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MAJOR ARTICLE

Serum HBV RNA as a Predictor of Peginterferon Alfa-2a Response in Patients With HBeAg-Positive Chronic Hepatitis B

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Background. Hepatitis B virus (HBV) RNA is a novel serum biomarker that has the potential to predict treatment response in patients with chronic hepatitis B. We explored whether HBV RNA serum levels can predict hepatitis B e antigen (HBeAg) seroconversion in patients treated with peginterferon alfa-2a.

Methods. Serum samples from HBeAg-positive patients previously treated with peginterferon alfa-2a in 2 large randomized controlled trials were retrospectively analyzed. HBV RNA levels were measured using a real-time polymerase chain reaction assay. Ability of individual biomarkers to predict HBeAg seroconversion at 24 weeks posttreatment was evaluated using receiver operating characteristics (ROC) analyses.

Results. The study included 131 subjects (70% male, 96% Asians, 35% HBV genotypes B, and 61% C), 76 treated with peginterferon alfa-2a alone and 55 in combination with lamivudine. Median HBV RNA levels were significantly lower, at all timepoints, in patients achieving HBeAg seroconversion. Levels of HBV RNA at treatment weeks 12 and 24 showed good ability to predict HBeAg seroconversion (area under ROC scores >0.75, $P < .001$). A HBV RNA cutoff of >5.5 $\log_{10}$ copies/mL identified 30% of nonresponders at week 12 (negative predictive value >90%).

Conclusion. Serum HBV RNA is an early predictor of HBeAg seroconversion in patients treated with peginterferon alfa-2a.

Clinical Trials Registration. NCT01705704.

Keywords. PegIFN; stopping rules; CHB; biomarkers; HBV RNA; life cycle.

Currently approved treatments for chronic hepatitis B virus (HBV) infection fall into 2 classes—nucleos(t)ide analogs (NAs), which are direct-acting antiviral agents, and nonpegylated or pegylated interferon (PegIFN) alfa, which are immunomodulators. NAs effectively suppress HBV replication and are well tolerated, but must be continued indefinitely by most patients. In contrast, treatment with PegIFN alfa-2a (Pegasys, Roche, Basel, Switzerland), which has a dual immunomodulatory and direct antiviral mode of action, is finite but it is associated with a significant treatment burden, including bothersome interferon-related adverse events.

The proportion of patients with chronic HBV infection achieving hepatitis B e antigen seroconversion (HBeAg SC) following treatment with PegIFN alfa-2a seems to be limited. Thus, HBeAg SC was achieved by approximately 32%–36% of patients following a 48-week treatment with PegIFN, with 12% achieving hepatitis B surface antigen (HBsAg) loss at 5 years posttreatment [1–3]. However, while of finite duration, PegIFN alfa therapy is associated with significant adverse events. Thus, the ability to predict treatment response would optimize PegIFN alfa therapy by minimizing treatment exposure in those unlikely to respond.

Although several host and HBV biomarkers have been identified to be associated with response to PegIFN alfa (eg, HBV genotype, alanine aminotransferase [ALT], HBV DNA, and HBeAg and HBsAg serum levels), baseline predictors (eg, HBeAg, HBV DNA, and HBsAg serum levels) have been of limited value in accurate and early identification of responders [1, 4–10]. While several treatment-stopping rules and response-guided therapy algorithms have been proposed [11], their main limitation is the relatively small proportion of nonresponders (approximately 20%) that are accurately identified [7, 8]. Thus, exploration of novel response-predicting biomarkers is needed in order to improve the efficacy of PegIFN alfa treatment [12].

HBV RNA is an intermediate of HBV replication that is detectable in serum [13–16]. Recent studies have shown that serum HBV RNA levels decline early in some patients during

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NA monotherapy or PegIFN alfa plus NA combination therapy, and that this decline is significantly stronger in those patients who subsequently achieve HBeAg SC [15, 16]. On this basis, we explored whether serum HBV RNA can be used as a reliable predictor of HBeAg SC in patients treated with PegIFN alfa-2a.

MATERIALS AND METHODS

Patients

Available stored serum samples from HBeAg-positive patients with chronic hepatitis B who had previously participated in 2 published large phase III/IV randomized controlled trials (WV16240 [NCT00048945] and WV19432 [NCT00435825]) were retrospectively analyzed [1, 2]. Briefly, patients enrolled had confirmed chronic hepatitis B monoinfection, absence of cirrhosis, were HBeAg-positive at screening with elevated serum ALT (>1–10 × upper limit of normal) and HBV DNA levels (>500 000 copies/mL [1] or >100 000 IU/mL [2]). All study participants provided written permission for future analyses of samples collected during the study. In the WV16240 study, patients were randomized to 48 weeks of treatment with PegIFN alfa-2a (40 kDa) 180 µg/week alone or in combination with lamivudine (LAM) or LAM monotherapy, while in the WV19432 study patients were randomized (in a 2 × 2 factorial fashion) to treatment with PegIFN alfa-2a at a dose of 90 or 180 µg/week for a duration of 24 or 48 weeks.

Only HBeAg-positive patients who were randomized to PegIFN alfa-2a 180 µg/week with or without LAM for 48 weeks, and who had available samples for baseline and the week 12 and 24 timepoints, were considered for this retrospective analysis. The study is registered at clinicaltrials.gov (NCT01705704).

Laboratory Assessments

Clinical and laboratory data were obtained from the study sponsor (F. Hoffmann-La Roche). Quantitative HBV DNA levels were measured using the Cobas Amplicor HBV Monitor Test (Roche Diagnostics, Pleasanton, CA; lower limit of quantification [LLQ], 200 copies/mL, or 38 IU/mL) in the WV16240 study and the Cobas TaqMan HBV Test (Roche Diagnostics; LLQ, 29 IU/mL) in the WV19432 study. Quantitative HBeAg was measured using a custom assay (LLQ 0.15 IU/mL) based on calibrating the AxSYM HBe 2.0 (Abbott, Wiesbaden, Germany) qualitative assay, which was validated using reference standards obtained from the Paul Ehrlich Institute. Quantitative HBsAg was measured using Architect HBsAg (Abbott; LLQ 0.05 IU/mL) assay in the WV16240 study, and Elecsys HBsAg II quant (Roche Diagnostics; LLQ 0.05 IU/mL) in the WV19432 study. Serum ALT levels were measured in local laboratories.

Quantification of HBV RNA in Serum

HBV RNA was quantified from serum samples stored at −80°C using a specific real-time polymerase chain reaction (PCR) technique that has been similarly described previously [16]. Briefly, reverse transcription of polyadenylated HBV RNA was performed using rapid amplification of cDNA ends (RACE) primer 5′-ACCACGCTATCG CTACTCAC(dT17)GWAGCTC-3′ with subsequent quantification of complementary DNA by real-time PCR according to van Bömmel et al [16]. The lower limit of detection (LLD) was 2800 (3.4 $\log_{10}$) copies/mL, with a corresponding linear range of 3.4 $\log_{10}$ to 9.4 $\log_{10}$ copies/mL. For statistical analysis, positive PCR results with quantitative values below the LLD were adjusted to 3.4 $\log_{10}$ copies/mL (LLD) and negative results were set to the minimal detectable quantity of 2.7 $\log_{10}$ copies/mL (corresponding to 1.0 copy per PCR reaction).

Statistical Analysis

Treatment response was defined as HBeAg SC at 24 weeks posttreatment. HBV DNA, HBsAg, HBeAg, HBV RNA, and ALT data were $\log_{10}$-transformed prior to analysis. Linear relationships between biomarker levels were evaluated with Pearson's correlation coefficient. Univariate relationships between biomarker levels and treatment response over time were visualized using boxplots. Differences between patients with and without HBeAg SC were tested with Wilcoxon test at each timepoint. Prediction performance of single biomarkers was illustrated with receiver operating characteristics (ROC) curves calculated with 10-fold cross-validation based on a logistic regression model.

Performance characteristics of biomarkers with moderate (0.6–0.75) to high (>0.75) area under the ROC curve (AUROC) scores were explored to identify biomarker cutoffs associated with high negative predictive values (NPV > 90%) for HBeAg SC. Where multiple cutoffs were identified, the cutoff with the highest specificity (ie, identifying the largest proportion of nonresponders) was selected, and cutoffs with low specificity (<10%) were disregarded. Statistical software used included MedCalc 14.8.1, SAS JMP 12.0.1, and R 3.1.2.

RESULTS

Patient Demographics and Baseline Characteristics

The study population comprised 131 subjects, 76 treated with PegIFN alfa-2a and 55 with PegIFN alfa-2a plus LAM. There were no significant differences in baseline characteristics between the 2 treatment groups ($P < .05$; Table 1).

Overall, most patients were male (70%), of Asian race (96%), and infected with HBV genotypes B (35%) or C (61%).

Similarly, there were no significant differences in the response rates between the 2 treatment groups, and the majority of responders (82%) achieved low HBV DNA levels (<2000 IU/mL) at 24 weeks posttreatment. However, HBeAg SC rates were higher in the current cohort than in the original trials (51% vs 32%–36% and 47% vs 27%, among those receiving monotherapy and combination therapy, respectively) indicating that this analysis included a responder-enriched subpopulation [1, 2].

Table 1. Baseline Characteristics and Response Rates by Treatment Regimen

Characteristic	PegIFN Alfa-2a n = 76	PegIFN Alfa-2a + LAM n = 55
Gender, male, n (%)	52 (68)	40 (73)
Race, n (%)		
Asian,	72 (95)	54 (98)
Caucasian	4 (5)	1 (2)
Age, years, mean ± SD	31.4 ± 8.1	30.4 ± 8.1
HBV genotype, n (%)		
A	2 (3)	1 (2)
B	21 (28)	25 (45)
C	51 (67)	29 (53)
D	2 (3)	0
HBV DNA, $\log_{10}$ IU/mL, mean ± SD	8.5 ± 1.8	9.0 ± 1.6
ALT, U/L, mean ± SD	149.2 ± 161.4	124.8 ± 91.9
HBeAg, $\log_{10}$ IU/mL, mean ± SD	2.4 ± 1.0	2.3 ± 0.9
HBsAg, $\log_{10}$ IU/mL, mean ± SD	4.0 ± 0.8	4.1 ± 0.7
HBV RNA, $\log_{10}$ copies/mL, mean ± SD	5.8 ± 1.6	5.5 ± 1.4
HBeAg SC, 24 weeks posttreatment, n (%)	39 (51)	26 (47)
HBeAg SC and HBV DNA <2000 IU/mL, 24 weeks posttreatment, n (%)	28/71[a] (39)	22/47[a] (47)

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LAM, lamivudine; PegIFN alfa-2a, peginterferon alfa-2a; SC, seroconversion.

[a]Thirteen subjects had missing HBV DNA levels at 24 weeks posttreatment.

HBV RNA Serum Levels Before and During Treatment With PegIFN Alfa-2a With or Without LAM

Before initiation of treatment, HBV DNA, HBsAg, HBeAg, and HBV RNA levels were similar between patients treated with PegIFN alfa-2a alone or in combination with LAM (Table 1) and the correlation between HBV RNA levels and HBV DNA or HBsAg levels was relatively high (r = 0.71–0.72, Figure 1), whereas the correlation between HBV RNA and HBeAg or ALT levels was moderate (r = 0.35–0.47). ALT levels had very low correlation with all other HBV markers (r = 0.12–0.2).

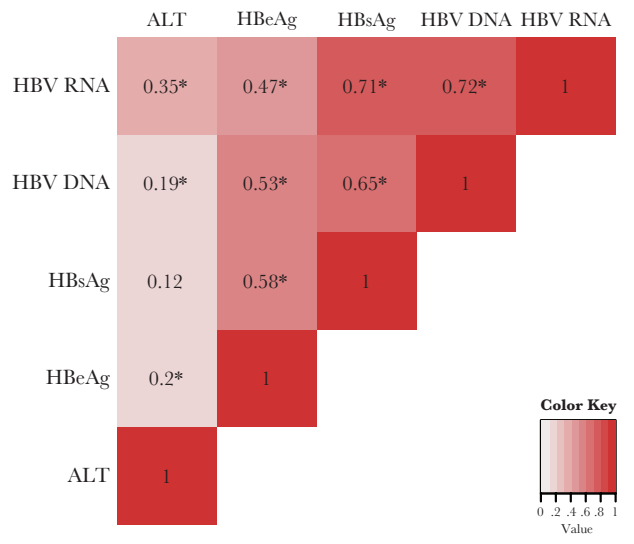

Figure 1. Heat map of pair-wise correlations among baseline biomarker levels. Fields in red indicate positive correlations. Numbers in fields represents Pearson correlation coefficients (r) and associated P values. *P < .05. Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

During treatment, mean declines in HBV RNA and HBV DNA levels were significantly larger in patients receiving PegIFN alfa-2a plus LAM combination therapy compared with those receiving PegIFN alfa-2a monotherapy (P < .05), although the difference was less pronounced for HBV RNA (Figure 2). In contrast, the mean declines in HBeAg, HBsAg, and ALT levels were similar across the 2 groups, with <0.5 $\log_{10}$ difference at all treatment timepoints.

In general, the proportions of patients with undetectable HBV RNA at treatment weeks 0, 12, 24, 48, and 72 were similar between PegIFN alfa-2a (1%, 46%, 57%, 75%, and 55%) and PegIFN alfa-2a plus LAM (1.8%, 58.2%, 80%, 80%, and 55.3%) treatment groups.

Kinetics of Serum HBV RNA According to PegIFN Alfa-2a Response

Before treatment initiation, HBV RNA serum levels were significantly lower in patients who subsequently achieved HBeAg SC than in nonresponders, and this pattern was similar in patients who received PegIFN alfa-2a as monotherapy (P = .004; Figure 3A) and in combination with LAM (P = .028; Figure 3B). During treatment and posttreatment follow-up, median HBV RNA levels were consistently lower in responders at all timepoints, regardless of treatment regimen. In contrast to nonresponders, the distribution of HBV RNA levels in responders was very narrow at weeks 12 and 24 (Figure 3). This was caused by the fact that in patients treated with PegIFN alfa-2a monotherapy HBV RNA became undetectable in 26 of 39 responders (67%) at week 12, and in 34 of 39 responders (87%) at week 24. Similarly, in patients treated with PegIFN alfa-2a plus LAM,

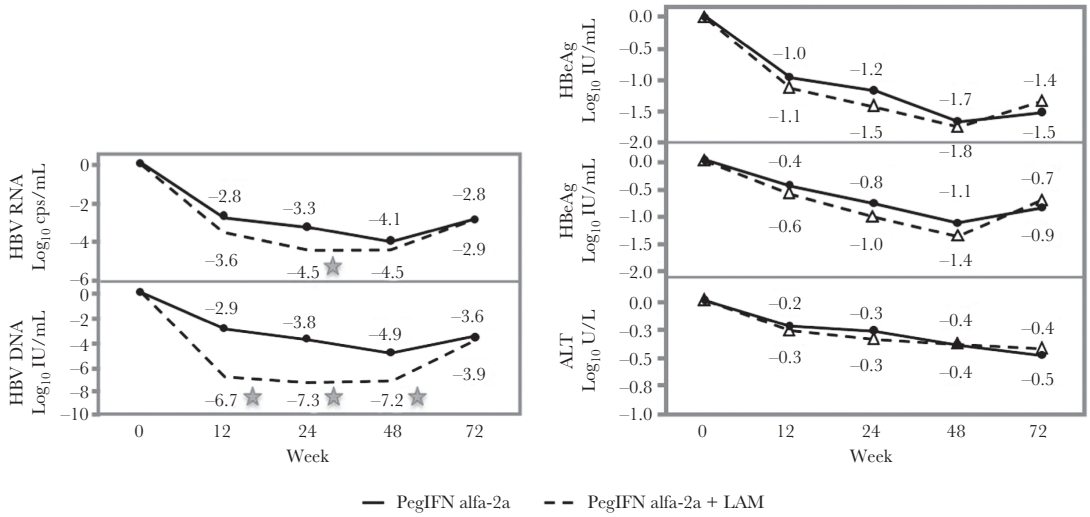

Figure 2. Mean change from baseline biomarker levels during peginterferon alfa-2a with or without lamivudine (LAM) therapy. Numbers show means at each timepoint according to regimen, with statistically significant differences beween means ($P < .05$, unpaired *t* test) indicated by stars. Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PegIFN alfa-2a, pegylated interferon alfa-2a.

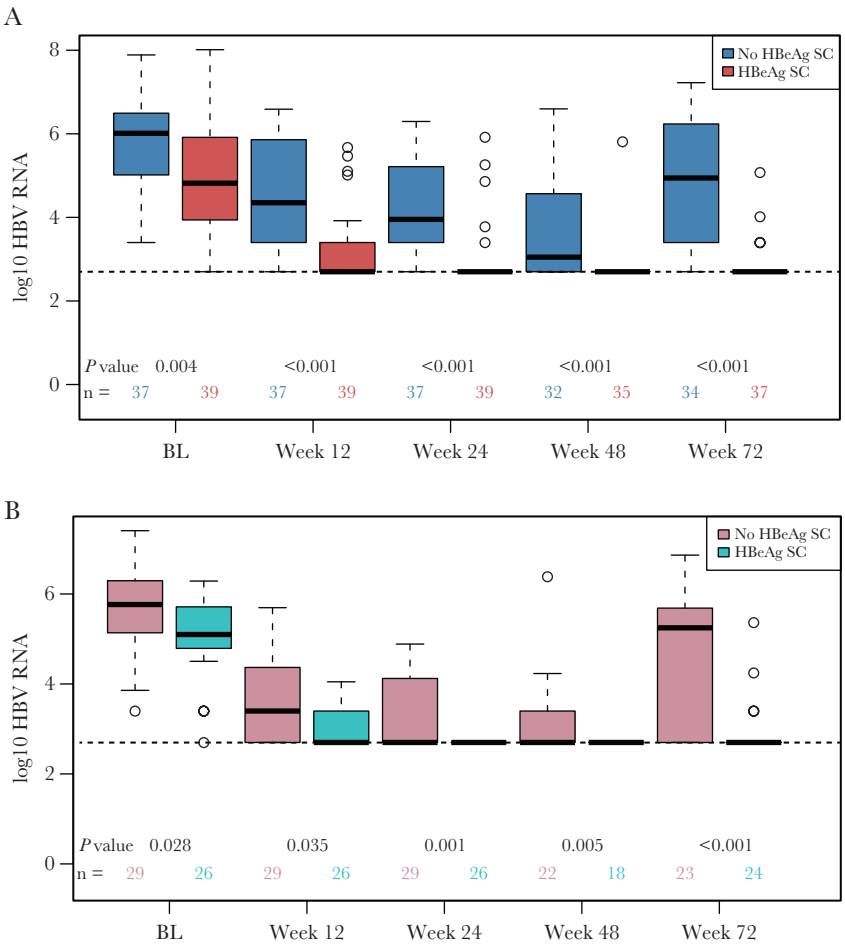

Figure 3. Boxplots of serum hepatitis B virus (HBV) RNA levels (log_{10} copies/mL) in patients receiving peginterferon alfa-2a alone (*A*) or in combination with LAM (*B*) according to hepatitis B e antigen seroconversion (HBeAg SC) at week 72. Boxes show 50% observations around median (interquartile range, IQR), median denoted by black solid line in the box. Whiskers represent median ± 1.5 × IQR. Dots above boxes depict outlier values. Number of patients at each timepoint are given below the boxes. The dotted horizontal line represents the lower limit of detection (2.7 log_{10}). Abbreviation: BL, baseline.

HBV RNA became undetectable in 18 of 26 responders (69%) at week 12, and in all 26 responders (100%) at week 24.

HBV Biomarkers as Predictors of PegIFN Alfa-2a Response

To illustrate the prediction performance of single biomarkers for HBeAg SC, ROC curves were estimated with Monte Carlo cross-validation and performed separately for each treatment regimen. The resulting ROC curves and AUROC scores of absolute and change from baseline biomarker levels for PegIFN alfa-2a monotherapy are shown in Figure 4 and Table 2, respectively.

At baseline, responders and nonresponders could be well discriminated by absolute levels of HBV DNA, HBV RNA, and HBsAg, with AUROC scores of 0.72, 0.77, and 0.81, respectively. Similarly, at weeks 12 and 24, absolute HBV DNA, HBeAg, HBsAg, and HBV RNA levels showed good discriminatory ability, with AUROC scores >0.77 (Table 2). In contrast, absolute ALT levels consistently showed poor discriminatory ability, with AUROC scores ≤0.6 at each timepoint.

In general, change from baseline biomarker levels at weeks 12 and 24 showed poorer discriminatory performance compared with absolute biomarker levels, with lower AUROC scores for each biomarker (Table 2).

A similar pattern of AUROC scores was observed for patients treated with PegIFN alfa-2a plus LAM (Supplementary Figure 1).

HBV Biomarker Cutoffs Predictive of Nonresponse

Biomarker cutoffs associated with high NPV (>90%) for PegIFN alfa-2a response were identified for absolute levels (Table 2) and change from baseline levels for HBV RNA, HBeAg, HBsAg, and HBV RNA, and their performance characteristics were compared.

Where multiple cutoffs were identified, the cutoff with the highest specificity was selected and cutoffs with low specificity (<10%) were disregarded. ALT cutoffs were not explored, given the ROC analysis finding that ALT was a poor predictor of HBeAg SC. No biomarker cutoffs were identified at baseline, indicating that HBV biomarkers do not accurately predict nonresponse pretreatment. At week 12, an HBV RNA cutoff of 5.5-$\log_{10}$ copies/mL identified a higher proportion of nonresponders (30%) than an HBV DNA cutoff of 8.9-$\log_{10}$ IU/mL (22%) or HBeAg cutoff of 2.7-$\log_{10}$ IU/mL (29%) (Figure 5 and Table 2). The HBsAg cutoff of 2.8-$\log_{10}$ IU/mL identified the largest proportion (41%) of nonresponders.

At week 24, HBV DNA cutoff of 8.9-$\log_{10}$ IU/mL identified the lowest proportion of nonresponders (11%), followed by HBV RNA 5.7-$\log_{10}$ copies/mL (24%). HBeAg and HBsAg cutoffs of 1.7 and 4.0 $\log_{10}$ IU/mL identified a larger proportion of nonresponders (52%–57%). Consistent with the ROC results of lower AUROC scores for change from baseline biomarker levels, biomarker cutoffs for change from baseline with an NPV >90% could only be identified for 2 of the 4 biomarkers considered (HBV DNA and HBeAg) at weeks 12 and 24. However, these cutoffs identified a significantly lower proportion of nonresponders (4%–16% at week 12, and 4%–30% at week 24) than the corresponding absolute biomarker cutoffs at these timepoints.

Biomarker cutoffs associated with NPVs >90% in patients receiving PegIFN alfa-2a plus LAM combination therapy are provided in the Supplementary Table 1.

DISCUSSION

In this retrospective analysis of 2 large randomized clinical trials, we evaluated, for the first time, the utility of serum HBV RNA as a novel biomarker to predict PegIFN alfa-2a response in HBeAg-positive patients. Furthermore, we compared the performance characteristics of HBV RNA serum levels with established serum biomarkers including ALT, HBV DNA, HBsAg, and HBeAg, that have previously been extensively studied as potential predictors of response to interferon-based treatments. Our results indicate that serum HBV RNA is an additional early on-treatment predictor of PegIFN alfa-2a response.

Prediction of nonresponse is desirable for therapies, such as PegIFN alfa, that achieve a treatment response in a subset of patients, but which are associated with clinically significant side effects. In addition to limiting treatment exposure in individual patients unlikely to achieve response, response-guided therapy has the potential to increase cost-effectiveness of PegIFN alfa-2a therapy at a population level, which is of particular relevance given the high prevalence of HBV infection in South-East Asia. Indeed, current treatment guidelines recommend using absolute HBsAg levels to decide whether to continue therapy beyond week 12 or week 24 for HBeAg-positive patients [4, 9]. European Association for the Study of the Liver and Asian Pacific Association for the Study of the Liver guidelines recommend that treatment with PegIFN alfa should not be considered if the absolute HBsAg level is ≥20 000 IU/mL (4.3-$\log_{10}$ IU/mL) before treatment, or if there is no decline in HBsAg level by treatment week 12, respectively [4, 9]. The absolute HBsAg level cutoff at week 12 (>4.5-$\log_{10}$ or >32 000 IU/mL) identified for accurately predicting nonresponse within this cohort differs from previously identified cutoffs, despite using data from the same clinical trials. This is likely due to the fact that the current cohort consists of a smaller and responder-enriched subpopulation, which would affect individual cutoff performance characteristics. For example, positive predictive value (PPV) and NPV metrics are known to be influenced by the prevalence of the event under study (eg, treatment response rate).

ROC analyses in our study showed that absolute biomarker levels were consistently superior to the change from baseline for predicting PegIFN response. AUROC values of at least 0.75 were obtained for absolute values of the 4 HBV biomarkers

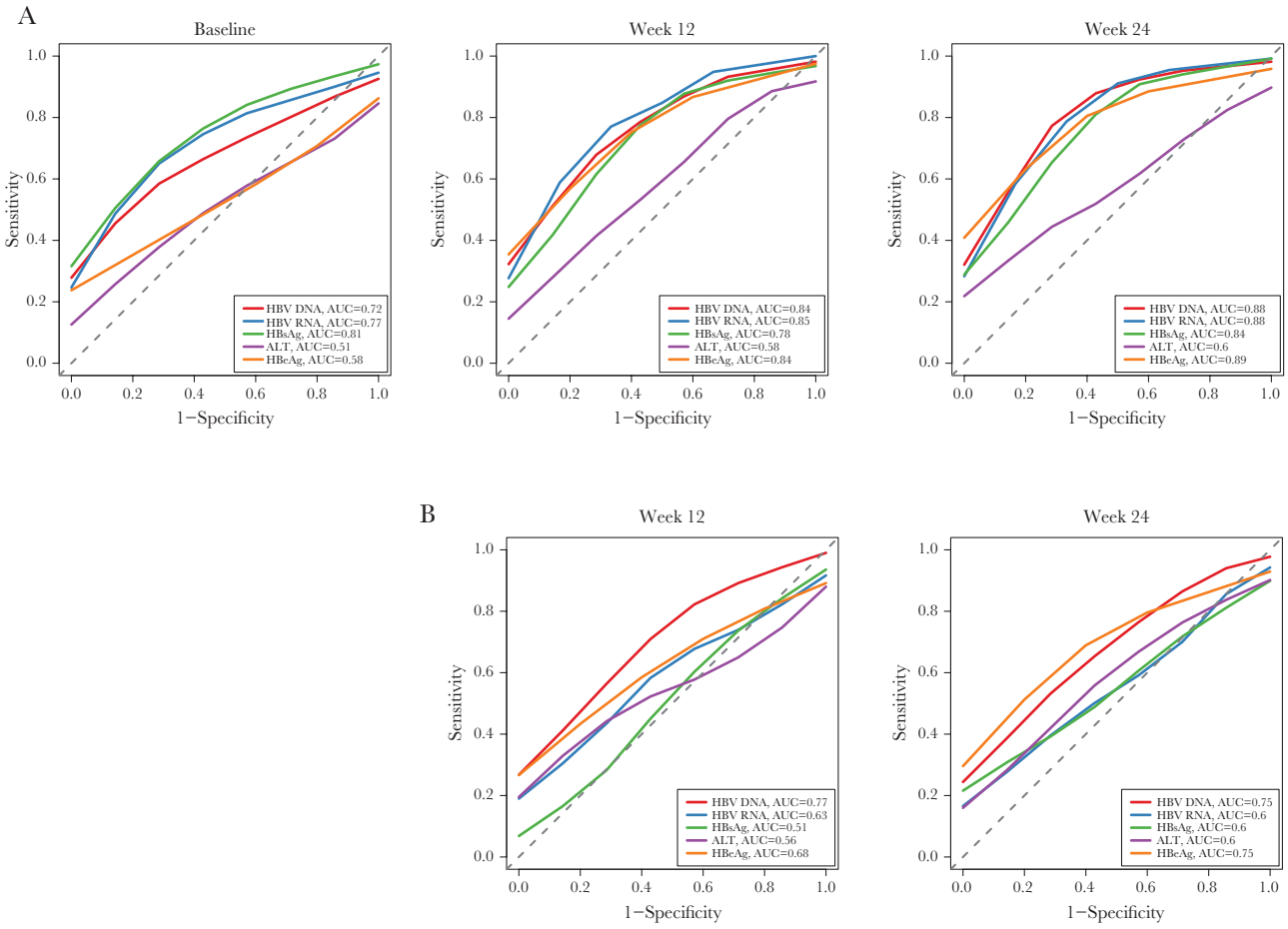

Figure 4. Receiver operating characteristics (ROC) curves at baseline, and after 12 and 24 weeks for peginterferon alfa-2a monotherapy for (*A*) absolute biomarker levels and (*B*) change from baseline in biomarker levels. Area under the ROC (AUC) scores (estimated with cross-validation) are shown in the legend. Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

(HBV RNA, HBV DNA, HBeAg, and HBsAg) at weeks 12 and 24, although no single biomarker appears to be a clearly superior predictor evidenced by the overlapping 95% CIs of the AUROC scores. Whilst absolute cutoffs with an NPV >90% at week 12 could be identified for all 4 biomarkers, HBV DNA and HBeAg cutoffs appeared inferior as they identified a lower proportion of nonresponders (24%–29%), when compared to HBV RNA and HBsAg cutoffs (30%–41%). However, larger nonresponder-enriched cohorts are needed to confirm these findings and establish which, if any, of these biomarkers is a superior

Table 2. Performance Characteristics of Absolute Biomarker Cutoffs Associated With High Negative Predictive Values for HBeAg SC and Corresponding AUROC Values in Patients Receiving PegIFN α-2a Monotherapy

Biomarker	Cutoff	Se, % (n)	Sp, % (n)	PPV, % (n)	NPV, % (n)	AUROC (95% CI)
Week 12						
HBeAg, $\log_{10}$ IU/mL	2.74	100 (28/28)	29 (7/24)	62 (28/45)	100 (7/7)	0.85 (0.81–0.88)
HBsAg, $\log_{10}$ IU/mL	4.40	97 (37/38)	41 (15/37)	63 (37/59)	94 (15/16)	0.76 (0.72–0.79)
HBV DNA, $\log_{10}$ IU/mL	8.89	97 (38/39)	24 (9/37)	58 (38/66)	90 (9/10)	0.83 (0.80–0.86)
HBV RNA, $\log_{10}$ copies/mL	5.47	97 (38/39)	30 (11/37)	59 (38/64)	92 (11/12)	0.77 (0.73–0.82)
Week 24						
HBeAg, $\log_{10}$ IU/mL	1.61	96 (27/28)	52 (13/25)	69 (27/39)	93 (13/14)	0.89 (0.86–0.92)
HBsAg, $\log_{10}$ IU/mL	3.90	95 (37/39)	57 (21/37)	70 (37/53)	91 (21/23)	0.78 (0.74–0.81)
HBV DNA, $\log_{10}$ IU/mL	8.89	100 (39/39)	11 (4/37)	54 (39/72)	100 (4/4)	0.86 (0.84–0.89)
HBV RNA, $\log_{10}$ copies/mL	5.26	97 (38/39)	24 (9/37)	58 (38/66)	90 (9/10)	0.82 (0.78–0.87)

Abbreviations: AUROC, area under the receiver operating characteristics curve; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NPV, negative predictive value; PegIFN alfa-2a, peginterferon α-2a; PPV, positive predictive value; SC, seroconversion; Se, sensitivity; Sp, specificity.

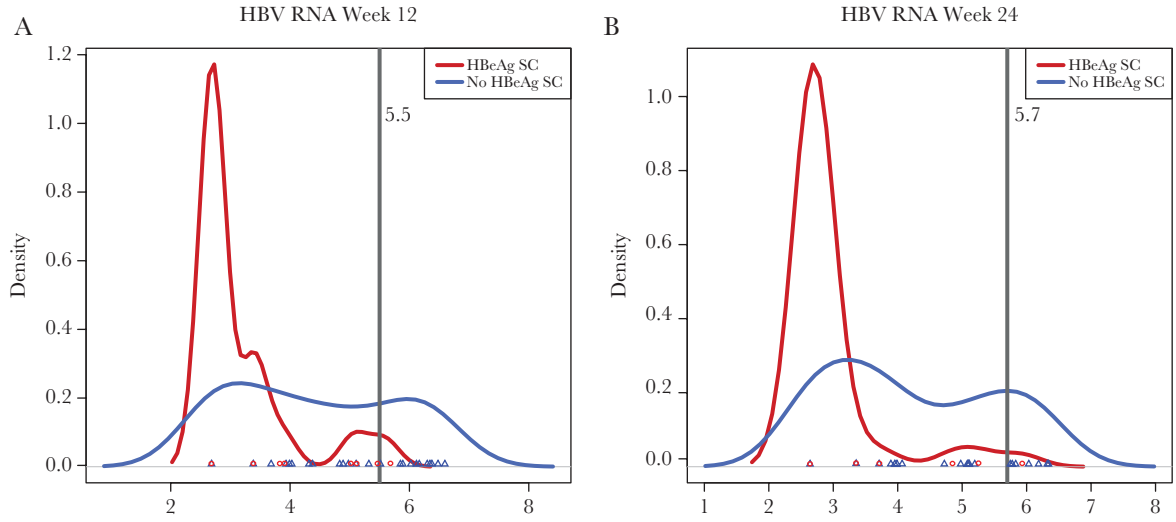

Figure 5. Visualization of serum hepatitis B virus (HBV) RNA cutoff levels for the prediction of hepatitis B e antigen seroconversion (HBeAg SC) in patients receiving peginterferon alfa-2a as monotherapy at (*A*) week 12 and (*B*) week 24. Circles and triangles indicate the measured data points in each group. Grey vertical line indicates cutoff associated with high negative predictive value (see Table 2).

predictor of PegIFN alfa-2a response. Our study defined PegIFN alfa-2a response as HBeAg SC at 24 weeks posttreatment, as treatment-induced HBeAg SC is an important milestone that characterizes the induction of a partial immune control and marks an end of the immunoactive HBeAg-positive phase [9]. An alternative endpoint that could have been explored is the combined serological/virologic endpoint of HBeAg SC and HBV DNA <2000 IU/mL at 24 weeks posttreatment. However, given that 82% of patients with HBeAg SC in our cohort also met the combined endpoint, we anticipate the biomarker performance characteristics for prediction of PegIFN alfa-2a response to be similar regardless of which of the 2 endpoints is used. Only 3 subjects in our cohort achieved HBsAg loss at 24 weeks posttreatment, which is insufficient for exploration of predictive biomarkers. Lastly, we would have liked to explore posttreatment response; however, longer follow-up data were not available. Previous studies have already provided evidence that HBV RNA is an early predictor of serologic response to NA treatment. In an analysis of data from 62 patients treated with NAs, decline in HBV RNA levels at 12 and 24 weeks of treatment was the strongest predictor of HBeAg SC [16]. In our present study, we could concordantly show that HBV RNA was a strong predictor of HBeAg SC, but in contrast to our previous study the performances of HBV RNA and other serum marker were more similar in predicting HBeAg SC. Those similarities in HBV biomarker kinetics may have been caused by the mechanism of action of PegIFN, which is thought to have a global effect on infected hepatocytes rather than on the HBV life cycle and which lead to suppression of HBV RNA also in nonresponders (Figure 3).

Although suppression of HBV DNA to undetectable levels is rare in PegIFN alfa-2a monotherapy-treated subjects, a decrease of HBV RNA to undetectability did appear to precede virological response in most cases. Thus, at week 12, the proportion of patients with HBV RNA clearance and HBV DNA <2000 IU/mL was 42% versus 28%, respectively (Supplementary Figure 2).

Further more, HBV RNA levels became undetectable at week 24 of treatment in the vast majority of responders (ie, 87% in mono and 100% in combination treatment), a phenomenon that could play an important role in the use of HBV RNA as biomarker in novel HBV treatments targeting different points of the HBV life cycle.

In our study, the decrease in serum HBV RNA was significantly greater at week 24 of treatment in the patients receiving combination therapy with PegIFN alfa plus NA than in patients receiving only PegIFN alfa (–4.5 vs –3.3 $\log_{10}$ copies/mL, $P < .05$). Consistent with this observation, in a previous study the decrease in HBV RNA during treatment was greater in patients treated with PegIFN alfa-2a plus adefovir than in those treated with NAs alone [16]. However, the proportion of patients achieving HBeAg SC was not higher after combination treatment, suggesting that its more pronounced effect of on HBV RNA levels does increase response [15].

Although serum HBV RNA can be used as a clinical marker, its biological role as well as its clinical utility are still unclear. For the prediction of HBeAg SC, HBV RNA was not superior to HBsAg levels in our patient population (Table 2). However, it may be that HBV RNA is a superior predictor of other efficacy endpoints (eg, HBsAg clearance or sustained immune control) or at earlier treatment timepoints than assessed in our study (eg, 4 weeks posttreatment). Also, the link between a reduction in serum HBV RNA levels and the occurrence of the immunological event of HBeAg SC still needs to be elucidated.

Several studies suggest that the large majority of secreted serum HBV RNA is present within enveloped encapsidated particles [15, 17, 18]. One study demonstrated that serum HBV RNA represents pregenomic HBV RNA that is associated with virus-like particles [19]. Another study showed that secreted HBV RNA contained spliced RNA variants [18]. A further study raised the possibility that hepatitis B virus X protein (HBx) RNA may be delivered by HBV-like particles into hepatocytes, thereby preventing Smc5/6 restriction of newly formed covalently closed circular DNA (cccDNA) [15]. If serum HBV RNA is able to infect hepatocytes, it may be able to maintain the cccDNA pool even during suppression of HBV DNA in patients undergoing long-term treatment with NAs. This may account for the minimal decline in HBsAg level observed in most of these patients. Accordingly, a strong correlation between serum HBV RNA and cccDNA during treatment with NAs as well as with PegIFN alfa-2a was demonstrated in a recent study by Giersch et al [20]. Thus, serum HBV RNA may represent a reliable serum marker for cccDNA activity, and monitoring of serum HBV RNA levels may be a useful additional marker, particularly for future treatments aimed at cccDNA eradication [12].

Our analysis has certain limitations, including its retrospective nature and predominance of Asian patients (96%) infected with HBV genotypes B (31%) or C (65%). Prospective analyses are required to validate our findings in large and diverse populations. In conclusion, our analysis demonstrates that serum HBV RNA levels are predictive of PegIFN alfa-2a response and that HBV RNA may be a useful biomarker in the development of treatment-stopping rules. HBV RNA serum levels should be included in future prospective studies of licensed or newly developing therapies for chronic HBV infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. F. V. B., S. B., C. W., and V. P. designed the study. S. B., D. D., and A. K. established and validated the HBV RNA assay. A. K. and D. D. measured HBV RNA in serum and A. V. B. and L. Y. acquired the data. F. V. B., A. V. B., L. Y., T. B., and V. P. analyzed and interpreted the data. F. V. B. and V. P. drafted the manuscript. All authors critically revised the manuscript.

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Potential conflicts of interest. F. V. B. has received research support and provided consultancy for Roche. C. W. is employed by Roche and has stockholdings in Roche. L. Y. is employed by Roche. V. P. was employed by Roche at the time this work was carried out. T. B. has received research support and provided consultancy for Roche. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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