Hepatitis B core-related antigen reflects viral replication and protein production in chronic hepatitis B patients

Jun Li[1], Zhao Wu[1], Gui-Qiang Wang[1,2,3], Hong Zhao[1,3]; China HepB-Related Fibrosis Assessment Research Group

[1]Department of Infectious Disease, Center for Liver Disease, Peking University First Hospital, Beijing 100034, China;
[2]The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Zhejiang University, Hangzhou, Zhejiang 310000, China;
[3]Department of Infectious Disease, Peking University International Hospital, Beijing 100034, China.

Abstract

Background: Hepatitis B core-related antigen (HBcrAg) is a promising disease-monitoring marker for chronic hepatitis B (CHB). We investigated correlations between HBcrAg with antiviral efficacy and virological and histological variables.

Methods: One hundred and forty-five CHB patients from the mainland of China between August 2013 and September 2016 who underwent liver biopsy received entecavir therapy and had paired liver biopsy at 78 weeks. We analyzed correlations between HBcrAg and virological and histological variables in hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients. We also explored the predictors of HBeAg loss after 78 weeks of antiviral therapy. Pearson correlation analysis and logistic forward stepwise regression were the main statistic methods.

Results: HBeAg-positive patients ($n = 93$) had higher baseline HBcrAg (median 7.4 *vs.* 5.3 $\log_{10}$ U/mL $P < 0.001$) and greater HBcrAg declines (median 1.6 *vs.* 0.9 $\log_{10}$ U/mL $P = 0.007$) than HBeAg-negative patients after 78 weeks of therapy. At baseline, HBcrAg correlated with hepatitis B virus (HBV) DNA in both HBeAg-positive ($r = 0.641$, $P < 0.001$) and -negative patients ($r = 0.616$, $P < 0.001$), with hepatitis B surface antigen (HBsAg) in HBeAg-positive patients ($r = 0.495$, $P < 0.001$), but not with anti-hepatitis B virus core antibody (anti-HBc). Weak correlations existed between HBcrAg, histology activity index (HAI; $r = 0.232$, $P = 0.025$), and Ishak fibrosis score ($r = -0.292$, $P = 0.005$) in HBeAg-positive patients. At 78 weeks, significant correlations existed only between HBcrAg and anti-HBc in HBeAg-positive ($r = -0.263$, $P = 0.014$) and HBeAg-negative patients ($r = -0.291$, $P = 0.045$). Decreased HBcrAg significantly correlated with reduced HBV DNA ($r = 0.366$, $P = 0.001$; $r = 0.626$, $P < 0.001$) and HBsAg ($r = 0.526$, $P = 0.001$; $r = 0.289$, $P = 0.044$) in HBeAg-positive and -negative patients, respectively, and with reduced HAI in HBeAg-positive patients ($r = 0.329$, $P = 0.001$). Patients with HBeAg loss ($n = 29$) showed a larger reduction in HBcrAg than those without (median 2.3 *vs.* 1.3 $\log_{10}$ U/mL, $P = 0.001$). In multivariate analysis, decreased HBcrAg was an independent predictor of HBeAg loss ($P = 0.005$).

Conclusions: HBcrAg reflects viral replication and protein production. Decreased HBcrAg could predict HBeAg loss after antiviral therapy.

Trial registration: Clinical Trials.gov: NCT01962155; https://www.clinicaltrials.gov/ct2/show/NCT01962155?term=NCT01962155&draw=2&rank=1

Keywords: Chronic hepatitis B; Hepatitis B core-related antigen; Hepatitis B e antigen; Antiviral therapy

Introduction

Chronic hepatitis B (CHB) affects approximately 260 million people worldwide, with an estimated 15% to 40% developing cirrhosis and/or hepatocellular carcinoma (HCC).[1] Antiviral therapy can achieve a virological response, hepatitis B e antigen (HBeAg) loss/seroconversion, histological improvement, and, preferably, hepatitis B surface antigen (HBsAg) loss/seroconversion.[2,3] However, it is challenging to completely eliminate hepatitis B virus (HBV) from infected hepatocytes because of the existence of intrahepatic covalently closed circular DNA (cccDNA).[4,5]

Hepatitis B core-related antigen (HBcrAg), which has been put into practical use as a virological marker in Japan, is coded by the precore/core region, which comprises three proteins: HBeAg; hepatitis B core antigen (HBcAg), mainly

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001418

Correspondence to: Dr. Hong Zhao, Department of Infectious Diseases and Center for Liver Diseases, Peking University First Hospital, No. 8 Xishiku Street, Xicheng District, Beijing 100034, China
E-Mail: zhaohong_pufh@bjmu.edu.cn

Chinese Medical Journal 2021;134(10)
Received: 19-12-2020 **Edited by:** Peng Lyu

comprising a Dane particle; and a 22,000 Da precore protein (p22cr), which is an HBV DNA-negative (empty) particle.[6] The use of HBcrAg as a surrogate marker for other virological agents has been suggested and could be used to predict the antiviral efficacy,[7] risk of HBV reactivation in occult HBV infections under immunosuppressive therapies,[8] risk of HCC development,[9] as well as post-operative HCC recurrence.[10]

Recent studies have shown that HBcrAg correlates with HBV DNA, HBsAg, HBV RNA, and intrahepatic cccDNA at baseline, especially in HBeAg-positive patients.[11,12] During antiviral therapy, HBcrAg presented a gradually decreasing trend, similar to HBV DNA, HBsAg, and intrahepatic cccDNA, in both HBeAg-positive and -negative patients.[13] A decline in HBcrAg also correlated with declines in HBV DNA, HBsAg, and intrahepatic cccDNA,[14] but correlations between HBcrAg and other virological markers after antiviral therapy were inconsistent.[14,15] The relationships between HBcrAg and grade of inflammation, stage of fibrosis, and their degrees of change before and after antiviral therapy also differed.[14,16] Furthermore, few studies have addressed the associations between HBcrAg and virological or histological variables after antiviral therapy or the relationships between the degrees of change for the markers, especially in HBeAg-negative patients.

While attempting antiviral therapy for HBeAg-positive patients, HBeAg loss is the first step toward immune control. HBeAg seroconversion is described as the primary therapy endpoint in all major guidelines.[2,17] However, there are no widely accepted predictors of HBeAg loss/seroconversion. Recently, HBcrAg alone, or in combination with HBsAg, at baseline or during therapy has been shown to predict spontaneous or therapy-induced HBeAg loss/seroconversion.[18,19]

In this multicenter study, treatment-naïve CHB patients who received 78 weeks of entecavir (ETV) therapy and a paired liver biopsy were investigated. We aimed to investigate the correlations between HBcrAg and virological and histological variables before and after 78 weeks of antiviral therapy in HBeAg-positive and -negative patients and to identify the predictors for HBeAg loss in HBeAg-positive patients.

Methods

Ethics

This study was approved by the Ethical Committees of Peking University First Hospital (No: 2013[639]). All patients gave informed consent for the use of their clinical data, serum, and liver biopsy samples in research.

Patients

We investigated a cohort of treatment-naïve CHB patients who had undergone a liver biopsy from 24 hospitals in the mainland of China between August 2013 and September 2016. The patients then received 78 weeks of ETV therapy and had a paired liver biopsy at 78 weeks. The inclusion criteria are summarized as follows: 18 to 65 years old, HBsAg-positive for at least 6 months, no history of antiviral therapy within the previous 6 months, and fulfilling the antiviral therapy criteria according to the Asian Pacific Association for the Study of Liver guidelines.[3] The exclusion criteria are summarized as follows: had other forms of chronic liver disease (such as hepatitis C virus infection, autoimmune hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease), had decompensated liver cirrhosis or HCC, pregnant, or lactating women. All patients gave informed consent for the use of their clinical data, serum, and liver biopsy samples in research.

Data collection and laboratory examination

Patient demographics and clinical parameters, including age, gender, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin, were collected up to 2 weeks before liver biopsy. The results of ALT and AST were expressed as ratios of the upper limit of normal.

The quantitative detection of HBsAg and anti-hepatitis B virus core antibody (anti-HBc) were performed using available enzyme immunoassays (Roche Diagnostics, Penzberg, Germany)[20] and sandwich enzyme-linked immunosorbent assays,[21] respectively. HBV DNA was measured by COBAS AmpliPrep/COBAS TaqMan (CAP-CTM; Roche, Branchburg, NJ, USA), with a minimum value of detection of 20 IU/mL, and samples below the lower limit were recorded as 20 IU/mL.[20] The levels of HBsAg, anti-HBc, and HBV DNA were expressed in $\log_{10}$ IU/mL.

Serum HBcrAg quantification

Serum HBcrAg was measured using the Lumipulse G HBcrAg assay on the Lumipulse G1200 Analyzer (Fujirebio, Tokyo, Japan). Briefly, 150 μL of pre-treatment solution was added to each of the serum samples (150 μL) and incubated for 30 min at 60°C. The subsequent reaction processes were carried out on the Lumipulse G1200 analyzer. HBcrAg had a dynamic range of 3.0 to 7.0 log U/mL, with a lower detection limit of 2.0 log U/mL. Samples with measurement values higher than the upper range of detection were diluted in HBV-negative human serum at a ratio of 1:1000 before reanalysis.[22,23]

Liver histological examination

Ultrasonographic-guided liver biopsies were performed at baseline and 78 weeks in each institute, according to a standardized protocol, after receiving the patients' informed consent. Liver biopsy specimens of a length of >2.0 cm and with at least 11 portal tracts were considered adequate for scoring. All liver biopsies were blindly reviewed by two hepatopathologists from Beijing You An Hospital affiliated with Capital Medical University. When discrepancies occurred (a kappa value of <0.9 in consistency checks), the samples were reassessed by another experienced pathologist, and the three hepatopathologists consulted and agreed on a final score. Hepatic inflammation was graded using the modified histology

activity index (HAI, ranging from 0 to 18), and fibrosis was staged using the Ishak fibrosis score (*F*, ranging from 0 to 6).[24]

Statistical analyses

Continuous variables were expressed as mean ± standard deviation or median (P25, P75) and were compared using Student's *t* test or non-parametric test (Wilcoxon rank sum test). Categorical variables were expressed as numbers and percentages and were compared using the Chi-square test. Pearson correlation analysis was performed to analyze the correlations between HBcrAg and other variables. We used logistic forward stepwise regression to analyze independent variables for HBeAg loss. Diagnostic accuracy was analyzed with receiver operating characteristic (ROC) curves and expressed as the area under the ROC curve (AUROC) and 95% confidence intervals (CI). Statistical analyses were conducted using SPSS 25.0 (SPSS, Chicago, IL, USA). All tests were two-tailed, and a *P* value of <0.05 was considered statistically significant.

Results

Baseline characteristics

The characteristics of the patients at baseline are summarized in Table 1. A total of 145 patients had undergone liver biopsy at baseline, including 93 HBeAg-positive (mean age 36.2 years) and 52 HBeAg-negative (mean age 43.0 years) patients. For the HBeAg-positive patients, the median levels were 7.4 $\log_{10}$ U/mL for HBcrAg, 6.6 $\log_{10}$ IU/mL for HBV DNA, 3.6 $\log_{10}$ IU/mL for HBsAg, 3.5 $\log_{10}$ IU/mL for anti-HBc, 6.0 for HAI, and 3.0 for *F* score. For the HBeAg-negative patients, the median levels were 5.3 $\log_{10}$ U/mL for HBcrAg, 5.6 $\log_{10}$ IU/mL for HBV DNA, 3.3 $\log_{10}$ IU/mL for HBsAg, 4.2 $\log_{10}$ IU/mL for anti-HBc, 5.5 for HAI, and 3.0 for *F* score. In general, an HBeAg-positive status was associated with a higher HBcrAg level compared with an HBeAg-negative status, and HBeAg-positive patients had higher levels of HBV DNA, HBsAg, and ALT than HBeAg-negative patients. In contrast, the anti-HBc levels

Table 1: Characteristics of CHB patients in the HBeAg-positive and HBeAg-negative groups.

Variables	HBeAg-positive (*N* = 93)	HBeAg-negative (*N* = 52)	Statistics	*P*
Baseline				
Age (years)	36.2 ± 9.2	43.0 ± 10.0	−4.140*	<0.001
Male	74 (80.0)	39 (75.0)	−0.634†	0.526
ALT (/ULN)	1.7 (1.0, 3.9)	1.3 (0.8, 2.4)	−1.892‡	0.058
AST (/ULN)	1.3 (0.9, 2.5)	1.1 (0.8, 2.4)	−1.354‡	0.176
TBIL (μmol/L)	13.8 (11.5, 19.6)	15.2 (12.3, 20.7)	−0.878‡	0.380
HBcrAg ($\log_{10}$ U/mL)	7.4 (6.4, 8.1)	5.3 (4.6, 6.0)	−7.934‡	<0.001
HBV DNA ($\log_{10}$ IU/mL)	6.8 (5.5, 7.6)	5.6 (4.5, 6.4)	−4.370‡	<0.001
HBsAg ($\log_{10}$ IU/mL)	3.6 (3.1, 4.0)	3.3 (3.1, 3.5)	−2.882‡	0.004
Anti-HBc ($\log_{10}$ IU/mL)	3.5 (3.2, 3.8)	4.2 (3.7, 4.5)	−4.836‡	<0.001
HAI score	6.0 (5.0, 7.5)	5.5 (5.0, 7.0)	−1.108‡	0.268
HAI 0–4/5–7/8–9/10–18	15/55/12/11	9/33/6/4	−1.108‡	0.268
F score	3.0 (2.0, 4.0)	3.0 (3.0, 4.0)	−1.351‡	0.177
F 0–1/2/3/4/5–6	7/26/28/24/8	2/8/21/18/3	−1.351‡	0.177
78-week				
HBcrAg ($\log_{10}$ U/mL)	5.6 (5.1, 6.1)	4.2 (3.9, 4.8)	−6.974‡	<0.001
HBcrAg <3	0	3 (5.8)	−3.974†	0.044
HBV DNA ($\log_{10}$ IU/mL)	1.3 (1.3, 1.5)	1.3 (0.5, 1.3)	−3.436‡	0.001
HBV DNA <1.3	58 (62.4)	44 (84.6)	−2.804†	0.005
HBsAg ($\log_{10}$ IU/mL)	3.3 (3.0, 3.5)	3.2 (3.1, 3.4)	−0.262‡	0.793
Anti-HBc ($\log_{10}$ IU/mL)	2.5 (2.1, 2.8)	2.8 (2.4, 3.2)	−3.771‡	<0.001
HAI score	3.0 (3.0, 4.5)	3.0 (3.0, 5.0)	−6.616‡	0.538
HAI 0–4/5–7/8–9/10–18	70/22/1/0	37/15/0/0	−0.616‡	0.538
F score	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	−0.905‡	0.365
F 0–1/2/3/4/5–6	12/26/27/22/6	3/16/15/13/5	−0.905‡	0.365
Decline in variables				
ΔHBcrAg ($\log_{10}$ U/mL)	1.6 (0.9, 2.3)	0.9 (0.3, 2.0)	−2.677‡	0.007
ΔHBV DNA ($\log_{10}$ IU/mL)	6.0 (4.7, 6.9)	5.4 (3.7, 6.3)	−2.344‡	0.019
ΔHBsAg ($\log_{10}$ IU/mL)	0.2 (−0.1, 0.7)	0.1 (0, 0.2)	−2.226‡	0.026
ΔAnti-HBc ($\log_{10}$ IU/mL)	1.0 (0.6, 1.5)	1.2 (1.0, 1.5)	−1.745‡	0.081
ΔHAI score	3.0 (1.0, 4.0)	2.0 (1.0, 3.8)	−1.332‡	0.183
Δ*F* score	0 (0, 1.0)	0 (−1.0, 1.0)	−0.388‡	0.698

Data are presented as *n* (%), *n*, mean ± SD, or median (IQR). *t value; †χ^2 value; ‡Z value. ALT: Alanine aminotransferase; Anti-HBc: Anti-hepatitis B virus core antibody; AST: Aspartate aminotransferase; CHB: Chronic hepatitis B; *F*: Ishak fibrosis score; HAI: Histological activity index; HBcrAg: Hepatitis B core-related antigen; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; IQR: Interquartile range; SD: Standard deviation; TBIL: Total bilirubin; ULN: Upper limit of normal.

in HBeAg-positive samples were significantly lower than those in HBeAg-negative samples. The mean levels and distributions of the HAI and *F* scores were similar between the groups.

Satisfactory antiviral therapy efficacy after 78 weeks of antiviral therapy

After 78 weeks of ETV therapy, all 145 patients had a second liver biopsy. As shown in Table 1, the median respective decreases in HBcrAg, HBV DNA, HBsAg, anti-HBc, HAI, and *F* score were 1.6 $\log_{10}$ U/mL, 6.0, 0.2, 1.0 $\log_{10}$ IU/mL, 3.0, and 0 in HBeAg-positive patients and 0.9 $\log_{10}$ U/mL, 5.4, 0.1, 1.2 $\log_{10}$ IU/mL, 2.0, and 0 in HBeAg-negative patients, respectively. In the HBeAg-positive and -negative groups, 0 and 3 (5.8%) patients had undetectable HBcrAg, and 58 (62.4%) and 44 (84.6%) individuals had undetectable HBV DNA after antiviral therapy, respectively. Of the 102 samples from patients with undetectable HBV DNA at 78 weeks, 99 (97.1%) showed detectable HBcrAg. Generally, HBeAg-positive patients had greater declines in HBcrAg, HBV DNA, and HBsAg, but not anti-HBc or histology scores, than HBeAg-negative patients.

Correlations between HBcrAg and virological and histological variables at baseline and 78 weeks

The correlations between HBcrAg and other variables among the HBeAg-positive and -negative patients are shown in Table 2. At baseline, HBcrAg correlated strongly with HBV DNA ($r = 0.641$, $P < 0.001$) and moderately with HBsAg ($r = 0.495$, $P < 0.001$), but there was no correlation with anti-HBc ($r = -0.102$, $P = 0.343$) among HBeAg-positive patients. A weak correlation was found between HBcrAg and HAI ($r = 0.232$, $P = 0.025$), but correlation with the *F* score was inverse ($r = -0.292$, $P = 0.002$). In the 52 HBeAg-negative patients, HBcrAg also strongly correlated with HBV DNA ($r = 0.616$, $P < 0.001$) but did not significantly correlate with HBsAg ($r = 0.221$, $P = 0.115$), anti-HBc ($r = 0.050$, $P = 0.614$), HAI ($r = 0.118$, $P = 0.405$), or Ishak fibrosis score ($r = 0.115$, $P = 0.417$).

At 78 weeks, no correlation was found between HBcrAg and HBV DNA ($r = -0.001$, $P = 0.992$; $r = -0.071$, $P = 0.630$). The samples in which HBV DNA were undetectable at 78 weeks were included in the correlation analysis. When we removed these samples, there were still no significant correlations in either the HBeAg-positive or -negative groups ($r = -0.122$, $P = 0.48$; $r = -0.218$, $P = 0.604$), and no correlations were found between HBcrAg and HBsAg, HAI, and *F* scores. A significant correlation existed between HBcrAg and anti-HBc for the HBeAg-positive and -negative patients ($r = -0.263$, $P = 0.014$; $r = -0.291$, $P = 0.045$), but the correlation coefficient was low.

Correlations between declines in HBcrAg and virological and histological variables after 78 weeks of therapy

As shown in Table 3, the decline in HBcrAg (ΔHBcrAg) significantly correlated with declines in HBV DNA ($r = 0.366$, $P = 0.001$; $r = 0.626$, $P < 0.001$) and HBsAg ($r = 0.526$, $P = 0.001$; $r = 0.289$, $P = 0.044$), but not with that of anti-HBc or *F* score, in the HBeAg-positive and -negative patients. However, when we removed the samples in which HBV DNA was undetectable at 78 weeks from the analysis, there was no significant correlation between ΔHBcrAg and reduced HBV DNA in the HBeAg-positive ($r = 0.071$, $P = 0.684$) or HBeAg-negative patients ($r = 0.129$, $P = 0.760$). There was a significant correlation between ΔHBcrAg and improvement of inflammation ($r = 0.329$, $P = 0.001$) in HBeAg-positive patients but not in HBeAg-negative patients ($r = -0.015$, $P = 0.920$).

A decline in HBcrAg was the only predictor of HBeAg loss

We identified the relevance of HBeAg loss using baseline variables and reductions in the variables for HBeAg-positive patients [Table 4]. In the 93 HBeAg-positive groups, 29 (31.2%) patients experienced HBeAg loss after 78 weeks of therapy. The baseline HBcrAg was similar among patients with and without HBeAg loss (7.2 *vs.* 7.4 $\log_{10}$ U/mL, $P = 0.872$), but patients who experienced HBeAg loss had greater ΔHBcrAg than those who

Table 2: Correlations between HBcrAg and HBV DNA, HBsAg, anti-HBc, HAI score, and Ishak fibrosis score at baseline and 78 weeks after ETV therapy.

	HBcrAg							
	HBeAg-positive				HBeAg-negative			
	Baseline		78-week		Baseline		78-week	
Variables	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HBV DNA	0.641	<0.001	−0.001	0.992	0.616	<0.001	−0.071	0.630
HBsAg	0.495	<0.001	0.164	0.116	0.221	0.115	0.208	0.151
Anti-HBc	−0.102	0.343	−0.263	0.014	0.050	0.614	−0.291	0.045
HAI	0.232	0.025	0.035	0.739	0.118	0.405	−0.157	0.283
F	−0.292	0.005	0.039	0.709	0.115	0.417	0.180	0.216

Anti-HBc: Anti-hepatitis B virus core antibody; *F*: Ishak fibrosis score; HAI: Histological activity index; HBcrAg: Hepatitis B core-related antigen; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

Table 3: Correlations between declines in HBcrAg and HBV DNA, HBsAg, anti-HBc, HAI score, and Ishak fibrosis score after 78 weeks of ETV therapy.

	Decline in HBcrAg (ΔHBcrAg)			
	HBeAg-positive		HBeAg-negative	
Decline in variables	*r*	*P*	*R*	*P*
ΔHBV DNA	0.366	<0.001	0.626	<0.001
ΔHBsAg	0.526	<0.001	0.289	0.044
ΔAnti-HBc	0.073	0.514	0.139	0.358
ΔHAI score	0.329	0.001	−0.015	0.920
Δ*F* score	0.120	0.251	0.083	0.571

Anti-HBc: Anti-hepatitis B virus core antibody; *F*: Ishak fibrosis score; HAI: Histological activity index; HBcrAg: Hepatitis B core-related antigen; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

Table 4: Univariate and multivariate analysis of variables and HBeAg loss after antiviral therapy in HBeAg-positive patients.

Variables	With HBeAg loss (*N* = 29)	Without HBeAg loss (*N* = 64)	Univariate analysis *P*	Multivariate analysis *P*
Baseline variables				
Age (years)	36.0 ± 8.3	36.2 ± 9.6	0.930	
Male	9 (75.0)	11 (64.7)	0.106	
ALT (/ULN)	4.4 (0.9, 7.2)	1.5 (1.1, 2.6)	0.006	0.056
AST (/ULN)	2.0 (0.9, 3.7)	1.2 (0.9, 1.8)	0.082	
TBIL (μmol/L)	13.2 (11.5, 19.8)	14.1 (11.6, 19.6)	0.813	
HBcrAg ($\log_{10}$ U/mL)	7.2 (6.1, 8.2)	7.4 (6.5, 8.0)	0.872	
HBV DNA ($\log_{10}$ IU/mL)	6.5 (4.3, 7.6)	6.9 (5.8, 7.7)	0.127	
HBsAg ($\log_{10}$ IU/mL)	3.6 (3.2, 4.3)	3.5 (3.1, 4.0)	0.331	
Anti-HBc ($\log_{10}$ IU/mL)	3.7 (3.2, 3.9)	3.4 (3.1, 3.8)	0.270	
Decline in variables				
ΔHBcrAg ($\log_{10}$ U/mL)	2.3 (1.5, 3.5)	1.3 (0.8, 2.1)	0.001	0.005
ΔHBV DNA ($\log_{10}$ IU/mL)	5.7 (4.2, 7.2)	6.0 (5.0, 6.9)	0.585	
ΔHBsAg ($\log_{10}$ IU/mL)	0.4 (0, 1.3)	0.1 (−0.2, 0.6)	0.024	0.661
ΔAnti-HBc ($\log_{10}$ IU/mL)	1.1 (0.6, 1.5)	1.0 (0.6, 1.5)	0.766	

Data are presented as *n* (%) or mean ± SD or median (IQR). ALT: Alanine aminotransferase; Anti-HBc: Anti-hepatitis B virus core antibody; AST: Aspartate aminotransferase; HBcrAg: Hepatitis B core-related antigen; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; IQR: Interquartile range; SD: Standard deviation; TBIL: Total bilirubin; ULN: Upper limit of normal.

remained HBeAg-positive (2.3 *vs.* 1.3 $\log_{10}$ U/mL, $P = 0.001$). Other variables associated with the probability of HBeAg loss in the univariate analysis were baseline ALT and a decline in HBsAg. In the multivariate analysis, only ΔHBcrAg remained as an independent predictor of HBeAg loss ($P = 0.005$). As a result, a cut-off value of 2.6 $\log_{10}$ U/mL for ΔHBcrAg was defined by optimizing sensitivity and specificity on the ROC curve (maximized Youden index). This threshold had a moderate predictive value for HBeAg loss (sensitivity 48.3% and specificity 92.2%), and the AUROC was 0.727 (95% CI: 0.602, 0.851).

Discussion

Previous studies showed that HBcrAg levels vary significantly during the different phases of HBV infection.[25,26] Maasoumy *et al*[26] studied HBcrAg levels during the natural history of HBV infection in a large European cohort. The results showed that the median HBcrAg levels were high in the HBeAg-positive immune-tolerance ($n = 30$) and HBeAg-positive immune-clearance phases ($n = 60$) (8.41 and 8.11 $\log_{10}$ U/mL, respectively), lower in HBeAg-negative hepatitis ($n = 50$) (4.82 $\log_{10}$ U/mL), and only 2.00 $\log_{10}$ U/mL in the HBeAg-negative inactive/quiescent carrier phase ($n = 109$).[26] In the present study, HBeAg-positive patients showed significantly higher HBcrAg levels than HBeAg-negative patients before therapy, which is consistent with the findings of previous studies. This phenomenon is understandable, as HBeAg is part of HBcrAg, and HBeAg-positive status was associated with more active HBV replication compared with HBeAg-negative status.

HBV DNA amplified from intrahepatic cccDNA is commonly used to evaluate viral replication. HBcrAg has been suggested as a surrogate marker of intrahepatic cccDNA and its transcriptional activity.[27] Testoni *et al*[27] analyzed 130 (36 HBeAg-positive) treatment-naïve CHB patients and found that HBcrAg strongly correlated with intrahepatic cccDNA ($r = 0.74$, $P < 0.001$) and its transcriptional activity (defined as the pre-genomic RNA/

cccDNA ratio, $r = 0.52$, $P < 0.001$). Studies also demonstrated a close correlation between HBcrAg and HBV DNA, irrespective of HBeAg status before therapy, with correlation coefficients ranging from 0.59 to 0.85,[15,28,29] which is similar to our results. We also found that a decline in HBcrAg significantly correlated with reduced HBV DNA, which is similar to a previous study's findings.[14] All these results suggest that HBcrAg levels are a reflection of viral replication.

However, patients often have undetectable HBV DNA after receiving antiviral therapy. Lam *et al*[30] investigated 222 Chinese CHB patients (90 HBeAg-positive patients) administered continuous ETV therapy and revealed an undetectable HBV DNA rate of 98.7%, whereas only 32.0% of patients had undetectable HBcrAg after 7 years of therapy. Wang *et al*[14] analyzed 76 HBeAg-positive CHB patients who received 96 weeks of lamivudine and adefovir therapy and showed that 48.7% (37/76) of patients had undetectable HBV DNA. While HBcrAg could be detected in all patients, no significant correlation existed between HBcrAg and HBV DNA after therapy when the samples in which HBV DNA was undetectable were removed ($r = 0.334$, $P = 0.071$).[14] The divergence between HBcrAg and HBV DNA can be explained by HBcrAg production only being derived from the expression of core mRNA/pre-genomic RNA and precore mRNA, which are transcribed from cccDNA, and HBcrAg production was unaffected by the inhibition of reverse transcription of pre-genomic RNA by a nucleos(t)ide analog.[31]

In the study, a significant correlation between HBcrAg and HBsAg existed in HBeAg-positive patients but not in HBeAg-negative patients at baseline or all patients at 78 weeks. However, a decline in HBcrAg significantly correlated with a decline in HBsAg in both HBeAg-positive and -negative patients. Previous studies also showed a moderate correlation between HBcrAg and HBsAg in HBeAg-positive patients[14,32]; however, the results were inconsistent among HBeAg-negative patients. By analyzing 121 HBeAg-negative patients who received 48 weeks pegylated interferon alone or with ETV, Chuaypen *et al*[7] demonstrated that HBcrAg did not correlate with HBsAg at baseline ($r = 0.07$, $P = 0.446$) but did correlate with HBsAg at 48 weeks ($r = 0.305$, $P = 0.001$). Furthermore, a reduction in HBcrAg correlated with reduced HBsAg ($r = 0.295$, $P = 0.001$). However, Wang *et al*[13] found that HBcrAg correlated with HBsAg among HBeAg-negative patients ($n = 25$, $r = 0.552$, $P = 0.007$). The inconsistent and weak correlation may be because HBsAg can be synthesized by using both cccDNA and sub-genomic fragments of HBV DNA integrated into the host genome, while HBeAg and HBcAg production require the presence of cccDNA, including the entire HBV genome.[33]

Anti-HBc levels, however, were higher in HBeAg-negative than in HBeAg-positive patients, which is the inverse of the trends seen with other virological markers, but this phenomenon is consistent with the results of a previous study.[34] The mechanism may be connected to the discovery that, in comparison with HBeAg-positive CHB, HBeAg-negative CHB is more likely to undergo disease activity fluctuation, which re-stimulates anti-HBc production.[34]

Only one study investigated the relationship between HBcrAg and anti-HBc. Liao *et al*[35] enrolled 57 CHB patients (16 HBeAg-positive patients) and showed that anti-HBc weakly correlated with HBcrAg ($r = 0.249$, $P < 0.001$) in all patients before therapy. This study demonstrated no significant correlation in all patients at baseline but showed an inverse and weak correlation at 78 weeks. Theoretically, HBcrAg also identifies HBcAg, and circulatory HBcAg should affect anti-HBc levels. Although HBcAg can be secreted into the blood as a component of virions, it is typically contained within the virus envelope and is not readily accessible by B cells. This containment of HBcAg may provide a mechanistic explanation for the non-parallel amounts of HBcrAg and anti-HBc.[34]

The relationships between HBcrAg and histological changes differed among studies.[14,16] Chang *et al*[16] revealed that higher HBcrAg levels were associated with a higher grade of inflammation in all patients, and HBcrAg negatively correlated with liver fibrosis staging ($r = -0.357$, $P < 0.001$) in HBeAg-positive patients but positively correlated ($r = 0.317$, $P < 0.001$) in HBeAg-negative patients. They also found that a greater reduction in HBcrAg was associated with improvements in inflammation and regression of fibrosis when they analyzed 320 CHB patients (164 of whom were HBeAg-positive) who received 72 weeks of ETV.[16] The study showed that there was a positive correlation between HBcrAg and the grade of inflammation and an inverse correlation between HBcrAg and the stage of fibrosis in HBeAg-positive patients but not in HBeAg-negative patients at baseline nor in all patients at 78 weeks. A decline in HBcrAg only correlated with improvements in inflammation in HBeAg-positive patients, not with the regression of fibrosis in all patients. However, all the above-mentioned relationships were weak. A possible reason is that HBcrAg may more closely reflect viral replication and that the regression of fibrosis may have been an outcome of a much longer therapy period.

HBeAg loss or seroconversion was defined as a primary endpoint for HBeAg-positive patients during the course of antiviral therapy. Several studies have shown that baseline levels and changes in HBcrAg while on antiviral therapy may predict HBeAg loss or seroconversion after patients receive nucleos(t)ide analog or peginterferon therapy.[18,19,36] Wang *et al*[18] discovered that a combination of HBcrAg and HBsAg levels at month 6 (with AUROC 0.769) or month 12 (with AUROC 0.807) had the greatest predictive value for HBeAg seroconversion, in which 36.4% (43/118) of patients achieved HBeAg seroconversion after a median of 39 months of treatment. This study also showed that a decline in HBcrAg was the only predictor of HBeAg loss after 78 weeks of therapy.

It should be noted that there were limitations to our research. HBcrAg was only measured at baseline and 78 weeks; therefore, we were lacking serial data for fixed time points during antiviral therapy. As a result, the

dynamics of HBcrAg during the 78 weeks of therapy are unknown. In addition, for independent predictors for HBeAg loss, HBcrAg levels or the degree of change during therapy, rather than the degree of change between baseline and 78 weeks, might be useful. Further investigations are needed to describe the dynamic changes in HBcrAg and to demonstrate whether ΔHBcrAg during treatment can still predict HBeAg loss.

We conclude that significant correlations between HBcrAg, HBV DNA, and HBsAg were observed among CHB patients at baseline, and the same correlations were also found between the decline in HBcrAg and HBV DNA and HBsAg after 78 weeks of therapy, which indicates that, as a marker for the number of infected hepatocytes, HBcrAg may reflect both viral replication and viral protein production. In addition, a decline in HBcrAg can be used to predict HBeAg loss after antiviral therapy. HBcrAg may become a practical clinical marker, thanks to its extensive and reliable application.

Acknowledgements

The authors gratefully acknowledge the members of the China HepB-Related Fibrosis Assessment Research Group for assisting patient recruitment and data acquisition.

Funding

The study was supported by the China Mega-Project for Infectious Diseases (Nos. 2017ZX10203202 and 2013ZX10002005) and China Mega-Project for Innovative Drugs (No. 2016ZX09101065).

Conflicts of interest

None.

References

1. Razavi-Shearer D, Gamkrelidze I, Nguyen MH, Chen DS, Van Damme P, Abbas Z, *et al*. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 2018;3:383–403. doi: 10.1016/S2468-1253(18)30056-6.
2. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; 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. doi: 10.1016/j.jhep.2017.03.021.
3. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HYL, Chen CJ, *et al*. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1–98. doi: 10.1007/s12072-015-9675-4.
4. Asselah T, Loureiro D, Boyer N, Mansouri A. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. Lancet Gastroenterol Hepatol 2019;4:883–892. doi: 10.1016/S2468-1253(19)30190-6.
5. Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 2015;64:1972–1984. doi: 10.1136/gutjnl-2015-309809.
6. Drafting Committee for Hepatitis Management Guidelines, the Japan Society of Hepatology. JSH guidelines for the management of hepatitis B virus infection. Hepatol Res 2014;44 (Suppl S1):1–58. doi: 10.1111/hepr.12272.
7. Chuaypen N, Posuwan N, Chittmittraprap S, Hirankarn N, Treeprasertsuk S, Tanaka Y, *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.e7–306.e13. doi: 10.1016/j.cmi.2017.07.016.
8. Seto W-K, Wong DK, Chan TS, Hwang Y-Y, Fung J, Liu KS, *et al*. Association of hepatitis B core-related antigen with hepatitis B virus reactivation in occult viral carriers undergoing high-risk immunosuppressive therapy. Am J Gastroenterol 2016;111:1788–1795. doi: 10.1038/ajg.2016.436.
9. Tseng T-C, Liu C-J, Hsu C-Y, Hong C-M, Su T-H, Yang W-T, *et al*. High level of hepatitis B core-related antigen associated with increased risk of hepatocellular carcinoma in patients with chronic HBV infection of intermediate viral load. Gastroenterology 2019;157:1518–1529.e3. doi: 10.1053/j.gastro.2019.08.028.
10. Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, *et al*. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int 2010;30:1461–1470. doi: 10.1111/j.1478-3231.2010.02344.x.
11. Inoue T, Tanaka Y. The role of hepatitis B core-related antigen. Genes (Basel) 2019;10:357. doi: 10.3390/genes10050357.
12. Mak L-Y, Wong DK-H, Cheung K-S, Seto W-K, Lai C-L, Yuen M-F, *et al*. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. Aliment Pharmcol Ther 2018;47:43–54. doi: 10.1111/apt.14376.
13. Wang M-L, Deng R, Chen E-Q, Tao C-M, Liao J, Zhou T-Y, *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. doi: 10.1016/j.clinre.2018.10.020.
14. Wang L, Cao X, Wang Z, Gao Y, Deng J, Liu X, *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–e01318. doi: 10.1128/JCM.01303-18.
15. Wong DK-H, Seto W-K, Cheung K-S, Chong C-K, Huang F-Y, Fung J, *et al*. Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA. Liver Int 2017;37:995–1001. doi: 10.1111/liv.13346.
16. Chang X-J, Sun C, Chen Y, Li X-D, Yu Z-J, Dong Z, *et al*. On-treatment monitoring of liver fibrosis with serum hepatitis B core-related antigen in chronic hepatitis B. World J Gastroenterol 2019;25:4764–4778. doi: 10.3748/wjg.v25.i32.4764.
17. Terrault NA, Lok ASF, McMahon BJ, Chang K-M, Hwang JP, Jonas MM, *et al*. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560–1599. doi: 10.1002/hep.29800.
18. Wang B, Carey I, Bruce M, Montague S, Dusheiko G, Agarwal K. HBsAg and HBcrAg as predictors of HBeAg seroconversion in HBeAg-positive patients treated with nucleos(t)ide analogues. J Viral Hepat 2018;25:886–893. doi: 10.1111/jvh.12889.
19. Sonneveld MJ, van Oord GW, van Campenhout MJ, De Man RA, Janssen HLA, de Knegt RJ, *et al*. Relationship between hepatitis B core-related antigen levels and sustained HBeAg seroconversion in patients treated with nucleo(s)tide analogues. J Viral Hepat 2019;26:828–834. doi: 10.1111/jvh.13097.
20. Jia W, Song L-W, Fang Y-Q, Wu X-F, Liu D-Y, Xu C, *et al*. Antibody to hepatitis B core antigen levels in the natural history of chronic hepatitis B: a prospective observational study. Medicine (Baltimore) 2014;93:e322. doi: 10.1097/MD.0000000000000322.
21. Li A, Yuan Q, Huang Z, Fan J, Guo R, Lou B, *et al*. Novel double-antigen sandwich immunoassay for human hepatitis B core antibody. Clin Vaccine Immunol 2010;17:464–469. doi: 10.1128/CVI.00457-09.
22. Tanaka E, Matsumoto A, Suzuki F, Kobayashi M, Mizokami M, Tanaka Y, *et al*. Measurement of hepatitis B virus core-related antigen is valuable for identifying patients who are at low risk of lamivudine resistance. Liver Int 2006;26:90–96. doi: 10.1111/j.1478-3231.2005.01200.x.
23. Matsumoto A, Tanaka E, Minami M, Okanoue T, Yatsuhashi H, Nagaoka S, *et al*. Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol Res 2007;37:661–666. doi: 10.1111/j.1872-034X.2007.00094.x.
24. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, *et al*. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–699. doi: 10.1016/0168-8278(95)80226-6.
25. Loggi E, Vukotic R, Conti F, Grandini E, Gitto S, Cursaro C, *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. doi: 10.1111/jvh.13054.
26. Maasoumy B, Wiegand SB, Jaroszewicz J, Bremer B, Lehmann P, Deterding K, *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.e1–606.e10. doi: 10.1016/j.cmi.2015.02.010.
27. Testoni B, Lebosse F, Scholtes C, Berby F, Miaglia C, Subic M, *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. doi: 10.1016/j.jhep.2018.11.030.
28. Rokuhara A, Sun X, Tanaka E, Kimura T, Matsumoto A, Yao D, *et al*. Hepatitis B virus core and core-related antigen quantitation in Chinese patients with chronic genotype B and C hepatitis B virus infection. J Gastroenterol Hepatol 2005;20:1726–1730. doi: 10.1111/j.1440-1746.2005.04087.x.
29. Wong DK-H, Tanaka Y, Lai C-L, Mizokami M, Fung J, Yuen M-F, *et al*. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol 2007;45:3942–3947. doi: 10.1128/JCM.00366-07.
30. Lam Y-F, Seto W-K, Wong D, Cheung K-S, Fung J, Mak L-Y, *et al*. Seven-year treatment outcome of entecavir in a real-world cohort: effects on clinical parameters, HBsAg and HBcrAg levels. Clin Transl Gastroenterol 2017;8:e125. doi: 10.1038/ctg.2017.51.
31. Kimura T, Ohno N, Terada N, Rokuhara A, Matsumoto A, Yagi S, *et al*. Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. J Biol Chem 2005;280:21713–21719. doi: 10.1074/jbc.M501564200.
32. Chen E-Q, Wang M-L, Tao Y-C, Wu D-B, Liao J, He M, *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. doi: 10.1111/jvh.13061.
33. Cornberg M, Wong VW-S, Locarnini S, Brunetto M, Janssen HLA, Chan HL-Y, *et al*. The role of quantitative hepatitis B surface antigen revisited. J Hepatol 2017;66:398–411. doi: 10.1016/j.jhep.2016.08.009.
34. Song L-W, Liu P-G, Liu C-J, Zhang T-Y, Cheng X-D, Wu H-L, *et al*. Quantitative hepatitis B core antibody levels in the natural history of hepatitis B virus infection. Clin Microbiol Infect 2015;21:197–203. doi: 10.1016/j.cmi.2014.10.002.
35. Liao H, Liu Y, Li X, Wang J, Chen X, Zou J, *et al*. Monitoring of serum HBV RNA, HBcrAg, HBsAg and anti-HBc levels in patients during long-term nucleoside/nucleotide analogue therapy. Antivir Ther 2018;24:105–115. doi: 10.3851/IMP3280.
36. Song G, Yang R, Rao H, Feng B, Ma H, Jin Q, *et al*. Serum HBV core-related antigen is a good predictor for spontaneous HBeAg seroconversion in chronic hepatitis B patients. J Med Virol 2017;89:463–468. doi: 10.1002/jmv.24657.

How to cite this article: Li J, Wu Z, Wang GQ, Zhao H; China HepB-Related Fibrosis Assessment Research Group. Hepatitis B core-related antigen reflects viral replication and protein production in chronic hepatitis B patients. Chin Med J 2021;134:1160–1167. doi: 10.1097/CM9.0000000000001418