

# Early HBeAg Loss During Peginterferon $\alpha$ -2b Therapy Predicts HBsAg Loss: Results of a Long-Term Follow-Up Study in Chronic Hepatitis B Patients

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- OBJECTIVES:** Treatment with pegylated interferon (PEG-IFN)  $\alpha$ -2b results in hepatitis B e antigen (HBeAg) loss in 36% of patients at 6 months post treatment. The aim of this study was to determine whether a long-term response to PEG-IFN is dependent on the timing of HBeAg loss.
- METHODS:** A total of 91 patients treated with PEG-IFN  $\alpha$ -2b alone (100  $\mu$ g per week) and 81 patients treated with PEG-IFN  $\alpha$ -2b and lamivudine (100 mg/day) for 52 weeks were enrolled in this study. Patients were initially followed up at 4-week intervals and had one additional long-term follow-up (LTFU) visit (mean: 3.03 $\pm$ 0.77 years 26 weeks post treatment).
- RESULTS:** Of the 172 patients included, 78 patients (46%) did not have loss of HBeAg, 47 (27%) lost HBeAg within 32 weeks, and 47 patients (27%) had loss of HBeAg after week 32. At LTFU, patients with HBeAg loss  $\leq$  32 weeks had hepatitis B virus DNA of <400 copies/ml significantly more often than did those who lost HBeAg after week 32 (47 vs. 21%, respectively;  $P=0.009$ ). Hepatitis B surface antigen (HBsAg) negativity was also observed significantly more often in patients with early HBeAg loss (36 vs. 4%, respectively,  $P<0.001$ ). Early HBeAg loss tended to occur more often in patients treated with PEG-IFN and lamivudine combination therapy than in those treated with PEG-IFN alone (35 vs. 21%;  $P=0.10$ ), as did HBsAg loss (15 vs. 8%;  $P=0.14$ ).
- CONCLUSIONS:** Early PEG-IFN-induced HBeAg loss results in a high likelihood of HBsAg loss and may be associated with more profound viral suppression during the first 32 weeks of therapy in patients treated with lamivudine combinations.

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## INTRODUCTION

Worldwide, more than 2 billion people have evidence of infection with hepatitis B virus (HBV), and chronic hepatitis B affects ~400 million people (1,2). It is estimated that between 500,000 and 1 million people die annually because of HBV-associated liver disease, largely because of cirrhosis and hepatocellular carcinoma. Despite the availability of safe and

effective vaccines for more than two decades, HBV infection is still a global health problem (3).

Treatment of chronic HBV infection has considerably improved over the last decade, with seven antiviral drugs being available currently (4). Treatment strategies can be divided into those providing sustained off-treatment response after a finite course of therapy (immunomodulatory drugs) and

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those aiming at maintaining on-treatment remission (direct antivirals). The effects of interferon (IFN) are predominantly immunomodulatory, but it also has a limited direct antiviral effect on HBV. Nucleos(t)ide analogs such as lamivudine, adefovir, entecavir, telbivudine, and tenofovir on the other hand are potent inhibitors of HBV replication. IFN-induced hepatitis B e antigen (HBeAg) loss is sustained in the majority of responders because of the immunomodulatory effects of the drugs (5–7).

Treatment with standard IFN or pegylated IFN (PEG-IFN) results in loss of HBeAg in about one-third of patients (8–10). Overall 29% of patients treated with PEG-IFN  $\alpha$ -2b lost HBeAg by the end of a 1-year treatment course, increasing to 36% at 6 months post treatment (8). A sustained HBeAg loss and hepatitis B surface antigen (HBsAg) loss were observed in 81 and 30% of these responders, respectively, after 3 years of additional follow-up. HBsAg loss was observed significantly more often in genotype A-infected responders than in those with genotype-non-A infection (58 vs. 6%, respectively,  $P < 0.001$ ) (11). Other factors associated with an increased likelihood of response to PEG-IFN therapy include a low initial viral load, high alanine transaminase (ALT) concentrations, absence of previous IFN therapy, and a low HBeAg level (8,12). The aim of this study is to determine whether long-term virological and biochemical outcome is dependent on the timing of HBeAg loss in HBeAg-positive patients treated with PEG-IFN  $\alpha$ -2b (PEG-IFN  $\alpha$ -2b).

## METHODS

### Participants and study design

In all, 172 of 266 patients (65%) enrolled in the HBV99-01 study were enrolled in this long-term follow-up (LTFU) study (8,11). The majority of the 94 patients who were not enrolled in the LTFU study did not participate because the local study site did, for variable reasons, not take part in this study ( $n = 52$ , 55%) or because the patient was lost to follow-up ( $n = 23$ , 24%). Informed consent was obtained from each participant, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the approval by the human research committee of all participating centers. Patients were treated with PEG-IFN  $\alpha$ -2b 100  $\mu$ g weekly (PegIntron, Schering-Plough, Kenilworth, NJ) in combination with placebo or lamivudine 100 mg daily (Zeffix, GlaxoSmith-Kline, Greenford, UK) for 52 weeks. After 32 weeks, the PEG-IFN  $\alpha$ -2b dosage was lowered to 50  $\mu$ g per week to prevent side effects and early treatment discontinuation.

The inclusion and exclusion criteria were previously reported (8,11). In short, patients were eligible if they had been HBsAg positive for more than 6 months, were HBeAg positive on two occasions within 8 weeks before randomization, had an elevated serum ALT of at least twice the upper limit of normal, and had serum HBV DNA  $> 1.0 \times 10^5$  copies/ml. Major exclusion criteria were antiviral therapy within 6 months before randomization, viral coinfections, preexistent cytopenia, or decompensated liver disease.

Patients were followed up at 4-week intervals during the initial study. For the LTFU study, patients were reevaluated by one additional visit to the local participating center. The local investigator assessed clinical signs and symptoms of liver disease, complications of liver disease (hepatocellular carcinoma, ascites, variceal bleeding, encephalopathy, or jaundice), liver transplantation, mortality, and administration of (other) antiviral therapy after the initial study, according to predefined criteria on standardized questionnaires. If the patient had been re-treated, local baseline data before re-treatment were also collected. Blood samples were obtained for hematology, biochemistry, and virology testing. Follow-up time was calculated from the end of the initial study (week 78) until the visit for the LTFU study. Patients were reevaluated at a mean interval of  $3.0 \pm 0.8$  years (range: 1.6–5.0) after the end of the initial study (week 78). Patients who were re-treated after the initial study were considered as nonresponders for all categorical outcome measures, except for the HBeAg response for which the local test result at the time of restarting antiviral therapy was taken into account. Patients were classified as nonresponders if they remained HBeAg positive throughout follow-up. Responders were those with HBeAg loss, and were subdivided depending on the timing of the first evidence of HBeAg loss, within the first 32 weeks of therapy or after week 32. ALT flares were defined as serum ALT of at least twice the nadir value and  $> 10 \times$  the upper limit of normal (13).

### Laboratory testing

During therapy and post treatment follow-up of the initial study, patients were monitored monthly by a routine physical examination and by biochemical and hematological assessments. ALT was assessed locally in accordance with standardized procedures and therefore, expressed as the number of times the upper limit of normal. Serology and quantification of serum HBV DNA were carried out at Erasmus MC in Rotterdam by staff unaware of treatment allocation and outcome at the end of treatment and at the end of initial study. For the initial study, serum HBV DNA levels were measured monthly using an in-house developed Taqman PCR assay (a lower limit of detection of 373 copies/ml) on the basis of the EuroHep standard (14). Quantification of serum HBV DNA levels for the LTFU study was carried out using the Cobas TaqMan HBV assay (Roche Molecular Systems, Branchburg, NJ), with a dynamic range of quantification of  $30$ – $1.1 \times 10^8$  IU/ml ( $174$ – $6.4 \times 10^8$  copies/ml). As serum HBV DNA was expressed in copies/ml in the initial study, all HBV DNA measurements for the LTFU study were recalculated to copies/ml (1 IU/ml = 5.8 copies/ml). The two HBV DNA quantification assays were comparable in the dynamic range (11). Testing for markers of HBV infection (HBeAg, anti-HBe, HBsAg, and anti-HBs) was carried out using the AxSYM radioimmunoassay (Abbott, Abbott Park, IL) at weeks 0, 32, 52, and 78, and with the commercially available enzyme-linked immunosorbent assays (ELISA) of DiaSorin (DiaSorin S.p.A., Saluggia, Italy) at LTFU. HBV genotype analysis was carried out by INNO-LiPA assay

(Innogenetics, Gent, Belgium). All initial responders who had serum HBV DNA  $>5.0 \times 10^3$  copies/ml after HBeAg loss were tested for the presence of core promoter (A1762T/G1764A) and precore mutations (G1896A) by sequence analysis. Histological scoring was carried out according to the modified histological activity index by one experienced pathologist, who was unaware of the chronological order of biopsies, treatment allocation, and outcome measures (15).

### Statistical analysis

For the comparison of frequencies between or within groups, the  $\chi^2$ -test and Fisher's exact test were used where appropriate. Student's *t*-test and the Mann-Whitney test were used to compare means between groups. The positive predictive value (percentage of response if the test is positive), negative predictive value (percentage of nonresponse if test is negative), sensitivity (percentage of responders identified by test), and specificity (percentage of nonresponders identified by test) of various patient characteristics and outcome measures for long-term HBsAg negativity were determined. The AUC (area under the receiver-operating characteristic curve) was used to assess the predictive value of these factors. Cox regression analysis was used for the identification of factors influencing HBsAg negativity at LTFU. Patients with missing data and those who were re-treated were considered nonresponders. A *P* value of 0.05 was considered to be statistically significant (all two-sided). Statistical analysis was carried out using the SPSS14.0 program (SPSS, Chicago, IL).

## RESULTS

### Patients and baseline characteristics

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

Patients with loss of HBeAg within 32 weeks were older, more often acquired HBV infection through sexual or parenteral transmission, had higher baseline ALT levels, and more often harbored genotype A than did patients who lost HBeAg after week 32 ( $P < 0.05$ ). The duration of follow-up was comparable in the three patient groups ( $3.09 \pm 0.77$ ,  $3.02 \pm 0.84$ , and  $2.95 \pm 0.70$  years for groups I, II, and III, respectively;  $P > 0.32$ ). Retreatment with other antiviral drugs was needed in 26% of patients with HBeAg loss within 32 weeks, in 30% of those

**Table 1. Baseline characteristics**

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

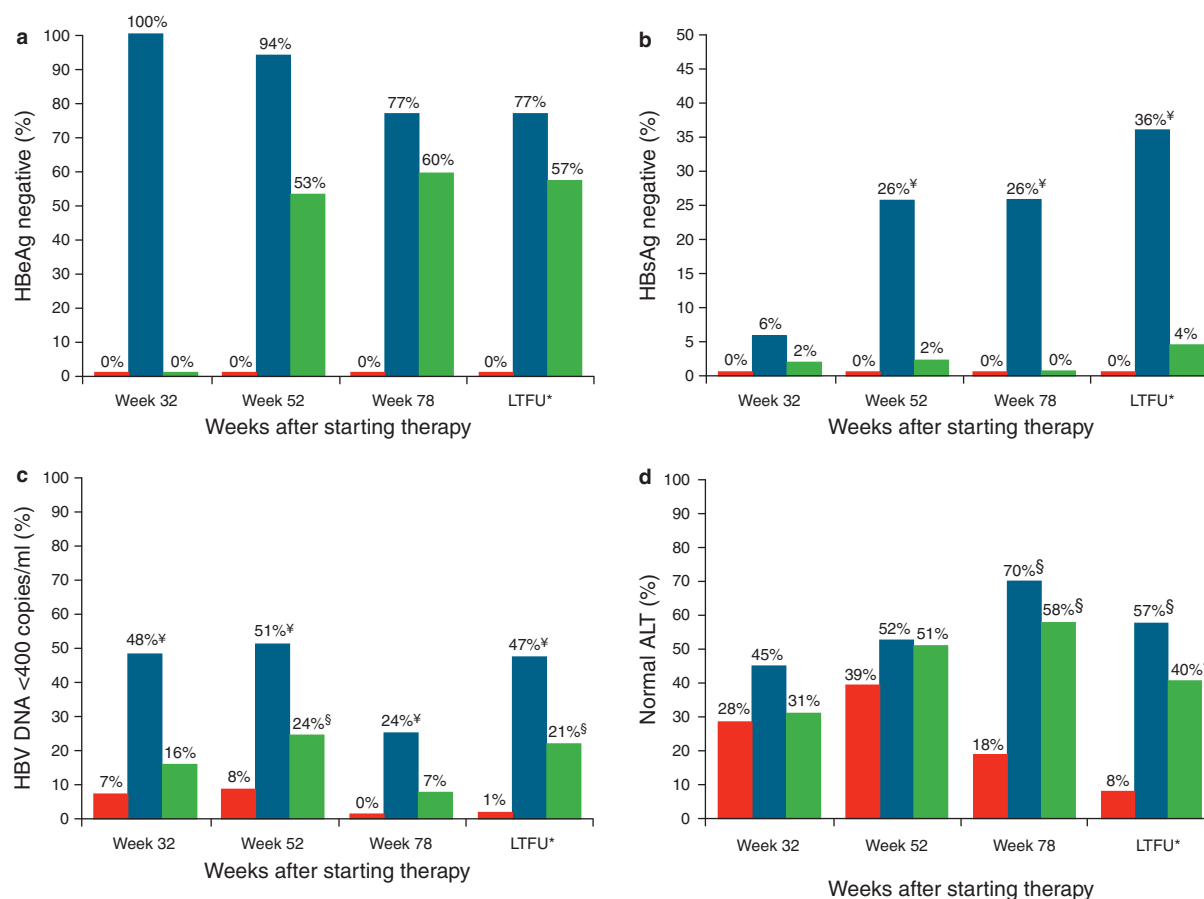
ALT, alanine transaminase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ULN, upper limit of normal.  
<sup>†</sup> $P < 0.001$  for group II vs. III; <sup>†</sup> $P < 0.05$  for group II vs. III.

with HBeAg loss after week 32, and in 71% of patients without HBeAg loss ( $P < 0.001$ ). Lamivudine resistance was observed in 9 of 81 (11%) patients in the combination therapy group (with a rate of 4, 9, and 19% among patients with HBeAg loss  $\leq 32$  weeks, HBeAg loss  $> 32$  weeks, and no HBeAg loss, respectively;  $P = 0.15$ ).

### HBeAg

The proportion of patients in each of the patient groups that was negative for serum HBeAg at various time points is shown in **Figure 1a**. At LTFU, serum HBeAg was still undetectable in 36 of 47 patients (77%) who were HBeAg negative within 32 weeks compared with 27 of 47 patients (57%) who lost HBeAg after week 32 ( $P = 0.05$ ). At LTFU, HBeAg negativity with seroconversion to anti-HBe was observed in 43 and 30% of patients with HBeAg loss within or after 32 weeks of therapy, respectively ( $P = 0.20$ ). HBeAg seroconversion was sustained in 16 of 29 patients (55%) who seroconverted within 32 weeks compared with 18 of 44 patients (41%) with HBeAg seroconversion after week 32 ( $P = 0.23$ ).

Early HBeAg loss tended to occur more often in patients treated with PEG-IFN and lamivudine combination therapy



**Figure 1.** Outcomes after 32 and 52 weeks of therapy, at 26 weeks post treatment, and at long-term follow-up. This figure shows the proportion of patients with (a) negative hepatitis B e antigen (HBeAg), (b) negative hepatitis B surface antigen (HBsAg), (c) hepatitis B virus (HBV) DNA <400 copies/ml, and (d) normal alanine transaminase (ALT) in patients without HBeAg loss (red, group I;  $n=78$ ), in patients with HBeAg loss within 32 weeks of therapy (green, group II;  $n=47$ ), and in patients with HBeAg loss after 32 weeks of therapy (blue, group III;  $n=47$ ). <sup>‡</sup> $P<0.02$  vs. group I; <sup>§</sup> $P<0.03$  vs. group I or III; <sup>#</sup> $P=0.05$  vs. group III.

than in those treated with PEG-IFN alone (35 vs. 21%, respectively;  $P=0.10$ ), whereas HBeAg loss after week 32 occurred equally in both treatment groups (27 vs. 28%, respectively). Among patients who were HBeAg negative at 26 weeks post treatment, core promoter and precore mutations were observed equally among patients who lost HBeAg within 32 weeks compared with those with HBeAg loss after week 32 (31 vs. 32%, respectively;  $P=0.89$ ).

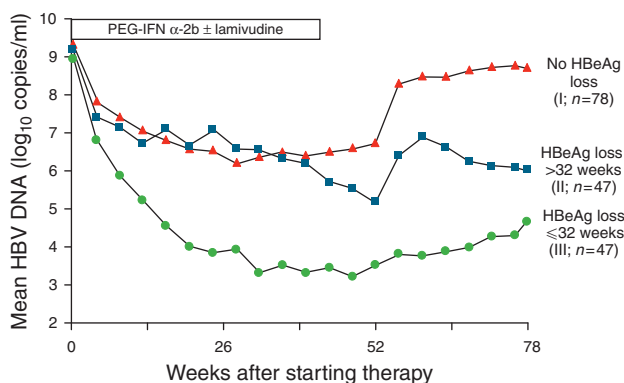
### HBsAg

In **Figure 1b**, the proportion of patients with loss of HBsAg at various time points is shown for the three patient groups. At LTFU, HBsAg negativity was observed in 17 of 47 (36%) patients with loss of HBeAg within 32 weeks compared with 2 of 47 (4%) patients who lost HBeAg after week 32 and none of 78 (0%) without HBeAg loss ( $P<0.001$ ). In all, 12 of 19 patients (63%) with HBsAg loss at LTFU also developed anti-HBs and 18 of them (95%) had undetectable HBV DNA. There was no significant difference in the HBsAg loss rate between patients with HBeAg loss after week 32 and those who

remained HBeAg positive ( $P=0.14$ ). HBsAg loss was observed in 12 of 29 patients (41%) and in 6 of 44 patients (14%) with HBeAg seroconversion within or after 32 weeks, respectively ( $P=0.007$ ). Anti-HBs was detectable in 12 of 19 patients (63%) with HBsAg loss. All but one of the HBsAg-negative patients had HBV DNA of <400 copies/ml at LTFU (the remaining patient had serum HBV DNA of  $1.3 \times 10^3$  copies/ml). At LTFU, HBsAg loss was observed in 15% of patients treated with PEG-IFN and lamivudine compared with 8% of those treated with PEG-IFN alone ( $P=0.14$ ). In both treatment groups, virtually all patients who were HBsAg negative at LTFU lost HBeAg within 32 weeks of therapy (92 and 86% of patients with HBsAg loss receiving combination therapy or monotherapy, respectively).

### HBV DNA

**Figure 1c** shows the proportion of patients with HBV DNA of <400 copies/ml for the three patient groups at weeks 32, 52, and 78, and at LTFU. At all time points, patients with loss of HBeAg within 32 weeks had HBV DNA of <400 copies/ml



**Figure 2.** The mean hepatitis B virus (HBV) DNA levels over time depending on time of hepatitis B e antigen (HBeAg) loss. Baseline serum HBV DNA was comparable in patients without HBeAg loss (group I), in patients with HBeAg loss within 32 weeks of therapy (group II), and in patients with HBeAg loss after 32 weeks of therapy (group III). Patients in group II had significantly lower HBV DNA than did patients in group I at weeks 32, 52, and 78 ( $P < 0.001$ ). Patients in group III had significantly lower HBV DNA than did patients in group I at weeks 52 and 78 only ( $P < 0.001$ ). At weeks 32 and 52, patients in group II had significantly lower HBV DNA than did those in group III ( $P < 0.001$ ), whereas mean HBV DNA tended to be different in these patient groups at week 78 ( $P = 0.08$ ). PEG-IFN, pegylated interferon.

significantly more often than did patients with loss of HBeAg after week 32 ( $P < 0.03$ ). At LTFU, HBV DNA of  $< 10,000$  copies was observed in 5% of patients without HBeAg loss, in 55% of patients with HBeAg loss within 32 weeks, and in 38% of patients with HBeAg loss after week 32 ( $P < 0.005$  for HBeAg loss within or after week 32 vs. no HBeAg loss).

The mean HBV DNA levels over time in the three patient groups are shown in **Figure 2**. At the start of treatment, mean serum HBV DNA was comparable in the three patient groups (see **Table 1**). Although mean serum HBV DNA was significantly lower in patients with HBeAg loss within 32 weeks than in those with loss of HBeAg after week 32 during therapy ( $3.14 \pm 0.96$  vs.  $5.82 \pm 2.41$ , respectively, and  $3.11 \pm 1.03$  vs.  $5.82 \pm 2.41$   $\log_{10}$  copies/ml, respectively, at week 32 and 52, respectively;  $P < 0.001$ ), the difference was no longer observed at week 78 ( $5.07 \pm 2.65$  vs.  $6.07 \pm 2.33$   $\log_{10}$  copies/ml, respectively;  $P = 0.08$ ).

In both treatment groups, the decline in HBV DNA at week 32 was significantly more profound in patients who lost HBeAg within 32 weeks than in those with delayed HBeAg loss, although the difference was most striking in patients who received PEG-IFN alone ( $-6.36$  vs.  $-5.02$   $\log_{10}$ , respectively, and  $-4.95$  vs.  $-1.12$   $\log_{10}$  copies/ml, respectively, in patients receiving combination therapy or monotherapy, respectively;  $P < 0.001$ ).

## ALT

The proportion of patients with normal ALT at weeks 32, 52, and 78, and at LTFU is shown in **Figure 1d** for the three patients groups. During therapy (weeks 32 and 52), normal ALT was observed equally in patients with HBeAg loss (within or after

32 weeks) and in those who did not have loss of HBeAg. At 26 weeks post treatment (week 78) and at LTFU, normal ALT was observed significantly more often in patients with loss of HBeAg within 32 weeks or after week 32 than in those without HBeAg loss ( $P < 0.02$ ). However, there was no difference in the proportion of patients with normal ALT in patients with loss of HBeAg within 32 weeks and in those with HBeAg loss after week 32 at any time point. The occurrence of ALT flares was not associated with early HBeAg loss ( $P = 0.63$ ).

## Predictors of HBsAg negativity at LTFU

On Cox regression analysis, older age, HBV genotype A infection, HBeAg negativity at weeks 4, 32, and HBV DNA clearance at weeks 24–32 were found to be predictors of HBsAg negativity at LTFU (**Table 2**). The occurrence of ALT flares did not predict HBsAg loss at LTFU ( $P = 0.15$ ). When correcting for age, HBV genotype, and week 32 HBV DNA level by multivariate Cox regression analysis, HBeAg at week 32 was found to independently predict HBsAg loss (hazard ratio: 13.15, 95% CI (95% confidence interval): 1.32–131.09).

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

## DISCUSSION

In this long-term prospective study of PEG-IFN  $\alpha$ -2b alone or in combination with lamivudine in HBeAg-positive patients, we showed that early loss of HBeAg is associated with a higher rate of undetectable HBV DNA by PCR assay and a higher likelihood of HBsAg loss compared with HBeAg loss later during therapy or after therapy. About one-third of patients who lost HBeAg within 32 weeks of therapy were negative for serum HBsAg at LTFU, thereby having a ninefold higher chance of HBsAg loss compared with patients with HBeAg loss after week 32. Prediction of long-term outcome on the basis of either HBeAg status or HBV DNA level at an earlier time point was not possible.

This is the first study that shows that early HBeAg loss during PEG-IFN therapy is associated with an increased likelihood of HBsAg loss. HBV genotype A was common among patients with early HBeAg loss, but accounted for only 60% of the cases with early response. A recent study by Hou *et al.* (16) also showed that genotype A-infected patients were more likely to clear HBeAg from serum early during IFN therapy than were patients harboring other genotypes. Other baseline factors associated with early HBeAg loss in our study were high ALT levels and older age. High ALT is a well-known predictor of response

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

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

ALT, alanine transaminase; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PEG-IFN, pegylated interferon; ULN, upper limit of normal.

to PEG-IFN (8,12). Age above 50 years has been described to be associated with HBsAg loss in lamivudine-treated patients (17). Results of a study investigating outcome after PEG-IFN  $\alpha$ -2a in HBeAg-positive patients showed that patients with loss of HBeAg within 24 weeks of therapy were more likely to have serum HBV DNA of < 10,000 copies/ml at 24 weeks post treatment than were patients with loss of HBeAg after week 24 (79

vs. 59%, respectively) (18). Interpretation of this finding was hampered by the fact that patients with HBeAg loss after week 24 had a relatively shorter duration of follow-up after HBeAg loss. Our study, which has a longer duration of follow-up compared with this previous study also shows that, in the long term, patients with early HBeAg loss still have a higher chance of HBeAg negativity, undetectable HBV DNA (< 400 copies/ml),

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

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

AUC, area under the (receiver-operating characteristic) curve; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

and HBsAg negativity than those with delayed HBeAg loss. Obviously, the duration of HBeAg negativity was somewhat shorter for patients with HBeAg loss after week 32 compared with those with early HBeAg loss in our study as well. However, as the difference in response rates between patients with HBeAg loss after week 32 and those with early HBeAg loss remained stable, these differences will most likely prevail if the duration of follow-up after the first evidence of HBeAg loss was comparable. In addition, in retrospect, a relatively low dosage of PEG-IFN  $\alpha$ -2b may have been used in our study (a starting dose of 100  $\mu$ g per week, reduced to 50  $\mu$ g per week after 32 weeks).

HBeAg loss was more durable in patients who lost HBeAg within 32 weeks than in those with HBeAg loss after week 32. Among patients with HBeAg loss after week 32, only 57% were still HBeAg negative at LTFU. Therefore, immune control over the virus may be more profound in patients with an early HBeAg loss, of whom 77% were still HBeAg negative at LTFU. Interestingly, the incidence of mutations in the core promoter or precore region in early responders who remained HBeAg negative was comparable with that observed in patients with HBeAg loss after week 32.

Patients with added lamivudine tended to have higher rates of early HBeAg loss and HBsAg loss than were those treated with PEG-IFN alone. Decline in HBV DNA was most profound in these patients. In previous studies of PEG-IFN in HBeAg-positive chronic hepatitis B with a shorter duration of follow-up,

these benefits of added lamivudine were not observed (8,9,19). In nucleoside analog-treated HBeAg-positive patients, low serum HBV DNA after 6 months of therapy was also associated with higher rates of long-term virological response and a lower risk of antiviral resistance (20). Patients who had HBV DNA of <300 copies/ml after 24 weeks of telbivudine or lamivudine therapy had a significantly higher likelihood of achieving HBeAg seroconversion, normalization of ALT, and undetectable HBV DNA at year 2 of therapy compared with patients with higher HBV DNA levels. In HBeAg-positive patients treated with standard IFN, a profound decline in serum HBV DNA during the first weeks of therapy was associated with a higher likelihood of HBeAg loss (21). We also found that low HBV DNA at week 32 of PEG-IFN therapy was strongly associated with long-term HBsAg negativity on Cox regression analysis. The magnitude of early HBV suppression by antiviral drugs is thus associated with long-term virological response in chronic hepatitis B-infected patients treated with either IFN-based therapy or nucleos(t)ide analogs. A recent study in PEG-IFN-treated patients showed that a decline in HBeAg level more accurately predicted HBeAg loss than a decline in HBV DNA (22). However, so far, quantitative HBeAg measurement is not routinely available in many institutions.

We found that early HBeAg loss independently predicted long-term HBsAg negativity. The overall test performance for the prediction of HBsAg negativity was best for HBeAg loss at week 32 compared with baseline factors and other

outcome measures at week 32, although a negative predictive value was good for these other factors as well. With a negative predictive value of 98%, testing for HBeAg at week 32 is particularly useful for the identification of those who will most likely not achieve HBsAg negativity after a course of PEG-IFN therapy. The rate of HBsAg negativity may still increase with a longer duration of follow-up, as has been previously observed in studies of standard IFN in chronic HBV (5,6,23–28). A delayed clearance of HBsAg has been observed in up to 65% of patients within 5 years after IFN-induced HBeAg loss.

In conclusion, early HBeAg loss during PEG-IFN therapy was associated with higher rates of sustained HBeAg and HBV DNA negativity in the long term. We found that HBsAg loss occurred in about one-third of patients with early HBeAg loss, virtually all patients who lost HBeAg after week 32 remained HBsAg positive. These findings are of interest for the therapeutic management of HBV infection, as they may contribute toward enhancing the predictive values of early outcome measures during PEG-IFN therapy in chronic hepatitis B patients. In addition, we showed that more a profound viral suppression during the first 32 weeks of therapy in patients with added lamivudine may have led to higher rates of early HBeAg loss and HBsAg loss compared with treatment with PEG-IFN alone. These findings may reopen the perspective of combination therapy of PEG-IFN and other nucleos(t)ide analogs in chronic hepatitis B patients.

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#### Clinical trial details

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#### CONFLICT OF INTEREST

**Guarantor of the article:** Harry L.A. Janssen, MD, PhD.

**Specific author contributions:** Analysis and interpretation of data, drafting of the manuscript, finalizing the manuscript: Erik H.C.J. Buster, Bettina E. Hansen; acquisition of data, critical revision of draft of manuscript, approval of the final version of the manuscript: Hajo J. Flink, Halis Simsek, E. Jenny Heathcote, Sachithanandan Sharmila, George E. Kitis, Guido Gerken, Maria Buti, Richard A. de Vries, Elke Verhey; study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, finalizing the manuscript: Harry L.A. Janssen.

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#### Study Highlights

##### WHAT IS CURRENT KNOWLEDGE

- ✓ Peginterferon-induced hepatitis B e antigen (HBeAg) loss was sustained in 81% of initial responders.
- ✓ Hepatitis B surface antigen (HBsAg) loss was observed in 11% of patients in the long term, with a rate of 30% among initial responders. Little is known about the predictive values of early outcome measures during pegylated interferon (PEG-IFN) therapy.

##### WHAT IS NEW HERE

- ✓ Early HBeAg loss was associated with increased rates of undetectable HBV DNA and HBsAg loss compared with delayed HBeAg loss.
- ✓ Loss of HBeAg within 32 weeks of therapy was the best predictor of HBsAg loss in the long term.
- ✓ A reliable prediction of long-term outcome on the basis of either HBeAg status or HBV DNA level at a time point earlier than week 32 was not possible.

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