Predictive value of serum HBV RNA on HBeAg seroconversion in treated chronic hepatitis B patients

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Purpose To investigate the predictive value of serum hepatitis B virus (HBV) RNA on HBeAg seroconversion in treated chronic hepatitis B (CHB) patients.
Methods Sixty-four HBeAg-positive CHB patients were selected. They were divided into HBeAg seroconversion group including 11 cases and HBeAg non-seroconversion group including 53 cases. HBV RNA levels and other laboratory results were measured at baseline and week 12, 24, 48, 72 during treatment in both groups. The predictive value of HBV RNA level for the seroconversion of HBeAg in patients treated for hepatitis B was analyzed.
Results Significant differences existed in serum HBV DNA and HBV RNA levels between the two groups at baseline while there was no significant difference in HBsAg. The correlation between HBV RNA and HBV DNA was significantly high ($r = 0.707$, $P < 0.05$), while the correlation between HBV DNA and HBsAg ($r = 0.474$, $P < 0.05$) or HBV RNA and HBsAg was poor ($r = 0.372$, $P < 0.05$). Patients with younger age and higher HBV RNA levels at baseline and week 24 were less likely to have HBeAg seroconversion. HBV RNA was better than HBV DNA and HBsAg in predicting HBeAg seroconversion whether at baseline or week 12 and week 24. The area under the curve of HBV RNA level at 24th week was the highest, which was 0.942, and the cutoff value was 4.145 $\log_{10}$ copies/ml.
Conclusion HBV RNA level may be a suitable serum marker to predict whether HBeAg seroconversion can occur. CHB patients with serum HBV RNA level lower than 4.145 $\log_{10}$ copies/ml at week 24 were more likely to achieve HBeAg seroconversion. Eur J Gastroenterol Hepatol 37: 738–744

Introduction

WHO estimates that 254 million people were living with chronic hepatitis B infection in 2022, with 1.2 million new infections each year [1]. If CHB was not effectively treated, it can eventually develop into liver fibrosis, liver cirrhosis, and even hepatocellular carcinoma (HCC). In 2022, hepatitis B resulted in an estimated 1.1 million deaths, mostly from liver cirrhosis and HCC [1]. In some countries, liver cirrhosis and HCC account for more than 50% of all deaths among people with HBV [2]. CHB is a serious world public health problem.

With the emergence and development of antiviral drugs, such as interferon and nucleoside/nucleotide analogues (NUCs), the treatment of CHB patients has been significantly improved [3,4]. NUCs control the progression of CHB by inhibiting the transcription of covalently closed circular DNA (cccDNA) into HBV DNA, and has little effect on cccDNA itself which is responsible for the persistence of the virus [5–8]. If cccDNA is not cleared, it is easy to cause the virus to rebound after stopping the drug. cccDNA exists in liver cells, if you want to detect it, you need to perform liver puncture, which is an invasive examination, so it is not practical to detect cccDNA regularly [8,9].

In 1996, Köck *et al.* demonstrated the presence of HBV RNA in free viruses firstly [10]. Wang *et al.* confirmed that serum HBV RNA is pre-HBV genomic RNA (pgRNA), which is present in virion-like particles [11]. The HBV genome is 3.2 kb long relaxed circular DNA (rcDNA) [12]. After HBV virion enters hepatocytes via taurocholate cotransport polypeptides, rcDNA translocations into the nucleus, where it is converted into cccDNA [13]. cccDNA can be used as a template for the transcription of five viral mRNAs, among which the 3.5 kb pgRNA is the template for the reverse transcription of the negative strand of HBV DNA. Su *et al.* [14] and Rokuhara *et al.* [15] found that HBV RNA level in peripheral blood can reflect cccDNA level in CHB patients treated with lamivudine. Since the only source of HBV RNA is the transcription of cccDNA, and it is not directly inhibited by NUCs, it can be used as an ideal serum index to reflect the reserve and transcriptional activity of cccDNA.

The standard of effective anti-HBV treatment is seroconversion specially HBeAg seroconversion for HBeAg-positive CHB patients, generally representing that HBV enters the stage of low-level replication, when infectivity is reduced, and the possibility of progressing to liver cirrhosis and HCC is also reduced [16]. Studies have confirmed that the prognosis of patients with HBeAg seroconversion is better than that of patients with persistent positive

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HBeAg [17]. HBeAg seroconversion is an important part of the elimination of viral antigen. The clinical significance of HBV RNA is currently one of the hot spots in clinical research. Previous studies have explored the monitoring and outcome prediction value of HBV RNA in antiviral therapy, drug withdrawal, and other aspects, but at present, the prediction value of HBV RNA in HBeAg seroconversion in CHB patients is still lacking in studies with large sample size and multiple time points. The purpose of this study was to analyze the predictive value of HBV RNA level on HBeAg seroconversion in treated CHB patients by measuring HBV RNA level and other laboratory indexes at different observation points during treatment.

Materials and methods

Research subjects and data collection

From August 2020 to December 2023, 64 cases clinically diagnosed as CHB in Infection Hospital Area of the First Affiliated Hospital of University of Science and Technology of China were selected. According to whether HBeAg seroconversion occurred after 24 weeks of treatment, the group was divided into HBeAg seroconversion group and HBeAg non-seroconversion group. Serum samples of the two groups at baseline and week 12, 24, 48 and 72 during treatment were collected for HBV RNA level detection. At the same time, laboratory indicators of patients at each observation point were collected, including HBV DNA, HBsAg, alanine aminotransferase (ALT), aspartic acid aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), total protein, albumin protein (ALB), etc.

Inclusion and exclusion criteria

The inclusion standard were: (1) Patients who were diagnosed with CHB according to the 2015 revised 'Guidelines for the Prevention and Treatment of Chronic Hepatitis B' [18]; (2) Patients whose serum HBeAg was positive at baseline; (3) Patients who were at least 18 years old. The exclusion criteria were: (1) Patients with coinfection with other pathogens, such as hepatitis C virus, hepatitis E virus, HIV, etc.; (2) Patients who had alcoholic liver damage, nonalcoholic steatohepatitis, drug-induced liver damage, or autoimmune liver disease; (3) Patients with malignant tumors or failure of other vital organs.

Instruments and reagents

Serum HBsAg was quantified using a chemiluminescence assay on the AutoLumo A2000plus with matching kit (Antu Biotechnology Co., Ltd, Zhengzhou, China). Biochemical indicators including ALT, AST, ALP, GGT, total protein, and ALB were detected by automatic chemical analyzer and matching kit (Beckman Coulter, Brea, California, USA). HBV DNA concentration was assessed by real-time PCR (Ampley Biotechnology Co., Ltd, Xiamen, China) with a lower detection limit of 100 IU/ml. HBV RNA detection was performed by real-time PCR using SLAN-96P fluorescence amplification instrument with matching extraction and amplification kit (Sansure Biotech, Changsha, China) and the operation was carried out in strict accordance with the instructions and the minimum detection limit was 100 copies/ml.

Statistical analysis

SPSS 22.0 software (IBM Corp., Armork, New York, USA) and Origin 2021 (OriginLab Corp., Northampton, Massachusetts, USA) were used for statistical analysis and making data analysis graphs. The quantitative data of normal distribution were expressed as $x \pm s$, and the differences between groups were analyzed by *t*-test. Data with non-normal distribution were expressed as *M* (P25, P75) percentiles, and Mann–Whitney *U* test was used for comparison between groups. Statistical data were compared using χ^2 test. Pearson correlation was used to analyze the correlation of continuity variables. Logistic regression was used to analyze the factors influencing the HBeAg seroconversion. Receiver operating characteristic (ROC) curve was used to evaluate the predictive value of HBV RNA. It is generally considered that the model with area under the curve (AUC) > 0.7 has clinical application value, and a *P*-value of <0.05 is considered to have statistical significance.

Results

Baseline clinical characteristics of enrolled patients

Among the 64 HBeAg-positive CHB patients, 43 were males and 21 were females, with an average age of 34.50 (29.00, 45.75) years, ranging from 19 to 67 years. Serum HBV DNA and HBV RNA levels at baseline were 6.84 (2.19, 8.00) $\log_{10}$ IU/ml and 6.62 (4.72, 7.31) $\log_{10}$ copies/ml, respectively. HBsAg, ALT, AST, ALP, GGT, total protein, and ALB levels were 6599.47 (1123.45, 13 678.09) IU/ml, 39.00 (23.75, 106.75) U/ml, 31.50 (20.75, 57.25) U/ml, 80.50 (61.75, 102.00) U/ml, 23.00 (13.75, 57.75) U/ml, 73.70 (70.80, 76.40) g/l, and 45.80 (42.05, 48.90) g/l, respectively. The correlation between HBV RNA and HBV DNA was significantly high at baseline ($r = 0.707$, $P < 0.05$). The correlation between HBV DNA and HBsAg ($r = 0.474$, $P < 0.05$) or HBV RNA and HBsAg ($r = 0.372$, $P < 0.05$) was low. In the HBeAg seroconversion group, there were eight males and three females, with an average age of 48.00 (34.00, 55.00) years, ranging from 25 to 67 years old. There were 35 males and 18 females in the HBeAg non-seroconversion group, with an average age of 33.00 (28.50, 43.00) years, ranging from 19 to 59 years old. Patients were older in the HBeAg seroconversion group than in the HBeAg non-seroconversion group. Serum HBV DNA level [7.15 (2.53, 8.00) vs 3.89 (1.30, 6.79) $\log_{10}$ IU/ml, $P < 0.05$] and HBV RNA level [7.01 (5.49, 7.37) vs 3.32 (2.70, 6.15) $\log_{10}$ copies/ml] in the HBeAg non-seroconversion group and HBeAg seroconversion group showed significant difference while there were no significant differences in HBsAg, ALT, AST, ALP, GGT, and ALB. But total protein level of the HBeAg seroconversion group was lower than that of HBeAg non-seroconversion group, which may be related to the older age of the HBeAg seroconversion group than that of HBeAg non-seroconversion group (Table 1, Fig. 1a).

Hepatitis B virus DNA, Hepatitis B virus RNA, and HBsAg levels at different observation points during treatment

At the 12th week of treatment, there were no significant differences in HBV DNA, HBV RNA, and HBsAg

Table 1. Baseline characteristics of HBeAg-positive CHB patients

Characteristic	All patients (n = 64)	HBeAg seroconversion (n = 11)	HBeAg non-seroconversion (n = 53)	P-value
Gender, male/female	43/21	8/3	35/18	
Age (years)	34.50 (29.00, 45.75)	48.00 (34.00, 55.00)	33.00 (28.50, 43.00)	0.005
HBV DNA ($\log_{10}$ IU/ml)	6.84 (2.19, 8.00)	3.89 (1.30, 6.79)	7.15 (2.53, 8.00)	0.023
HBV RNA ($\log_{10}$ copies/ml)	6.62 (4.72, 7.31)	3.32 (2.70, 6.15)	7.01 (5.49, 7.37)	0.002
HBsAg (IU/ml)	6599.47 (1123.45, 13 678.09)	991.06 (290.66, 9850.63)	7165.55 (1367.57, 21 731.33)	0.104
ALT (U/l)	39.00 (23.75, 106.75)	30.65 (20.50, 245.00)	44.50 (24.50, 108.25)	0.579
AST (U/l)	31.50 (20.75, 57.25)	30.00 (20.00, 167.75)	31.50 (21.00, 57.75)	0.916
ALP (U/l)	80.50 (61.75, 102.00)	89.50 (59.50, 108.50)	79.50 (62.00, 101.75)	0.681
GGT (U/l)L	23.00 (13.75, 57.75)	19.00 (12.50, 105.77)	26.00 (14.00, 57.00)	0.767
TP (g/l)	73.70 (70.80, 76.40)	70.15 (66.50, 72.97)	74.60 (71.60, 76.80)	0.012
ALB (g/l)	45.80 (42.05, 48.90)	43.40 (38.60, 47.42)	46.00 (42.30, 49.00)	0.280

ALB, albumin protein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartic acid aminotransferase; CHB, chronic hepatitis B; GGT, γ-glutamyl transpeptidase; HBV, hepatitis B virus; TP, total protein.

between the two groups ($P > 0.05$). HBV RNA and HBV DNA were positively correlated ($r = 0.587$, $P < 0.05$). There was a high correlation between HBV DNA and HBsAg ($r = 0.702$, $P < 0.05$). There was no correlation between HBV RNA and HBsAg ($P > 0.05$). At week 24, there was no significant difference in HBV DNA between the two groups ($P > 0.05$). HBV RNA level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [2.77 (2.49, 3.81) vs 5.34 (4.35, 6.84) $\log_{10}$ copies/ml, $P < 0.05$]. HBsAg level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [2.40 (2.32, 3.29) vs 3.69 (3.02, 4.00) $\log_{10}$ IU/ml, $P < 0.05$]. HBV RNA and HBV DNA were positively correlated ($r = 0.569$, $P < 0.05$). There was significant high correlation between HBV DNA and HBsAg ($r = 0.842$, $P < 0.05$). There was a certain correlation between HBV RNA and HBsAg ($r = 0.606$, $P < 0.05$). At the 48th week of treatment, HBV DNA level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [1.30 (1.30, 1.31) vs 1.60 (1.30, 2.59) $\log_{10}$ IU/ml, $P < 0.05$]. HBV RNA level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [2.65 (2.24, 3.50) vs 5.06 (4.65, 6.56) $\log_{10}$ copies/ml, $P < 0.05$]. HBsAg level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [2.40 (2.23, 3.16) vs 3.79 (3.06, 4.44) $\log_{10}$ IU/ml, $P < 0.05$]. There was no correlation between HBV RNA and HBV DNA ($P > 0.05$). There was a certain correlation between HBV DNA and HBsAg ($r = 0.697$, $P < 0.05$). There was a certain correlation between HBV RNA and HBsAg ($r = 0.626$, $P < 0.05$). At week 72, HBV DNA level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [1.30 (1.30, 1.31) vs 1.85 (1.30, 2.34) $\log_{10}$ IU/ml, $P < 0.05$]. There was no significant difference in HBV RNA level between the two groups ($P > 0.05$). HBsAg level in the HBeAg seroconversion group was significantly lower than that in the HBeAg non-seroconversion group [2.43 (1.61, 3.18) vs 3.94 (3.49, 4.28) $\log_{10}$ IU/ml, $P < 0.05$]. There was no correlation between HBV RNA and HBV DNA ($P > 0.05$). There was a certain correlation between HBV DNA and HBsAg ($r = 0.683$, $P < 0.05$). There was no correlation between HBV RNA and HBsAg ($P > 0.05$). The results showed that there was a good correlation between HBV DNA and HBV RNA at the baseline level, and that the correlation between the two became smaller with longer treatment (Fig. 1b–e; Fig. 2).

Univariate logistic regression analysis of HBeAg seroconversion by each laboratory index

The univariate logistic regression analysis was analyzed with the following variables: age, HBV DNA, HBV RNA, HBsAg, and ALT at each observation point, whether there was HBeAg seroconversion. The result showed that age, HBV RNA level at baseline, and HBV RNA level at 24th week after treatment were independent correlated factors for HBeAg seroconversion. Patients with younger age and higher HBV RNA levels at baseline and 24th week after treatment were less likely to have HBeAg seroconversion. In addition, HBV DNA level at baseline and HBV RNA level at 12th week were also associated with HBeAg seroconversion ($P < 0.2$, Table 2).

Receiver operating characteristic curve analysis of HBeAg seroconversion by each laboratory index

ROC curve analysis of HBeAg seroconversion was performed based on the levels of HBV DNA, HBV RNA, HBsAg, and ALT at baseline, 12th, and 24th week after treatment. It was found that the AUC of HBV RNA after 24 weeks of treatment was the largest (0.942). The cut-off value was 4.145 $\log_{10}$ copies/ml, the sensitivity was 84.6%, and the specificity was 100% (Table 3).

Discussion

Today, it is important to individualize therapy, assess each patient's baseline status at the start of treatment. Serum HBV RNA is a very important serum factor during NUCs treatment and has great application potential. It may be better than HBV DNA in monitoring the efficacy of NUCs, and it may be better than HBsAg in reflecting the transcriptional activity of cccDNA and guiding drug withdrawal. Serum HBsAg is used in the domestic and foreign guidelines for the prevention and treatment of CHB, as a substitute index for cccDNA to formulate the ideal endpoint for clinical cure of antiviral therapy at present, that is, serum HBsAg disappears for a long time after the cessation of antiviral therapy, with or without HBsAb seroconversion. However, more and more studies have shown that the proportion of serum HBsAg clearance in CHB patients after NUCs treatment

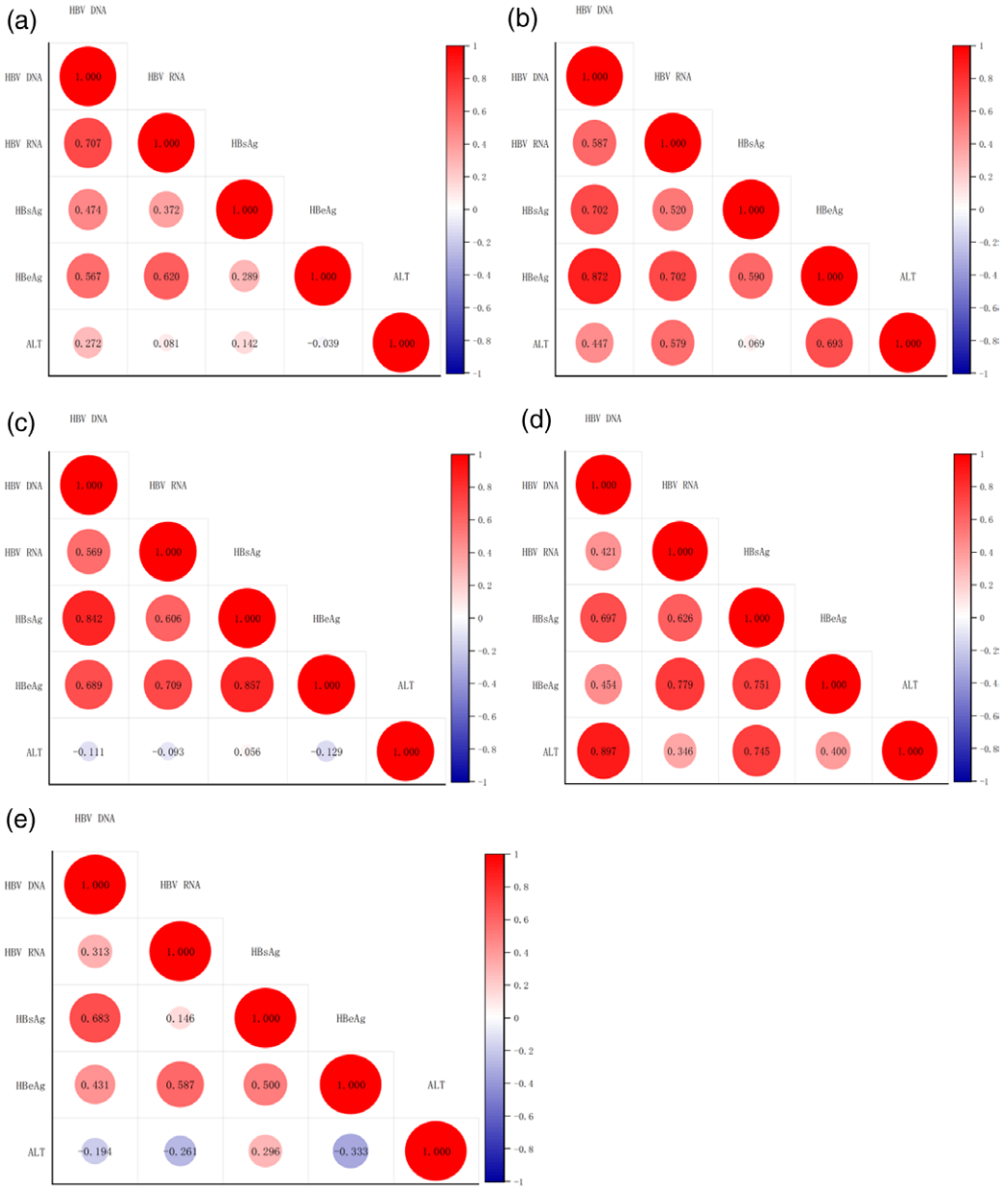

Fig. 1. Correlation coefficients for each laboratory indicator at (a) baseline, (b) week 12, (c) week 24, (d) week 48, and (e) week 72.

is not high [19–22]. If HBsAg clearance is the endpoint of treatment, it means that most patients need long-term or even lifelong antiviral therapy. Serum HBeAg is a soluble viral protein in the plasma of chronic HBV-infected persons, and the negative transfer of serum HBeAg in HBeAg-positive CHB patients usually indicates that HBV enters the stage of low replication, and the risk of progression to cirrhosis and liver cancer is reduced, and the infectivity is also lower [16]. However, the negative conversion rate of serum HBeAg in HBeAg-positive CHB patients is still relatively low [23]. Therefore, it is of great significance to explore the related factors affecting the seroconversion of serum HBeAg in HBeAg-positive CHB patients for the formulation of treatment plan and prognosis evaluation. HBV RNA is a new biomarker of HBV that has not been widely promoted in clinic. Luo *et al*. found that serum HBV RNA was an independent indicator for predicting HBeAg seroconversion and virological response through regression analysis [24]. Jansen *et al*. also showed that low serum HBV RNA level at baseline predicted the response to combination therapy in HBeAg-negative patients (odds ratio: 0.44; $P = 0.19$) [25]. However, the predictive value of HBV RNA on HBeAg seroconversion in CHB patients is still lacking in studies with large sample size and multiple time points. This study included 64 untreated CHB patients, who were divided into conversion group and non-conversion group according to whether HBeAg seroconversion occurred after 24 weeks of treatment. HBV RNA level and other serologic indicators of the two groups were observed and detected at baseline, 12th, 24th, 48th, and 72nd week after treatment. The correlations between HBV RNA and other serological markers at different observation points were analyzed, focusing on the predictors of HBeAg seroconversion in CHB patients.

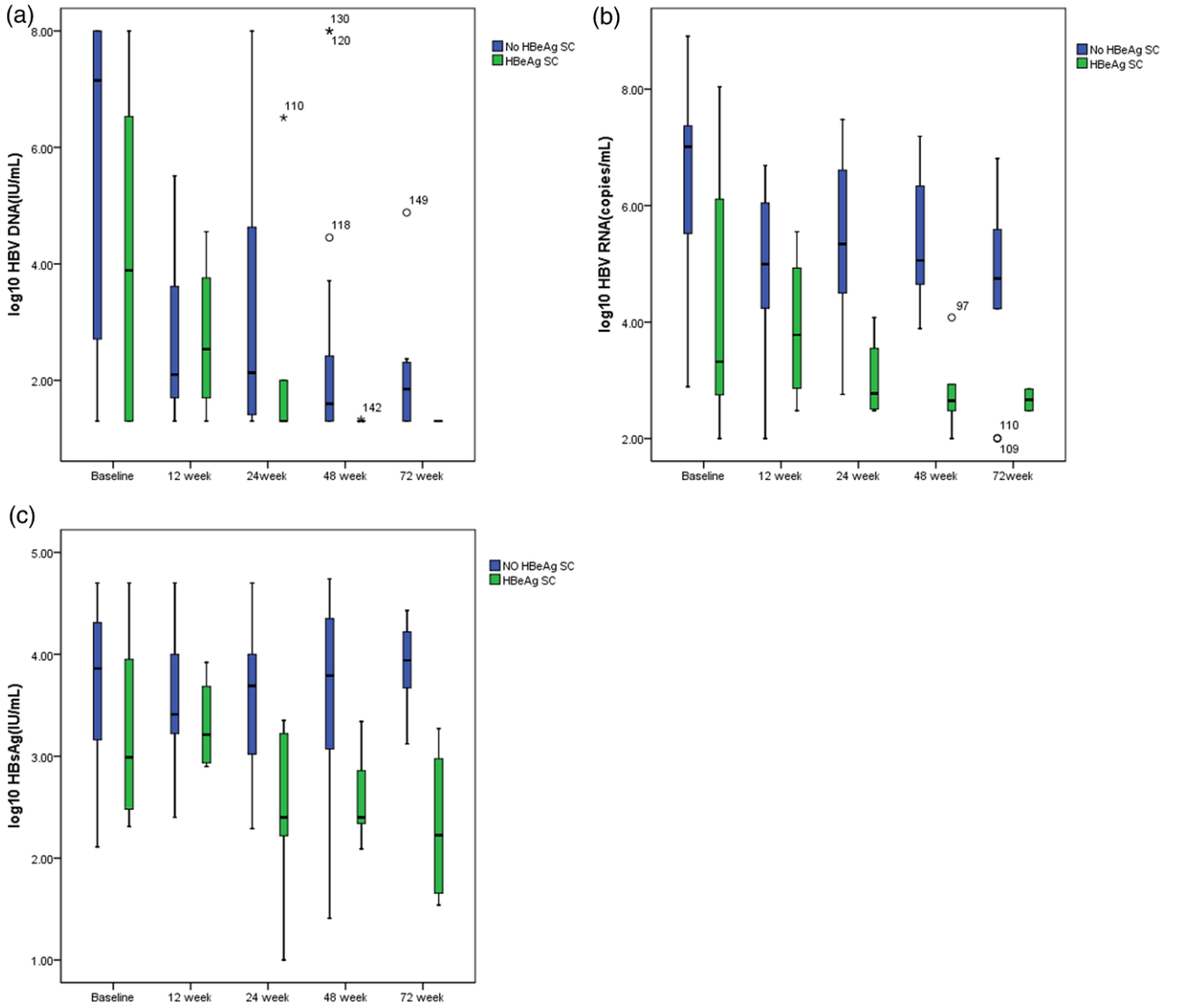

Fig. 2. (a) HBV DNA, (b) HBV RNA, and (c) HBsAg levels in the two groups at each observation point during treatment. HBV, hepatitis B virus.

Table 2. Univariate analyses of factors associated with HBeAg seroconversion

Dependent variable	*B*-Value	SE value	Wald	OR	OR, 95% CI	*P*-Value
Age (years)	–0.102	0.036	8.148	0.903	[0.842–0.968]	0.004
Baseline						
HBV DNA (log_{10} IU/ml)	0.216	0.122	3.124	1.241	[0.977–1.576]	0.077
HBV RNA (log_{10} copies/ml)	0.730	0.237	9.476	2.075	[1.304–3.302]	0.002
HBsAg (IU/ml)	0.001	0.001	1.012	1.000	[1.000–1.001]	0.314
ALT (U/l)	–0.001	0.001	0.380	0.999	[0.997–1.001]	0.538
Week 12						
HBV DNA (log_{10} IU/ml)	0.023	0.416	0.003	1.024	[0.453–2.312]	0.955
HBV RNA (log_{10} copies/ml)	0.972	0.639	2.317	2.644	[0.756–9.248]	0.128
HBsAg (IU/ml)	0.001	0.001	0.598	1.000	[1.000–1.001]	0.440
ALT (U/l)	–0.007	0.007	0.821	0.993	[0.979–1.008]	0.365
Week 24						
HBV DNA (log_{10} IU/ml)	0.247	0.261	0.896	1.280	[0.768–2.135]	0.344
HBV RNA (log_{10} copies/ml)	1.965	0.976	4.056	7.137	[1.054–48.329]	0.044
HBsAg (IU/ml)	0.001	0.001	1.557	1.001	[1.000–1.002]	0.212
ALT (U/l)	–0.008	0.021	0.150	0.992	[0.952–1.034]	0.698

ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; OR, odds ratio.

The results showed that the level of HBV DNA in the conversion group was always lower than that in the non-conversion group except at the 12th week after treatment. HBV RNA and HBsAg levels in the conversion group were always lower than those in the non-conversion group at all observation points. In addition,

Table 3. ROC curve analysis of HBeAg seroconversion by each laboratory index

	Cutoff value	Se (%)	Sp (%)	AUROC
Baseline				
HBV DNA (log_{10} IU/ml)	6.805	58.5	81.8	0.717 [0.550–0.883]
HBV RNA (log_{10} copies/ml)	6.320	66.7	90.0	0.812 [0.632–0.992]
HBsAg (log_{10} IU/ml)	3.058	81.6	60.0	0.664 [0.467–0.861]
Week 12				
HBV DNA (log_{10} IU/ml)	4.645	20.0	100.0	0.500 [0.190–0.810]
HBV RNA (log_{10} copies/ml)	4.375	77.8	100.0	0.852 [0.628–1.000]
HBV DNA (log_{10} IU/ml)	3.962	36.4	100.0	0.614 [0.310–0.917]
Week 24				
HBV DNA (log_{10} IU/ml)	1.350	82.6	66.7	0.743 [0.502–0.984]
HBV RNA (log_{10} copies/ml)	4.145	84.6	100.0	0.942 [0.832–1.000]
HBV DNA (log_{10} IU/ml)	3.476	57.1	100.0	0.819 [0.624–1.000]

AUROC, area under the ROC curve; HBV, hepatitis B virus; Se, sensitivity; Sp, Specificity;

we also analyzed the correlations between HBV RNA and other serum markers, and found that there was a good correlation between HBV RNA and HBV DNA at baseline level ($r = 0.707$, $P < 0.05$) while there was a normal correlation between HBV DNA and HBsAg ($r = 0.474$, $P < 0.05$) as well as the correlation between HBV RNA and HBsAg ($r = 0.372$, $P < 0.05$), supporting the view that HBV RNA is a marker of HBV replication [15,26,27]. However, with the extension of treatment time, the correlation between HBV RNA and HBV DNA became smaller ($r = 0.587$ at 12th week, $r = 0.0.569$ at 24th week, $r = 0.421$ at 48th week). Furthermore, the decline rate of HBV DNA was significantly higher than that of HBV RNA, indicating that after NUCs treatment, HBV DNA replication was inhibited, but intrahepatic cccDNA was not completely consumed, and HBV RNA could still be transcribed. Therefore, the decline rate of HBV RNA and HBV DNA is not consistent, which further indicates that HBV RNA may better reflect the transcriptional activity of cccDNA than HBV DNA.

Univariate logistic regression analysis was performed on age, HBV DNA, HBV RNA, HBsAg, and ALT levels at different observation points, respectively. It was found that age, HBV RNA level at baseline, and 24th week were independent correlation factors for HBeAg seroconversion ($P < 0.05$). HBV DNA level at baseline and HBV RNA level at 12th week were also correlated with the occurrence of HBeAg seroconversion ($P < 0.2$).

ROC curve analysis showed that HBV RNA was better than HBV DNA and HBsAg in predicting HBeAg seroconversion at all observation points. The AUC of HBV RNA at 24th week after treatment was the highest (AUC = 0.942). The cutoff value was 4.145 log_{10} copies/ml. This suggested that HBeAg seroconversion was more likely to occur if HBV RNA level was below 4.145 log_{10} copies/ml at 24th week after treatment.

Conclusion

HBV RNA, as a novel biomarker, is of great significance for the treatment of CHB patients. For HBeAg-positive CHB patients, HBV RNA level may be a suitable serum marker to predict the HBeAg seroconversion. Patients with HBV RNA level below 4.145 log_{10} copies/ml at 24th week during treatment were more likely to have HBeAg seroconversion. HBV RNA can be used to judge the disease progression and clinical outcome of CHB patients and provide references for clinical diagnosis and treatment to a certain extent.

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Our study was approved by the Institutional Review Board of the First Affiliated Hospital of University of Science and Technology of China. We could not obtain written informed consent from the patients who have been discharged from hospital because this study is a retrospective study. But we obtained oral informed consent from all participants during telephone follow-up and the approval of the Institutional Review Board of the First Affiliated Hospital of the University of Science and Technology of China. We insure that all data that could indicate the identity of the patients were kept strictly confidential in this study. Work on human beings is conducted in accordance with the Declaration of Helsinki.

All data and materials were in full compliance with the journal's policy.

Conflicts of interest

There are no conflicts of interest.

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