Editorial

Hepatitis B virus core-related antigen is a serum prediction marker for hepatocellular carcinoma

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Introduction

Serum hepatitis B virus (HBV)-DNA is a useful measurement for chronic hepatitis B (CH-B) patients and can help determine when to start antiviral therapies, such as nucleos(t)ide analogues (NAs) and interferon (IFN), as well as monitoring of the antiviral effects of therapy. It is reported that the development of advanced chronic liver disease, cirrhosis, and the incidence of hepatocellular carcinoma (HCC) can be prevented by decreasing serum HBV-DNA levels. Serum HBV-related markers can be conveniently measured along with other serum liver function markers such as AST, ALT, rGTP, and ALP at regular intervals from small serum samples. However, serum HBV-DNA levels in many cases during NAs treatment are below detection limits despite the presence of HBV-related proteins in patients' hepatocytes. HBV-DNA decrease is not the aim of therapy in recent years; rather, the absence of HBs antigen has become essential. This indicates that HBs antigen loss is associated with intrahepatic HBV loss, so a HBV marker that reflects the production of intrahepatic HBV is needed. Kimura *et al*. reported that HBV core-related antigen (HBcrAg) reflects HBcAg in Dane particles, p22cr in empty particles, and HBeAg, and is detected by CLEIA method after pretreatment to inactivate the antibodies (1). The results of the study suggested that most Dane particles lack viral HBV-DNA and core capsids, but contain p22cr (2). This study additionally provided a model for the formation of the HBV-DNA-negative Dane particles. The precore proteins, which lack the arginine-rich nucleotide-binding domain, form viral RNA/DNA-negative capsid-like particles and are enveloped and released as empty particles. To measure HBcrAg, antibodies such as HBeAb and HBcAb are inactivated and HBV core antigen (HBcAg) is contained within the Dane particles. However, HBcAg cannot be directly detected in serum, unlike HBcAb, which indicates only past and current infection status. The lower limit of detection of HBcrAg is 3.0 logU/mL and the upper limit is 7.0 logU/mL, and detection reflects the production of intrahepatic HBV including HBV cccDNA, which if eradicated, corresponds with decreased HBcrAg in serum. HBcrAg includes HBeAg but is of little use in HBeAg-positive patients. On the other hand, measurement is useful when serum HBV-DNA cannot be detected and the patient has a negative HBeAg and positive HBeAb status. Previous reports showed that there is a significant correlation between intrahepatic HBV cccDNA and the detection of serum HBcrAg (3,4). Moreover, HBcAg staining in liver tissue has been found to be associated with serum HBcrAg (2).

HBcrAg is a useful serum marker for estimating the intrahepatic replication of HBV, and if it cannot be detected during NAs therapy, HBV is considered to be completely suppressed, meaning that therapy can be discontinued. For example, this may occur when HBV-DNA is suppressed and the patient's transaminase levels remain within the normal range after several months of NAs treatment. Conversely, if the value is above the normal range for HBcrAg, it shows that HBV-related proteins are being produced and NAs treatment should not be discontinued because hepatitis would recur. Some reports have shown that discontinuation of NAs does not lead to reactivation in

Table 1 HBcrAg in clinical studies of HBV-related HCC

Study	Publication year	Population	HCC cases	NAs treatment	Mean duration (years)	HCC incidence in HBcrAg-positive patients compared with negative patients
Hosaka *et al*. (14)	2010	1,149	55 (recurrence: 21)	Yes	2.2	NR (analyzed for recurrence: P<0.01)
Akuta *et al*. (15)	2014	1,610	270 (AFP: >11 mg/L)	No	6.0	P<0.05 (AFP >11 mg/L, HCC incidence for AFP high groups: P<0.001)
Honda *et al*. (16)	2016	109	36	Yes	6.5	P<0.05
Tada *et al*. (17)	2016	1,031	78	No	10.7	P<0.001

AFP, α-fetoprotein; HBV, hepatitis B virus; HBcrAg, HBV core-related antigen; HCC, hepatocellular carcinoma; NAs, nucleos(t)ide analogues; NR, not reported.

cases of low HBcrAg (5,6). In these patients, HBV cccDNA may be at a low level and unable to produce HBV-related antigens including HBcAg in Dane particles. In fact, very little HBV cccDNA can be detected in liver tissue in these cases, and it is undetectable by RTD-PCR-based assays after the isolation of genomic DNA (3). Rokuhara *et al*. compared levels of HBcrAg with HBV-DNA levels during lamivudine treatment and found higher viremia in HBcrAg-positive patients. The authors concluded that HBcrAg assay is a sensitive and useful test for assessing HBV load (7). Shinkai *et al*. reported that HBcrAg is useful for predicting relapses after discontinuation of lamivudine treatment (6). They found that HBcrAg levels of <3.4 logU/mL when lamivudine was discontinued represented the only independent factor that predicted the absence of post-treatment relapse. Furthermore, Matsumoto *et al*. reported in 2007 that HBcrAg could be a useful marker for identifying patients who are not at risk of reactivation of severe hepatitis after discontinuation of lamivudine (5). The authors found that HBcrAg serum levels were significantly higher (P<0.01) in patients who experienced reactivation, and lower levels indicated a lower risk. Matsuzaki *et al*. reported that HBcrAg is a useful HBV re-infection marker after liver transplantation (4). Moreover, they highlighted that maintaining negative levels of HBcrAg and HBV cccDNA might contribute to long-term graft survival. Seto *et al*. analyzed and characterized both linearized hepatitis B surface antigen (HQ-HBsAg) and HBcrAg during the natural history of CH-B (8). They showed that the measurement of these markers is crucial and they can be detected even in cases of HBsAg sero-clearance. Maasoumy *et al*. suggested that HBcrAg might be an additional marker of HBV infection (9). The group performed a study of a European cohort in HBeAg-negative patients predominantly infected with genotypes A and B, and concluded that HBcrAg might help to distinguish between inactive carriers and cases of active disease. In 2015, Matsumoto *et al*. conducted a retrospective study of factors associated with the patient outcome of IFN sequential therapy, which was established so that NAs could be discontinued (10). The authors concluded that the combined detection of HBsAg and HBcrAg levels might be useful for predicting the 24-month outcome of sequential therapy. Chuaypen *et al*. compared HBcrAg against quantitative HBsAg in patients with HBeAg-positive CH-B receiving PEG-IFN therapy (11). They concluded that HBcrAg levels measured during PEG-IFN therapy might help identify patients with a very low probability of response, which is comparable with, if not better than, quantitative HBsAg. van Campenhout *et al*. measured HBcrAg among patients treated with NAs with or without PEG-IFN add-on therapy (12). The result showed that the decline in HBcrAg was stronger in patients given combined therapy than in those given NAs alone. The authors also mentioned that HBcrAg was associated with a combined response but was not superior to quantitative HBsAg. Zhang *et al*. compared HBcrAg values and pathological status using clinical liver tissues (13). They concluded that HBcrAg could predict severe necroinflammation and advanced fibrosis in HBeAg-positive patients, as well as significant necroinflammation and fibrosis in HBe-negative patients.

HBcrAg measurement is reported to be useful for predicting HCC incidence in CH-B patients (*Table 1*). The reports show a direct or indirect association between HBcrAg and HCC incidence, whether under NAs treatment, or via observation of the natural course of the disease. Hosaka *et al*. first reported the association of HBcrAg with HCC and analyzed the risk factors recurrent during NAs treatment (14). An analysis of intrahepatic HBV cccDNA showed that high levels were associated with HCC incidence, and the authors concluded that HBcrAg was a risk indicator of HCC recurrence. Akuta *et al*. investigated the relationship between HCC and HBsAg (15). The authors compared HBsAg and

AFP and found an association between low HBsAg and low HBcrAg; in these cases, HCC did not develop. The group measured AFP at the start of their observations and reported that initial high levels (>10 μg/L) are associated with HCC incidence (P<0.001).

We introduced the significance of measuring HBcrAg and related it to intra-hepatic HBV replication and the development of HCC (16). We could not find any significant association between HBcrAg values and cancer incidence before NAs treatment. On the other hand, we observed a greater incidence of HCC in HBcrAg-positive cases compared with negative cases during NAs treatment, and HCC incidence was associated with liver fibrosis status using the FIB-4 index. The incidence of HCC cannot be accurately assessed if patients are still HBeAg-positive after NAs administration and if minor seroconversion has not taken place. However, many of our cases exhibited minor seroconversion, and we were able to compare HCC and non-HCC cases with HBeAg-negative status. Using microarray analysis, we found that transcriptional factors including HNF4α, PPARα, and LRH1 are upregulated in HBcrAg-positive livers. The overexpression of HBV precore/core in HepG2 increased *in vitro*, but these transcription factors were reduced by Metformin in HBV-infected primary hepatocytes.

In an analysis of CH-B patients not treated with antivirals, Tada *et al*. reported that the development of HCC is associated with the elevation of HBcrAg levels (17). They found that the elevation of both HBcrAg and basic core promoter (BCP) mutation were independently associated with the incidence of HCC. Moreover, time-dependent ROC analysis showed that HBcrAg is superior to HBV-DNA in terms of its prediction of the development of HCC during the follow-up period. This data indicated that HBcrAg is better at predicting HCC incidence at all times compared with serum HBV-DNA. These results suggest that HBcrAg is closely associated with the production of intrahepatic HBV proteins.

Tada *et al*. collected information on 3,122 HBV-positive patients and excluded patients under treatment before conducting a final analysis on 1,031 patients that fulfilled the criteria. The subjects were 473 female and 558 male patients. During the follow-up period, 162 developed cirrhosis and 78 developed HCC. The cumulative HCC incidence was 2.0% for 5 years, 8.3% for 10 years, 10.7% for 15 years, and 12.5% for 20 years. In addition, they conducted multivariate analysis and found that HBcrAg (HR 5.05; 95% CI, 42.40–10.63; P<0.001) and BCP mutation (HR 28.85; 95% CI, 4.00–208.20; P<0.001) were associated with the incidence of HCC. They then analyzed the cumulative incidence of HCC combined with HBcrAg and BCP status and found that patients with HBcrAg <2.9 logU/mL and BCP wild status represented 0% of the incident rate of HCC even up to 20 years later. Moreover, HBcrAg >2.9 logU/mL and BCP mutant status was 6.4% for 5 years, 25.0% for 10 years, 30.1% for 15 years, and 30.4% for 20 years, suggesting that positivity for both represents a definite risk for HCC. They also analyzed HBV-DNA and found that values of >5 log copies/mL were significantly associated with the incidence of HCC; however, values of <5 log copies/mL showed no significant correlation. Multivariate analysis of HBV genotypes, HBV-DNA, HBcrAg (cut off >2.9 logU/mL), HBeAg, and BCP mutation, showed that HBcrAg of >2.9 logU/mL and BCP mutation were associated with the risk of HCC incidence. In an analysis of HCC incidence among patient subgroups of those with low HBsAg levels (<3 logU/mL) and HBV genotype C, it was found that in both subgroups, HBcrAg levels of >2.9 logU/mL were significantly associated with the incidence of HCC. They characterized a subgroup that had HBV-DNA levels <4.0 log copies/mL, and were HBeAg negative and non-cirrhotic (n=581). Among this subgroup, 17 patients developed HCC and had HBcrAg levels of >3.7 logU/mL, but not HBcrAg >2.9 logU/mL or HBsAg >3 logU/mL, showing that high HBcrAg levels were significantly associated with the incidence of HCC. On the other hand, among 376 patients with HBV-DNA >4 log copies/mL, any HBeAg status, and an FIB-4 index of <3.6, 36 developed HCC and there was significant association with HBcrAg >2.9 and >3.7 logU/mL groups. A time-dependent ROC curve illustrated the levels of HBcrAg and HBV-DNA alongside the incidence of HCC at 2, 3, 4, 5, 6, 7, 8, 9, and 10 years, and they found that the AUCs were greater for HBcrAg than HBV-DNA at all points in time, suggesting that HBcrAg provides a superior estimation of HCC incidence than HBV-DNA. However, one limitation of the study was that it was conducted within a single hospital-based population and they suggested that data should be collected from community-based populations. Another limitation was that none of the CH-B patients received NAs, which means that this study involved a low-risk cohort. They concluded that HBcrAg levels are associated with the development of HCC in CH-B patients not receiving antiviral therapy.

More than 10 years have passed since the introduction of HBcrAg measurement in Japan, and its value in patients under NA treatment via the analysis of many HBV-infected patients has been reported. The most useful purpose

of these evaluations has been to help clarify when to discontinue NAs in inactive hepatitis patients. However, the advantage of this marker is diminished in cases of persistent HBeAg positivity, because the assay detects HBeAg in addition to HBcAg. This marker is associated with HCC incidence, especially in cases of HBeAg-negative and HBeAb-positive patients. As this paper has reported, even in non-treated cases, HBcrAg is more useful for estimating cancer risk than other markers, such as HBV-DNA using ROC analysis.

Conclusions

Serum HBcrAg reflects intrahepatic HBV production more accurately than HBV-DNA. This marker is associated with HCC incidence, and is applicable to NAs or IFN non-treated patients. Tada *et al.* measured and compared serum HBcrAg and HBV-DNA annually in follow-up using a ROC curve, and discovered that the AUC value of HBcrAg was superior compared with serum HBV-DNA. Although it is a serum assay, several reports have shown that the measurement of HBcrAg is advantageous in the evaluation of intrahepatic HBV.

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Footnote

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