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Review Article

Emerging Serum Biomarkers for Chronic Hepatitis B: Focus on Serum HBV RNA and HBcrAg

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Abstract

Chronic hepatitis B virus (HBV) infection remains a major cause of liver diseases, including cirrhosis and hepatocellular carcinoma. Reliable biomarkers for assessing viral replication, liver damage, and predicting clinical outcomes are essential for effective patient management. This review focuses on two promising biomarkers: serum HBV RNA and hepatitis B core-related antigen, both of which show strong correlations with viral replication and disease progression. Serum HBV RNA levels reflect the quantity and transcriptional activity of intrahepatic covalently closed circular DNA, providing insights into viral replication. They also correlate with other markers of replicative activity and have predictive value for key clinical outcomes, including hepatitis B e antigen and hepatitis B surface antigen seroconversion, relapse after therapy cessation, and liver fibrosis. Similarly, hepatitis B core-related antigen is closely associated with covalently closed circular DNA levels, correlates with markers of viral replication, and shows promise in predicting liver fibrosis, cirrhosis, and the risk of hepatocellular carcinoma. This review highlights the potential of both biomarkers for monitoring disease progression and guiding therapeutic decisions, particularly in the context of personalized treatment strategies and risk assessment for liver-related complications.

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Introduction

Hepatitis B virus (HBV) remains a significant global health challenge, with over 250 million people worldwide living with chronic HBV infection despite the widespread promotion of vaccination for more than 40 years.[1] If left untreated or inadequately managed, chronic HBV infection can progress to severe complications, including cirrhosis, liver failure, and hepatocellular carcinoma (HCC).[2] HBV is a hepatotropic virus with a 3.2-kb partially double-stranded relaxed circular DNA (rcDNA) genome.[3] During the HBV life cycle, rcDNA is released into the nucleus, where it is converted into covalently closed circular DNA (cccDNA) via host DNA repair mechanisms.[4] The cccDNA serves as a transcriptional template, generating a 3.5-kb pregenomic RNA (pgRNA) and multiple subgenomic RNAs (including preCore, preS1, preS2/S, and X mRNAs).[4] The pgRNA is subsequently reverse-transcribed into rcDNA within nucleocapsids composed of hepatitis B core antigen (HBcAg). These nucleocapsids are then enveloped by hepatitis B surface antigen (HBsAg) to form mature virions, which are secreted from the cell.[5,6] Notably, during pgRNA reverse transcription, 5–20% of transcripts undergo failed primer translocation, resulting in the formation of double-stranded linear DNA.[7] These double-stranded linear DNA molecules can integrate into the host genome.[8,9] Once integrated, they act as persistent transcriptional templates for HBsAg production,[10,11] contributing to immune tolerance in the host.[12–14]

Existing antiviral drugs cannot directly eliminate cccDNA or integrated HBV DNA within liver cells, making it very difficult to achieve a "complete cure" of hepatitis B.[15–18] Given this limitation, one of the core goals of current antiviral treatment is to suppress the transcriptional activity of cccDNA and inhibit the functionality of integrated HBV DNA, ultimately achieving a "clinical cure" of hepatitis B.[19] Therefore, accurately monitoring the activity levels of cccDNA and integrated HBV DNA is crucial for evaluating treatment efficacy.[20–23] Since these viral forms are exclusively confined to hepatocytes, liver biopsy remains the gold standard for evaluation. However, its clinical utility is severely limited by invasiveness and the lack of standardized procedural guidelines.[24]

Recent studies have demonstrated that serum HBV RNA and hepatitis B core-related antigen (HBcrAg) can reliably reflect the transcriptional activity of cccDNA, though their association with the transcriptional activity of integrated HBV DNA requires further investigation. Moreover, they have shown potential clinical value in predicting antiviral treatment response, assessing liver fibrosis progression, and estimating HCC risk, offering novel tools for the precision management of chronic HBV infection.[25–28] Therefore, this review systematically summarizes the clinical applications and research ad-

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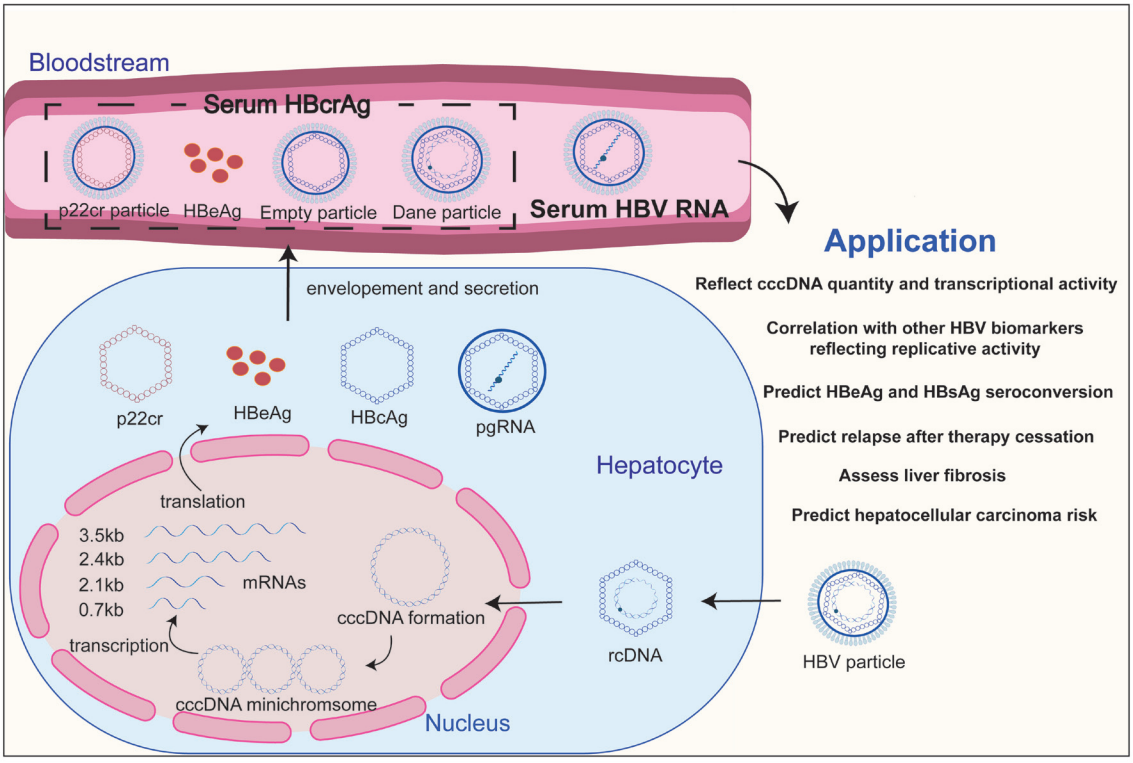

Fig. 1. Production and clinical application of Serum HBV RNA and HBcrAg. Created with BioRender. cccDNA, covalently closed circular DNA; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA; rcDNA, relaxed circular DNA; p22cr, 22-kDa core-related antigen (precore protein).

vances of serum HBV RNA and HBcrAg in HBV infection and related diseases (Fig. 1).

Serum HBV RNA

In 1996, German researchers first discovered the presence of HBV RNA in the peripheral blood of chronic hepatitis B (CHB) patients.[29] These RNA molecules primarily consist of non- or partially reverse-transcribed pgRNA, with significant heterogeneity due to diverse post-transcriptional processing.[30] Current research confirms five predominant classes of serum HBV RNA: (1) Full-length pgRNA (3.5 kb)[31]: The major component, transcribed directly from cccDNA in the hepatocyte nucleus, containing the complete coding region with a polyadenylated tail.[28,32–35] (2) 3′-Truncated pgRNA: Generated by incomplete template degradation mediated by the RNase H domain of the viral polymerase during reverse transcription; it lacks the 3′ polyadenylated tail but retains the 5′ ε packaging signal.[15,36–38] Its proportion increases significantly with the duration of nucleos(t)ide analogue (NA) therapy.[32,39] (3) pgRNA splicing variants: Arising from aberrant host cell splicing mechanisms, with at least 20 distinct secreted isoforms (sp1–sp20) identified[15,36–38,40,41]; sp1 is the most abundant, constituting up to 30% of total pgRNA in HBV-infected HCC cell lines.[16,42] (4) HBx transcripts: Generally present at low levels,[15,28,43] including full-length or truncated HBx open reading frames as well as an ultra-long variant containing the polymerase (P) open reading frame.[15,28,44] (5) HBV-human chimeric RNAs: Recently detected in serum samples from CHB patients harboring integrated HBV DNA. These include both 5′-HBV-human-3′ and 5′-human-HBV-3′ transcripts, although they represent only a minimal proportion of total serum HBV RNA.[28,45] Overall, the composition of serum HBV RNA is highly dynamic and complex. The specific RNA species present and their relative proportions are influenced by multiple factors, including infection stage, antiviral treatment status, and detection methodologies.[32]

In addition, mounting evidence suggests that each distinct RNA species has its own clinical implications. For instance, splice variants can regulate HBV replication and promote the migration and invasion of liver cancer cells[46–48]; 3′-truncated pgRNA, which accumulates during long-term NA therapy, is implicated in viral persistence[32,39]; and the newly discovered HBV-human chimeric RNAs are closely associated with the development and progression of HCC.[49,50] Nevertheless, current clinical research primarily focuses on detecting total serum pgRNA levels, which encompass full-length, 3′-truncated, and spliced variants.[30] This strategy is based on three key factors. Firstly, the total amount of pgRNA constitutes the majority of serum HBV RNA, providing a stable foundation for detection. Secondly, the relative proportions and contents of different components exhibit highly dynamic changes, and existing mainstream detection techniques[51] make it difficult to analyze individual components accurately. Most importantly, as a direct transcription product of cccDNA, changes in serum pgRNA levels specifically reflect the transcriptional activity of cccDNA.[31] Therefore, monitoring overall serum pgRNA levels is helpful for assessing viral replication status and the progression of HBV-related liver disease.[52–54]

Serum HBV RNA quantification techniques

Quantitative detection of serum HBV RNA is critically important for understanding viral activity, assessing cccDNA transcriptional activity, and guiding clinical management. Cur-

rently, several molecular biology techniques are employed, primarily including reverse transcription (RT)-quantitative polymerase chain reaction (qPCR), RT-droplet digital polymerase chain reaction (PCR), fluorescence-based nucleic acid isothermal amplification testing (SAT), and rapid amplification of cDNA ends (RACE). These methods have distinct principles, advantages, and limitations, and differ in their capability to detect various HBV RNA isoforms.[30]

Widely used serum HBV RNA quantification methods in clinical practice and research, such as RT-qPCR and SAT assays, cannot effectively differentiate between distinct HBV RNA isoforms.[51] This limitation arises from their core technical principle: they target conserved, shared regions within the HBV genome (e.g., the X, preC/C, or S regions) rather than unique molecular markers specific to individual isoforms.[31,55] RT-qPCR detects the sum of all RNA molecules containing the target sequence within the amplified region by reverse transcription and real-time amplification.[51] While highly sensitive, this approach indiscriminately captures pgRNA, splice-generated SP1 variants, and truncated RNAs. SAT utilizes primers incorporating T7 promoters to achieve isothermal amplification, offering operational simplicity and eliminating the need for DNase treatment.[56] However, its amplification products similarly represent a mixed signal from all isoforms within the primer-binding region.

In contrast, RACE and droplet digital PCR possess inherent potential for isoform differentiation. RACE selectively amplifies polyadenylated transcripts by anchoring to the polyadenylated tail, enabling separation of full-length pgRNA generated by the canonical polyadenylation signal from truncated RNAs produced by the cryptic polyadenylation signal.[57–60] However, this method cannot detect truncated RNAs lacking a polyadenylated tail. Droplet digital PCR leverages microfluidic partitioning for single-molecule isolation and Poisson distribution principles.[37] When combined with isoform-specific primer design, it enables absolute quantification of different splice variant proportions. Notably, conventional RT-qPCR also theoretically holds differentiation potential if designed with probes targeting isoform-specific markers; however, due to high sensitivity requirements, complex primer/probe design, and lack of standardization, it is rarely applied in routine clinical testing.[51,61]

Given that distinct HBV RNA isoforms have unique biological properties and clinical significance, the inability of current mainstream methods to differentiate them obscures critical biological insights within composite detection signals.[62] This represents a major obstacle to achieving precision medicine in HBV management. To overcome this bottleneck, future research must urgently focus on: first, developing novel detection platforms capable of highly sensitive, simultaneous differentiation of core isoforms; second, establishing international reference materials and unified detection standards encompassing these major isoforms to ensure result comparability and accelerate clinical translation.

Serum HBV RNA reflects cccDNA quantity and transcriptional activity

Unlike traditional HBV biomarkers such as HBV DNA and HBsAg, serum HBV RNA originates directly from the cccDNA template, making it a more precise indicator of intrahepatic cccDNA levels and transcriptional activity.[63] Wang *et al*. (2018) investigated this relationship in the natural history of CHB, finding a moderate correlation between serum HBV RNA and intrahepatic cccDNA ($r = 0.596$, $P < 0.001$).[64]

Further studies have corroborated this correlation in untreated chronic HBV infections, with serum HBV RNA levels showing variable correlations with intrahepatic cccDNA ($r = 0.25–0.89$).[58,64–66] These variations may arise from differences in patient characteristics or detection methodologies for circulating HBV RNA and intrahepatic cccDNA.[63]

In patients undergoing antiviral therapy, serum HBV RNA also correlates with cccDNA levels. For example, in Peg-interferon-treated patients, serum HBV RNA demonstrated a stronger correlation with cccDNA than other HBV biomarkers after 48 weeks of treatment, irrespective of hepatitis B e antigen (HBeAg) seroconversion.[67] Similarly, in NAs-treated patients, serum HBV RNA, derived predominantly from cccDNA and minimally affected by antiviral drugs, has proven to be a reliable marker for monitoring cccDNA levels and activity.[68] Even in patients with suppressed HBV replication under NAs therapy, a significant correlation between serum HBV RNA and cccDNA transcriptional activity remains evident ($r = 0.78$, $P < 0.0001$).[69]

In summary, serum HBV RNA, as a direct downstream product of cccDNA, serves as a robust biomarker reflecting intrahepatic cccDNA levels and activity. Its utility has been validated in both untreated CHB patients and those receiving antiviral therapy, highlighting its potential for infection monitoring and treatment response evaluation.

Correlation between serum HBV RNA and integrated DNA

In recent years, there has been growing research interest in the association between serum HBV RNA and integrated viral DNA. Studies reveal that integrated HBV DNA can transcribe two forms of replication-independent RNAs: (i) 5′-HBV-human-3′ chimeric RNAs (integrant-derived RNAs, id-RNAs) initiated from viral promoters and polyadenylated using human polyadenylation signals, and (ii) 5′-human-HBV-3′ RNAs initiated from upstream human promoters. Although this suggests the potential presence of integrant-derived envelope protein (cps) RNAs in serum, a systematic analysis of their complete molecular profiles remains lacking.[28] Furthermore, whether these RNAs accurately reflect the transcriptional activity and integration levels of integrated DNA remains unclear.

Given that the relevant molecular mechanisms have not been fully elucidated, an in-depth exploration of the characteristics of integrant-derived RNA in serum and its relationship with the transcriptional activity of viral integration sites will help elucidate the molecular mechanisms of HBV persistent infection and provide potential biomarkers for clinical monitoring.

Serum HBV RNA predicts HBeAg and HBsAg seroconversion

Serum HBV RNA levels and their dynamic changes are emerging as potential predictors of HBeAg and HBsAg seroconversion. During the immune-active phase, a rapid decline in serum HBV RNA strongly correlates with higher rates of spontaneous HBeAg seroconversion. Specifically, when HBV pgRNA levels at week 28 are below 5.63 $\log_{10}$ copies/mL or when the reduction in HBV pgRNA from baseline exceeds 1.85 $\log_{10}$ copies/mL, the likelihood of spontaneous HBeAg seroconversion within 48 weeks reaches approximately 87%. In contrast, patients with higher pgRNA levels or smaller reductions exhibit significantly lower conversion rates of 10–12%.[70]

In treated patients, HBV RNA status after therapy also closely predicts HBeAg seroconversion. Studies have shown that patients remaining HBV RNA-positive after 48 weeks of NA therapy experience a prolonged time to seroconversion and a reduced likelihood of achieving it (hazard ratio (HR)

= 6.69, 95% CI: 1.88–23.84).[71] Additionally, early dynamic changes in HBV RNA levels serve as reliable indicators of HBeAg seroconversion. For instance, HBV RNA levels at week 12 are significantly predictive of HBeAg seroconversion at 96 weeks of NAs therapy, with a threshold of 6.18 $\log_{10}$ copies/mL (sensitivity 81%, specificity 80%, OR = 3.560, 95% CI: 1.39–9.110, P = 0.008). Fluctuations in HBV RNA at later stages, such as weeks 24 and 48, as well as three and six months post-treatment, further enhance predictive accuracy in HBeAg-positive patients.[72–74]

Beyond HBeAg seroconversion, HBV RNA levels may also predict HBsAg seroconversion. HBV RNA-negative patients exhibit significantly lower quantitative HBsAg levels compared to HBV RNA-positive patients (2.2 vs. 3.1 $\log_{10}$ IU/mL, P < 0.001).[75] Furthermore, patients with HBV RNA levels below 1,000 copies/mL at treatment cessation demonstrate a significantly higher cumulative HBsAg clearance rate over six years (30.9% vs. 1.6%, P = 0.007).[76]

In conclusion, serum HBV RNA levels and their changes are useful predictors of both HBeAg and HBsAg seroconversion. These findings highlight the potential of serum HBV RNA as a valuable tool for guiding individualized treatment strategies and monitoring therapeutic outcomes in CHB.

Serum HBV RNA predicts relapse after therapy cessation

Current cessation criteria for HBV antiviral therapy continue to face clinical challenges, with over 40% of patients discontinuing treatment requiring reinitiation due to virological relapse.[77] The underlying mechanism lies in the persistent viral reservoir maintained by cccDNA, which serves as the fundamental source of post-treatment recurrence. Serum HBV RNA, as a direct transcriptional product of cccDNA, dynamically reflects the transcriptional activity of this viral reservoir in real time.[54] Leveraging this biological characteristic, multiple clinical investigations have demonstrated the potential utility of serum HBV RNA quantification in predicting post-therapeutic viral rebound and optimizing personalized treatment cessation strategies.

Multiple studies have consistently demonstrated a significant association between serum HBV RNA levels at the end of treatment (EOT) and the risk of virological relapse.[78,79] A study involving 74 patients who completed one year of NAs therapy revealed a marked disparity in relapse rates between the EOT HBV RNA-negative and -positive groups (25.4% vs. 71%, P = 0.011).[80] This correlation was further quantified in a cohort of 114 entecavir-treated patients, where an HBV RNA cutoff ≥ 44.6 U/mL demonstrated over 90% predictive power for post-treatment relapse.[81]

Furthermore, emerging evidence supports the enhanced prognostic capacity of combined virological biomarker profiling for post-treatment recurrence prediction. Fan *et al*. conducted a four-year longitudinal follow-up study of 130 treatment-naïve HBeAg-positive patients, demonstrating that dual negativity of HBV DNA and RNA at EOT conferred a fourfold lower clinical relapse rate compared to double-positive cases (8.0% vs. 31.4%, P = 0.018).[82] Innovatively, the Seto research group developed a novel composite criterion integrating HBV RNA negativity with HBsAg < 10 IU/mL, which reliably identifies candidates with minimal rebound risk post-cessation.[81] Current studies suggest that integrating multidimensional viral markers may enable precise risk stratification in clinical practice. However, attention should be paid to the biological heterogeneity of different markers (e.g., HBV RNA reflecting cccDNA activity vs. HBsAg characterizing host immune response) when setting thresholds. Future validation through multicenter cohorts and dynamic marker combination models is warranted.

In summary, serum HBV RNA holds significant translational medical value in optimizing antiviral treatment endpoints for CHB and predicting recurrence risk after drug withdrawal. However, for routine clinical application, large-scale, multicenter prospective cohort studies are needed to address key issues: (1) determining optimal predictive thresholds at different treatment stages (e.g., NAs therapy, interferon therapy); (2) establishing standardized protocols for dynamic monitoring; and (3) validating combined biomarker prediction models across diverse populations. These efforts will provide a solid evidence base for developing individualized drug withdrawal strategies guided by biomarkers.

Serum HBV RNA assesses liver fibrosis

The early diagnosis and monitoring of liver fibrosis are critical for effective management of CHB. Although liver biopsy remains the gold standard for fibrosis evaluation, its invasiveness and risk of sampling errors limit routine clinical use.[83] Recent studies highlight serum HBV RNA as a non-invasive biomarker that correlates strongly with both the progression and regression of liver fibrosis, offering an alternative diagnostic tool.[84–86]

Wang *et al*. (2017) found significant correlations between serum HBV RNA levels and histopathological scores for necroinflammation and fibrosis (r = 0.665, P <0.001 for grading; r = 0.722, P <0.001 for staging).[87] Using a cutoff value of 2.45 $\log_{10}$ copies/mL, serum HBV RNA effectively differentiates samples with inflammation activity scores and fibrosis scores of <2 versus ≥2, achieving AUROCs of 0.88 and 0.85, respectively, surpassing the diagnostic accuracy of HBsAg.[87] Furthermore, Huang *et al*. (2020) demonstrated that serum HBV RNA levels are independent predictors of liver fibrosis in both HBeAg-positive (OR = 0.514, P < 0.001) and HBeAg-negative patients (OR = 3.574, P < 0.001), outperforming traditional indices such as APRI and FIB-4.[83]

In addition to assessing fibrosis progression, serum HBV RNA levels are also effective in predicting fibrosis regression. Lower HBV RNA levels are observed in patients with regression compared to those without.[84,85] Notably, a reduction in HBV RNA levels exceeding 0.63 $\log_{10}$ copies/mL within the first six months of treatment predicts fibrosis regression at 60 months, with a sensitivity of 53.8% and specificity of 92.3%.[84] This likely reflects decreased cccDNA transcriptional activity in hepatocytes, a key factor in fibrosis resolution.[65,88]

In summary, serum HBV RNA serves as a pivotal biomarker for evaluating liver fibrosis in CHB patients. Its ability to assess both fibrosis progression and regression provides valuable insights for optimizing treatment strategies and improving patient outcomes.

HBV RNA predicts HCC risk and prognosis

The strong relationship between HBV RNA and cccDNA has positioned serum HBV RNA as a promising biomarker for predicting HCC risk and prognosis in CHB patients. A 2021 case-control study from Hong Kong first demonstrated significantly higher serum HBV RNA positivity and levels in HCC patients compared to non-HCC patients during prior treatment (undetectable pgRNA: 9.6% vs. 36.5%, P < 0.001).[89] These findings were corroborated by a large prospective cohort study that established elevated serum HBV RNA levels as a significant risk factor for HCC in CHB patients.[90]

In CHB patients undergoing long-term antiviral therapy with undetectable serum HBV DNA, elevated serum pgRNA levels were associated with poorer overall survival and higher

recurrence rates following hepatectomy. Conversely, pgRNA-negative patients exhibited significantly improved overall survival ($P < 0.001$).[91] Cox multivariate analysis further identified high serum HBV RNA expression as an independent predictor of HCC recurrence, with HRs of 2.1 ($P = 0.003$) in Cohort A and 1.6 ($P = 0.033$) in Cohort B. The underlying mechanism may involve multiple carcinogenic pathways: (1) elevated HBV pgRNA levels promote expression of oncoproteins[91]; (2) HBV RNA can act as a microRNA sponge, sequestering and inhibiting tumor-suppressing host microRNAs, thereby promoting HCC growth and invasion.[92]

Interestingly, patients with poorly differentiated HCC or lymphovascular invasion were found to have lower serum HBV RNA levels, particularly one year after diagnosis (1.71 [IQR 1.71–2.37] vs. 2.14 [IQR 1.71–3.59] l $\log_{10}$ IU/mL, $P = 0.076$).[89,93] This may be due to the high metabolic state in poorly differentiated HCC being unfavorable to HBV survival.[94]

Overall, comprehensive analysis indicates that elevated serum HBV RNA levels are positively correlated with HCC risk, postoperative recurrence, and poor prognosis in CHB patients. However, patients with highly aggressive or poorly differentiated advanced HCC exhibit markedly reduced serum HBV RNA levels. This paradox suggests that the predictive value of HBV RNA may be disease stage-specific: during early HCC stages, active viral replication may directly drive tumorigenesis, whereas in advanced disease, dynamic tumor microenvironment remodeling might suppress viral replication, resulting in an inverse correlation between HBV RNA levels and tumor malignancy. Future studies should systematically elucidate the dynamic fluctuation patterns of HBV RNA according to HCC clinical staging and molecular subtyping. Additionally, developing multidimensional predictive models integrating clinicopathological features, imaging findings, and molecular biomarkers is essential to enhance precision in HCC risk prediction and prognosis evaluation.

HBcrAg

HBcrAg is a composite biomarker comprising three viral proteins—HBcAg, HBeAg, and the 22 kDa truncated precursor core protein—which share an identical 149-amino acid sequence but differ in their processing pathways.[95] HBcAg is produced by translation of the 3.5 kb pgRNA transcribed from cccDNA; HBeAg is generated via translation of precore mRNA into the precore protein, followed by N-terminal specific proteolytic processing to remove the signal peptide; and the 22 kDa truncated precursor core protein represents a distinct processing form of the precore protein undergoing both N-terminal and C-terminal modifications.[96–100]

Because these proteins are almost entirely dependent on cccDNA-driven transcription and translation, HBcrAg quantitatively reflects the transcriptional activity of this viral reservoir, making it a potential clinical marker for evaluating HBV persistence and therapeutic efficacy.

HBcrAg correlates with intrahepatic cccDNA

HBcrAg has been demonstrated as a reliable surrogate marker for intrahepatic cccDNA due to its strong and consistent correlation with cccDNA levels.[101–105] Studies show serum HBcrAg is significantly associated with intrahepatic cccDNA, independent of HBeAg status. In HBeAg-positive patients, multivariate regression analysis revealed that serum HBcrAg correlated more strongly with intrahepatic cccDNA than HBsAg (β = 0.563 vs. 0.328, both $P < 0.001$). In contrast, among HBeAg-negative patients, serum HBcrAg was the only biomarker significantly correlated with intrahepatic cccDNA levels (β = 0.774, $P < 0.001$).[102]

Further evidence indicates that, in patients undergoing NA therapy, reductions in serum HBcrAg closely parallel decreases in intrahepatic cccDNA. This correlation is stronger than that between serum HBsAg and intrahepatic cccDNA, both before and during treatment.[101,103–105] While prolonged NA therapy often results in undetectable serum HBV DNA in most patients, serum HBcrAg remains detectable in approximately 78% of cases.[106] This persistence is attributed to NAs' ability to inhibit HBV DNA replication with minimal effect on cccDNA transcription and HBcrAg synthesis.[107] Even after five years of entecavir therapy, a moderate correlation between serum HBcrAg and intrahepatic cccDNA persists (r = 0.419, $P = 0.005$).[106,108]

Overall, HBcrAg demonstrates superior and more consistent correlation with intrahepatic cccDNA compared to HBsAg and HBV DNA across diverse clinical contexts. These findings establish HBcrAg as a reliable surrogate marker for cccDNA in clinical practice. With the advent of novel therapies targeting cccDNA, HBcrAg holds significant potential as a non-invasive indicator for directly assessing cccDNA activity, thereby guiding precision therapeutic interventions.

HBcrAg correlates with other HBV markers reflecting replicative activity

Suzuki *et al*. (2019) first reported a significant correlation between HBcrAg and other virological markers in a study involving 57 CHB patients, observing a strong positive correlation between serum HBcrAg and HBV DNA levels (r = 0.713, $P < 0.001$).[109] Subsequent studies confirmed that this correlation remains consistent regardless of HBeAg status (r = 0.59–0.85, $P < 0.001$)[104,110] or treatment history (r = 0.786–0.820, $P < 0.001$).[105,111]

While the correlation between HBcrAg and HBV DNA is well established, evidence suggests a comparatively weaker relationship between HBcrAg and HBsAg. A cohort study of 2,666 patients in Taiwan found a strong correlation between HBcrAg and HBV DNA (r = 0.83, $P < 0.001$), whereas the correlation with HBsAg was moderate (r = 0.59, $P < 0.001$).[112] This discrepancy may be explained by the distinct origins of these markers: both HBcrAg and HBV DNA derive from cccDNA, whereas HBsAg can also originate from integrated viral genomes.[113] Additionally, a moderate correlation between HBcrAg and HBeAg levels has been reported (r = 0.491, $P < 0.001$).[102]

In summary, HBcrAg exhibits strong correlations with HBV DNA and moderate correlations with HBsAg and HBeAg, effectively reflecting HBV replicative activity. These findings highlight its potential utility in monitoring viral dynamics in CHB patients.

HBcrAg predicts HBeAg and HBsAg seroconversion

Emerging evidence suggests HBcrAg may serve as a supplementary biomarker for monitoring HBeAg and HBsAg seroconversion in CHB management. Studies show that patients with lower HBcrAg levels or significant reductions in HBcrAg are more likely to achieve HBeAg seroconversion, either spontaneously or during NA therapy.[114,115] For example, at the third month of treatment, an HBcrAg level of 6.20 $\log_{10}$ U/mL moderately predicts HBeAg seroconversion (AUROC = 0.663).[116] In patients undergoing combined NAs and interferon-αtherapy, baseline HBcrAg levels > 4.5 log U/mL predict non-response and failure to achieve HBeAg seroconversion at 24 months ($P < 0.003$).[117]

Additionally, HBcrAg may serve as a valuable indicator in predicting HBsAg clearance. In cases of spontaneous HBsAg

clearance, 79% of patients had undetectable HBcrAg levels.[118,119] Further research demonstrated that baseline HBcrAg levels are closely associated with virological response rates and HBsAg seroconversion in treated patients. Specifically, a baseline HBcrAg level of 2.550 $\log_{10}$ U/mL moderately predicts HBsAg seroconversion (AUROC = 0.552).[120] At the EOT, HBcrAg levels below 2 $\log_{10}$ U/mL significantly associate with higher virological response rates and HBsAg conversion ($P < 0.001$).[121] These findings suggest that lower HBcrAg levels during treatment may indicate a greater likelihood of successful HBsAg conversion.

In conclusion, existing studies suggest HBcrAg levels provide supplementary insights for anticipating HBeAg and HBsAg seroconversion. Further investigations should focus on validating these findings through standardized multicenter studies, exploring integration with emerging biomarkers, and establishing long-term clinical correlations to refine its prognostic utility.

HBcrAg evaluates the risk of HBV recurrence after treatment

HBcrAg, as a marker of cccDNA transcriptional activity, has been extensively validated for its role in predicting HBV relapse after discontinuation of antiviral therapy. Numerous studies have demonstrated a strong correlation between baseline HBcrAg levels and the risk of virological relapse. A prospective study in Hong Kong revealed that the rate of HBV reactivation in patients with baseline HBcrAg positivity was significantly higher than in those with HBcrAg negativity (71.8% vs. 31%, $P = 0.002$). Multivariate analysis further identified baseline HBcrAg positivity as an independent risk factor for HBV reactivation ($P = 0.004$; HR = 2.94; 95% CI: 1.43–6.07).[122]

In addition to baseline levels, HBcrAg at the EOT has emerged as a critical predictor of relapse risk. Studies consistently show that patients who experience reactivation after antiviral therapy have significantly higher HBcrAg levels at EOT compared to those who remain relapse-free (4.9 log U/mL vs. 3.2 log U/mL, $P = 0.009$).[123] Multiple analyses have confirmed that HBcrAg levels at EOT are an independent risk factor for HBV recurrence, with optimal cutoff values ranging from 3.7 to 4.0 log U/mL ($P = 0.002$–0.024).[124–126]

Shinkai *et al*. (2006) further highlighted the predictive value of HBcrAg at treatment cessation, showing that an HBcrAg level below 3.4 log U/mL at EOT was the only independent factor effectively predicting non-recurrence after therapy ($P = 0.042$).[127] Moreover, the Japanese Society of Hepatology has incorporated HBcrAg into clinical guidelines for relapse risk assessment, recommending a cutoff of <3.0 log U/mL to define low relapse risk.[128]

In summary, both baseline and EOT HBcrAg levels are valuable for predicting HBV reactivation and recurrence. Regular monitoring of these levels enables clinicians to stratify patients by relapse risk, optimize treatment discontinuation strategies, and improve long-term clinical management.

HBcrAg predicts liver fibrosis and cirrhosis risk

Severe necroinflammatory activity is widely recognized as the initial stage in liver fibrosis progression. Studies have shown that HBcrAg independently predicts both significant necroinflammation ($P = 0.000$; OR = 2.290; 95% CI: 1.524–3.441) and significant liver fibrosis ($P = 0.000$; OR = 2.456; 95% CI: 1.631–3.699). The predictive accuracy for necroinflammation and fibrosis, measured by the area under the curve, is 0.807 (95% CI: 0.707–0.885) and 0.804 (95% CI: 0.703–0.883), respectively.[129] These associations remain consistent across different HBeAg statuses (both $P < 0.001$).[105,129,130]

Notably, HBcrAg demonstrates high diagnostic accuracy for liver fibrosis with optimal cutoff values specific to HBeAg status. For HBeAg-positive patients, a cutoff of $\leq 2.45 \times 10^4$ kU/mL effectively excludes severe lesions, while for HBeAg-negative patients, a cutoff of ≥4.02 kU/mL is more suitable for confirming significant liver damage (area under the curve > 0.70 for both). Based on the Youden index, HBcrAg thus serves distinct roles in fibrosis diagnosis depending on HBeAg status.[129]

As liver fibrosis progresses, cirrhosis represents its end stage, often accompanied by severe liver damage and functional impairment.[131] A Japanese study first established the association between HBcrAg levels and cirrhosis progression, identifying HBcrAg ≥ 3.7 log U/mL as an independent risk factor for cirrhosis (HR = 3.28; 95% CI: 1.60–6.75).[132] In a long-term follow-up study of 1,673 CHB patients, Tseng *et al*. confirmed a significant correlation between elevated HBcrAg levels and cirrhosis risk ($P < 0.001$). Risk stratification analysis revealed that compared to patients with HBcrAg < 10 kU/mL, those with HBcrAg levels of 10–99 kU/mL and ≥100 kU/mL had HRs of 3.32 (95% CI: 1.99–5.52) and 7.35 (95% CI: 4.28–12.64), respectively.[133]

In summary, HBcrAg is a promising non-invasive biomarker for assessing the risk of liver fibrosis and cirrhosis. Its strong predictive capabilities, especially when stratified by HBeAg status, highlight its clinical value. However, further research is needed to validate its applicability across diverse patient populations and clinical settings.

HBcrAg predicts HCC risk

HCC accounts for 75–85% of primary liver cancers and is associated with poor prognosis.[134] The limited sensitivity of traditional imaging and liver function tests for early diagnosis underscores the urgent need for novel serological markers.[135,136] Recent studies have identified HBcrAg as a promising biomarker for predicting HCC risk, offering potential for early diagnosis and individualized risk assessment.

Kumada *et al*. (2013) first demonstrated a significant association between serum HBcrAg levels and HCC occurrence, reporting that elevated HBcrAg was independently associated with HCC development (HR = 2.77; 95% CI: 1.07–7.17; $P = 0.036$).[137] Subsequent studies have corroborated this finding, demonstrating that high serum HBcrAg levels are closely linked to HCC risk regardless of treatment status. For example, Tada *et al*. conducted a retrospective cohort study in untreated patients, revealing that baseline serum HBcrAg levels exceeding 2.9 log U/mL were associated with a fivefold increased risk of HCC compared to lower levels.[138]

In patients receiving NA therapy, serum HBcrAg levels also strongly correlate with HCC risk. Research indicates that patients with serum HBcrAg levels exceeding 3.89 log U/mL after treatment exhibit a threefold higher risk of developing HCC.[139] Additionally, Hosaka *et al*. (2019) reported that patients with continuously rising HBcrAg levels during treatment face significantly greater HCC risk compared to those with stable or declining levels.[140]

Overall, serum HBcrAg has been established as an independent predictive biomarker for HCC, with elevated levels significantly correlating with HCC risk in both untreated and antiviral-treated patients. Dynamic monitoring of HBcrAg facilitates evaluation of disease progression and prognosis, supporting early risk stratification. Future research should focus on integrating HBcrAg with other biomarkers to develop predictive models, while elucidating its mechanistic links to HBV-related carcinogenesis.

Conclusions

Serum HBcrAg and HBV RNA have emerged as promising biomarkers for the monitoring and prognostication of CHB. Their applications span a broad range of clinical scenarios, including predicting HBeAg and HBsAg seroconversion, assessing the risk of HBV recurrence following treatment cessation, evaluating liver fibrosis and cirrhosis, and forecasting the development of HCC. These biomarkers hold significant potential to advance personalized treatment strategies and improve clinical outcomes in CHB management. Despite their promise, further validation studies are necessary to establish standardized cutoff values and refine their clinical utility across diverse patient populations.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization: YT; Drafting of the manuscript: YT; Critical review and editing of the manuscript: HY and JC. All authors have approved the final version and publication of the manuscript.

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