Original Article

HBcrAg Identifies Patients Failing to Achieve HBeAg Seroconversion Treated with Pegylated Interferon Alfa-2b

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Abstract

Background: We aimed to evaluate the usefulness of serum hepatitis B virus core-related antigens (HBcrAg) for predicting hepatitis B e antigen (HBeAg) seroconversion in HBeAg-positive chronic hepatitis B patients treated with conventional interferon (IFN) alfa-2b or pegylated IFN.

Methods: Fifty-eight patients were enrolled: 29 for the training group and 29 for the validating group. HBcrAg was measured at baseline, week 12, end of the treatment, and 12- and 24-week follow-ups. Sixteen patients in the training group were enrolled in the long-term follow-up (LTFU), during which time the dynamics of the HBcrAg was monitored.

Results: The serum HBcrAg level gradually declined during treatment among the HBeAg seroconversion patients of the training group (from baseline, week 12, end of the treatment, 12-week follow-up to 24-week follow-up were 110,245 kU/ml, 3760 kU/ml, 7410 kU/ml, 715 kU/ml, 200 kU/ml, respectively). HBcrAg <19,565 kU/ml at week 24, HBcrAg <34,225 kU/ml at 12-week follow-up, and HBcrAg decrease ≥0.565 $\log_{10}$ kU/ml from the baseline to the end of treatment (EOT) had negative predictive values (NPVs) of 100% for HBeAg seroconversion at the end of follow-up, whereas the positive predictive values (PPVs) were 30.77%, 26.67%, and 25.00%, respectively. The patients with HBeAg seroconversion at the end of 24-week follow-up remained in seroconversion during the LTFU, during which time their serum HBcrAg levels steadily declined or even became undetectable, ranging from 0 to 2.1 kU/ml.

Conclusions: Effective antiviral treatment can decrease HBcrAg levels in the serum. The NPVs of HBcrAg for predicting HBeAg seroconversion at 24-week follow-up was 100%, but the PPVs were not satisfactory (all <31%). The serum HBcrAg levels of the patients with HBeAg seroconversion at the end of the 24-week follow-up steadily declined or even became undetectable during the LTFU.

Key words: Chronic Hepatitis B; HBeAg; HBcrAg; Pegylated Interferon Alfa-2b

Introduction

An estimated 240 million people worldwide are chronically infected with hepatitis B virus (HBV).[1] Carriers of HBV are at increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC).[2] At present, seven therapeutic agents have been approved for the treatment of adults with chronic hepatitis B (CHB), and pegylated interferon (PegIFN) remains one of the first-line options for CHB patients without liver cirrhosis.[3,4] In hepatitis B e antigen (HBeAg) positive patients, HBeAg seroconversion is the primary endpoint of treatment.

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The strongest predictor of HBeAg seroconversion to conventional and PegIFN-alfa is the pretreatment alanine aminotransferase (ALT) level. Other factors include high histologic activity index and low HBV DNA level, and some studies have suggested that persons infected with HBV genotypes A and B respond better than those with genotypes C and D.[4-7]

Results from Fried *et al*.[8] study suggested that quantitative HBeAg is a useful measurement for predicting HBeAg seroconversion in patients treated with PegIFN. Results from Lau *et al*.[9] study suggested that hepatitis B surface antigen (HBsAg) decline is associated with HBeAg seroconversion 1 year posttreatment and that on-treatment HBsAg levels could be used as an early predictor of durable off-treatment response to PegIFN-based therapy. Our previous study showed that quantitative serum HBsAg and HBeAg were strong predictors of sustained HBeAg seroconversion to PegIFN alfa-2b in HBeAg-positive patients.[10]

Recently a new quantitative assay, the HBV core-related antigen (HBcrAg) assay, has been developed. It measures serum HBV core, e, and precore (also referred to as p22cr) antigens simultaneously using the monoclonal antibodies that recognize the common epitopes of these three denatured antigens.[11,12] Because all of the antigens are transcribed from the precore/core gene, they are regarded as the HBcrAg.[13]

Okuhara *et al*.[14] study demonstrated that HBcrAg is a predictive marker of the virological response (VR) to entecavir therapy in CHB patients. Before treatment, elevated interleukin (IL)-22 and lower HBsAg and HBcrAg, but not HBV DNA, were associated with a favorable treatment outcome. The levels of IL-22, HBsAg, and HBcrAg all decreased from baseline to 24 months of treatment in virological responders. Rokuhara *et al*.[15] study suggests that HBcrAg assay is a sensitive and useful test for the assessment of a patient's HBV load. When monitoring the antiviral effect of lamivudine (LAM), HBcrAg provided a viral marker that was independent of HBV DNA. Chuaypen *et al*.[16] study demonstrated that the quantitative HBcrAg represents a reliable marker of intrahepatic covalently closed circular DNA (cccDNA). Monitoring HBcrAg levels during PegIFN therapy might help to identify patients with a very low probability of response comparable to, if not better than, quantitative HBsAg.

One of our aims was to investigate the usefulness of HBcrAg for predicting HBeAg seroconversion in HBeAg-positive CHB patients treated with PegIFN or conventional IFN alfa-2b. Moreover, we aimed to evaluate the correlation between the HBcrAg and the long-term outcome of the CHB patients.

Methods

Patients

We enrolled two groups of patients: the training group and the validating group. Patients of the training group met the following criteria: adults (18–70 years old), positive HBsAg for more than 6 months, positive HBeAg, HBV DNA level >2 × 10^4 IU/ml, and elevated serum ALT value 2–10 times the upper limit of the normal range. Patients with any causes of liver diseases other than CHB and with decompensated or compensated cirrhosis were excluded. Patients of the validating group met the same criteria.

The study was conducted in agreement with the Ethics Committee of Peking University People's Hospital.

Study design

The study of the training group comprised 24 weeks of treatment and 24 weeks of follow-up. Patients meeting the entry criteria were randomized to receive subcutaneous PegIFN alfa-2b (12,000 Da) 1 μg/kg once a week (PegIntron, Schering-Plough, Kenilworth, NJ, USA), or conventional IFN alfa-2b 3.0 Million International Units (MIU) three times weekly (IntronA, Schering-Plough, Kenilworth, NJ, USA). Patients of the validating group received PegIFN alfa-2b 1 μg/kg once a week for 24 weeks, 1.5 μg/kg for 24 weeks, or 1.5 μg/kg for 48 weeks, and then received 24 weeks of follow-up. The HBeAg seroconversion was assessed at the end of the follow-up. HBV genotype was determined at baseline using the direct sequencing method.[17]

HBcrAg of the training group was measured retrospectively on frozen sera from patients at baseline, week 12, the end of 24 weeks of treatment (week 24), 12-week follow-up (week 36), and 24-week follow-up (week 48). HBcrAg of the validating group was measured at baseline, week 12, the end of treatment (EOT), 12-week follow-up, and 24-week follow-up, respectively.

HBcrAg was measured using the Lumipulse G HBcrAg assay (Lumipulse System; Fujirebio, Tokyo, Japan). This assay was a quantitative chemiluminescent enzyme immunoassay, with an analytical measurement range of 1–10,000 kU/ml. A 30 min pretreatment incubation with the detergent solution at 60°C was included to disassociate and expose the target proteins in the serum samples. For quantitative results >10,000 kU/ml, the original serum was diluted with human negative sera and then retested. The detailed testing procedures were previously described.[12]

Long-term follow-up study

Sixteen patients of the training group participated in the long-term follow-up (LTFU) study in 2009, 2010, and 2013. We assessed complications of liver disease (HCC, ascites, variceal bleeding, encephalopathy), and administration of other antiviral therapy after the initial study. Follow-up time was calculated from the end of the initial study to the visit for the LTFU study. Laboratory investigations included ALT, aspartate aminotransferase, total bilirubin, albumin, alpha-fetoprotein, ultrasonography, HBV DNA, and quantification of HBsAg, HBeAg, and HBcrAg.

Statistical analysis

The Chi-squared test and Mann-Whitney *U*-test were carried out as appropriate. Spearman's rank correlation coefficients

were adopted to evaluate the relationship between pairs of markers. The accuracy of serum HBcrAg to predict HBeAg seroconversion was assessed using the receiver operating characteristic (ROC) curve. A value of $P < 0.05$ was considered statistically significant. The statistical analysis and representation were performed using the SPSS 13.0 software (SPSS, Chicago, IL, USA).

Results

Patient characteristics

From August 2003 to October 2004, 29 patients were randomized to receive PegIFN alfa-2b in 15 cases and IFN alfa-2b in 14 cases. All patients completed the 24-week treatment phase. One patient in the IFN alfa-2b group dropped out during follow-up. Baseline characteristics of the 29 patients are shown in Table 1.

Twenty-nine patients were enrolled in the validating group from January 2008 to October 2009. Ten patients received PegIFN alfa-2b 1 μg/kg for 24 weeks. Nine patients received 1.5 μg/kg for 24 weeks. Ten patients received 1.5 μg/kg for 48 weeks once weekly. All the patients completed the treatment phase. One patient treated with 1.5 μg/kg for 48 weeks dropped out during follow-up. Baseline characteristics of the 29 patients are shown in Table 1.

Clinical efficacy of patients

In the training group, HBeAg seroconversion occurred in four patients. In the validating group, HBeAg seroconversion also occurred in four patients.

Hepatitis B virus core-related antigen level gradually declined through treatment in hepatitis B e antigen seroconversion patients, but not in nonhepatitis B e antigen seroconversion patients of the training group

Median HBcrAg concentrations over time in the two groups of patients are shown in Figure 1. At week 24, week 36, and week 48, HBcrAg levels in the HBeAg seroconversion group were lower than those in the non-HBeAg seroconversion group. Among four patients who achieved HBeAg seroconversion, the HBcrAg level decreased consistently during treatment and remained at lower levels during the follow-up. Conversely, HBcrAg levels in the patients of the non-HBeAg seroconversion group showed diversified patterns, and most of them experienced a rebound after treatment [Figure 2].

Positive correlation was observed between the serum hepatitis B virus core-related antigen and the serum hepatitis B e antigen levels

We examined the correlation between the serum HBcrAg and HBeAg levels. The results are listed in Table 2. There was a positive correlation between the HBcrAg and HBeAg quantifications at each time point.

Hepatitis B virus core-related antigen <19,565 kU/ml at week 24 (end of treatment) showed strong potential of hepatitis B e antigen seroconversion at the end of follow-up in the training group

The HBcrAg levels at the baseline and week 12 were not significantly different between the HBeAg seroconversion and nonseroconversion groups ($P > 0.05$), showing no predictive value for the HBeAg seroconversion at the end of follow-up. However, among 11 patients with HBcrAg levels <19,565 kU/ml at week 24, four patients had HBeAg seroconversion at the end of follow-up. Among 18 patients with HBcrAg levels ≥19,565 kU/ml at week 24, no patient achieved HBeAg seroconversion ($\chi^2 = 7.593$, $P = 0.006$). The accuracy of the cutoff value of 19,565 kU/ml in serum HBcrAg level at week 24 of PegIFN or IFN therapy to predict HBeAg seroconversion was assessed using ROC. The area under the curve was 0.854 ($P = 0.026$, 95% confidence interval [*CI*], 0.706–1.000).

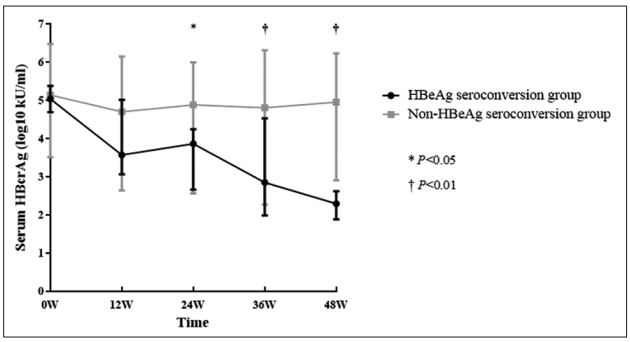

Figure 1: The median HBcrAg levels over time in the training group patients with and without HBeAg seroconversion. The minimum, median, and maximum of the HBcrAg concentration at each time point are represented using the vertical bar. HBcrAg: Hepatitis B virus core-related antigens; HBeAg: Hepatitis B e antigen; W: Week.

Table 1: Baseline characteristics of the patients in the training and validating groups

Characteristics	Training group ($n = 29$)	Validating group ($n = 29$)	Statistics	*P*
Male, *n* (%)	22 (75.86)	24 (82.76)	0.420*	0.517
Age (years), mean ± SD	32.38 ± 10.53	28.59 ± 8.94	1.217†	0.229
ALT (U/L), median (range)	152.00 (82–384)	156.00 (82–390)	−0.016‡	0.988
HBV DNA ($\log_{10}$ IU/ml), median (range)	7.47 (6.00–9.23)	7.98 (4.35–9.03)	−1.252‡	0.211
Genotype, *n* (%)				
B	5 (17.24)	13 (44.83)	5.156*	0.023
C or B/C	24 (82.76)	16 (55.17)	5.156*	0.023

*χ^2 value; †*t* value; ‡*Z* value; ALT: Alanine transaminase; HBV: Hepatitis B virus; SD: Standard deviation.

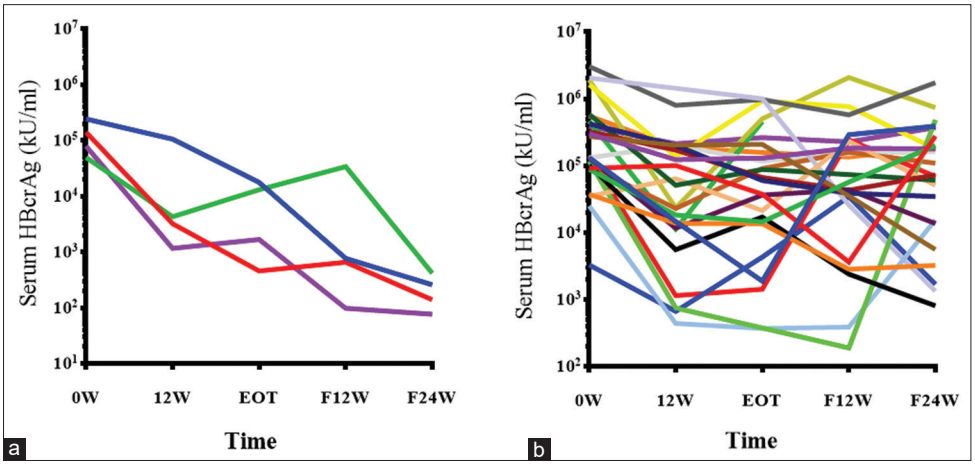

Figure 2: The individual dynamics of the serum HBcrAg levels in patients with HBeAg seroconversion (a) and those without HBeAg seroconversion (b). Lines in different colors represent the dynamics of serum HBcrAg from the different CHB patients. HBcrAg: Hepatitis B virus core-related antigens; HBeAg: Hepatitis B e antigen; CHB: Chronic hepatitis B; W: Week; EOT: End of treatment; F12W: 12-week follow-up; F24W: 24-week follow-up.

Table 2: Correlation between the HBcrAg and the HBeAg levels

Time	Baseline	Week 12	EOT	F12W	F24W
r	0.800	0.864	0.947	0.940	0.950
P	<0.01	<0.01	<0.01	<0.01	<0.01

HBcrAg: Hepatitis B virus core-related antigens; HBeAg: Hepatitis B e antigen; EOT: End of treatment; F12W: 12-week follow-up; F24W: 24-week follow-up.

Hepatitis B virus core-related antigen <34,225 kU/ml at 12-week follow-up can predict hepatitis B e antigen seroconversion at the end of follow-up

Among ten patients with HBcrAg levels <34,225 kU/ml at 12-week follow-up, four patients had HBeAg seroconversion at the end of the follow-up. Among 19 patients with HBcrAg levels ≥34,225 kU/ml at 12-week follow-up, no patient achieved HBeAg seroconversion (χ^2 = 8.816, P = 0.003). The area under the curve was 0.896 (P = 0.013, 95% *CI*, 0.766–1.000).

Hepatitis B e antigen <2.695 Paul-Ehrlich-Institute Unit (PEI-U)/ml at 12-week follow-up can predict hepatitis B e antigen seroconversion at the end of follow-up

Among seven patients with HBeAg levels below 2.695 Paul-Ehrlich-Institute unit (PEI-U/ml) at 12-week follow-up, four patients had HBeAg seroconversion at the end of follow-up. Among 22 patients with HBeAg levels ≥2.695 PEI-U/ml at 12-week follow-up, no patient achieved HBeAg seroconversion (χ^2 = 14.583, P = 0.000). The area under the curve was 0.958 (P = 0.0004, 95% *CI*, 0.883–1.000).

Hepatitis B virus core-related antigen decrease ≥0.565 $\log_{10}$ kU/ml from the baseline to 24 weeks of therapy showed strong potential of hepatitis B e antigen seroconversion in the training group at the end of the follow-up

The accuracy of the decrease in HBcrAg level from baseline to 12 weeks, 24 weeks, and 12-week follow-up to predict HBeAg seroconversion at the end of follow-up was assessed. We found that only the P value of the decrease in the HBcrAg level from baseline to 24 weeks of therapy <0.05. Among 13 patients with ≥0.565 $\log_{10}$ kU/ml decrease in HBcrAg level from baseline to 24 weeks of therapy, four patients had HBeAg seroconversion at the end of follow-up. Among 16 patients with <0.565 $\log_{10}$ kU/ml decrease in HBcrAg level from baseline to 24 weeks of therapy, no patient achieved HBeAg seroconversion (χ^2 = 5.711, P = 0.017). The area under the curve was 0.849 (P = 0.028, 95% *CI*, 0.683–1.000).

Hepatitis B surface antigen decrease ≥0.825 $\log_{10}$ IU/ml from baseline to 24 weeks of therapy showed a strong potential of hepatitis B e antigen seroconversion in the training group at the end of the follow-up

The accuracy of the decrease in HBsAg level from baseline to 12 weeks, 24 weeks, and 12-week follow-up to predict HBeAg seroconversion at the end of follow-up was assessed. We found that only the P value of the decrease in HBsAg level from baseline to 24 weeks of therapy <0.05.

Among eight patients with ≥0.825 $\log_{10}$ IU/ml decrease in HBsAg level from the baseline to 24 weeks of therapy, four patients had HBeAg seroconversion at the end of the follow-up. Among 21 patients with <0.825 $\log_{10}$ IU/ml decrease in HBsAg level from the baseline to 24 weeks of therapy, no patient achieved HBeAg seroconversion (χ^2 = 12.180, P = 0.000). The area under the curve was 0.880 (P = 0.016, 95% *CI*, 0.757–1.000).

Clinical outcome of the patients during the long-term follow-up

Sixteen patients of the training group were followed up for an average of 8.8 years (range: 8.5–9.0 years) after the end of the initial study. No patient achieved HBsAg loss during LTFU. HCC, variceal bleeding, and encephalopathy were not observed. The serial HBcrAg, HBsAg, HBeAg, and HBV DNA quantification results in 16 patients are listed in Figure 3.

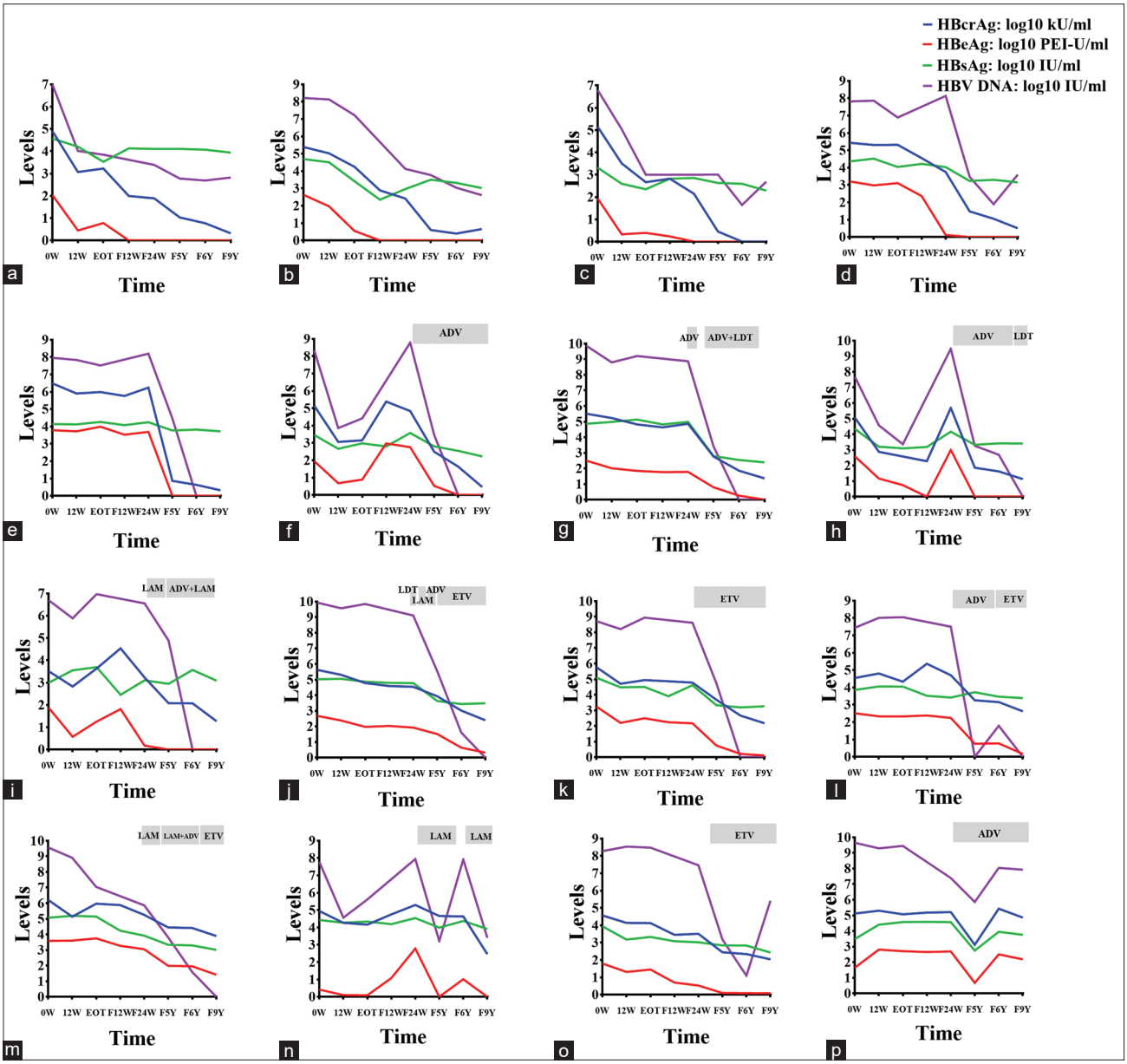

Figure 3: The dynamics of the HBcrAg, HBeAg, HBsAg, and HBV DNA of the CHB patients during the initial study and LTFU (n = 16, a-p). The gray bar at the top-right corner (f-p) indicates the antiviral agents and the treatment duration. Patient f was treated with ADV from 2005 to 2013; patient g was treated with ADV from the end of 24-week follow-up to 2006, then treated with ADV and LDT from 2008 to 2013; patient h was treated with ADV from 2007 to February 2013, then treated with LDT from February 2013 to July 2013; patient i was treated with LAM from the end of 24-week follow-up to 2009, then ADV was added on from 2009 to 2013; patient j was sequentially treated with LDT, LAM, and ADV from the end of 24-week follow-up to 2009, followed by ETV from 2009 to 2013; patient k was treated with ETV from the end of 24-week follow-up to 2013; patient l was treated with ADV from 2006 to 2010, then treated with ETV from 2010 to 2013; patient m was treated with LAM from 2004 to 2007, then ADV was added on from 2007 to 2012, followed by ETV monotherapy from 2012 to 2013; patient n was treated with LAM from the end of 24-week follow-up to August 2010, after a short-term cessation the treatment was continued from October 2010 to 2013; patient o was treated with ETV from the end of 24-week follow-up to 2013; patient *P* was treated with ADV from 2005 to 2013. HBcrAg: Hepatitis B virus core-related antigens; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; CHB: Chronic hepatitis B; LTFU: Long-term follow-up; W: Week; EOT: End of treatment; F12W: 12-week follow-up; F24W: 24-week follow-up; F5Y: 5-year follow-up; F6Y: 6-year follow-up; F9Y: 9-year follow-up; LAM: Lamivudine; LDT: Telbivudine; ADV: Adefovir dipivoxil; ETV: Entecavir.

Three patients with HBeAg seroconversion at the end of 24-week follow-up remained seroconversion during the LTFU, and no patient progressed to cirrhosis. Their serum HBcrAg levels steadily declined or even became undetectable during the LTFU (0–2.1 kU/ml, shown in Figure 3a-3c). In addition, two female patients with abnormal ALT and positive HBeAg at the end of the 24-week follow-up achieved HBeAg seroconversion during LTFU although neither of them received any antiviral treatment during LTFU, and both of whom had a very low level of serum

HBcrAg, ranging from 2.1 to 3.2 kU/ml [Figure 3d and 3e]. Their ALT levels were normal, and their HBV DNA levels were below 1000 IU/ml.

Eleven patients with positive HBeAg at the end of 24-week follow-up received antiviral treatment during LTFU. Three patients progressed to cirrhosis. They had high serum HBcrAg levels, ranging from 250 to 71,990 kU/ml; one patient was complicated with autoimmune hepatitis. In the remaining eight patients, one patient once had ascites resulting from LAM resistance.

The cutoff value of the hepatitis B virus core-related antigen levels at the end of treatment and 12-week follow-up from the training group had similar positive predictive value and negative predictive value for the validating group

At the end of 24 weeks of follow-up in the validating group, HBeAg seroconversion occurred in four patients. The cutoff value of 19,565 kU/ml of HBcrAg at the EOT, 34,225 kU/ml at 12-week follow-up, and ≥0.565 $\log_{10}$ kU/ml decrease in HBcrAg level from the baseline to EOT had 25–30.77% positive predictive value (PPV), and their negative predictive values (NPVs) were 100%. The cutoff value of 2.695 PEI-U/ml of HBeAg at 12-week follow-up had a PPV of 66.67% and an NPV of 100%. HBsAg decrease ≥0.825 $\log_{10}$ IU/ml from the baseline to the EOT had a PPV of 60% and an NPV of 95.83%. The results are shown in Figure 4.

Discussion

Our previous study evaluated the usefulness of quantitative HBsAg and HBeAg for predicting HBeAg seroconversion in HBeAg-positive CHB patients treated with PegIFN and conventional IFN alfa-2b.[10] In this study, we investigated the usefulness of HBcrAg for predicting HBeAg seroconversion in HBeAg-positive CHB patients treated with PegIFN or conventional IFN alfa-2b.

Our study found that in patients with HBeAg seroconversion, HBcrAg levels decreased consistently during treatment and remained at lower levels during the posttreatment follow-up. HBcrAg in patients without HBeAg seroconversion, however, experienced a slight decrease during therapy and rebounded when the treatment was discontinued. Thus, effective antiviral treatment can decrease HBcrAg levels in serum, which was consistent with the findings of Okuhara *et al*.[14] and Chuaypen *et al*.[16]

Kimura *et al*.[13] demonstrated that HBcrAg levels correlated well with HBV DNA levels. Chuaypen *et al*.[16] and Suzuki *et al*.[18] showed that the serum HBcrAg concentration was related to the level of intrahepatic cccDNA. Our result showed that HBcrAg levels correlated very well with HBeAg at different time points [Table 2], indicating that the majority of the serum HBcrAg in the HBeAg-positive patients was HBeAg and that HBcrAg might have similar predictive values as the HBeAg. Therefore, the predictive values of the HBeAg were also assessed in our study.

Chuaypen *et al*.[16] showed that serum HBcrAg at week 12 was identified as a predictor of VR. The optimal cutoff value for HBcrAg ($\log_{10}$ 8.0 U/ml) provided NPVs of achieving VR at weeks 12 and 24 of 94.4% and 100%, respectively. We found that the HBcrAg cutoff value of 19,565 kU/ml at week 24 had a PPV of 36.36% and an NPV of 100% for predicting the HBeAg seroconversion of week 48. The cutoff value of 34,225 kU/ml at 12-week follow-up (week 36) had a PPV of 40% and an NPV of 100% for predicting the HBeAg seroconversion of week 48. This result suggests that physicians should pay close attention to the patients who fail to achieve HBeAg seroconversion at the 12-week follow-up. If their serum HBcrAg level is <34,225 kU/ml, they have a 40% possibility of gaining HBeAg seroconversion at the 24-week follow-up even without any additional antiviral treatment. Moreover, the NPV of the cutoff value at week 24 or the 12-week follow-up were both 100% and could help to guide physicians to stop PegIFN alfa-2b at week 24 or switch to other treatment regimens at the 12-week follow-up.

We also found that the cutoff value HBeAg of 2.695 PEI-U/ml at 12-week follow-up had a PPV of 57.14% and an NPV of 100% for predicting the HBeAg seroconversion of week 48. The area under the curve was 0.958. Therefore, the cutoff value HBeAg 2.695 PEI-U/ml at 12-week follow-up had a slightly higher predictive value than the cutoff value HBcrAg 34,225 kU/ml at 12-week follow-up.

Moucari *et al*.[19] found that a decrease of 0.5 and 1 $\log_{10}$ IU/ml in serum HBsAg levels at weeks 12 and 24 of therapy, respectively, had high predictive values of sustained VR (SVR). Thus, early serum HBsAg drop had high predictive values of SVR to PegIFN 2a in HBeAg-negative CHB patients. We found that HBcrAg decrease ≥0.565 $\log_{10}$ kU/ml from the baseline to 24 weeks of therapy showed the strong potential of HBeAg seroconversion at the end of the follow-up (PPV of 30.77%, NPV of 100%). We also found that HBsAg decrease ≥0.825 $\log_{10}$ IU/ml from the baseline to 24 weeks of therapy showed the strong potential of HBeAg seroconversion at the end of the follow-up (PPV of 50%, NPV of 100%). Thus, compared with serum HBcrAg drop, serum HBsAg drop at the EOT had higher PPV.

We enrolled an additional 29 HBeAg-positive CHB patients treated with PegIFN alfa-2b for further validation. We found that in the validating group, the NPVs of HBcrAg for predicting HBeAg seroconversion was 100%, which was equal to those of the HBeAg and higher than that of the HBsAg; however, the PPVs of the HBcrAg were all <31%, which were lower than those of the serum HBeAg and that of serum HBsAg.

In our LTFU study, we found that patients with HBeAg seroconversion at week 48 maintained negative HBeAg at 9-year follow-up. Thus, HBeAg seroconversion after treatment with PegIFN alfa-2b was sustained, and their serum HBcrAg levels steadily declined or even became

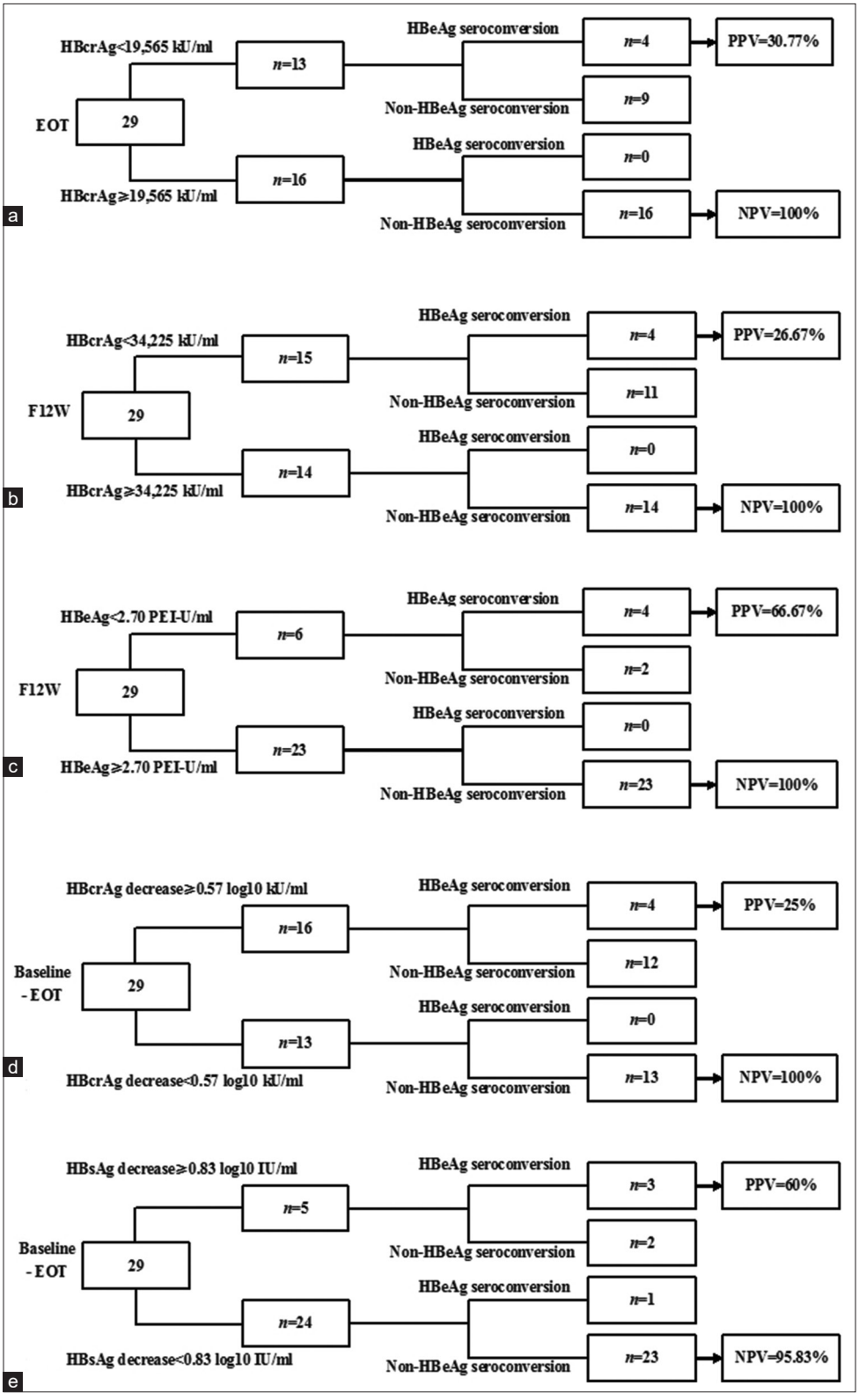

Figure 4: Predictive values of the HBcrAg, HBeAg, and HBsAg for predicting the HBeAg seroconversion at the end of follow-up in the validating group ($n = 29$). (a and b) The predictive values of the HBcrAg level at EOT and F12W, respectively; (c) the predictive values of the HBeAg level at F12W; (d) the predictive values of the decrease of the HBcrAg level from baseline to EOT; (e) the predictive values of the decrease of the HBsAg level from baseline to EOT. HBcrAg: Hepatitis B virus core-related antigens; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; EOT: End of treatment; F12W: 12-week follow-up; PPV: Positive predictive value; NPV: Negative predictive value.

undetectable during the LTFU (0–2.1 kU/ml). Two additional patients with positive HBeAg at the end of the 24-week follow-up achieved HBeAg seroconversion during LTFU although neither of them received any antiviral treatment; therefore, they achieved a delayed response, both having a very low level of serum HBcrAg, ranging from 2.1 to 3.2 kU/ml. The remaining 11 patients with positive HBeAg at the end of the 24-week follow-up received other antiviral treatment regimens during the LTFU. Three patients progressed to cirrhosis. It should be noted that their serum HBcrAg levels were high, ranging from 250 to 71,990 kU/ml.

The limitations of this study should be considered. It is a retrospective study. Approximately a decade ago, we enrolled the CHB patients from the real-world practice.[10] Considering the high cost and potential adverse events of pegylated and conventional IFN, we initially chose a lower dose than usual dose (3.0 MIU three times weekly versus 5.0 MIU three times weekly for conventional IFN, and 1 μg/kg once a week versus 1.5 μg/kg once a week for PegIFN, respectively), as well as a shorter duration of treatment (24 weeks vs. 48 weeks). We acknowledged that the heterogeneity of the subjects remains an issue although the validating results were highly consistent.

In conclusion, our study found that effective antiviral treatment can decrease HBcrAg levels in serum. HBcrAg at week 24 and at 12-week follow-up can predict HBeAg seroconversion at 24-week follow-up. The NPVs of HBcrAg for predicting HBeAg seroconversion was 100%, but the PPVs were not so satisfactory. Therefore, we suggest that the HBcrAg is more useful for identifying the HBeAg-positive patients without the chance of achieving HBeAg seroconversion under IFN treatment. HBeAg seroconversion after treatment with PegIFN alfa-2b is sustained during LTFU, during which time their serum HBcrAg levels steadily declined or even became undetectable.

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Conflicts of interest

There are no conflicts of interest.

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