow-up and investigate baseline markers that predict the outcome of treatment.

Methods

Study design

This investigator-initiated, prospective and open-label study was carried out in two hospitals in the Netherlands; the Academic Medical Centre (AMC) in Amsterdam and the Erasmus University Medical Centre (EMC) in Rotterdam.

All patients received a combination of PEG-IFN-α2a (Pegasys®; Hoffman La Roche, Basel, Switzerland) 180 μg subcutaneously once a week and adefovir dipivoxil (Hepsera®; Gilead Sciences, Foster City, CA, USA) 10 mg daily for 48 weeks. We offered patients a liver biopsy before treatment. After 48 weeks, we discontinued treatment and began a follow-up period of up to 5 years. Patients attended the outpatient clinic every 4–6 weeks for routine examination and laboratory tests up to week 72, and every 24 weeks afterwards (for schedule of assessments see Additional file 1).

Patients

Patients with chronic HBV infection (HBeAg-positive and -negative) aged 18 years or older were enrolled after assessment of eligibility. To be eligible, patients must have had documented HBsAg positivity for longer than 6 months. Other inclusion criteria were HBV DNA>100,000 copies/ml (17,182 IU/ml), normal or increased alanine aminotransferase (ALT) levels, but ≤10× upper limit of normal (ULN) or histological signs of chronic active hepatitis. Exclusion criteria were concurrent infection with HCV, hepatitis Delta virus or HIV; decompensated liver disease, HCC or a history of bleeding from oesophageal varices; direct antiviral or immune modulatory therapy within previous 6 months; women with ongoing pregnancy or breast feeding; a history of autoimmune-related disease, significant cardiac disease or renal impairment; evidence of chronic liver disease other than HBV; neutrophil count <1,500 cells/mm^3, platelet count <90,000 platelets/mm^3 or total serum bilirubin >2×ULN; evidence of current hard drug(s) and/or alcohol abuse.

The study complied with the Declaration of Helsinki and with the principles of Good Clinical Practice and was approved by the ethical committees of the corresponding sites (controlled-trials.com; ISRCTN 77073364). All patients gave written informed consent.

Laboratory assays

Biochemical and virological analyses

Local laboratories carried out biochemical and haematological analyses in accordance with good laboratory practice. ALT levels were expressed relative to the ULN range. ALT reference values were 45 U/l for males and 34 U/l for females. Plasma HBV DNA was extracted by COBAS® Ampliprep (F. Hoffmann-La Roche Ltd, Diagnostics Division, Basel, Switzerland) according to the manufacturer's instructions. Quantitation of plasma HBV DNA levels was done by the Roche COBAS® TaqMan 48® assay (F. Hoffmann-La Roche Ltd), with a dynamic range between 20 and 1.70×10^8 IU/ml. HBV genotype was determined using the INNO-LiPA assay (Innogenetics, Gent, Belgium) or by sequencing a part of the polymerase gene with dideoxynucleotide technology. Presence of precore (PC) or basal core promoter (BCP) mutations was

determined by sequencing the PC and BCP regions. Qualitative detection of serum hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg) and antibody to hepatitis B e antigen (anti-HBe) was performed by enzyme immunoassay (AxSYM; Abbott Laboratories, Abbott Park, IL, USA), and expressed as sample to cutoff ratio (S/CO) with a lower limit of detection of HBsAg 0.05 IU/ml, HBeAg 1.0 S/CO and anti-HBe 1.0 S/CO. Serum HBsAg quantitation was performed by the Abbott Architect (Abbott Diagnostics), with a dynamic range between 0.05 and 250 IU/ml [14]. If HBsAg levels were above 250 IU/ml, we diluted 10-fold with Abbott Manual Diluent (Abbott Diagnostics) until a quantitative value was achieved.

Histological analyses

For histological assessment of liver biopsies, we used the modified Ishak scoring system, based on a 0 to 6 score for fibrosis (fibrosis score) in which score 5 and 6 represent marked bridging (incomplete cirrhosis) and cirrhosis, respectively [15].

Response definitions

Response was determined after 48 weeks of treatment (end of treatment; week 48), after 24 weeks of treatment-free follow-up (short-term follow-up; week 72) and after 96 weeks of treatment-free follow-up (long-term follow up; week 144).

We defined responses to comply with the most recent AASLD and EASL guidelines [16,17]. HBeAg loss was defined as undetectable HBeAg. HBeAg seroconversion was defined as HBeAg loss with formation of anti-HBe. HBsAg loss was defined as undetectable HBsAg (Abbott AxSYM: HBsAg<0.05 IU/ml). HBsAg seroconversion was defined as HBsAg loss with formation of anti-HBs (anti-HBs>10 IU/ml).

Combined response (CR) was defined as combination of virological (HBeAg negativity and HBV DNA levels ≤2,000 IU/ml) and biochemical response (persistent normal ALT levels) in both HBeAg-positive and -negative patients. Patients were considered non-responders when not meeting one or both criteria for combined response, or when re-treatment with NUCs had been initiated.

Statistical analyses

Statistical comparisons were tested with IBM SPSS Statistics, version 19.0.0.1 (SPSS Inc., Chicago, IL, USA). Analyses were based on the intention-to-treat (ITT) model, in which patients who prematurely discontinued treatment were scored as non-responders. Differences by normally distributed variables (age, log HBsAg and log HBV DNA) were tested using the Student's *t*-test, whereas differences in variables with skewed distribution (ALT) were tested using the Mann–Whitney U test. For comparison of categorical variables, the χ^2 test or Fisher's exact test was used. The associations between variables as potential predictors of combined response or HBsAg loss as dependent variables were examined by logistic regression analysis. We used multivariable logistic regression analysis, including all variables with a *P*-value below 0.05, to determine independent predictive factors for response. All *P*-values are two sided and values below 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 96 patients signed informed consent (Additional file 1). Four patients did not meet inclusion criteria at (re-)screening, all four because of a viral load <17,182 IU/ml. Ultimately, 92 patients (82 from AMC and 10 from EMC) received at least one dose of combination therapy (ITT). Six patients prematurely discontinued treatment as a result of adverse events and were considered as non-responders in all analyses. One patient stopped because of general side effects at week 18 (fatigue and flu-like symptoms), one became pregnant while on therapy at week 10, one had a myocardial infarction at week 10, one developed autoimmune hepatitis at week 25, one had severe neutropenia despite dose reduction from week 2 and one developed Child–Pugh B cirrhosis at week 4. Common side effects included fatigue, myalgia, other flu-like symptoms, pruritus and insomnia (Additional file 1). This side effect pattern was similar to that seen before in interferon treatment, and no new side effects that could be attributable to combination therapy were reported. In sum, 91 of 92 patients (99%) completed at least 2 years of treatment-free follow-up. One patient with combined response at week 72 was lost to follow-up after virological relapse at week 96 and was regarded as a non-responder at week 144.

Table 1 shows baseline characteristics of HBeAg-positive and -negative patients. HBeAg-positive patients were younger, were more frequently Caucasian or Asian, had higher ALT, higher baseline HBV DNA and HBsAg levels and a lower prevalence of PC and BCP mutations. Although patients with genotype A were more frequently HBeAg-positive, and genotype D and E more frequently HBeAg-negative, this difference was not significant in our cohort. Data on the association of IL28B polymorphisms and treatment outcome in these patients has been published earlier by de Niet *et al.* in 2012 [18].

HBeAg-positive patients

Response

Table 2 shows an overview of clinical outcomes at week 48, week 72 and week 144 in HBeAg-positive

Table 1. Baseline characteristics of all HBeAg-positive and -negative patients included in intention to treat analysis

Characteristics	HBeAg-positive (*n*=44)	HBeAg-negative (*n*=48)	*P*-value
Mean age, years (SD; range)[a]	35.8 (9.5; 19–54)	43.1 (9.7; 24–69)	**<0.001**
Female sex, *n* (%)[b]	9 (20.5)	15 (31.3)	0.24
Ethnicity[b]			**0.02**
Caucasian, *n* (%)	16 (36)	12 (25)	
Asian, *n* (%)	20 (46)	14 (29)	
African, *n* (%)	8 (18)	22 (46)	
Median ALT×ULN (range)[c]	2.3 (0.6–27.9)	1.6 (0.5–9.2)	**0.04**
Interferon-treatment naive, *n* (%)[b]	35 (79.5)	33 (68.8)	0.24
NUC–treatment naive, *n* (%)[b]	41 (93)	44 (92)	0.78
Viral characteristics			
Mean HBV DNA, $\log_{10}$ IU/ml (SD; range)[a]	8.1 (1.2; 4.8–10.4)	5.5 (1.1; 3.6–7.7)	**<0.001**
Mean HBsAg, $\log_{10}$ IU/ml (SD; range)[a]	4.3 (0.7; 1.9–5.3)	3.3 (0.7; 1.6–4.5)	**<0.001**
HBV genotype[b]			0.10
A, *n* (%)	18 (42)	11 (23)	
B, *n* (%)	8 (18)	7 (15)	
C, *n* (%)	7 (16)	5 (10)	
D, *n* (%)	9 (21)	18 (38)	
E, *n* (%)	2 (5)	7 (15)	
Pre-core mutation, *n* (%)[b]	6 (14)	36 (78)	**<0.001**
Basal core promoter mutation, *n* (%)[b]	16 (38)	29 (62)	**0.03**
Baseline liver biopsy, *n* (%)[d]	31 (81)	39 (71)	0.23
Median fibrosis score, (range)[c]	1 (0–6)	1 (0–6)	0.48
Cirrhosis, *n* (%)[b]	2 (7)	9 (23)	0.06

P-values <0.05 are bold. [a]*P*-value for Student's *t*-test. [b]*P*-value for Fisher's exact or χ^2 test. [c]*P*-value for Mann–Whitney U test. [d]70 patients had baseline liver biopsy material available for evaluation. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; NUC, nucleoside/nucleotide analogue; ULN, upper limit of normal.

patients. In total, 13 of 44 patients (30%) lost HBeAg at week 48, which increased to 18 (41%) at week 144. The majority also had HBeAg seroconversion: 11 of 44 patients (25%) at week 48, and 15 of 44 (34%) at week 144. In none of the patients, reversion to HBeAg positivity was documented throughout follow-up. Of 18 patients with HBeAg loss, 12 had a combined response at week 144. We considered the other six patients who lost HBeAg as non-responders. This included three with increased HBV DNA levels after HBeAg loss, and three with HBeAg loss after the initiation of re-treatment with NUCs. In addition, two patients lost HBeAg after premature discontinuation of study treatment (drop outs) and counted as non-responders. 20 of 32 non-responders (63%) were re-treated with NUCs before week 144.

At the end of week 144, 5 of 44 patients (11%) had lost HBsAg, 4 of them with HBsAg seroconversion before week 48. The other remained HBsAg-negative, without detectable anti-HBs. One patient with HBeAg loss and HBsAg loss reverted to an HBsAg-positive state at week 68.

Baseline predictors for combined response at week 144 in HBeAg-positive patients

The additional information shows baseline characteristics in relation to combined response. We found no significant differences between the baseline characteristics of patients who had combined response at week 144 and those with no response. Patients with combined response tended to have higher ALT levels (median 3.6×ULN versus 1.9×ULN; *P*=0.17).

Baseline predictors for HBsAg loss at week 144 in HBeAg-positive patients

Tables 3 shows the baseline characteristics in relation to HBsAg loss at week 144. An overview of all evaluated characteristics is shown in Additional file 1. The five patients who lost HBsAg at week 144 tended to be older than those with HBsAg persistence (mean 42 years versus 35 years; *P*=0.12) and had a relatively high proportion of HBV genotype A (80% versus 36%; *P*=0.06). Liver biopsies were available for four patients with HBsAg loss, which showed higher Ishak fibrosis scores than those with HBsAg persistence (median 2.5 versus 1.0; *P*=0.03).

HBeAg-negative patients

Response

Table 2 shows an overview of the clinical outcomes at week 48, week 72 and week 144 in HBeAg-negative patients. In HBeAg-negative patients, 37 of 48 (77%) attained combined response at week 48, decreasing to 17 (35%) and 12 (25%) during treatment-free follow-up (week 72 and week 144, respectively). Re-treatment with NUCs was initiated in 15/31 (48%) patients with no response at week 72 and in 27/36 (75%) patients with no response at week 144. Five non-responder patients at week 144 did not meet the treatment criterion of HBV DNA>20,000 IU/ml. One patient developed HCC during follow-up and was re-treated with NUC therapy.

The percentage of patients with HBsAg loss increased during 2 years of follow-up. At week 48, 3 (6%) had achieved HBsAg loss, increasing to 8 (17%) at week 144. All but one patient with HBsAg loss had developed HBsAg seroconversion at week 144. Of note, the one patient without HBsAg seroconversion at week 144 did develop anti-HBs at week 177.

Table 2. Response rates of HBeAg-positive patients and HBeAg-negative patients at 48 weeks of treatment[a], 24 weeks of treatment-free follow-up[b] and 96 weeks of treatment-free follow-up[c]

Response	HBeAg-positive (*n*=44)			HBeAg-negative (*n*=48)		
	Week 48	Week 72	Week 144	Week 48	Week 72	Week 144
Biochemical response						
ALT normalization, *n* (%)[d]	33 (75)	22 (50)	17 (39)	37 (77)	25 (52)	14 (29)
Virological response						
HBV DNA<2,000 IU/ml, *n* (%)	29 (66)	14 (32)	12 (27)	47 (98)	17 (35)	12 (25)
Non-response, *n* (%)	12 (27)	27 (61)	29 (66)	8 (17)	28 (58)	33 (69)
NUC re-treatment, *n* (%)	–	3 (7)	20 (45)	–	12 (25)	24 (50)
Drop out, *n* (%)	3 (7)	3 (7)	3 (7)	3 (6)	3 (6)	3 (6)
Lost to follow-up, *n* (%)	0	0	0	0	0	1 (2)
Serological response						
HBeAg loss, *n* (%)	13 (30)	14 (32)	18 (41)	–	–	–
HBeAg loss after NUC re-treatment, *n* (%)	–	0	3 (7)	–	–	–
HBeAg seroconversion, *n* (%)	11 (25)	12 (27)	15 (34)	–	–	–
HBeAg seroconversion after NUC re-treatment, *n* (%)	–	0	2 (5)	–	–	–
HBsAg loss, *n* (%)	4 (9)	4 (9)	5 (11)	3 (6)	4 (8)	8 (17)
HBsAg seroconversion, *n* (%)	4 (9)	4 (9)	4 (9)	3 (6)	3 (6)	7 (15)
HBsAg loss after NUC re-treatment, *n* (%)	–	0	0	–	0	0
Combined response, *n* (%)[e]	29 (66)	14 (32)	12 (27)	37 (77)	17 (35)	12 (25)

[a]End of treatment; week 48. [b]Short-term follow-up; week 72. [c]Long-term follow-up; week 144. [d]Alanine aminotransferase (ALT) reference values: male 45 U/l, female 34 U/l. [e]Combined response was defined as hepatitis B e antigen (HBeAg) negativity, HBV DNA<2,000 IU/ml and ALT normalization. HBsAg, hepatitis B surface antigen; NUC, nucleoside/nucleotide analogue.

Table 3. Baseline characteristics of HBeAg-positive and -negative patients who lost HBsAg at long-term follow-up[a] compared with those with HBsAg persistence

Characteristics	HBsAg loss at long-term follow-up (week 144)		*P*-value
	HBsAg loss	HBsAg persistence	
HBeAg-positive, *n* (%)	**5 (11)**	**39 (52)**	**–**
Mean age, years (SD)[b]	42.0 (6.9)	35.0 (9.6)	0.12
IFN-treatment naive, *n* (%)[c]	4 (80)	31 (80)	0.98
Median ALT×ULN (IQR)[d]	5.3 (1.8–6.7)	2.2 (1.1–4.8)	0.23
Mean HBV DNA, $\log_{10}$ IU/ml (SD)[b]	8.09 (1.1)	8.04 (1.2)	0.94
Mean HBsAg, $\log_{10}$ IU/ml (SD)[b]	4.16 (1.0)	4.33 (0.7)	0.64
HBV genotype A, *n* (%)[c]	4 (80)	14 (36)	0.06
Baseline liver biopsy, *n* (%)[e]	4 (80)	27 (69)	0.62
Median fibrosis score (range)[d]	2.5 (2–6)	1 (0–5)	**0.03**
HBeAg-negative, *n* (%)	**8 (17)**	**40 (83)**	**–**
Mean age, years (SD)[b]	46.9 (12.9)	42.3 (9.0)	0.22
IFN-treatment naive, *n* (%)[c]	2 (25)	31 (78)	**0.02**
Median ALT×ULN (IQR)[d]	1.1 (0.8–2.4)	1.8 (1.1–2.9)	0.18
Mean HBV DNA, $\log_{10}$ IU/ml (SD)[b]	4.76 (0.9)	5.69 1.1)	**0.03**
Mean HBsAg, $\log_{10}$ IU/ml (SD)[b]	2.35 (0.6)	3.55 (0.5)	**<0.001**
HBV genotype A, *n* (%)[c]	3(38)	8 (20)	0.28
Baseline liver biopsy, *n* (%)[e]	7 (88)	32 (80)	0.62
Median fibrosis score (range)[d]	2 (0–6)	1 (0–6)	0.65

P-values <0.05 are bold. [a]Week 144. [b]*P*-value for Student's *t*-test. [c]*P*-value for Fisher's exact or χ^2 test. [d]*P*-value for Mann–Whitney U test. [e]70 patients had baseline liver biopsy material available for evaluation. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ULN, upper limit of normal.

Baseline predictors for combined response at week 144 in HBeAg-negative patients

The additional information shows all baseline characteristics in relation to combined response in HBeAg-negative patients. HBV DNA and HBsAg levels were lower in patients achieving a combined response at week 144, as compared with non-responders (mean HBV DNA 4.85 $\log_{10}$ IU/ml versus 5.76 $\log_{10}$ IU/ml;

Table 4. Multivariable analysis

HBeAg-negative patients[a]	HBsAg loss at long-term follow-up (week 144), multivariable logistic regression (adjusted)		
	OR	95% CI	*P*-value
HBV DNA, $\log_{10}$ IU/ml	0.40	0.09, 1.73	0.21
HBsAg, $\log_{10}$ IU/ml	0.02	0.00, 0.39	**0.01**
IFN-treatment naive	0.05	0.02, 3.86	0.32

P-values <0.05 are bold. [a]Multivariable analysis was performed in hepatitis B e antigen (HBeAg)-negative patients only (*n*=48). HBsAg, hepatitis B surface antigen; IFN, interferon.

P=0.01, and mean HBsAg 2.87 $\log_{10}$ IU/ml versus 3.50 $\log_{10}$ IU/ml; *P*=0.04). Higher levels of HBV DNA correlated with higher levels of HBsAg (Pearson's correlation coefficient 0.334; *P*=0.02).

Using multivariable logistic regression only the HBsAg level was an independent predictor of combined response at week 144 (OR 0.30 per 1 $\log_{10}$ IU/ml HBsAg decrease [95% CI 0.09, 0.93]; *P*=0.04).

Baseline predictors for HBsAg loss at week 144 in HBeAg-negative patients

Table 3 shows baseline characteristics in relation to HBsAg loss in HBeAg-negative patients. An overview of all evaluated characteristics is shown in Additional file 1. Both HBV DNA and HBsAg levels were significantly lower in HBeAg-negative patients who had lost HBsAg at week 144 (mean HBV DNA 4.76 $\log_{10}$ IU/ml versus 5.69 $\log_{10}$ IU/ml; *P*=0.03, and mean HBsAg 2.35 $\log_{10}$ IU/ml versus 3.55 $\log_{10}$ IU/ml; *P*<0.001). Moreover, fewer patients with HBsAg loss were naive to interferon treatment than those with HBsAg persistence; 2/8 (25%) versus 31/40 (78%); *P*=0.02. In multivariable analysis, HBsAg level was the only independent predictor of HBsAg loss at week 144 (OR 0.02 per 1 $\log_{10}$ IU/ml increase [95% CI 0.00, 0.39]; *P*=0.01) as shown in Table 4.

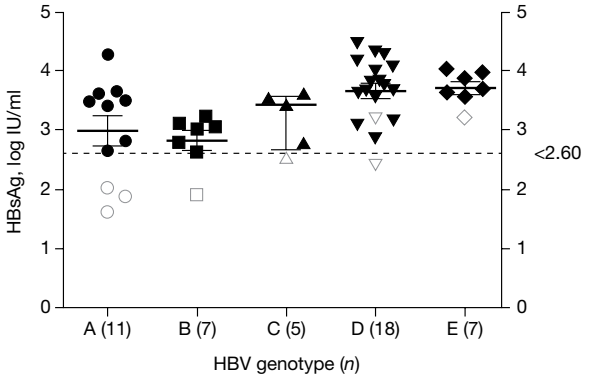
Figure 1 shows the distribution of baseline HBsAg levels in all HBeAg-negative patients of different genotypes. In total, 6 of 8 (75%) patients with HBsAg loss had a baseline HBsAg level below 2.60 $\log_{10}$ IU/ml (400 IU/ml), corresponding to a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 95%.

HBsAg kinetics – on treatment and during follow-up

HBeAg-positive patients

Figure 2A shows the course of serum HBsAg during treatment and long-term follow-up in patients with combined response and those who did not respond. Patients with combined response at week 144 had significantly lower HBsAg levels than patients with no response at all time points, except at baseline.

Figure 1. Baseline serum HBsAg levels in HBeAg-negative patients according to viral genotype



Patients with hepatitis B surface antigen (HBsAg) loss at long-term follow-up (week 144) are marked in grey and HBsAg persistence is marked in black. Error bars represent the mean and SEM.

Non-responder patients receiving NUC therapy were censored in this analysis.

At end of week 72, seven HBeAg-positive patients had a serum HBsAg level below 20 IU/ml, including five with HBsAg loss at week 144. By contrast, none of the 35 patients with a serum HBsAg level of >20 IU/ml at week 72 had lost HBsAg at week 144. This corresponds to a PPV of 71% and an NPV of 100% for HBsAg loss at week 144.

Individual figures of HBsAg kinetics in all HBeAg-positive patients (*n*=5) with HBsAg loss at week 144 are shown in Additional file 1.

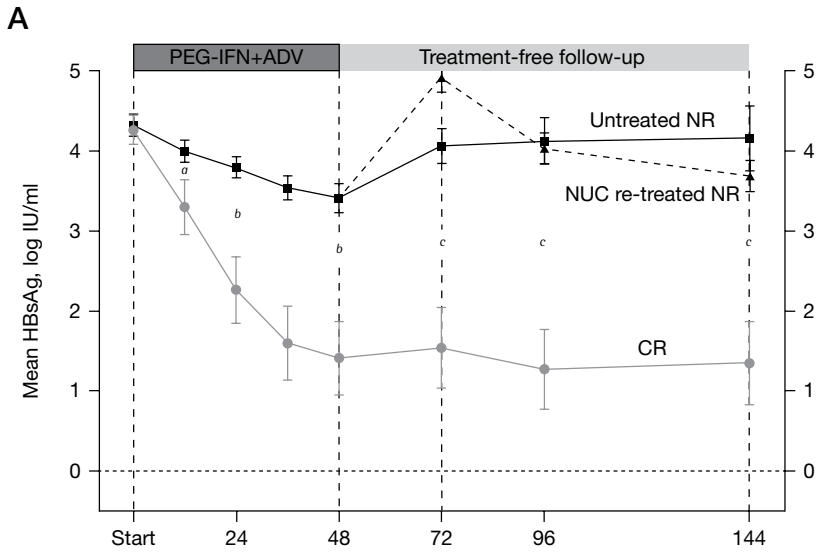
HBeAg-negative patients

Figure 2B shows the course of serum HBsAg during treatment and long-term follow-up in patients with combined response and non-response. Those with combined response at week 144 had significantly lower HBsAg levels than non-responding patients at all time points, including baseline. Non-responder patients receiving NUC therapy were censored in this analysis.

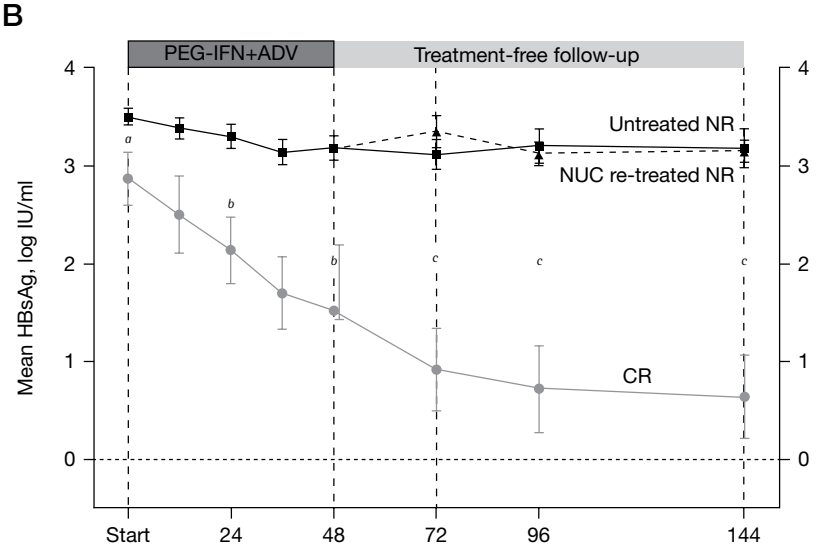
At end of week 72, nine HBeAg-negative patients had a serum HBsAg level below 20 IU/ml, including eight with HBsAg loss at week 144. Of note, the one patient who had not lost HBsAg at week 144 had lost it after 174 weeks with no anti-HBs formation up to 296 weeks. By contrast, none of the 36 patients with a serum HBsAg level of >20 IU/ml at week 72 had lost HBsAg at week 144. Using a cutoff of 20 IU/ml at week 72, we observed a PPV of 89% and an NPV of 100% for predicting HBsAg loss at week 144.

Individual figures of HBsAg kinetics in all HBeAg-negative patients (*n*=8) with HBsAg loss at week 144 are shown in Additional file 1.

Figure 2. Mean serum HBsAg levels in HBeAg-positive and -negative patients with combined response and in those with no response



NR						
Untreated	32	30	30	26	12	10
NUC re-treated	–	–	–	3	16	20
CR	12	12	12	11	10	11



NR						
Untreated	36	33	33	21	13	7
NUC re-treated	–	–	–	12	18	25
CR	12	12	12	12	11	11

Mean serum hepatitis B surface antigen (HBsAg) levels in hepatitis B e antigen (HBeAg)-positive **(A)** and -negative **(B)** patients with combined response (CR) and in those with no response. The lines represent mean serum HBsAg levels in patients with no response (black lines), and patients with CR at week 144 (grey line). CR was defined as a combination of HBV DNA<2,000 IU/ml and alanine aminotransferase (ALT) normalization. Patients with no response were further subdivided into patients receiving retreatment with nucleotide analogues (black dashed line) or not (black line). *P*-values of the difference in HBsAg level between patients with CR and untreated non-responders are represented as ^{a}(P<0.05), ^{b}(P<0.005) and ^{c}(P<0.0005), respectively. Error bars represent the SEM. Total number of available patient samples at each time point is given in the table below each figure. NR, non-response; NUC, nucleoside/nucleotide analogues; PEG-IFN+ADV, pegylated interferon plus adefovir.

Discussion

Here, we report the results of a prospective study of predictive markers for response in HBeAg-positive and -negative chronic hepatitis B patients treated with PEG-IFN and adefovir. In our study, we found a high rate of HBsAg loss in both HBeAg-positive (11%) and HBeAg-negative (17%) patients at 2 years of treatment-free follow-up (week 144). The main outcome of the study was that HBeAg-negative patients with a low baseline HBsAg level were more likely to achieve HBsAg loss.

In HBeAg-negative patients in particular, the extent of HBsAg loss (17%) and HBsAg seroconversion (15%) at 2-year follow-up was high compared with those treated with PEG-IFN for 48 weeks as monotherapy or combined with lamivudine. For example, Marcellin *et al.* [19] reported 4% and 5% HBsAg loss, respectively, in 116 PEG-IFN and 114 PEG-IFN+ lamivudine-treated HBeAg-negative patients after a follow-up of 2 years. Although we can make no direct comparison because of the absence of a monotherapy arm in our study, the high rate of HBsAg loss suggests an additive therapeutic effect of adefovir. Next to antiviral activity, adefovir enhances innate immune functions in mice [20,21], indicating that the immune modulatory effect of adefovir might be synergistic when combined with PEG-IFN. Three earlier studies have involved combination treatment of PEG-IFN and adefovir. Two studies found a relatively high rate of HBsAg loss in HBeAg-positive patients but were either small [22] or used a different treatment regimen [23]. In contrast to our study, a relatively small randomized trial in HBeAg-negative patients (n=30), mainly infected with viral genotype D, did not show an increase in HBsAg loss using PEG-IFN and adefovir combination treatment [10].

We observed that HBsAg level at baseline was significantly lower in HBeAg-negative patients with HBsAg loss compared with those with HBsAg persistence (P<0.001). To our knowledge, this is the first prospective study indicating that HBsAg at baseline is an independent predictor of HBsAg loss in HBeAg-negative patients. Only one retrospective study associated lower baseline HBsAg in a subset of HBeAg-negative patients treated with IFN-α2b with HBsAg loss over time [24]. Interestingly, all HBeAg-negative patients in our study with a baseline HBsAg level below 400 IU/ml (n=6) had lost HBsAg at week 144. Although most HBeAg-negative patients had HBsAg levels above this value, demographic studies on quantitative HBsAg showed that 2–13% of HBeAg negative patients with high viral load had HBsAg levels below 400 IU/ml and, thus, may be good candidates for PEG-IFN-based combination therapy [25–27].

As a result of a lack of studies and the usually low rate of HBsAg loss, there is a paucity of clear mechanistic insight into HBsAg loss in PEG-IFN-based treatment. The decrease in serum HBsAg seems to be associated with eradication of cccDNA through clearance of infected hepatocytes by cytotoxic T-cells [22,28]. There is evidence that HBsAg impairs antigen presenting cells by repetitive Toll-like receptor triggering [29,30]. Lower antigenic loads of HBsAg may, therefore, be associated with a less refractory innate immune system, thus lowering the threshold for response to exogenous IFN. Interestingly, a higher proportion of HBeAg-negative patients with HBsAg loss were IFN-experienced, compared with patients not losing HBsAg. We also showed that both responders and non-responders had a sustained decrease in HBsAg levels after therapy. This may suggest that lower baseline HBsAg levels in some patients could be explained by an earlier IFN-related decrease in HBsAg. Based on these findings, HBeAg-negative patients who relapse after PEG-IFN treatment but retain low levels of serum HBsAg may be good candidates for re-treatment with PEG-IFN-based therapy.

In contrast to our findings in HBeAg-negative patients, neither baseline HBsAg nor HBV DNA predicted HBeAg or HBsAg loss in HBeAg-positive patients. HBeAg-positive patients with HBsAg loss did have higher fibrosis scores in liver biopsy specimen (P=0.03), although only four biopsies of patients with HBsAg loss were available for analysis.

Both HBeAg-positive and -negative patients with combined response showed a significant decrease in serum HBsAg level during treatment, which was sustained during long-term follow-up. The definition of combined response (HBV DNA<2,000 IU/ml with normal ALT at end of follow-up) is accepted by recent international guidelines and relates to epidemiological studies showing that progression of cirrhosis and HCC significantly diminishes when HBV DNA is below 2,000 IU/ml [31–33]. However, it is a relatively weak end point because after longer follow-up many patients relapse. Those with HBeAg-negative disease in particular may show increased HBV DNA and increased ALT necessitating re-treatment, most often with nucleoside/nucleotide analogues [19]. For example, in our study, 5/13 (38%) HBeAg-negative patients with combined response at week 72, but without HBsAg loss, relapsed during long-term follow-up. Conversely, a large proportion of HBeAg-negative patients who did achieve a combined response at week 144 (n=19) experienced HBsAg loss (9/19; 47%) and subsequent HBsAg seroconversion (8/19; 42%), which is regarded as the closest outcome to clinical cure in HBV treatment [17]. In addition, we found that HBeAg-negative patients with HBsAg levels <20 IU/ml at 24 weeks of treatment-free follow-up had high predictive values for HBsAg loss at long-term follow-up (PPV of 89% and NPV of 100%).

Overall, these findings suggest that HBsAg loss with HBsAg seroconversion may be an important objective for future intervention studies to focus on.

This is a non-randomized study on a limited number of patients. Therefore, we have to be careful with our conclusions. We cannot exclude that differences in study population characteristics may account, in part, for the high rate of HBsAg loss found this study. In our study, for example, a relatively high percentage of patients with genotype A were present, known to easier clear HBsAg in PEG-IFN-based therapy. However, in HBeAg-negative patients, in whom higher rates of HBsAg loss occurred specifically, we observed HBsAg loss across all major genotypes. Nevertheless, our results have to be validated in a comparable cohort of patients treated with PEG-IFN-based combination therapy. Partly for this reason, large randomized studies comparing PEG-IFN and tenofovir or entecavir in combination versus monotherapies have been initiated. Next to therapeutic and viral factors, other yet undefined host and viral factors may play a role in inducing HBsAg loss in HBeAg-positive and -negative patients. In this regard, it would be of interest to study additional viral, host genetic and immunological markers that could be associated with HBsAg loss.

In conclusion, in HBeAg-negative patients with active disease and treated with PEG-IFN and adefovir combination therapy, a low baseline HBsAg is an excellent predictor for sustained HBsAg loss. Thus, selection of patients by baseline HBsAg levels may substantially increase the rate of HBsAg loss and avoid unnecessary IFN-related adverse events in patients with a poor chance of responding to PEG-IFN and nucleoside/nucleotide analogue combination therapy.

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Disclosure statement

The study was designed by HWR. RBT, AdN, VR and LJ performed the acquisition of data. RBT and LJ were involved in the analysis and interpretation of data and drafting the manuscript. HWR, CJW, PLMJ and AdN critically revised the manuscript. HWR, HLAJ and HLZ contributed to the study supervision and interpretation of data. VT, RM, MK and MGHMB provided technical and material support, and participated in data analysis. MGWD supervised the statistical analysis.

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Additional file

Additional file 1: Supplementary data can be found at http://www.intmedpress.com/uploads/documents/2786_Takkenberg_Add_file1.pdf

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