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Hepatitis B e antigen levels and response to peginterferon: Influence of precore and basal core promoter mutants

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ABSTRACT

Hepatitis B e antigen (HBeAg) levels may predict response to peginterferon (PEG-IFN) but are also influenced by presence of precore (PC) and core promoter (BCP) mutants.

HBeAg was measured in 214 patients treated with PEG-IFN ± lamivudine for 52 weeks. Patients were classified at baseline as wildtype (WT) or non-WT (detectable PC/BCP mutants). Combined response (HBeAg loss with HBV DNA < 2000 IU/mL), HBeAg response (HBeAg loss with HBV DNA > 2000 IU/mL) or non-response was assessed at week 78.

Mean baseline HBeAg levels were 2.65 logIU/mL in combined responders, 2.48 in non-responders and 2.24 in HBeAg responders ($p = 0.034$). Baseline HBeAg levels were not associated with combined response after stratification by WT/non-WT. Within the PEG-IFN monotherapy group ($n = 104$), patients with HBeAg < 1 logIU/mL at week 24 had a higher probability of combined response (29% versus 12%, $p = 0.041$). After stratification by WT/non-WT, WT patients with HBeAg < 1 logIU/mL at week 24 had a probability of combined response of 78% (versus 19% in patients with >1 logIU/mL, $p < 0.001$), whereas no difference in response rates was observed in non-WT patients ($p = 0.848$).

The relationship between HBeAg levels and response to PEG-IFN depends upon the presence of PC/BCP mutants. HBeAg levels should therefore not be routinely used to select patients for PEG-IFN, nor for monitoring of therapy.

1. Introduction

Chronic hepatitis B virus (HBV) infection may ultimately result in severe liver-related morbidity and mortality, and treatment of chronic hepatitis B (CHB) is therefore indicated in patients with signs of persistent liver inflammation (European Association For The Study Of The, 2009). Current treatment options for CHB consist of nucleo(s)tide analogues (NA) and (pegylated) interferons (PEG-IFN). Despite the recent registration of potent NA that are able to maintain undetectable HBV DNA levels through prolonged therapy, PEG-IFN remains an important first-line treatment option, especially in hepatitis B e antigen (HBeAg)-positive disease, as finite PEG-IFN therapy results in an off-treatment sustained response in about 25–30% of patients (Buster et al., 2008; Janssen et al.,

Abbreviations: HBeAg, Hepatitis B e Antigen; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; ULN, upper limit of normal; AUC, area under the receiver-operating characteristic curve.

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2005; Lau et al., 2005; Sonneveld and Janssen, 2010). Response to PEG-IFN therapy in these patients is accompanied by high rates of hepatitis B surface antigen (HBsAg) seroclearance, a reduced incidence of hepatocellular carcinoma (HCC) and prolonged survival (Niederau et al., 1996; van Zonneveld et al., 2004).

The clinical application of PEG-IFN is limited by high costs, frequent occurrence of side-effects, and relatively low probability of response (Sonneveld and Janssen, 2010). Selecting patients for PEG-IFN based on an individual's probability of response would help optimize application of this agent, but published prediction models, incorporating host and viral factors, provide only limited discrimination (Buster et al., 2009b; Sonneveld et al., 2012c). Recent studies have therefore focused on on-treatment predictors of response, including serum HBV DNA and HBsAg levels (Fried et al., 2008; Hansen et al., 2010; Janssen et al., 2012; Sonneveld et al., 2010, 2011, 2012a). Yet another study focused on the use of HBeAg levels to predict response to PEG-IFN for HBeAg-positive CHB, and showed that patients with low HBeAg levels at baseline had a higher probability of HBeAg seroconversion 6 months after treatment discontinuation (Fried et al., 2008). Unfortunately, several independent studies have shown that a considerable number of patients who achieve clearance of HBeAg have persistently high HBV DNA levels (Buster et al., 2008; Wong et al., 2010). We recently showed that presence of viral mutants harboring mutations in the precore (PC) and basal core promoter (BCP) regions may prohibit achievement of virological response after HBeAg clearance (Sonneveld et al., 2012b). Presence of these mutants may also influence HBeAg production and thus HBeAg levels in serum (Thompson et al., 2010). We therefore hypothesized that presence of PC and BCP mutants influences the association between HBeAg levels and response to PEG-IFN therapy.

The aim of the current study was therefore to investigate the association between HBeAg levels and response to PEG-IFN in relation to the presence of PC and BCP mutants.

2. Patients and methods

2.1. Patients

HBeAg levels were assessed in HBeAg-positive CHB patients who were previously enrolled in an investigator-initiated international multicenter randomized controlled trial (Buster et al., 2008; Janssen et al., 2005). In- and exclusion criteria for this study have previously been described elsewhere (Janssen et al., 2005). Patients were treated with PEG-IFN alfa-2b 100 μg weekly (PegIntron, Schering-Plough, Kenilworth, NJ, USA) in combination with placebo or lamivudine (LAM) 100 mg (Zeffix, GlaxoSmithKline, Greenford, UK) daily for 52 weeks. Inclusion criteria for the present analysis were completion of the 26-week follow-up phase of the main study, availability of data on presence of PC or BCP mutants at baseline and availability of a baseline serum sample for HBeAg measurements. Of the 266 patients in the initial study, 214 fulfilled these criteria.

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.

2.2. Laboratory measurements

Serum HBeAg was quantified in samples taken at baseline, during the treatment period (4, 8, 12, 24 and 52 weeks) and during follow-up (week 78) using the ELECSYS HBeAg assay (Roche Diagnostics, range 0.2–100 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. HBsAg was measured using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05–250 IU/mL) (Deguchi et al., 2004; Sonneveld et al., 2010). The presence of PC (at nucleotide position G1896) and BCP (at nucleotide positions A1762 and G1764) mutants was assessed using the INNO-LiPA HBV PreCore assay (Innogenetics, Ghent, Belgium) (Sonneveld et al., 2012b).

2.3. Statistical analysis

Response was defined as HBeAg loss with HBV DNA < 2000 IU/mL (~10,000 copies/mL; combined response), HBeAg loss with HBV DNA > 2000 IU/mL at week 78 (HBeAg response) or non-response (Buster et al., 2009b). Because therapy outcomes did not differ across the treatment arms, associations between baseline HBeAg levels and response were analysed for the pooled cohort. For on-treatment analyses only the patients treated with PEG-IFN monotherapy were analysed. 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. The ability of HBeAg levels to discriminate between patients with and without a response was evaluated by Radio Operator Charateristic (ROC) analyses, and quantified using the area under the ROC curve (AUROC).

3. Results

3.1. Baseline characteristics

Patients were predominantly male (78%), of Caucasian (73%) or Asian origin (19%), and harboured HBV genotypes A (35%), B (9%), C (14%) or D (40%). Other characteristics are shown in Table 1. Median baseline HBeAg levels were 501.8 IU/mL (IQR 125–924) in the 214 patients, and HBeAg levels were log transformed for further analysis. Mean HBeAg levels significantly varied by HBV genotype; 2.59 log IU/mL for genotype A, 2.68 for B, 2.56 for C and 2.30 log IU/mL for genotype D ($p = 0.021$ by ANOVA). Baseline HBeAg levels did not correlate with age ($r = 0.11$, $p = 0.10$), or ALT ($r = -0.01$, $p = 0.95$), but did correlate with baseline HBV DNA ($r = 0.39$, $p < 0.001$) and HBsAg levels ($r = 0.31$, $p < 0.001$).

3.2. Relationship between presence of PC and/or BCP mutants and HBeAg levels

Baseline HBeAg levels were highest in patients with WT (2.77 log IU/mL), and 2.56 log IU/mL in patients with PC mutants, 2.04 log IU/mL in those with only BCP mutants, and 2.26 in patients with both PC and BCP mutants ($p < 0.001$ by ANOVA, $p < 0.001$ for WT versus non-WT). When adjusted for HBV genotype distribution, HBeAg levels were still higher in patients with WT versus those with PC and/or BCP mutants (2.81 versus 2.33 log IU/mL, $p < 0.001$). To further explore the relationship between baseline HBeAg levels and presence of PC and/or BCP mutants, patients were allocated into groups according to baseline HBeAg levels: quartile 1 (baseline HBeAg < 2.098 log IU/mL), quartile 2 (>2.098,<2.70 log IU/mL), quartile 3 (>2.70,<2.97 log IU/mL) and quartile 4 (>2.97 log IU/mL). Patients were also classified as WT only ($n = 76$), or non-WT (PC and/or BCP mutants, $n = 138$). As shown in Fig. 1A, the proportion of patients harboring only WT virus increased as baseline HBeAg level was higher (Fig. 1A).

Table 1
Characteristics of the study cohort.

Characteristics	PEG-IFN (n = 104)	PEG-IFN + LAM (n = 110)	Overall (n = 214)
Demography			
Mean (SD) age, years	34.3 (13)	33.4 (12)	33.8 (13)
Male	84 (81%)	83 (76%)	167 (78%)
Race			
Caucasian	77 (74%)	80 (73%)	157 (73%)
Asian	21 (20%)	19 (17%)	40 (19%)
Other	6 (6%)	11 (10%)	17 (8%)
Laboratory results			
Mean (SD) ALT*	4.4 (3.1)	4.2 (3.0)	4.3 (3.0)
Mean (SD) HBV DNA, log c/mL	9.2 (0.80)	9.1 (0.98)	9.1 (0.90)
Mean (SD) HBsAg, log IU/mL	4.4 (0.54)	4.4 (0.65)	4.4 (0.60)
Mean (SD) HBeAg, log IU/mL	2.5 (0.70)	2.4 (0.70)	2.5 (0.70)
HBV genotype			
A	40 (39%)	34 (31%)	74 (35%)
B	9 (9%)	10 (9%)	19 (9%)
C	15 (14%)	14 (13%)	29 (14%)
D	39 (38%)	46 (42%)	85 (40%)
Other/mixed	1 (1%)	6 (6%)	7 (3%)
INNO-LiPA result			
Wildtype	40 (39%)	36 (33%)	76 (36%)
Precore	25 (24%)	31 (28%)	56 (26%)
Core promoter	20 (19%)	27 (25%)	47 (22%)
Precore and core	19 (18%)	16 (15%)	35 (16%)
Response at week 78			
Combined response#	18 (17%)	23 (21%)	41 (19%)
HBeAg loss	36 (35%)	41 (37%)	77 (36%)
HBsAg loss	6 (6%)	11 (10%)	17 (8%)

All comparisons of peginterferon alone versus peginterferon with lamivudine $p > 0.23$.
* Multiples of upper limit of the normal range $p < 0.231$.
# HBeAg loss and HBV DNA < 2000 IU/mL at week 78.

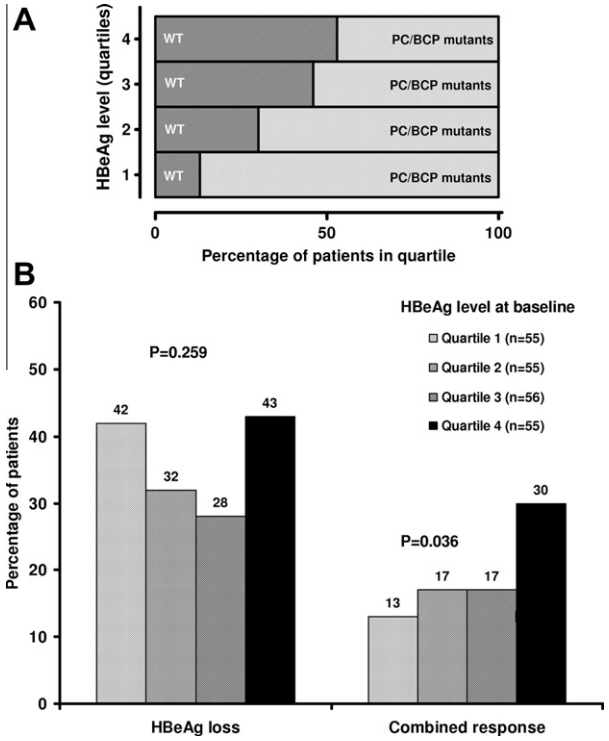

Fig. 1. Baseline HBeAg levels; relation to presence of mutants and probability of response. The proportion of wildtype (WT) and non-WT (precore and/or core promoter mutants) by baseline HBeAg level (quartile 1 (baseline HBeAg < 2.098 log IU/mL), quartile 2 (>2.098, <2.701 log IU/mL), quartile 3 (>2.701, <2.966 log IU/mL) and quartile 4 (>2.966 log IU/mL)) (Fig. 1A) and relationship between baseline HBeAg levels and response to PEG-IFN at 6 months post-treatment (Fig. 1B). Combined response was defined as HBeAg loss with HBV DNA < 2000 IU/mL at 6 months post-treatment.

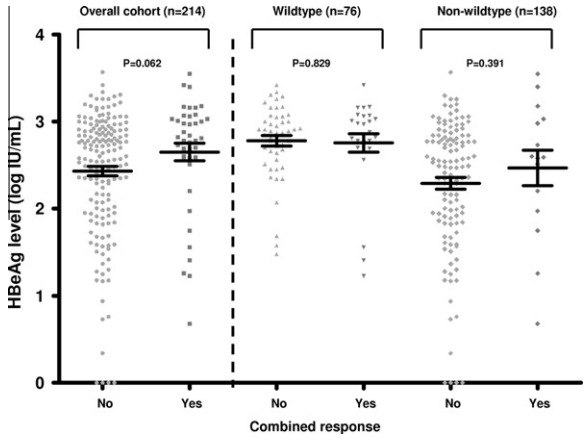

Fig. 2. Baseline HBeAg levels and combined response. Relationship between baseline HBeAg levels and combined response (HBeAg loss with HBV DNA < 2000 IU/mL) at week 78, stratified by presence of wildtype virus or non-wildtype (precore and/or core promoter mutants). Combined response was defined as HBeAg loss with HBV DNA < 2000 IU/mL at 6 months post-treatment. WT, wildtype.

3.3. Baseline HBeAg levels in relation to response

Among the total population of 214 patients 77 (36%) cleared HBeAg, 41 (19%) achieved a combined response of HBeAg loss with HBV DNA < 2,000 IU/mL, and 17 (8%) cleared HBsAg at week 78. Baseline HBeAg levels were highest in patients who achieved a combined HBeAg and HBV DNA response (2.65 log IU/mL), and lowest in patients who cleared HBeAg but did not achieve HBV DNA < 2000 IU/mL (HBeAg responders, 2.24 log IU/mL). Non-responders had intermediate levels of 2.48 log IU/mL ($p = 0.034$

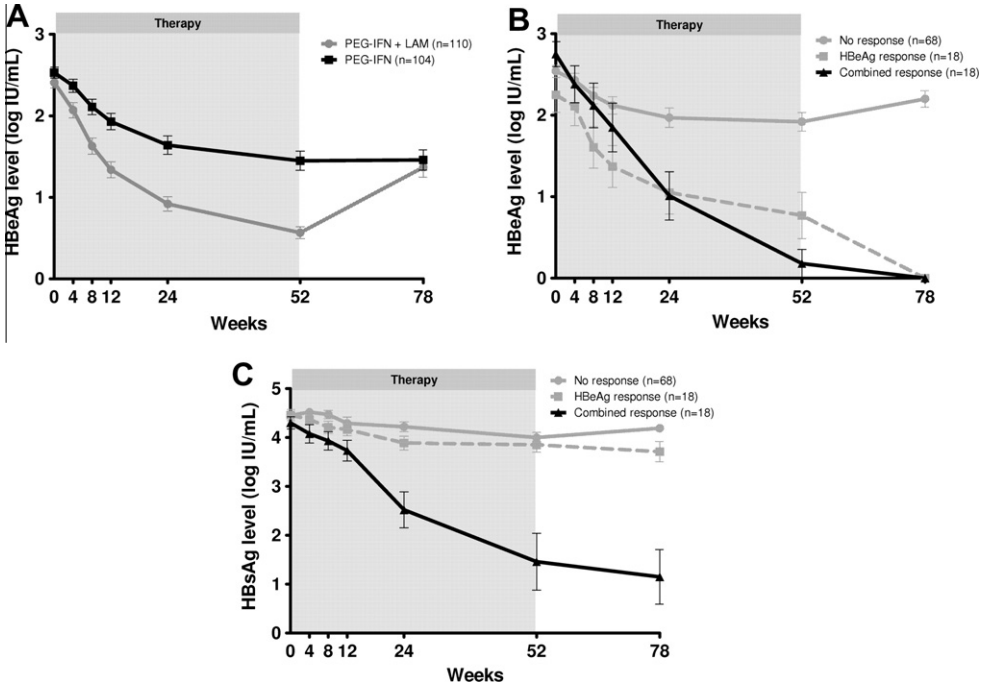

Fig. 3. On-treatment HBeAg and HBsAg levels by treatment and response. HBeAg levels at baseline and during therapy across treatment arms (A), and levels of HBeAg (B) and HBsAg (C) in combined responders (HBeAg clearance with HBV DNA < 2000 IU/mL at week 78), HBeAg responders (HBeAg loss with HBV DNA > 2000 IU/mL) and nonresponders for patients treated with PEG-IFN monotherapy.

by ANOVA). Analysis of the probability of response across HBeAg quartiles revealed that patients with the lowest (quartile 1) and the highest (quartile 4) HBeAg levels at baseline had a somewhat higher probability of HBeAg clearance at week 78 (42% and 43%, respectively), compared to 32% and 28% for patients in quartiles 2 and 3 (Fig. 2, $p = 0.259$ by Chi-square). However, combined HBeAg and HBV DNA response was most frequently achieved in patients in the highest quartile of HBeAg levels at baseline (30%, $p = 0.036$, Fig. 1B). Similarly, patients in the highest quartile of HBeAg levels tended to have a higher probability of HBsAg clearance; probabilities were 4%, 7%, 9% and 11% across quartiles 1 through 4, respectively ($p = 0.14$). Stratification by WT versus non-WT showed that within these groups, baseline HBeAg levels were not associated with combined HBeAg and HBV DNA response to PEG-IFN (Fig. 2).

3.4. HBeAg levels during therapy

Mean baseline HBeAg levels were 2.53 and 2.42 log IU/mL in the monotherapy and combination therapy groups ($p = 0.23$). HBeAg levels declined in both treatment groups, and end of treatment levels were 1.45 and 0.57 log IU/mL in the monotherapy and combination therapy groups, respectively ($p < 0.001$, Fig. 3A). The more pronounced reduction in the patients receiving combination therapy was not sustained post-treatment; week 78 levels were 1.46 and 1.37 log IU/mL in the monotherapy and combination therapy groups, respectively ($p = 0.61$).

3.5. On-treatment changes in HBeAg level according to response: comparison with HBsAg levels

The relationship between on-treatment changes in HBeAg level and off-treatment response at week 78 was analysed only in patients treated with PEG-IFN monotherapy ($n = 104$). Baseline HBeAg levels were highest in patients who achieved a combined response, and declined progressively through 52 weeks of therapy and during the off-treatment follow-up phase. Importantly, HBeAg levels remained higher in combined responders versus non-responders during the first 3 months and HBeAg levels were lower in combined responders versus HBeAg responders only after week 24 of treatment (Fig. 3B). HBeAg levels could therefore not be used to differentiate between HBeAg responders versus those with a combined response. For comparison, the HBsAg levels at the same time-points(Sonneveld et al., 2012a) are shown in Fig. 3C. Serum levels of HBsAg only showed a pronounced decline in patients with a combined response, whereas no or only a limited decline was observed in HBeAg responders or non-responders (Sonneveld et al., 2012a).

3.6. Predicting response using on-treatment HBeAg or HBsAg levels

The use of on-treatment HBeAg levels for prediction of response at week 78 was again only evaluated in patients treated with PEG-IFN monotherapy. By ROC analysis, week 24 HBeAg levels were superior to week 12 levels (AUC 0.694 versus 0.503). Of the 97 patients with available serum at week 24, 31 (32%) had an HBeAg level less than 1 log IU/mL. Patients with an HBeAg level less than 1 log IU/mL at week 24 had a higher probability of HBeAg clearance (65% versus 23%, $p < 0.001$), HBeAg seroconversion (58% versus 18%, $p < 0.001$), combined response (29% versus 12%, $p = 0.041$) and HBsAg clearance at week 78 (10% versus 3%, $p = 0.167$). However, stratification by WT/non-WT status at baseline revealed that the higher probability of combined response in patients with HBeAg levels under 1 log IU/mL at week 24 was confined to those with WT virus at baseline, and absent in those with PC and/or BCP mutant virus (Fig. 4A). In contrast, low levels of HBsAg at week 24 (<1500 IU/mL (Liaw et al., 2011; Piratvisuth et al., 2011)) were associated with a higher rate of combined response, irrespective of the presence of mutants (Fig. 4B).

4. Discussion

In this investigator initiated study, we have shown that the relationship between HBeAg levels and response to PEG-IFN therapy is

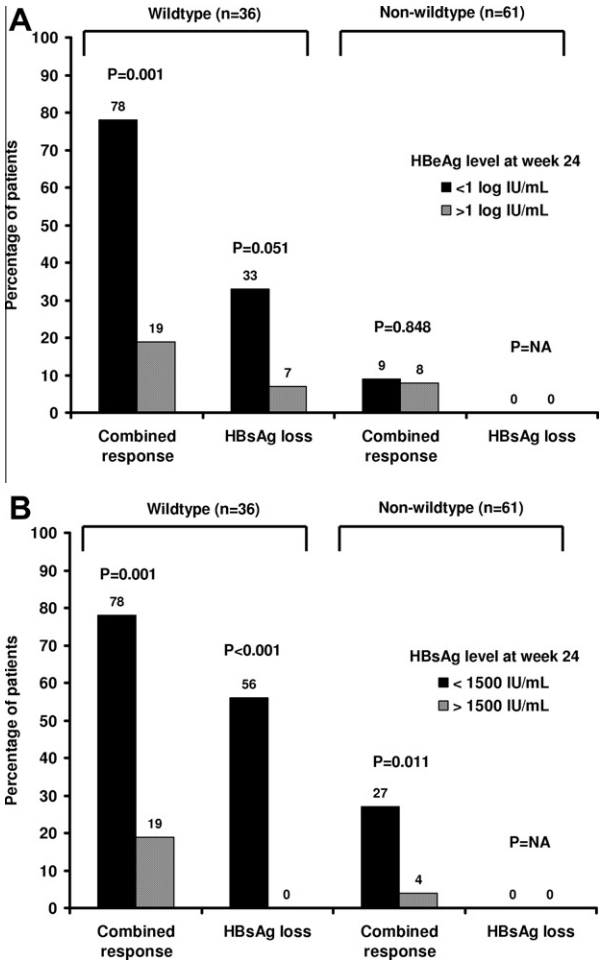

Fig. 4. HBeAg and HBsAg levels at week 24 and response to treatment. Probability of response at week 78 by HBeAg (A) or HBsAg (B) level at week 24 stratified by presence of wildtype or non-wildtype (precore and/or core promoter mutants) at baseline.

highly dependent upon the presence of viral strains with mutations in the PC and BCP domains. HBeAg levels should therefore not be routinely used to monitor PEG-IFN therapy.

PEG-IFN is an effective treatment option for HBeAg-positive CHB, but the limited probability of response to this agent necessitates careful selection of patients.(European Association For The Study Of The, 2009; Sonneveld and Janssen, 2010) Baseline serum HBeAg levels were previously shown to be associated with HBeAg seroconversion after PEG-IFN therapy, suggesting they may be used to select patients for PEG-IFN treatment.(Fried et al., 2008) Several long-term follow-up studies of patients treated with PEG-IFN have however shown that a considerable number of patients do not achieve low (<2000 IU/ml) HBV DNA levels after HBeAg seroconversion (Buster et al., 2008; Wong et al., 2010). These patients are considered to have progressed to HBeAg-negative CHB,(Frelin et al., 2009; Kawabe et al., 2009) and are thus at risk for the development of cirrhosis, liver cancer and death.(Chen et al., 2006; Fattovich et al., 2008a,b, 2004; Iloeje et al., 2006).

For this reason we investigated whether serum HBeAg levels predict achievement of a combined HBeAg and HBV DNA response to PEG-IFN. Our study shows that patients who will achieve such a combined response have higher baseline HBeAg levels than patients who fail to clear HBeAg or those who achieve HBeAg loss but have persistent HBV DNA more than 2000 IU/mL. These somewhat counter-intuitive findings may be explained by the influence of PC and BCP mutant strains. We have previously shown that these mutants can be detected in the majority of HBeAg-positive patients (Sonneveld et al., 2012b), and the current study shows that patients with detectable PC and BCP mutants have lower pre-treatment HBeAg levels than those who do not. Because patients with only WT virus (i.e. no detectable mutants) at baseline have a high probability of HBV DNA and HBsAg clearance after HBeAg loss (Sonneveld et al., 2012b), absence of PC and BCP mutants may explain the association between high HBeAg levels before treatment and a high probability of subsequent response to PEG-IFN. Furthermore, we also hypothesize that the patients with the lowest HBeAg levels were already experiencing spontaneous transition of HBeAg-positive to HBeAg-negative CHB, with selection for PC and BCP mutants. This translated to the high rate of HBeAg loss, but low rate of subsequent HBV DNA clearance, in these patients. Our findings therefore show that HBeAg levels cannot be used to select patients for PEG-IFN therapy without considering the presence of viral mutants.

Considering the difficulty of selecting patients with a high probability of response, several recent studies have focussed on on-treatment predictors. We have previously reported that patients who achieve a combined response to PEG-IFN achieve strong HBsAg declines during treatment, and that patients who fail to achieve a decline have a low probability of response.(Janssen et al., 2012; Sonneveld et al., 2010, 2011, 2012a) Fried et al reported a very strong association between low HBeAg levels at week 24 of treatment and HBeAg seroconversion at 6 months post-treatment. In concurrence with that study, we showed that a reduction of HBeAg levels to less than 1 log IU/mL by week 24 confers an increased probability of HBeAg clearance or seroconversion, as well as combined response and HBsAg clearance. These findings are in line with a previous report that suggested that early HBeAg clearance during PEG-IFN therapy predicts subsequent HBsAg clearance (Buster et al., 2009a). However, the current study also points out that the relationship between low HBeAg levels at week 24 of therapy and subsequent off-treatment response is confined to patients with WT virus, and virtually absent in those with PC and/or BCP mutants at baseline. Importantly, low serum levels of HBsAg remained a strong predictor of response, even among patients with PC and BCP mutants. Taken together, our findings do not support the routine use of HBeAg levels when monitoring response to PEG-IFN therapy, whereas they provide further evidence for the excellent predictive capabilities of HBsAg levels.

A possible caveat of our study pertains to the method we used to classify patients as WT/non-WT. The INNO-LiPA line probe assay is a sensitive method, but can only detect a few often encountered and widely acknowledged mutations, while others less frequently observed in previous studies are ignored.

In conclusion, we have shown that HBeAg levels do not adequately predict a combined HBeAg and HBV DNA response to PEG-IFN for HBeAg-positive CHB. Our findings are probably explained by the influence of PC and BCP mutant strains which are associated with both low HBeAg levels and failure to achieve a combined response. The relationship between HBeAg levels and response to PEG-IFN is therefore unpredictable, limiting the use of HBeAg levels as a sole predictor of treatment response in CHB. In contrast, serum levels of HBsAg were able to predict response in patients with and without detectable mutants.

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6. Disclosures

Milan J. Sonneveld has received speaker's fee from Roche. Vincent Rijckborst is a consultant for Roche. Roeland Zoutendijk has received speaker's honoraria from Bristol Myers Squibb. Krzysztof Simon, Ulus S. Akarca, Louwerens Zwang, Suzan Pas and Bettina E. Hansen have nothing to disclose. Stefan Zeuzem is a consultant for: BMS, Biolex, HGS, Merck/Schering-Plough, Novartis and Roche. E. Jenny Heathcote received funds for research and fees for consulting from Roche and Gilead Sciences. Prof. Dr. H.L.A. Janssen received grants from and is a consultant for: Bristol Myers Squibb, Gilead Sciences, Novartis, Roche, Merck, Innogenetics.

7. Author contributions

Study coordination and design, data collection, data analysis, writing of manuscript, approval of final version: MS, VR.

Study coordination and design, data collection, critical review of the manuscript, approval of final version: HLAJ.

Statistical analysis, critical review of the manuscript, approval of final version: BEH.

Patient enrolment, critical review of the manuscript, approval of final version: SZ, EJH, KS, RZ, USA.

Performance and coordination of assays, critical review of the manuscript, approval of final version: SDP, LZ.

HLAJ, BEH, VR and MS had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study guarantor: HLA Janssen.

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