Correlations between serum hepatitis B core-related antigen and hepatitis B surface antigen in patients with hepatitis B cirrhosis and a hepatitis B virus-DNA-negative status: a retrospective study

Baiguo Xu[1],* ⓘ, Anjing Liu[2],*, Ying Liu[1],*, Tao Han[1], Hua Guo[3], Xian Ding[4] and Huiling Xiang[1]

Abstract

Objective: This study aimed to examine the correlations between serum hepatitis B core-related antigen (HBcrAg) and hepatitis B surface antigen (HBsAg) titers in patients with hepatitis B cirrhosis and a hepatitis B virus (HBV)-DNA-negative status.

Methods: We retrospectively analyzed the data and blood samples of patients who were diagnosed with HBV liver cirrhosis and an HBV-DNA negative status. These patients were hospitalized between October 2018 and October 2019 at one hospital.

Results: A total of 180 patients were included. The median (interquartile range) HBsAg and HBcrAg concentrations were $2.77 \log_{10}$ IU/mL (1.60–3.15) and $3.96 \log_{10}$ U/mL (2.70–4.97), respectively. A non-linear significant relationship was found between HBsAg and HBcrAg

[4]Department of Clinical Laboratory, The Third Central Hospital of Tianjin, Tianjin, China; Institute of Hepatobiliary Disease of Tianjin, Tianjin, China

*These authors contributed equally to this work.

Corresponding author:
Huiling Xiang, Department of Hepatology and Gastroenterology, The Third Central Clinical College of Tianjin Medical University, #83 Jintang Road, Hedong District, Tianjin 300170, China.
Email: huilingxiang@163.com

[1]Department of Hepatology and Gastroenterology, The Third Central Clinical College of Tianjin Medical University, Tianjin, China; Tianjin Institute of Hepatobiliary Disease, Tianjin, China
[2]Center of Infectious Diseases, Tanggu Infectious Disease Hospital of Tianjin, Tianjin, China
[3]Institute of Hepatobiliary Disease of Tianjin, Tianjin, China; The Third Central Hospital of Tianjin, Tianjin, China

concentrations. The inflection point was 0.58. The effect size and confidence interval on the left and right sides of the inflection point were 0.10 ($-$0.23–0.42) and 0.62 (0.46–0.78), respectively. When HBsAg concentrations were $\geq$0.58 $\log_{10}$ IU/mL, HBsAg concentrations were positively correlated with HBcrAg concentrations. When HBsAg concentrations increased by 1 $\log_{10}$ IU/mL, HBcrAg concentrations increased by 0.62 $\log_{10}$ U/mL (95% confidence interval: 0.46, 0.78).
Conclusions: There might be a non-linear relationship between HBcrAg and HBsAg concentrations in patients with hepatitis B cirrhosis and an HBV-DNA-negative status.

Introduction

The increasingly common use of the hepatitis B virus (HBV) vaccine combined with hepatitis B immunoglobulin and the application of powerful anti-HBV agents has led to considerable progress in the prevention and treatment of HBV infection.[1,2] Nevertheless, even after patients test negative for serum HBV-DNA, some can still develop cirrhosis and liver cancer. Most patients treated with nucleoside/tide analogs continue to have detectable covalently closed circular DNA (cccDNA), although most of them have undetectable serum HBV-DNA.[3] Therefore, continuous surveillance of HBV replication activity is one of the most important methods of monitoring disease progression. Detecting intrahepatic cccDNA requires a liver biopsy. Therefore, identifying novel and convenient surrogate markers of intrahepatic viral replication activity has been a research hotspot in the past decades,[4] especially in HBV-DNA-negative patients. Serum hepatitis B surface antigen (HBsAg) and HBV core-related antigen (HBcrAg) titers can be used as virological markers in patients who are HBV-DNA-negative. The relationship between HBsAg and HBcrAg has already been well studied in chronic hepatitis B (CHB),[5,6] but their correlation has not been characterized yet in patients with hepatitis B cirrhosis who are HBV-DNA-negative. Therefore, we examined the relationship between serum HBcrAg and HBsAg concentrations in this population. Understanding this relationship could help determine the application value of two serological markers in patients with hepatitis B cirrhosis. In addition, establishing prediction models of hepatic adverse events, especially in the case of liver cirrhosis, would be helpful.

Methods

Study design and patients

We retrospectively analyzed the data and blood samples from patients who were diagnosed with HBV liver cirrhosis and had an HBV-DNA negative status. The patients were hospitalized between October 2018 and October 2019 at our hospital.

All consecutive patients had hepatitis B cirrhosis with an HBV-DNA-negative status (HBV-DNA $<$ 20 IU/mL), regardless

of the type and duration of use of anti-HBV agents. Cirrhosis was diagnosed according to the Chinese Guidelines on the Management of Liver Cirrhosis (2019 version) released by the Chinese Society of Hepatology, Chinese Medical Association. Participants with hepatitis C, human immunodeficiency virus co-infection, missing virological data, no surplus serum samples for HBcrAg measurement, serum samples not appropriately stored at -20°C, alcoholic liver disease (ALD), primary hepatic carcinoma, and/or diabetes mellitus were excluded.

This study was carried out in accordance with the Declaration of Helsinki. Retrospective testing of stored surplus clinical samples was approved by the medical ethics committee of The Third Central Hospital of Tianjin (date of approval: 27 December 2019; approval number: IRB2019-040-01). Written informed consent was obtained from each patient. All data were analyzed anonymously. The reporting of this study conforms to the STROBE guidelines.[7]

HBV serological markers

Serum HBsAg quantification. HBsAg concentrations were quantified using the Abbott ARCHITECT i4000SR chemiluminescent microparticle immunoassay (Abbott Diagnostics, Abbott Park, IL, USA), which has a detection range of 0.05 to 250 IU/mL. The samples with concentrations >250 IU/mL were used at a dilution of 1: 500 and retested.

Hepatitis B e antigen and HBV-DNA quantification. Hepatitis B e antigen (HBeAg) concentrations were tested using commercially available enzyme immunoassay kits (Abbott Diagnostics). HBV-DNA in serum samples was detected and quantified using the Amplly real-time polymerase chain reaction HBV assay performed on an Anadas9850 platform (Amplly Engineering Co. Ltd., Xiamen, China), which is a fully-automated nucleic acid extraction and real-time polymerase chain reaction system. Using the Amplly assay, HBV DNA was extracted from 200 µL of serum and amplified using the Anadas9850. The limit of detection was approximately 20 IU/mL. The samples with <20 IU/mL were regarded as undetectable in accordance with the manufacturer's instructions.

HBcrAg quantification. Surplus serum samples from patients were stored at -20°C and assayed using a fully automatic chemiluminescent enzyme immunoassay system (Lumipulse G1200; Fujirebio, Tokyo, Japan) according to the manufacturer's instructions. Briefly, the assay provided a linear range of 3 to $7\log_{10}$ U/mL. However, the system indicates concentrations $<3\log_{10}$ U/mL down to $2\log_{10}$ U/mL in HBcrAg-positive samples. HBcrAg concentrations $<2\log_{10}$ U/mL were treated as $2\log_{10}$ U/mL for statistical analysis. Samples with HBcrAg concentrations $>7\log_{10}$ U/mL were used at a dilution of 1:100 or 1:1000, according to the results of HBeAg, with the sera of healthy controls and retested to quantify HBcrAg concentrations.

Statistical analysis

Serum HBcrAg and HBsAg concentrations were $\log_{10}$-transformed. Continuous variables with a normal distribution are expressed as the mean $\pm$ standard deviation. Continuous variables with a skewed distribution are expressed as the median (interquartile range). Categorical variables are expressed as the percentage or frequency. The Kruskal–Wallis H-test (when the data had a skewed distribution), one-way analysis of variance (when the data had a normal distributions), and the chi-square test

(for categorical variables) were used to determine statistical differences between the groups.

A univariable linear regression was used to evaluate the associations between HBcrAg and HBsAg. Non-adjusted and multivariable-adjusted models are presented in this study, and show the results of unadjusted, minimally adjusted, and fully adjusted analyses. Whether the covariance was adjusted was determined by the following principle: if the covariance changed the matched odds ratio by at least 10% when added to the model, the covariance was adjusted.[8] In addition, the generalized additive model (GAM) was used to identify a non-linear relationship between HBcrAg and HBsAg. When the ratio between HBcrAg and HBsAg appeared in the form of a smoothed curve, the inflection point was calculated by the recursive method, where the method of the maximum model likelihood was used.[9] Subgroup analyses were performed by stratified linear regression models. The subgroup interaction and modification were tested by the method of the likelihood ratio test. Spearman's rank correlation analysis was also performed.

All data were analyzed using the Statistical packages R (The R Foundation; http://www.r-project.org; version 3.4.3) and Empower (R) (www.empowersATTs.com; X&Y Solutions Inc., MA, USA). In all analyses, $P < 0.05$ (two-sided) indicates statistical significance.[10]

Results

Baseline characteristics

In this study, 180 eligible patients who were negative for HBV-DNA and diagnosed with hepatitis B liver cirrhosis were analyzed. The baseline characteristics of the patients are summarized in Table 1. The mean age of the patients was 58.6 ± 9.8 years, and 82.8% were men. The median (interquartile range) HBsAg and HBcrAg concentrations were $2.77 \log_{10} IU/mL$ (1.60–3.15) and $3.96 \log_{10} U/mL$ (2.70–4.97), respectively. When HBsAg concentrations increased by $1 \log_{10} IU/mL$, HBcrAg concentrations increased by $0.62 \log_{10} U/mL$ (95% confidence interval [CI]: 0.46, 0.78) ($P < 0.001$). Eighty-two patients were diagnosed with primary hepatic carcinoma with hepatitis B liver cirrhosis. HBsAg measurement required dilution in 90 patients, and HBcrAg measurement required dilution in one patient. To clarify the relationship between serum HBsAg and HBcrAg concentrations, the patients were divided into two groups according to HBeAg concentrations. The median (Q1–Q3) HBcrAg concentrations were 3.48 (2.48–4.39) and 5.32 (4.88–5.73) $\log_{10} U/mL$ in the HBeAg negative and positive groups, respectively ($P < 0.010$). Because the type of anti-HBV agents widely varies, the patients were divided into the treatment-naïve, entecavir (ETV)-treated, and other anti-HBV agent groups.

Univariable analysis

The results of the univariable analysis are shown in Table 2. HBsAg, HBeAg, and anti-HBV agents were significantly correlated with HBcrAg (all $P < 0.05$). Among the continuous variables, only age was significantly negatively correlated with HBcrAg concentrations ($P = 0.014$). Among the categorical variables, only diabetes mellitus was significantly negatively correlated with HBcrAg concentrations ($P = 0.002$).

Subgroup analysis among stratification variables

Because 13 patients had HBsAg seroclearance in this study, the total number of participants was 167 for each stratification variable. The variation trend in β was

Table 1. Baseline characteristics of the patients (n = 180).

| Characteristics | All patients | HBeAg (log$_{10}$ IU/mL) | |
		Negative	Positive
Number of patients	180	142	38
Age, mean ± SD	58.62 ± 9.81	58.80 ± 9.76	57.92 ± 10.08
Sex, n (%)			
Male	149 (82.78)	116 (81.69)	33 (86.84)
Female	31 (17.22)	26 (18.31)	5 (13.16)
PLT (× 10^9/L), median (Q1–Q3)	68.00 (44.75–111.75)	66.00 (44.00–114.75)	72.50 (51.25–106.75)
ALT (U/L), median (Q1–Q3)	22.00 (17.00–33.00)	21.50 (16.25–33.00)	23.50 (19.25–33.75)
AST (U/L), median (Q1–Q3)	29.00 (22.00–43.00)	29.00 (22.00–43.75)	27.50 (23.00–37.75)
TBIL (μmol/L), median (Q1–Q3)	19.30 (14.67–29.32)	21.15 (15.12–31.60)	15.40 (12.88–24.10)
INR, mean ± SD	1.25 ± 0.28	1.27 ± 0.28	1.18 ± 0.26
ALB, g/L, mean ± SD	37.59 ± 7.18	37.02 ± 7.19	39.69 ± 6.80
HBcrAg (log$_{10}$ U/mL), median (Q1–Q3)	3.96 (2.70–4.97)	3.48 (2.48–4.39)	5.32 (4.88–5.73)
HBsAg (log$_{10}$ IU/mL), median (Q1–Q3)	2.77 (1.60–3.15)	2.28 (1.29–3.11)	3.14 (2.77–3.35)
Primary hepatic carcinoma, n (%)			
Yes	82 (45.56)	63 (44.37)	19 (50.00)
No	98 (54.44)	79 (55.63)	19 (50.00)
Child–Pugh class, n (%)			
A	126 (70.00)	94 (66.20)	32 (84.21)
B	43 (23.89)	38 (26.76)	5 (13.16)
C	11 (6.11)	10 (7.04)	1 (2.63)
Family history of hepatitis B, n (%)			
No	128 (71.11)	100 (70.42)	28 (73.68)
Yes	52 (28.89)	42 (29.58)	10 (26.32)
ALD, n (%)			
No	130 (72.22)	105 (73.94)	25 (65.79)
Yes	50 (27.78)	37 (26.06)	13 (34.21)
Diabetes mellitus, n (%)			
No	130 (72.22)	98 (69.01)	32 (84.21)
Yes	50 (27.78)	44 (30.99)	6 (15.79)
Anti-HBV agent, n (%)			
No	45 (25.00)	43 (30.28)	2 (5.26)
ETV	114 (63.33)	83 (58.45)	31 (81.58)
Others	21 (11.67)	16 (11.27)	5 (13.16)
Duration of anti-HBV agents (years)	1.00 (0.00–3.00)	0.50 (0.00–2.34)	2.00 (1.00–3.48)

Data are presented as mean ± SD, n (%), or median (Q1–Q3).
"Others" for anti-HBV agents were as follows: lamivudine (n = 5); adefovir dipivoxil (n = 4); telbivudine (n= 1); lamivudine + adefovir dipivoxil (n = 3); tenofovir disoproxil fumarate (n = 2); tenofovir alafenamide (n = 1); ETV and tenofovir disoproxil fumarate (n = 2); adefovir dipivoxil, telbivudine, and ETV (n = 1); and adefovir dipivoxil and ETV (n = 2).
HBeAg, hepatitis B e antigen; SD, standard deviation; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; INR, international normalized ratio; ALB, albumin; HBcrAg, hepatitis B virus core-related antigen; HBsAg, hepatitis B surface antigen; ALD, alcohol-related liver disease; HBV, hepatitis B virus; ETV, entecavir.

Table 2. Univariate analysis of HBcrAg concentrations ($\log_{10}$ U/mL).

Covariates	Value	β (95% CI)	P value
Age (years)	58.62 ± 9.81	−0.03 (−0.05, 0.01)	0.014
Sex, n (%)			
Male	149 (82.78)	Reference	
Female	31 (17.22)	−0.02 (−0.54, 0.50)	0.946
HBsAg ($\log_{10}$ IU/mL)	2.21 ± 1.33	0.59 (0.47, 0.71)	<0.001
PLT (×10^9/L)	68.00 (44.75–111.75)	−0.002 (−0.005, 0.001)	0.112
ALT (U/L)	22.00 (17.00–33.00)	0.001 (−0.003, 0.006)	0.576
AST (U/L)	29.00 (22.00–43.00)	−0.002 (−0.007, 0.002)	0.298
TBIL (mol/L)	19.30 (14.67–29.32)	−0.005 (−0.013, 0.003)	0.241
ALB (g/L)	37.59 ± 7.18	−0.001 (−0.03, 0.03)	0.936
INR	1.25 ± 0.28	−0.08 (−0.63, 0.79)	0.827
HBeAg, n (%)			
Negative	142 (78.89)	Reference	
Positive	38 (21.11%	1.72 (1.31, 2.12)	<0.001
Primary hepatic carcinoma, n (%)			
No	98 (54.44)	Reference	
Yes	82 (45.56)	0.02 (−0.37, 0.41)	0.904
Child–Pugh class, n (%)			
A	126 (70.00)	Reference	
B	43 (23.89)	−0.07 (−0.53, 0.40)	0.777
C	11 (6.11)	0.08 (−0.75, 0.90)	0.859
Family history of hepatitis B, n (%)			
No	128 (71.11)	Reference	
Yes	52 (28.89)	0.37 (−0.06, 0.79)	0.093
ALD, n (%)			
No	130 (72.22)	Reference	
Yes	50 (27.78)	0.29 (−0.15, 0.72)	0.194
Diabetes mellitus, n (%)			
No	130 (72.22)	Reference	
Yes	50 (27.78)	−0.68 (−1.10, −0.26)	0.002
Anti-HBV agent, n (%)			
No	45 (25.00)	Reference	
ETV	114 (63.33)	1.33 (0.92, 1.75)	<0.001
Others	21 (11.67)	0.93 (0.31, 1.56)	0.004
Duration of anti-HBV agents	1.00 (0.00–3.00)	0.05 (−0.01, 0.11)	0.100

Values are presented as mean ± standard deviation or n (%). In the univariate analysis of PLT, TBIL, ALT, and AST, the values for β and the 95% CI were small. Therefore, they are shown to three decimal places.

"Others" for anti-HBV agents were as follows: lamivudine (n = 5); adefovir dipivoxil (n = 4); telbivudine (n= 1); lamivudine + adefovir dipivoxil (n = 3); tenofovir disoproxil fumarate (n = 2); tenofovir alafenamide (n = 1); ETV and tenofovir disoproxil fumarate (n = 2); adefovir dipivoxil, telbivudine, and ETV (n = 1); and adefovir dipivoxil and ETV (n = 2).

CI, confidence interval; HBsAg, hepatitis B surface antigen; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin; INR, international normalized ratio; HBeAg, hepatitis B e antigen; ALD, alcohol-related liver disease; HBV, hepatitis B virus; ETV, entecavir.

consistent in all stratified variables, such as sex, primary hepatic carcinoma, HBeAg, a family history of hepatitis B, ALD, diabetes mellitus, Child–Pugh class, Barcelona Clinic Liver Cancer (BCLC) stage, and the type of use of anti-HBV agents (Table 3). There were significant differences (all $P < 0.05$) between HBsAg and HBcrAg concentrations among all of the stratification variables, except for the other anti-HBV agent group. We adjusted for aspartate aminotransferase (AST) concentrations, HBeAg concentrations, a family history of hepatitis B, Child–Pugh class, ALD, anti-HBV agents, and the duration of use of antiviral agents for each stratification variable, except for when the variable itself was a stratification variable.

Correlation between HBsAg and HBcrAg concentrations according to primary hepatic carcinoma

Regardless of whether the population was diagnosed with primary hepatic carcinoma, the correlation between HBsAg and HBcrAg concentrations was positive and significant (both $P < 0.001$) after adjusting for AST concentrations, HBeAg concentrations, a family history of hepatitis B, Child–Pugh class, ALD, the type of use of anti-HBV agents, and the duration of use of anti-HBV agents. The β (95% CI) were 0.41 (0.20, 0.62) and 0.57 (0.43, 0.71) in the primary hepatic carcinoma and cirrhosis groups, respectively (Table 3).

Correlation between HBsAg and HBcrAg concentrations according to HBeAg concentrations

The β (95% CI) and P values were 0.59 (0.25, 0.94) and 0.002 and 0.49 (0.36, 0.62) and <0.001, respectively, in the HBeAg positive and negative groups. We adjusted for AST concentrations, HBeAg concentrations, a family history of hepatitis B,

Table 3. Effect size of hepatitis B surface antigen ($\log_{10}$ IU/mL) on hepatitis B virus core-related antigen ($\log_{10}$ U/mL) in prespecified and exploratory subgroups.

Characteristics	n	β (95% CI)	P value
Sex			
Male	137	0.50 (0.37, 0.64)	<0.001
Female	30	0.57 (0.38, 0.77)	<0.001
Primary hepatic carcinoma			
Yes	79	0.41 (0.20, 0.62)	<0.001
No	88	0.57 (0.43, 0.71)	<0.001
HBeAg			
Negative	130	0.49 (0.36, 0.62)	<0.001
Positive	37	0.59 (0.25, 0.94)	0.002
Family history of hepatitis B			
No	116	0.55 (0.41, 0.68)	<0.001
Yes	51	0.36 (0.10, 0.62)	0.011
ALD			
No	119	0.54 (0.41, 0.68)	<0.001
Yes	48	0.53 (0.29, 0.77)	0.001
Diabetes mellitus			
No	123	0.55 (0.42, 0.68)	<0.001
Yes	44	0.38 (0.07, 0.69)	0.023
Child–Pugh class			
A	119	0.44 (0.29, 0.58)	<0.001
B	39	0.52 (0.22, 0.82)	0.002
C	9	–	–
BCLC stage			
0	4	–	–
A	39	0.50 (0.10, 0.90)	0.021
B	21	0.52 (0.22, 0.81)	0.007
C	13	–	–
D	2	–	–
Anti-HBV agent			
No	34	0.40 (0.19, 0.60)	0.001
ETV	112	0.60 (0.44, 0.76)	<0.001
Others	21	0.84 (–0.11, 1.79)	0.115

"Others" for anti-HBV agents were as follows: lamivudine (n = 5); adefovir dipivoxil (n = 4); telbivudine (n = 1); lamivudine + adefovir dipivoxil (n = 3); tenofovir disoproxil fumarate (n = 2); tenofovir alafenamide (n = 1); ETV and tenofovir disoproxil fumarate (n = 2); adefovir dipivoxil, telbivudine, and ETV (n = 1); and adefovir dipivoxil and ETV (n = 2).
The following variables were adjusted for: aspartate aminotransferase concentrations , HBeAg concentrations, a family history of hepatitis B, Child–Pugh class, ALD, anti-HBV agents, and the duration of antiviral agents for each stratification variable except for itself.
The number of participants was 167 rather than 180 because 13 participants were hepatitis B surface antigen-negative in this study.
CI, confidence interval; HBeAg, hepatitis B e antigen; ALD, alcohol-related liver disease; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus; ETV, entecavir.

Child–Pugh class, ALD, and the type and duration of use of anti-HBV agents for this stratification variable (Table 3).

Correlation between HBsAg and HBcrAg concentrations according to the Child–Pugh class

Only nine patients were diagnosed with Child–Pugh class C in our study. Therefore, the β, 95% CI, and P values were not statistically analyzed in this class. HBsAg and HBcrAg concentrations were positively significantly correlated (both $P < 0.01$ for classes A and B) after adjusting for AST concentrations, HBeAg concentrations, a family history of hepatitis B, ALD, anti-HBV agents, and the duration of use of anti-HBV agents (Table 3).

Correlation between HBsAg and HBcrAg concentrations according to the BCLC stage

The β (95% CI) and P values were 0.50 (0.10, 0.90) and 0.021 and 0.52 (0.22, 0.81) and 0.007, respectively, in stages A and B of BCLC. We adjusted for AST concentrations, HBeAg concentrations, a family history of hepatitis B, ALD, and the type and duration of use of anti-HBV agents. Because of the lack of a sufficient number of patients in stages 0, C, and D, statistical analysis could not be performed (Table 3).

Correlation between HBsAg and HBcrAg concentrations according to the type of anti-HBV agent

As mentioned above, the patients were divided into three groups according to categories of anti-HBV agents. HBsAg and HBcrAg concentrations were positively and significantly correlated (all $P < 0.01$), except for the "others" group, after adjusting for AST concentrations, HBeAg concentrations, a family history of hepatitis

B, ALD, and the type and duration of use of anti-HBV agents. The results of subgroups of other categorical variables, such as sex, ALD, diabetes mellitus, and a family history of hepatitis B, are shown in Table 3.

Analysis of the relationship between HBcrAg and HBsAg concentrations

To clarify the relationship between serum HBcrAg and HBsAg concentrations and their trend, the patients were divided into three groups on the basis of HBsAg concentrations as follows: T1 (-2.00 to $2.01 \log_{10}$ U/mL), T2 (2.04 to $3.04 \log_{10}$ U/mL), and T3 (3.06 to $3.95 \log_{10}$ U/mL). A univariable linear regression model was used to evaluate the correlation between HBcrAg and HBsAg concentrations. The non-adjusted and adjusted models are shown in Table 4. In the crude model, HBcrAg concentrations were correlated with HBsAg concentrations ($\beta = 0.59$, 95% CI: [0.47, 0.70], $P < 0.001$), and the results using the preliminary adjusted model (adjusted only for age and sex) were similar to those in the crude model ($\beta = 0.59$ [0.47, 0.71], $P < 0.001$). We also detected an association using the adjusted model II ($\beta = 0.48$ [0.37, 0.60], $P < 0.001$), which was adjusted for AST concentrations, HBeAg concentrations, the type and duration of use of anti-HBV agents, primary hepatic carcinoma, Child–Pugh class, ALD, and diabetes mellitus. In a sensitivity analysis, HBsAg was considered to be a categorical variable (tripartite), and a significant trend was observed (all P values for trend <0.001).

Analyses of non-linear relationships

In this study, the non-linear relationship between HBsAg and HBcrAg concentrations was analyzed ($P = 0.010$ in the log-likelihood ratio test) (Figure 1), with HBsAg as the continuous variable. The relationship between HBcrAg and HBsAg

Table 4. Relationship between HBsAg (log_{10} IU/mL) and hepatitis B virus core-related antigen (log_{10} U/mL) in different models.

Variable	Crude model		Model I		Model II	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
HBsAg (log_{10} IU/mL)	0.59 (0.47, 0.70)	<0.001	0.59 (0.47, 0.71)	<0.001	0.48 (0.37, 0.60)	<0.001
HBsAg (tripartite)						
T1 (−2.00–2.01)	Reference		Reference		Reference	
T2 (2.04–3.04)	1.31 (0.92, 1.70)	<0.001	1.30 (0.91, 1.70)	<0.001	1.05 (0.69, 1.40)	<0.001
T3 (3.06–3.95)	1.77 (1.39, 2.15)	<0.001	1.76 (1.38, 2.15)	<0.001	1.35 (0.99, 1.72)	<0.001
P for trend	<0.001		<0.001		<0.001	

HBsAg values were divided into small to large values (T1, T2, and T3) to observe its changing trend with hepatitis B virus core-related antigen values in different models.
Model I was adjusted for sex and age. Model II was adjusted for aspartate aminotransferase concentrations, hepatitis B e antigen concentrations, anti-hepatitis B virus agents, the duration of anti-hepatitis B virus agents, primary hepatic carcinoma, Child–Pugh class, alcohol-related liver disease, and diabetes mellitus.
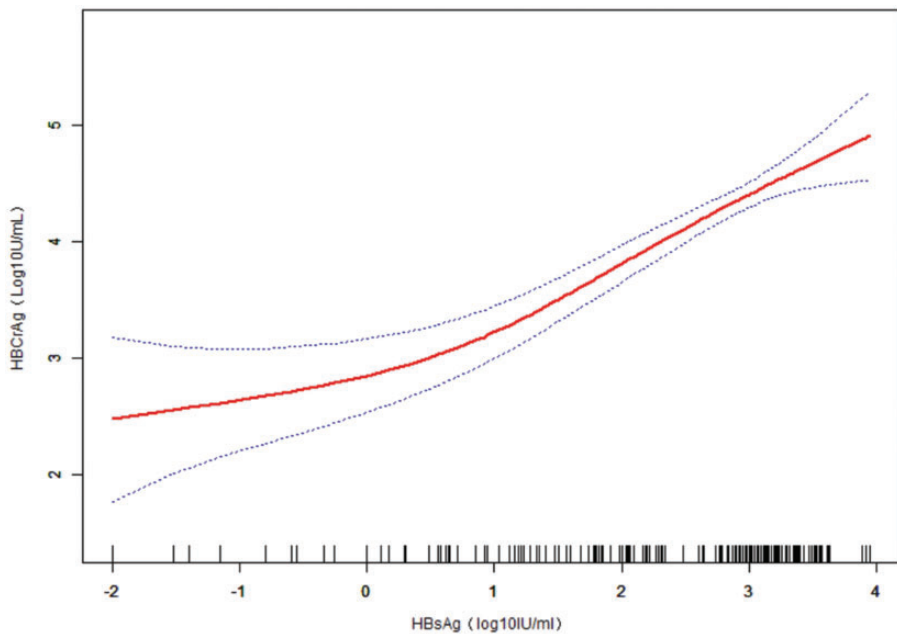HBsAg, hepatitis B surface antigen; CI, confidence interval.

Figure 1. Association between HBsAg and HBcrAg concentrations. A non-linear association between HBcrAg (log_{10} U/mL) and HBsAg (log_{10} IU/mL) concentrations was found ($P < 0.01$) in the generalized additive model. The solid red line represents the linear fit between variables. Blue bands represent the 95% confidence interval from the fit. We adjusted for sex, age, primary hepatic carcinoma, hepatitis B e antigen concentrations, Child–Pugh class, alcohol-related liver disease, diabetes mellitus, anti-hepatitis B virus agents, and the duration of anti-hepatitis B virus agents.
HBsAg, hepatitis B surface antigen; HBCrAg, hepatitis B virus core-related antigen.

concentrations was non-linear in the GAM (after adjusting for sex, age, primary hepatic carcinoma, HBeAg concentrations, Child–Pugh class, ALD, diabetes mellitus, and the type and duration of use of anti-HBV agents). Using a two-piecewise linear regression model, the inflection point was calculated as 0.58. On the left of the inflection point, the effect size, (95% CI), and P value were 0.100, (−0.23, 0.42), and 0.555, respectively. We observed a positive relationship between HBsAg and HBcrAg concentrations on the right side of the inflection point (0.62, [0.46–0.78], P < 0.001) (Table 5).

Spearman's correlation coefficient

Spearman's rank correlation analysis showed that the correlation coefficient between HBcrAg and HBsAg concentrations was 0.613 (P < 0.001) in the whole population.

Diagnostic value of HBcrAg for HBsAg negativity

Not all HBsAg-negative patients were HBcrAg negative. Among the 13 HBsAg-negative patients, 2 (15.39%) patients were

Table 5. Relationship between hepatitis B surface antigen ($\log_{10}$ IU/mL) and hepatitis B virus core-related antigen ($\log_{10}$ U/mL) (two-piecewise linear regression).

Inflection point	Effect size (β)	95% CI	P value
<0.58	0.10	(−0.23, 0.42)	0.555
≥0.58	0.62	(0.46, 0.78)	<0.001

A non-linear relationship between hepatitis B surface antigen and hepatitis B virus core-related antigen concentrations was detected after adjusting for sex, age, primary hepatic carcinoma, hepatitis B e antigen concentrations, Child–Pugh class, alcohol-related liver disease, diabetes mellitus, anti-hepatitis B virus agents, and the duration of anti-hepatitis B virus agents.
CI, confidence interval.

HBcrAg-positive. A receiver operator characteristic curve analysis showed that the area under the curve of HBcrAg for an HBsAg-negative status was 0.918 (95% CI: 0.873, 0.956; P < 0.001), with a Youden index of 0.799 and a cut-off of $2.815 \log_{10}$ IU/mL (Figure 2).

Discussion

This study showed a non-linear relationship between HBsAg and HBcrAg concentrations in patients with hepatitis B cirrhosis and an HBV-DNA-negative status. This finding could help establish predictive models of hepatic adverse events, especially in the case of liver cirrhosis.

HBsAg and HBcrAg can reflect the replication of cccDNA,[5,11–18] which is associated with the progression of liver disease in HBV infection, including cirrhosis and hepatocellular carcinoma. HBsAg is already widely applied as a virological monitoring marker in clinical practice. HBcrAg is also considered a tool to monitor disease progression[14] and is a novel serological marker of HBV replication.[19] Therefore, examining the relationship between these two serological markers is important to understand their respective clinical values, particularly in patients with hepatitis B cirrhosis who have an HBV-DNA-negative status.

The correlation between HBsAg and HBcrAg has already been well studied in CHB.[5,6] This study showed that HBsAg concentrations were positively associated with HBcrAg concentrations after adjusting for other covariables in patients with hepatitis B cirrhosis and an HBV-DNA-negative status. The β value remained stable in the crude and adjusted models after the regression analysis. Furthermore, the relationship between them was non-linear. To the best of our knowledge, this is the first study on the correlation between HBsAg and HBcrAg concentrations in patients with
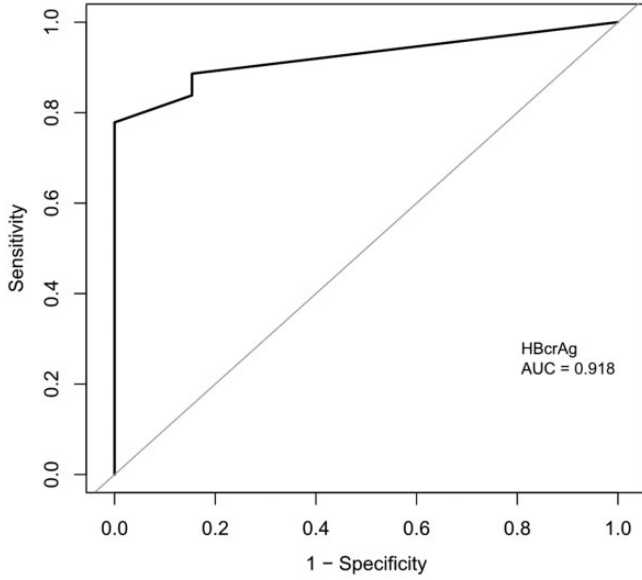
Figure 2. Diagnostic value of HBcrAg ($\log_{10}$ U/mL) for hepatitis B surface antigen negativity. HBCrAg, hepatitis B virus core-related antigen; AUC, area under the curve.

hepatitis B cirrhosis and an HBV-DNA-negative status.

The univariable analysis showed that HBsAg concentrations were significantly correlated with HBcrAg concentrations in patients with hepatitis B cirrhosis and an HBV-DNA-negative status. Age, HBeAg concentrations, diabetes mellitus, and the type of anti-HBV agent also showed a significant correlation with HBcrAg concentrations. Although the β value was decreased in the multivariable linear regression models after adjusting for all other correlated covariables, the trend of HBcrAg concentrations gradually increasing was still significant with an increase in HBsAg concentrations. The Spearman's correlation coefficient was 0.613, which indicated that there was a moderate correlation between HBsAg and HBcrAg concentrations in this population, but it is weaker than that found in treatment-naive patients with CHB.[5] Seto et al. found that serum HBcrAg concentrations were strongly correlated with serum HBsAg concentrations (r = 0.703, P < 0.001) in Asian treatment-naive patients with CHB.[5] The correlation between HBsAg and HBcrAg can be weakened by anti-HBV agents. A previous study showed that serum HBcrAg concentrations were positively associated with serum HBsAg concentrations (r = 0.713) at baseline in all patients with CHB, but this correlation was remarkably weakened during treatment.[20] Another study, which mainly focused on HBeAg-positive patients with CHB, showed that serum HBcrAg concentrations were significantly correlated with HBsAg concentrations (r = 0.696, P < 0.001) at baseline, but this correlation was weakened at 96 weeks after nucleotide analog therapy (r = 0.452, P < 0.001).[21]

The conclusions of previous studies on this correlation were not always consistent. One study, which mainly focused on pegylated interferon-based therapy for patients with CHB, showed that HBcrAg

concentrations were not correlated with HBsAg concentrations, even at baseline.[22] Another study suggested that HBcrAg concentrations were weakly correlated with HBsAg concentrations (Spearman's correlation coefficient: $r = 0.471$, $P < 0.001$) in treatment-naive HBeAg-negative patients.[23] There is a weak correlation between HBcrAg and HBsAg concentrations in the natural history of patients with hepatitis B virus infection.[6]

Previous studies have shown that the correlation between HBsAg and HBcrAg concentrations is also affected by HBeAg concentrations in CHB.[23,24] Chen et al. showed that HBcrAg concentrations were positively correlated with HBsAg concentrations ($r = 0.564$, $P < 0.001$) among HBeAg-positive patients rather than HBeAg-negative patients in treatment-naive CHB.[24] Our study showed a moderate correlation between HBcrAg and HBsAg concentrations in patients with hepatitis B cirrhosis and an HBV-DNA-negative status after adjusting for other covariables (including HBeAg). In addition, a non-linear association was found between HBcrAg and HBsAg concentrations using the GAM after adjusting for other covariables (including HBeAg), which is different from previous studies on CHB.[5,20]

Subgroup analysis, which can promote a better understanding of the true relationship between HBcrAg and HBsAg, is crucial for scientific research. In this study, there was a stable correlation in nearly all covariables. The variation trend in the β value was consistent in all of the stratified variables (e.g., sex, primary hepatic carcinoma, HBeAg concentrations, ALD, diabetes mellitus, Child–Pugh Class, BCLC stage, and the type and duration of use of anti-HBV agents). Previous studies showed that anti-HBV agents could weaken the correlation between HBcrAg and HBsAg concentrations.[13,20] Consistent with this result, there was a moderate correlation between

HBcrAg and HBsAg concentrations, and most patients experienced a long-term history of HBV infection and/or use of anti-HBV agents. Nevertheless, the results are inconsistent in most studies targeting treatment-naive patients with CHB.[5,20] The likely reason for this discrepancy between studies is that HBsAg is translated from mRNAs transcribed from cccDNA and/or from HBV sequences integrated into the host genome. HBsAg concentrations can be independent of the transcriptional activity of cccDNA.[25,26] Therefore, HBsAg concentrations might be affected by age, HBV genotype, and HBV-DNA levels.[27] HBcrAg can exist stably and reflect the activity of cccDNA, and is not affected by preS/S variants. The production of HBcrAg depends on mRNA transcription from cccDNA and is barely affected by nucleotide analog transcriptase inhibition.[20,28] In this study, most patients had a long history of HBV infection and/or anti-HBV agent use. Therefore, the relationship between HBsAg and HBcrAg concentrations might be weakened. Nevertheless, in this study, the correlation between serum HBcrAg and HBsAg concentrations in hepatitis B liver cirrhosis was clear.

The moderate correlation of serum HBcrAg and HBsAg concentrations suggested that these two indicators might not be replaced by each other regarding their clinical value in this population. This possibility was verified by a previous study that evaluated the antiviral effects of long-term antiviral therapy.[25] Another reason HBcrAg and HBsAg cannot replace each other is because of the unique patterns of their distribution at different stages of HBV infection, which results in different possibilities for their applicability in clinical practice.[5] Furthermore, different sources of generation also determine different clinical values. HBcrAg is the coding product of the pre-C/C region. As the second most

important viral marker,[29] HBsAg not only originates from the presence of abundant circulating empty DNA, but is also synthesized from integrated HBV DNA. Therefore, serum HBsAg concentrations reflect not only cccDNA transcription or mRNA translation, but also host immune control over HBV infection.[30,31]

In the present study, all included patients had compensated and decompensated hepatitis B cirrhosis and hepatitis B cirrhosis-induced primary liver cancer. All HBsAg- or HBV-DNA-positive patients were recommended to have long-term antiviral therapy.[32–34] Although some of these patients were able to achieve HBsAg negativity after long-term antiviral therapy, a large proportion remained HBcrAg-positive. Therefore, the status of antiviral therapy for hepatitis B virus can be monitored by HBcrAg. The relationship between HBsAg and HBcrAg concentrations could be useful for the monitoring of antiviral therapy in patients.

HBcrAg is a novel viral serum indicator with a relatively expensive price, which is not widely used clinically. In this study, when HBsAg concentrations were $\geq 0.58 \log_{10} IU/mL$, the relationship between HBsAg and HBCrAg concentrations was linear. Therefore, in this case, one indicator is sufficient for clinical detection. However, when HBsAg concentrations are $< 0.58 \log_{10} IU/mL$ after antiviral therapy or for other reasons, HBcrAg is associated with drug withdrawal recurrence and the occurrence of hepatocellular carcinoma owing to the non-significant relationship between them. In this case, HBcrAg and HBsAg concentrations need to be monitored simultaneously. These data could be useful for providing a cut-off value of HBsAg to monitor HBcrAg concentrations.

This study has many strengths. First, we used a generalized linear model and the GAM to evaluate the linear or non-linear relationships between HBcrAg and HBsAg concentrations. Second, we used strict statistical adjustments to minimize residual confounding, which was not performed in previous studies. Third, to the best of our knowledge, this is the first study on the relationship between HBsAg and HBcrAg concentrations in patients with hepatitis B cirrhosis and an HBV-DNA-negative status.

This study also has some limitations. First, this was an analytical, cross-sectional, single-center investigation in a limited number of patients. Therefore, the applicability of our findings to other ethnic groups requires further verification. Second, the dynamic correlation was not investigated between HBcrAg and HBsAg concentrations, which could have further confirmed their correlation. Third, because of the limited availability of tissue from patients with cirrhosis, we could not investigate the relationship between HBsAg, HBcrAg, and intrahepatic cccDNA in this population. Fourth, the spectrum of cirrhosis was highly discrete. Pathological cirrhosis and clinically diagnosed cirrhosis are not identical and cannot be completely interchanged. We could not perform stratified analyses of reversible and irreversible cirrhosis. Therefore, the generalization of the conclusions still requires further study in stratified reversible and irreversible cirrhosis. We had already obtained some liver biopsy samples from this population and are continuing to collect samples. These samples will be used for future research to obtain more evidence.

Conclusion

There is a non-linear relationship between HBcrAg and HBsAg concentrations in patients with hepatitis B cirrhosis and an HBV-DNA-negative status. Our findings enhanced our understanding of the relationship between HBcrAg and HBsAg concentrations in this population with a long history of HBV infection. This study was

adjusted for various confounders and clarified the relationship between them in a cirrhosis population through a real-world study, which provides a basis for the establishment of prediction models. Future research should be performed to explore and validate the combined application of HBcrAg and HBsAg in predicting adverse hepatic events and the monitoring of antiviral therapy in patients with hepatitis B cirrhosis and an HBV-DNA-negative status.

Author contributions

Tao Han and Huiling Xiang designed the study. Baiguo Xu, Ying Liu, and Anjing Liu collected the patients' clinical data and co-wrote the manuscript. Hua Guo and Xian Ding performed blood sample tests. All authors read and approved the final manuscript.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Data Availability

This manuscript has already been shown online as a preprint. The preprint version (DOI) is available at the below link: https://doi.org/10.21203/rs.3.rs-156751/v1

ORCID iD

Baiguo Xu https://orcid.org/0000-0002-0269-5155

References

1. Liu F, Wang XW, Chen L, et al. Systematic review with meta-analysis: development of hepatocellular carcinoma in chronic hepatitis B patients with hepatitis B surface antigen seroclearance. *Aliment Pharmacol Ther* 2016; 43: 1253–1261.

2. Kim GA, Lee HC, Kim MJ, et al. Incidence of hepatocellular carcinoma after HBsAg seroclearance in chronic hepatitis B patients: a need for surveillance. *J Hepatol* 2015; 62: 1092–1099.

3. Wong DK, Seto WK, Fung J, et al. Reduction of hepatitis B surface antigen and covalently closed circular DNA by nucleos(t)ide analogues of different potency. *Clin Gastroenterol Hepatol* 2013; 11: 1004–1010 e1001.

4. Lin CL and Kao JH. New perspectives of biomarkers for the management of chronic hepatitis B. *Clin Mol Hepatol* 2016; 22: 423–431.

5. Seto WK, Wong DK, Fung J, et al. Linearized hepatitis B surface antigen and hepatitis B core-related antigen in the natural history of chronic hepatitis B. *Clin Microbiol Infect* 2014; 20: 1173–1180.

6. Maasoumy B, Wiegand SB, Jaroszewicz J, et al. Hepatitis B core-related antigen (HBcrAg) levels in the natural history of hepatitis B virus infection in a large European cohort predominantly infected with genotypes A and D. *Clin Microbiol Infect* 2015; 21: 606 e601–610.

7. Von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; 147: 573–577.

8. Vandenbroucke JP, Von Elm E, Altman DG, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Med* 2007; 4: e297.

9. Kernan WN, Viscoli CM, Brass LM, et al. Phenylpropanolamine and the risk of hemorrhagic stroke. *N Engl J Med* 2000; 343: 1826–1832.

10. Liu Y, Zhao P, Cheng M, et al. AST to ALT ratio and arterial stiffness in non-fatty liver

Japanese population: a secondary analysis based on a cross-sectional study. *Lipids Health Dis* 2018; 17: 275.

11. Wong DK, Seto WK, Cheung KS, et al. Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA. *Liver Int* 2017; 37: 995–1001.

12. Suzuki F, Miyakoshi H, Kobayashi M, et al. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. *J Med Virol* 2009; 81: 27–33.

13. Liao H, Liu Y, Li X, et al. Monitoring of serum HBV RNA, HBcrAg, HBsAg and anti-HBc levels in patients during long-term nucleoside/nucleotide analogue therapy. *Antivir Ther* 2019; 24: 105–115.

14. Testoni B, Lebosse F, Scholtes C, et al. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. *J Hepatol* 2019; 70: 615–625.

15. Chen EQ, Feng S, Wang ML, et al. Serum hepatitis B core-related antigen is a satisfactory surrogate marker of intrahepatic covalently closed circular DNA in chronic hepatitis B. *Sci Rep* 2017; 7: 173.

16. Chan SL, Wong VW, Qin S, et al. Infection and Cancer: The Case of Hepatitis B. *J Clin Oncol* 2016; 34: 83–90.

17. Wong GL and Chan HL. Use of quantitative hepatitis B surface antigen with hepatitis B virus DNA in clinical practice. *Clin Liver Dis (Hoboken)* 2013; 2: 8–10.

18. Cornberg M, Wong VW, Locarnini S, et al. The role of quantitative hepatitis B surface antigen revisited. *J Hepatol* 2017; 66: 398–411.

19. Kimura T, Rokuhara A, Sakamoto Y, et al. Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 2002; 40: 439–445.

20. Wang ML, Deng R, Chen EQ, et al. Performance of serum HBcrAg in chronic hepatitis B patients with 8-year nucleos(t)ide analogs therapy. *Clin Res Hepatol Gastroenterol* 2019; 43: 301–309.

21. Wang L, Cao X, Wang Z, et al. Correlation of HBcrAg with Intrahepatic Hepatitis B Virus Total DNA and Covalently Closed Circular DNA in HBeAg-Positive Chronic Hepatitis B Patients. *J Clin Microbiol* 2019; 57: e01303–e0130318.

22. Chuaypen N, Posuwan N, Chittmittraprap S, et al. Predictive role of serum HBsAg and HBcrAg kinetics in patients with HBeAg-negative chronic hepatitis B receiving pegylated interferon-based therapy. *Clin Microbiol Infect* 2018; 24: 306 e307–306 e313.

23. Loggi E, Vukotic R, Conti F, et al. Serum hepatitis B core-related antigen is an effective tool to categorize patients with HBeAg-negative chronic hepatitis B. *J Viral Hepat* 2019; 26: 568–575.

24. Chen EQ, Wang ML, Tao YC, et al. Serum HBcrAg is better than HBV RNA and HBsAg in reflecting intrahepatic covalently closed circular DNA. *J Viral Hepat* 2019; 26: 586–595.

25. Wooddell CI, Yuen MF, Chan HL, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med* 2017; 9: eaan0241.

26. Cornberg M and Manns MP. Hepatitis: No cure for hepatitis B and D without targeting integrated viral DNA? *Nat Rev Gastroenterol Hepatol* 2018; 15: 195–196.

27. Nakamura E, Kakuda H, Matsuura K, et al. [Quantitative analysis of hepatitis B surface antigen as a clinical marker]. *Rinsho Byori* 2011; 59: 838–843.

28. Zhang ZQ, Zhang XN, Lu W, et al. Distinct patterns of serum hepatitis B core-related antigen during the natural history of chronic hepatitis B. *BMC Gastroenterol* 2017; 17: 140.

29. Papatheodoridi M and Papatheodoridis G. Emerging Diagnostic Tools to Decide When to Discontinue Nucleos(t)ide Analogues in Chronic Hepatitis B. *Cells* 2020; 9: 493.

30. Chan HL, Thompson A, Martinot-Peignoux M, et al. Hepatitis B surface antigen quantification: why and how to use it in 2011 – a core group report. *J Hepatol* 2011; 55: 1121–1131.

31. Su TH, Hsu CS, Chen CL, et al. Serum hepatitis B surface antigen concentration

correlates with HBV DNA level in patients with chronic hepatitis B. *Antivir Ther* 2010; 15: 1133–1139.

32. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; 67: 1560–1599.

33. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; 67: 370–398.

34. Wang GQ, Wang FS, Cheng J, et al. The guideline of prevention and treatment for chronic hepatitis B: a 2015 update. *Chin J Hepatol* 2015; 23: 888–905. (In Chinese).