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ORIGINAL ARTICLE

Performance of serum HBcrAg in chronic hepatitis B patients with 8-year nucleos(t)ide analogs therapy

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KEYWORDS
Hepatitis B;
Serum hepatitis B core-related antigen;
Kinetics;
Nucleos(t)ide analogs therapy

Summary
Aim: This study aimed to investigate long-term kinetics of serum hepatitis B core-related antigen (HBcrAg) and its correlation with serum hepatitis B surface antigen (HBsAg) in a real-world cohort of patients who had received over 8 years of nucleos(t)ide analogs(NAs) therapy.
Methods: This was a retrospective study. All patients were recruited from our previous published study, who started therapy with NAs between 2007 and 2008. Serum HBcrAg and HBsAg levels were quantitatively measured at baseline, the sixth month and each year of follow-up, using the stored serum samples.
Results: Among the 94 patients, serum HBcrAg presented a gradually decreasing trend from baseline to year 8, either in HBeAg-negative or HBeAg-positive patients. After 8 years of NAs treatment, 21.3% of patients achieved serum HBcrAg < 3 log 10 U/mL, and only baseline HBcrAg was an independent predictor. Additionally, good correlation of HBcrAg and HBsAg was observed at baseline, but this correlation weakened remarkably during treatment.
Conclusion: Serum HBcrAg is decreasing gradually with the duration of antiviral therapy, and baseline HBcrAg level is an independent predictor of long-term HBcrAg below the limit of detection.

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Introduction

Chronic hepatitis B (CHB) affects approximately 240 million individuals worldwide, with an estimated 15%—40% progressing to end-stage liver diseases [1—3]. Due to the presence of covalently closed circular DNA (cccDNA) in the nuclei of infected hepatocytes, hepatitis B virus (HBV) cannot be completely eradicated and the persistent hepatic viral replication has been regarded as the most important risk factor for the progression of HBV-related liver diseases [4,5]. Currently, the treatment of CHB with nucleos(t)ide analogs(NAs) is often required over a prolonged period to achieve durable virologic suppression and minimal liver damage. Thus, the accurate dynamic evaluation of active viral replication in the liver would be extremely helpful to reflect the presence and risk of disease progression [6].

Because of invasive procedure and potential for sampling error, dynamic liver biopsy is not well performed in real-world clinical practice, and it has brought great difficulties to the measurement of the intrahepatic cccDNA and intrahepatic HBV DNA at different stages of the natural history of CHB [7]. Searching for convenient surrogate markers of intrahepatic viral replication activity is always a hot spot in past decades [8]. In recent years, serum hepatitis B core-related antigen (HBcrAg) is reported to be useful in reflecting intrahepatic cccDNA and predicting therapeutic effect [8—12]. HBcrAg consists of 3 species of related proteins sharing an identical 149 amino acid sequence: hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), and a truncated 22 kDa precore protein (p22Cr) [9]. Like HBeAg, p22cr is also a processed product of the precore protein, but with protein processing at both the N- and C-terminals [9]. In our previous studies, early on-treatment quantitative HBcrAg is found to be a good biomarker for predicting off-treatment HBeAg seroconversion in patients receiving PEG-IFN therapy [13]; and as compared to HBsAg, serum HBcrAg also has a better correlation with intrahepatic cccDNA [14]. In addition, among patients who undergo treatment-induced HBeAg seroconversion, low serum HBcrAg (< 3 log 10 U/mL) is closely related low relapse risk after cessation of NAs therapy, and the value of HBcrAg and HBsAg combination in predicting off-treatment viral relapse is also recommended in Japanese CHB guideline [15].

However, most of current findings of serum HBcrAg come from cross-sectional studies or cohort studies with short observation periods, and head-to-head comparison of serum HBcrAg and HBsAg kinetics is relatively rare worldwide. This study aimed to investigate the kinetics and influence factors of long-term serum HBcrAg, and also reveal the correlation between serum HBcrAg and HBsAg a real-world cohort of patients who had received over 8 years of NAs therapy.

Methods

Study design and patients

This was a retrospective study. The primary aim was to investigate the detailed dynamics of serum HBcrAg and predictor of long-term low serum HBcrAg (< 3 log 10 U/mL) in a cohort of patients who had received over 8 years of NAs therapy. The secondary aim was to reveal the correlation between serum HBcrAg and HBsAg before and during NAs therapy. All patients were recruited from our previous published study [16], who started therapy with NAs (entecavir 0.5 mg daily or adefovir dipivoxil 10 mg daily) between 2007 and 2008. Patients were followed up every 3—6 months for clinical assessment and measurement of general laboratory parameters, and serum samples were also collected at baseline and each visit time-point.

This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Approval of this study was obtained from the Ethics Committee of West China Hospital of Sichuan University, and verbal informed consent was obtained from each patient.

Laboratory measurements

Serum biochemical indexes (such as ALT and Cr levels) were measured according to standard procedures (Olympus AU5400, Olympus Corporation, Tokyo, Japan). Serum HBeAg status was determined by the electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA). Serum HBV DNA was measured using the Cobas Taqman assay (Roche Diagnostics, Branchburg, NJ). HBV genotypes were determined by direct S-gene sequencing. The PC/BCP mutations of HBV (including A1762T and G1764A) were detected using commercially available Line Probe Assays (INNOGENETICS, Belgium).

The quantification of HBcrAg was performed using fully automated Lumipulse chemiluminescence enzyme immunoassay (CLEIA) analyzer (Fujirebio Inc., Tokyo, Japan), as described previously. Because the general analytic measurement range of this assay was between 1000 U/mL (3 log10 U/mL) and 10,000,000 U/mL (7 log10 U/mL), serial dilutions of the serum sample is needed when serum HBcrAg level above the detection limit of the assay.

Serum HBsAg were quantified using the Elecsys® HBsAg II Quant Assay (Roche Diagnostics, Penzberg, Germany), with a dynamic range of 20 to 52,000 IU/mL. If qHBsAg levels > 52,000 IU/mL, samples were retested with a stepwise dilution of 1:4000.

Statistical analyses

Data were expressed as mean and standard deviation for continuous variables and as counts and percentages for categorical variables. The differences between continuous variables were analyzed using the Student's *t*-test or Mann—Whitney test, as appropriate; and the comparison of continuous variables before and after antiviral therapy was analyzed using paired samples T test, considering a *P*-value < 0.05 as statistically significant. The correlation between two continuous variables was analyzed using Spearman's bivariate correlation, and the correlation is significant at the 0.01 level (2-tailed). In this study, the area under receiver operating characteristics (AUROC) was also used to assess the accuracy of the independent factors and to identify optimal cutoff value for the prediction of serum HBcrAg < 3 log 10 U/mL at the 8-year of therapy; and the optimal cutoff values were chosen based on a maximum sum

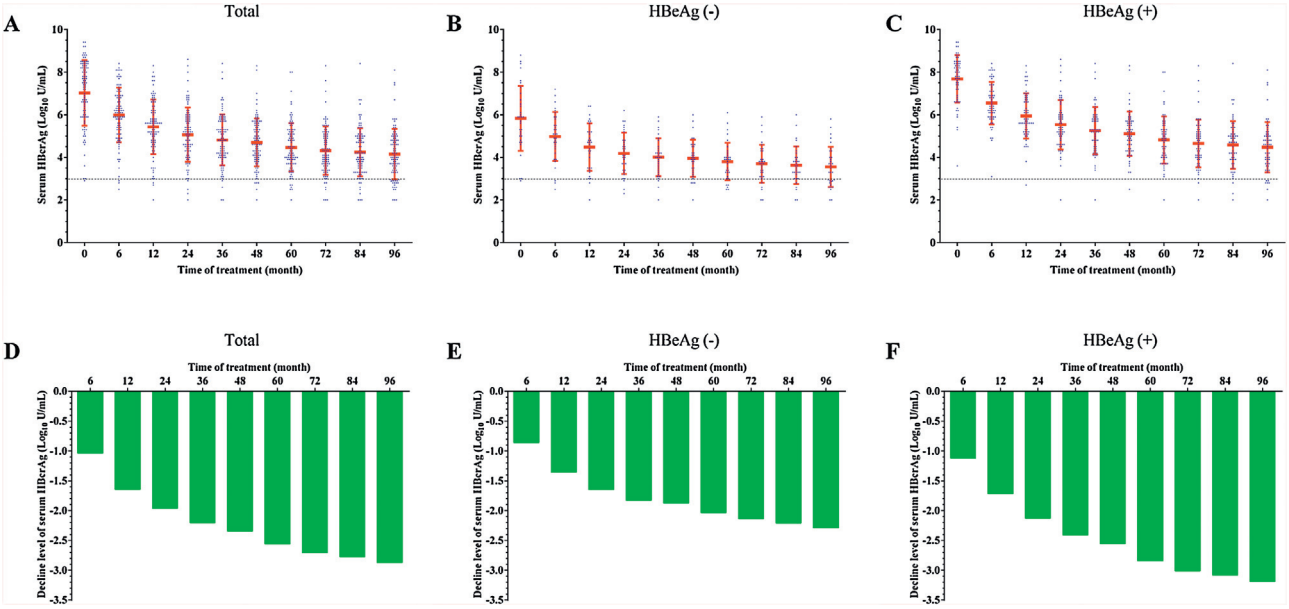

Figure 1 Long-term kinetics of serum hepatitis B core-related antigen (HBcrAg) from baseline to year 8 among patients receiving nucleos(t)ide analogs(NAs) therapy. Either total (A and D), hepatitis B e antigen (HBeAg)-negative (B and E) or HBeAg-positive (C and F) patients, the serum HBcrAg presented a gradually decreasing trend from baseline to year 8.

of sensitivity and specificity. A P-value < 0.05 was considered to indicate statistical significance. All statistical analyses were done with SPSS Version 18.0 (SPSS, Chicago, IL), and figures were drawn using GraphPad Prism 6 (GraphPad Software Inc., California, USA).

Results

General information

A total of 94 patients were included, including 46 patients with ETV and 48 patients with ADV therapies. The mean age was 36 years, 69 (73.40%) patients were male, 53 (56.4%) patients had family history of HBV infection, and 10 (10.6%) patients had cirrhosis. Sixty-one (64.9%) patients were HBeAg-positive, mean HBV DNA was 7.4 log10 copies/mL, and mean HBsAg was 3.80 log 10 IU/mL. In this cohort, the genotype and PC/BCP mutations of HBV were also detected. There were 63 (67.0%) patients with genotype B and 31 (33.05) patients with genotype C HBV infection, and BCP/C mutations were observed in 32 (34.0%) patients. After 8 years of therapy, 89.36% (84/94) patients achieved HBV DNA negativity and ALT normalization; and HBeAg seroconversion was achieved in 65.57% (40/61) of patients with positive-HBeAg at baseline. And the kinetic of HBV DNA negativity and ALT normalization were shown in Appendix A, Supplemental Figure 1.

Kinetics of serum HBcrAg from baseline to year 8 among patients receiving NAs therapy

The total serum HBcrAg presented a gradually decreasing trend from baseline to year 8. As shown in Fig. 1, the mean level of serum HBcrAg was 7.02 log10 U/mL at baseline; and the mean level of serum HBcrAg at month 6, 12, 24, 36, 48, 60, 72, 84 and 96 was 5.99, 5.43, 5.06, 4.82, 4.70, 4.46, 4.32, 4.25, and 4.15 log10 U/mL, respectively (P < 0.05 for all paired comparisons between each two time-points, except for month 72 and 84) (Fig. 1A). Compared to baseline, serum HBcrAg levels decreased by a mean of 1.03, 1.59, 1.96, 2.20, 2.32, 2.56, 2.71, 2.77 and 2.87 log10 U/mL at 6, 12, 24, 36, 48, 60, 72, 84 and 96 months of antiviral therapy, respectively (Fig. 1D).

Either in HBeAg-negative or HBeAg-positive patients, the serum HBcrAg also presented a gradually decreasing trend from baseline to year 8. In HBeAg-negative patients, the mean level of serum HBcrAg was 5.84 log10 U/mL at baseline; and the mean level of serum HBcrAg at month 6, 12, 24, 36, 48, 60, 72, 84 and 96 was 4.98, 4.48, 4.19, 4.01, 3.96, 3.80, 3.70, 3.63, and 3.55 log10 U/mL, respectively (Fig. 1B). Compared to baseline, serum HBcrAg levels decreased by a mean of 0.86, 1.36, 1.65, 1.83, 1.88, 2.04, 2.14, 2.21 and 2.29 log10 U/mL at 6, 12, 24, 36, 48, 60, 72, 84 and 96 months of antiviral therapy, respectively (Fig. 1E).

In HBeAg-positive patients, the mean level of serum HBcrAg was 7.66 log10 U/mL at baseline; and the mean level of serum HBcrAg at month 6, 12, 24, 36, 48, 60, 72, 84 and 96 was 6.54, 5.94, 5.53, 5.25, 5.10, 4.82, 4.65, 4.58, and 4.48 log10 U/mL, respectively (Fig. 1C). Compared to baseline, serum HBcrAg levels decreased by a mean of 1.12, 1.72, 2.13, 2.41, 2.56, 2.84, 3.01, 3.08 and 3.19 log10 U/mL at 6, 12, 24, 36, 48, 60, 72, 84 and 96 months of antiviral therapy, respectively (Fig. 1F).

Kinetics of serum HBcrAg among patients with or without HBeAg seroconversion

As shown in Fig. 2, the mean levels of serum HBcrAg in patients with HBeAg seroconversion were lower than those

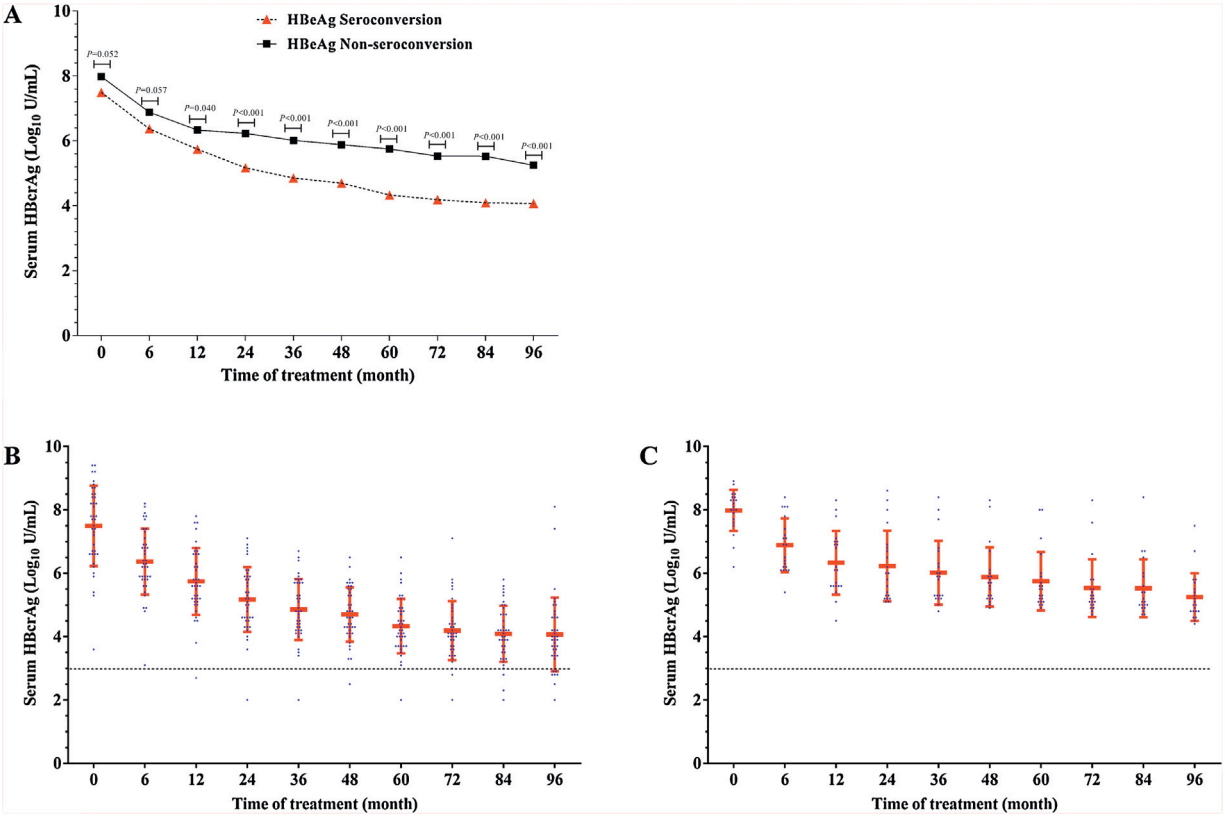

Figure 2 Kinetics of serum hepatitis B core-related antigen (HBcrAg) among patients with or without hepatitis B e antigen (HBeAg) seroconversion. Mean serum HBcrAg levels in patients with HBeAg seroconversion were lower than those without HBeAg seroconversion from baseline to month 96 of antiviral therapy (A). The distribution of serum HBcrAg levels in patients with HBeAg seroconversion (B) and without HBeAg seroconversion (C) were also shown.

without HBeAg seroconversion from baseline to month 96 of antiviral therapy (Fig. 2A). Among the 40 patients who achieved HBeAg seroconversion at year 8, the mean level of serum HBcrAg was 7.50 log10 U/mL at baseline; and the mean level of serum HBcrAg at month 6, 12, 24, 36, 48, 60, 72, 84 and 96 was 6.37, 5.74, 5.17, 4.86, 4.70, 4.33, 4.19, 4.09, and 4.07 log10 U/mL, respectively (Fig. 2B). While among the 21 patients who did not achieve HBeAg seroconversion at year 8, the mean level of serum HBcrAg was 7.98 log10 U/mL at baseline; and the mean level of serum HBcrAg at month 6, 12, 24, 36, 48, 60, 72, 84 and 96 was 6.88, 6.33, 6.23, 6.01, 5.88, 5.75, 5.53, 5.52, and 5.25 log10 U/mL, respectively (Fig. 2C). Between patients with or without HBeAg seroconversion, the difference of serum HBcrAg levels were not significantly at baseline ($P = 0.052$) and at month 6 ($P = 0.057$) time-points, but significantly from month 12 to 96 (all $P < 0.05$).

Kinetics of serum HBcrAg stratified by demographic, clinical, and laboratory variables

As shown in Table 1, the levels of serum HBcrAg were similar between patients with different age (≥ 40 vs. < 40), gender (male vs. female), family history of hepatitis B (yes vs. no), HBV genotype (B vs. C), HBV BCP/C mutation (yes vs. no) and ALT levels (≥ 5*ULN vs. < 5*ULN), either at baseline or at year 8 of antiviral therapy (all $P > 0.05$). However, baseline HBcrAg levels were significantly lower in patients with low HBV DNA as compared to patients with high HBV DNA (6.04 vs. 7.69, $P < 0.001$); and similar lower HBcrAg was also observed in patients with low HBsAg than patients with high HBsAg (5.99 vs. 7.93, $P < 0.001$). While at year 8 of antiviral therapy, serum HBcrAg were still significantly lower in patients with low baseline HBsAg than with high baseline HBsAg, but not significantly between patients with high baseline HBV DNA and patients with low baseline HBV DNA. Those findings suggested that the baseline HBsAg levels may affect the kinetics of serum HBcrAg during long-term NAs therapy.

Predictor of serum HBcrAg < 3 log 10 U/mL at year 8 of antiviral therapy

The univariate and multivariate analysis results were shown in Table 2. As presented in univariate analysis, lower HBV DNA, lower HBsAg, lower HBcrAg and high frequency of HBeAg-negative status at baseline were significantly more likely in patients with HBcrAg < 3.0 log 10 U/mL than HBcrAg $\geq$ 3.0 log 10 U/mL at year 8. However, there were no statistically significant differences in age, gender, BMI, family history of hepatitis B, cirrhosis, antiviral agents, HBV preC/BCP mutation, baseline ALT, baseline Cr, extent of HBcrAg decline at month 6, and extent of HBcrAg decline at month 12 between patients with HBcrAg < 3.0 log 10 U/mL

Table 1 Kinetics of serum HBcrAg stratified by demographic, clinical, and laboratory variables.

Variables	Baseline		Year 8	
	HBcrAg (log10 U/mL)	*P*-value	HBcrAg (log10 U/mL)	*P*-value
Age (years)				
≥ 40 (*n* = 29)	6.70 ± 1.75	0.183	3.89 ± 1.25	0.164
< 40 (*n* = 65)	7.16 ± 1.43		4.26 ± 1.15	
Gender				
Male (*n* = 69)	7.01 ± 1.56	0.944	4.26 ± 1.26	0.138
Female (*n* = 25)	7.04 ± 1.51		3.85 ± 0.91	
Family history of hepatitis B				
Yes (*n* = 53)	7.03 ± 1.36	0.928	4.17 ± 1.05	0.880
No (*n* = 41)	7.00 ± 1.76		4.13 ± 1.35	
HBV genotype				
B(*n* = 64)	7.02 ± 1.51	0.996	4.13 ± 1.23	0.798
C(*n* = 30)	7.02 ± 1.62		4.20 ± 1.09	
HBV BCP/C mutation				
Yes(*n* = 32)	6.69 ± 1.52	0.132	3.80 ± 0.96	0.039
No (*n* = 62)	7.19 ± 1.53		4.33 ± 1.25	
Serum ALT (∗ULN, IU/L)				
≥ 5 (*n* = 19)	7.06 ± 1.20	0.908	4.25 ± 1.00	0.689
< 5 (*n* = 75)	7.01 ± 1.62		4.12 ± 1.23	
Serum HBV DNA (log10 copies/mL)				
≥ 7.3 (*n* = 56)	7.69 ± 1.16	0.000	4.34 ± 1.19	0.064
< 7.3 (*n* = 38)	6.04 ± 1.51		3.88 ± 1.13	
Serum HBsAg (IU/mL)				
≥ 5000 (*n* = 50)	7.93 ± 0.97	0.000	4.53 ± 1.23	0.001
< 5000 (*n* = 44)	5.99 ± 1.41		3.72 ± 0.98	

HBcrAg: hepatitis B core-related antigen; HBsAg: hepatitis B surface antigen.

Table 2 Analysis of factors influencing serum HBcrAg levels at year 8 of NAs therapy using univariate and multivariate analysis.

Variables	Serum HBcrAg		Univariate		Multivariate	
	< 3 log 10 U/mL (*n* = 20)	≥ 3 log 10 U/mL (*n* = 74)	t/χ^2	*P*-value	OR (95% CI)	*P*-value
Age (years)	38.35 ± 10.06	35.86 ± 6.45	−1.048	0.305	—	—
Male gender	14 (70.0)	55 (74.3)	0.151	0.698	—	—
Body mass index (kg/m^2)	23.56 ± 2.82	22.68 ± 2.43	1.274	0.214	—	—
Family history of hepatitis B	8 (40.0)	45 (60.8)	2.773	0.096	—	—
Cirrhosis	4 (20.0)	6 (8.1)	2.342	0.212	-	-
Antiviral agents (ETV/ADV)	11 (55.0)/9 (45.0)	37(50.0)/37 (50.0)	0.158	0.691	—	—
HBV genotype (B/C)	13 (65.0)/7 (35.0)	51 (68.9)/23 (31.1)	0.111	0.739	—	—
HBV preC/C mutation	9 (45.0)	23 (31.1)	1.358	0.244	—	—
Baseline ALT (∗ULN, U/L)	3.35 ± 1.84	3.84 ± 2.43	0.842	0.402	—	—
Baseline Cr (μmol/L)	69.40 ± 12.35	68.74 ± 9.50	−0.257	0.798	—	—
Baseline HBV DNA (log10 copies/mL)	6.40 ± 1.50	7.73 ± 1.44	3.612	< 0.001	0.732	0.224
Baseline HBsAg (log10 IU/mL)	3.31 ± 0.57	3.93 ± 0.62	4.002	< 0.001	0.196	0.353
Baseline negative HBeAg	13 (65.0)	20 (27.0)	9.965	0.002	0.835	0.825
Serum HBcrAg (log 10 U/mL)						
Baseline	5.58 ± 1.69	7.41 ± 1.25	5.401	< 0.001	0.277	0.005
Extent of decline at month 6	0.96 ± 0.85	1.05 ± 0.78	−0.430	0.668	0.359	0.439
Extent of decline at month 12	1.46 ± 1.14	1.63 ± 1.19	−0.563	0.575	0.460	0.386

HBcrAg: hepatitis B core-related antigen; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen.

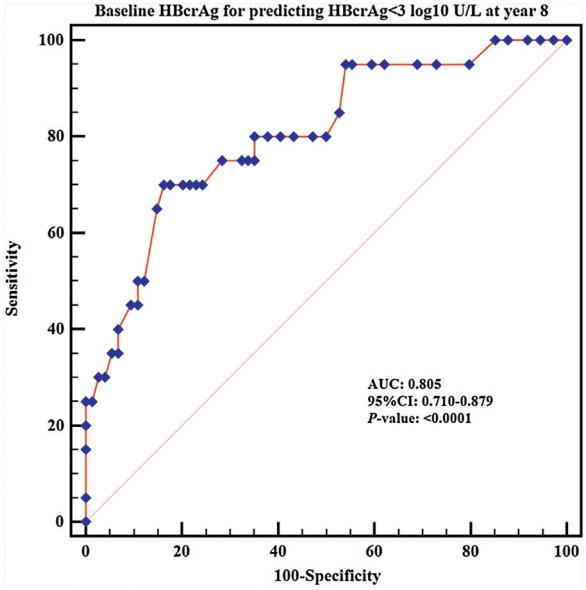

Figure 3 AUROC of baseline hepatitis B core-related antigen (HBcrAg) for predicting HBcrAg < 3.0 log 10 U/mL at year 8.

and ≥ 3.0 log 10 U/mL at year 8. In multivariate analysis, only baseline HBcrAg was the significant independent factor for predicting HBcrAg < 3.0 log 10 U/mL at year 8 of antiviral therapy. An unexpected observation in the multivariate analysis was that the baseline HBV DNA, HBsAg and HBeAg-negative status were not predictors of the outcomes of HBcrAg < 3.0 log 10 U/mL at year 8 of antiviral therapy.

As shown in Fig. 3, the AUROC of baseline HBcrAg for predicting HBcrAg < 3.0 log 10 U/mL at year 8 was 0.805 (95% CI: 0.710—0.879), and the optimal cutoff values of baseline HBcrAg suggested by ROC curves analysis was 6.0 log 10 U/mL. The cutoff value of baseline HBcrAg ≤ 6.0 log 10 U/mL (70.0% sensitivity and 83.8% specificity) had a positive predictive value (PPV) of 53.8% and a negative predictive value (NPV) of 91.2%.

Correlation of serum HBcrAg and HBsAg

The correlations of serum HBcrAg and HBsAg before and during antiviral therapy were shown in Fig. 4. Among the total 94 patients at baseline, the serum HBcrAg level was positively associated with serum HBsAg level ($r = 0.713$). And the good correlation of serum HBcrAg level and serum HBsAg level was also observed in either HBeAg-negative patients ($r = 0.687$) or HBeAg-positive patients ($r = 0.665$) at baseline.

At year 4 of antiviral therapy, the positive correlations of serum HBcrAg and HBsAg were weakened ($r = 0.326$ for total patients, $r = 0.327$ for HBeAg-negative patients, and $r = 0.401$ for HBeAg-positive patients). Similar poor correlations of serum HBcrAg level and HBsAg were also observed at year 8 of antiviral therapy ($r = 0.351$ for total patients, $r = 0.411$ for HBeAg-negative patients, and $r = 0.385$ for HBeAg-positive patients).

Discussion

In recent years, quantification of serum HBV-related proteins (such as HBsAg, HBcrAg and anti-HBc) has been regarded as complementary tools for comprehensive evaluation of on-treatment responses and identifying patients at risk of disease progression and viral relapse after discontinuing therapy [8,17,18]. Importantly, serum qHBcrAg is also a satisfactory surrogate of intrahepatic HBV cccDNA in CHB patients [14,19]. In this retrospective study, the detailed 8-year kinetics of serum HBcrAg in 94 patients was analyzed. The major findings from present study are:

- serum HBcrAg presented a gradually decreasing trend from baseline to year 8, either in HBeAg-negative or HBeAg-positive patients;
- On-treatment HBcrAg levels were significantly lower in patients with HBeAg seroconversion than patients without HBeAg seroconversion;
- Serum HBcrAg < 3 log 10 U/mL was observed in 21.3% of patients at year 8, and baseline HBcrAg was an independent predictor;
- Good correlation of HBcrAg and HBsAg was observed at baseline, but this correlation weakened remarkably during treatment.

As we known, serum HBV DNA is well correlated with intrahepatic cccDNA in the natural course but not under NAs therapy, because intrahepatic cccDNA decline did not parallel the rapid decrease of serum HBV DNA during a relative short duration of NAs therapy. In contrast, we have observed that serum HBcrAg has a significant positive correlation with intrahepatic cccDNA regardless of antiviral therapy, and this correlation is superior to that of serum HBsAg with intrahepatic cccDNA [14]. And results from other studies also have suggested that serum HBcrAg is a reliable and very sensitive surrogate marker to reflect the cccDNA content [19,20]. Part of the reason for this good performance of HBcrAg lies in the production of HBcrAg depending on the transcription of mRNA from cccDNA and little affection by NA transcriptase inhibition. Thus, the marked decline of serum qHBcrAg observed in present study indicated a satisfactory control of intrahepatic cccDNA and reduced risk of disease progression. In addition, the results of 7-year treatment outcome of entecavir in another real-world cohort suggested that the decline of HBsAg with treatment was suboptimal, and the decline of serum HBcrAg was better than that of serum HBsAg [21]. In recent years, serum HBV RNA as a new serum marker of HBV also has been widely concerned. Though the baseline serum HBV RNA level or its decline after 96 weeks of NAs therapy correlated with the corresponding intrahepatic cccDNA level, it was less than that seen with serum HBV DNA at baseline and HBsAg (or its decline) at 96 weeks after treatment, respectively [22].So, the gradually decreasing of on-treatment HBcrAg may also suggest a sustained and cumulative therapeutic benefit of long-term NAs therapy.

The therapeutic HBeAg seroconversion is widely considered a satisfactory endpoint for CHB treatment, which has been mentioned in several professional guidelines [23,24]. Additionally, the time to achieving HBeAg seroconversion also has a great influence on the long-term prognosis of patients [25,26]. Thus, to ensure maximum benefit from antiviral therapy, for those who have obtained sustained HBV DNA suppression but persistent HBeAg-positive state, treatment strategy needs to be adjusted appropriately for achieving HBeAg seroconversion as fast as possible [27,28].

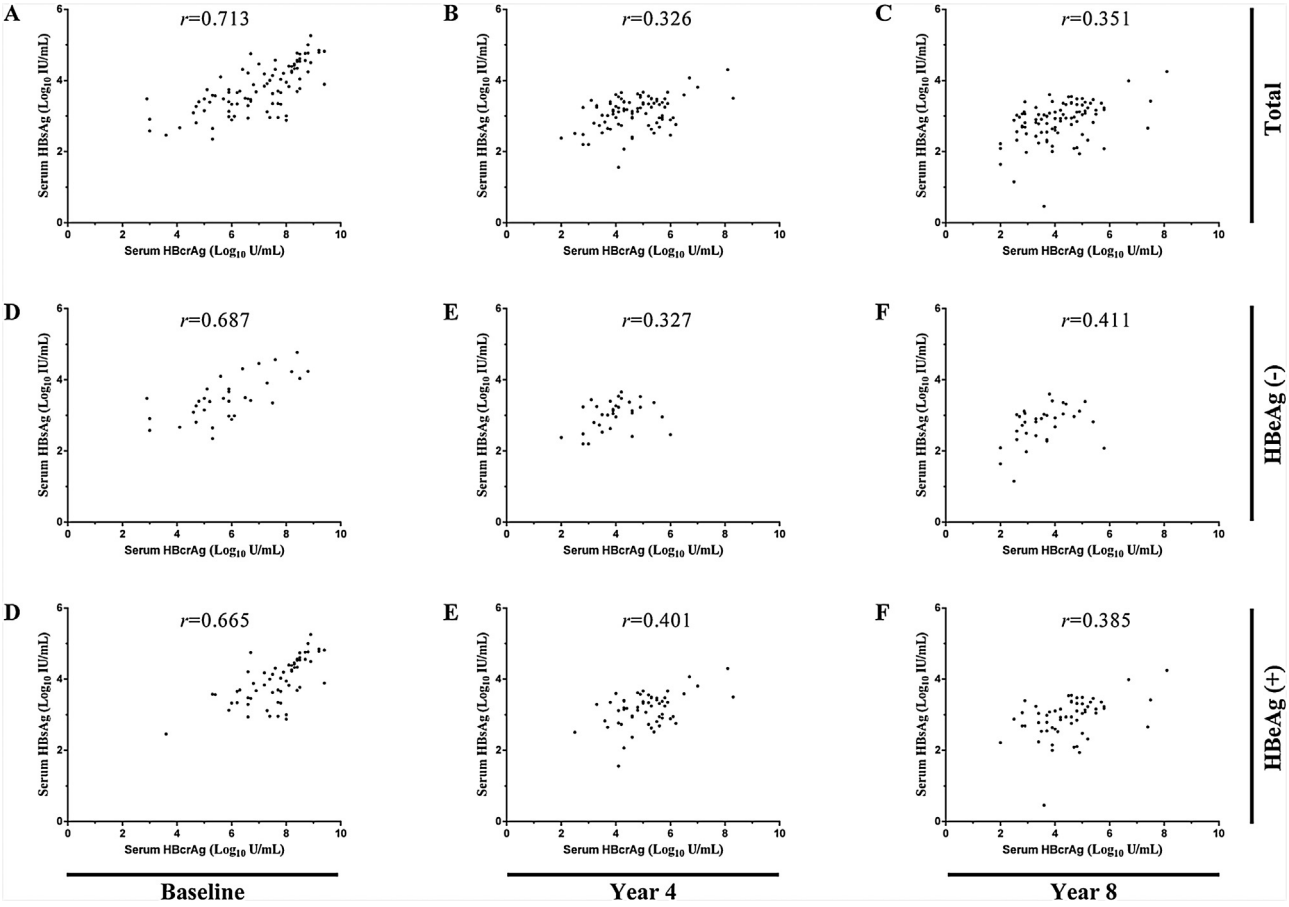

Figure 4 Correlation of serum hepatitis B core-related antigen (HBcrAg) and hepatitis B surface antigen (HBsAg) at baseline, year 4 and 8 of antiviral therapy [A, B and C for total patients; D, E and F for hepatitis B e antigen (HBeAg)-negative patients; and D, E and F for HBeAg-positive patients].

Besides, it is also very important to recognize CHB patients who are prone to develop therapeutic HBeAg seroconversion [29]. In present study, we found that the on-treatment serum HBcrAg were significantly lower in patients who achieved HBeAg seroconversion than those without HBeAg seroconversion. Additionally, our study of 31 HBeAg-positive patients showed that the kinetics of early on-treatment serum HBcrAg during PegIFN-α therapy was a stronger and earlier predictor of serological response than serum HBsAg [13]. Thus, to some extent, our finding indicated that significant reduction of on-treatment HBcrAg may suggest a high probability of HBeAg seroconversion in future. And monitoring the dynamic alteration of serum HBcrAg levels would also help to reflect the host immune responses against HBV infection.

In present study, the poor correlations of serum HBcrAg and HBsAg at year 4 or 8 of antiviral therapy suggested the two indicators cannot be replaced by each other in evaluation of antiviral effects. For patients who fulfilled the conditions required for cessation of NAs therapy, viral relapse risk was reported to be lower for those with low HBcrAg levels. In Japan CHB guideline [15], serum HBcrAg and HBsAg levels at therapy cessation had been recommended for assessing the risk of relapse in future. Patients with serum HBcrAg < 3 log10 U/mL and HBsAg < 80 IU/mL were regarded as low risk group of relapse [15]. In present study, we found that high to 21.3% (20/94) of patients achieved serum HBcrAg < 3.0 log 10 U/mL at year 8; and baseline serum HBcrAg level was a good independent predictor of serum HBcrAg < 3.0 log 10 U/mL at year 8 and the optimal cutoff values of baseline HBcrAg was 6.0 log 10 U/mL. In this cohort, after 8 years of antiviral therapy, only 3.2% of patients were graded as low risk of relapse after NAs withdrawal, but high to 62.8% of patients were still graded as high risk of relapse (data unshown). Thus, for more patients obtaining a chance to stop treatment with low relapse risk, the antiviral strategy may be optimized for those NAs-treated patients with potential poor response to HBcrAg and HBsAg (such as pegIFNα add-on or switch-to pegIFNα).

Our study has several limitations. This was a retrospective single-center study with relatively small sample size. So, large multicenter cohort studies are required to confirm present findings. Though PreC/BCP mutation showed no significant impact on serum HBcrAg distribution at baseline, patients with PreC/BCP mutation had significantly lower levels of HBcrAg at year 8 than patients without PreC/BCP mutation. As PreC/BCP mutations may influence the expression of HBeAg, the precise impact of PreC/BCP mutations on serum HBcrAg still needed to be clarified in future.

Conclusion

Our results indicate that serum HBcrAg presents a gradually decreasing trend from baseline to year 8 in CHB patients receiving NAs therapy, and baseline HBcrAg is an independent predictor of HBcrAg < 3 log 10 U/mL at year 8. In addition, serum HBcrAg and HBsAg is significantly correlated at baseline, but this correlation is weakened remarkably during treatment, which suggests that serum HBcrAg should be a new indicator in evaluation of antiviral effects, independent of serum HBsAg. Though some questions still need to be clarified, the value of HBcrAg is highlighted in predicting therapeutic effect and clinical outcome of disease.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.clinre.2018.10.020.

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