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

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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 log10 U/mL, P < 0.001) and greater HBcrAg declines (median 1.6 vs. 0.9 log10 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 log10 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.

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). 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. How-ever, it is challenging to completely eliminate hepatitis B virus (HBV) from infected hepatocytes because of the existence of intrahepatic covalently closed circular DNA (cccDNA). 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

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

Recent studies have shown that HBcrAg correlates with HBV DNA, HBsAg, HBV RNA, and intrahepatic cccDNA at baseline, especially in HBeAg-positive patients. During antiviral therapy, HBcrAg presented a gradually decreasing trend, similar to HBV DNA, HBsAg, and intrahepatic cccDNA, in both HBeAg-positive and -negative patients. A decline in HBcrAg also correlated with declines in HBV DNA, HBsAg, and intrahepatic cccDNA, but correlations between HBcrAg and other virological markers after antiviral therapy were inconsistent. The relationships between HBcrAg and grade of inflammation, stage of fibrosis, and their degrees of change before and after antiviral therapy also differed. 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. 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.

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. 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 compensated 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), 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) and sandwich enzyme-linked immunosorbent assays, 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. 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.

**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 assessed using 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 log10 IU/mL for HBcrAg, 6.6 log10 IU/mL for HBV DNA, 3.6 log10 IU/mL for HBSAg, 3.5 log10 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 log10 IU/mL for HBcrAg, 5.6 log10 IU/mL for HBV DNA, 3.3 log10 IU/mL for HBSAg, 4.2 log10 IU/mL for anti-HBC, 5.5 for HAI, and 3.0 for F score. In general, an HBeAg-positive status was associated with higher HBcrAg levels 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

| Variables | HBBeAg-positive (N=93) | HBBeAg-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  |
| TBL (μmol/L) | 13.8 (11.5, 19.6)    | 15.2 (12.3, 20.7)      | -0.878§  | 0.380  |
| HBcrAg (log10 U/mL) | 7.4 (6.4, 8.1) | 5.3 (4.6, 6.0) | -7.934§  | <0.001 |
| HBV DNA (log10 IU/mL) | 6.5 (5.5, 7.6) | 5.6 (4.5, 6.4) | -4.370§  | <0.001 |
| HBSag (log10 IU/mL) | 3.6 (3.1, 4.0) | 3.3 (3.1, 3.5) | -2.882‡  | 0.004  |
| Anti-HBc (log10 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  |
| F score  | 15/55/12/11            | 9/33/6/4               | -1.108‡  | 0.268  |
| 78-week  |                        |                        | 2/8/21/18/3 |   |
| HBcrAg (log10 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 (log10 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)              | -2.304†  | 0.065  |
| HBSag (log10 IU/mL) | 3.2 (3.0, 3.5) | 3.2 (3.1, 3.4) | -0.262‡  | 0.793  |
| Anti-HBc (log10 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  |
| F score  | 70/22/1/0              | 37/15/0                | -0.616‡  | 0.538  |
| 78-week  |                        |                        | 3/16/15/3/5 |   |
| Decline in variables |                        |                        |            |   |
| ΔHBcrAg (log10 U/mL) | 1.6 (0.9, 2.3) | 0.9 (0.3, 2.0) | -2.677‡  | 0.007 |
| ΔHBV DNA (log10 IU/mL) | 6.0 (4.7, 6.9) | 5.4 (3.7, 6.3) | -2.344‡  | 0.019 |
| ΔHBSag (log10 IU/mL) | 0.2 (-0.1, 0.7)      | 0.1 (0, 0.2)           | -2.226‡  | 0.026 |
| ΔAnti-HBc (log10 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  |
| ΔF 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; ‡ χ² 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; TBLI: 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 log10 U/mL, 6.0, 0.2, 1.0 log10 IU/mL, 3.0, and 0 in HBeAg-positive patients and 0.9 log10 U/mL, 5.4, 0.1, 1.2 log10 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 log10 U/mL, P = 0.872), but patients who experienced HBeAg loss had greater ΔHBcrAg than those who

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**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.**

| Variables | HBeAg-positive | | | HBeAg-negative | | |
|-----------|----------------|-------------------|-------------------|-------------------|-------------------|
|           | Baseline       | 78-week           | Baseline          | 78-week           | Baseline          | 78-week           |
|           | 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          | 0.023             | 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.
remained HBeAg-positive (2.3 vs. 1.3 log10 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 \( \Delta HBcrAg \) remained as an independent predictor of HBeAg loss (\( P = 0.005 \)). As a result, a cut-off value of 2.6 log10 U/mL for \( \Delta HBcrAg \) 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 log10 U/mL, respectively), lower in HBeAg-negative hepatitis (\( n = 50 \)) (4.82 log10 IU/mL), and only 2.00 log10 U/mL in the HBV-ag-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,\[12,28,29\] 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 HBcAg 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.\[14\] 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.\[33\] 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
HBcrAg may reflect both viral replication and viral protein production. In addition, a decline in HBcrAg can be used to predict HBsAg loss after antiviral therapy. HBcrAg may become a practical clinical marker, thanks to its extensive and reliable application.

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 HBsAg loss after antiviral therapy. HBcrAg may become a practical clinical marker, thanks to its extensive and reliable application.

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Conflicts of interest
None.

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