

Editorial

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Promise and pitfalls of new viral biomarkers for hepatocellular carcinoma risk prediction in patients with chronic hepatitis B

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Chronic hepatitis B virus (CHB) infection - estimated to affect 290 million individuals - remains the leading global cause of liver-related mortality and a risk factor for hepatocellular carcinoma (HCC)^[1]. Importantly, 56% of liver cancers worldwide were attributable to CHB based on GLOBOCAN estimates from 2012^[2]. Current first-line therapies for CHB reduce but do not eliminate the risk of HCC, and for those fortunate enough to experience clearance of hepatitis B surface antigen (HBsAg), either spontaneous or on-treatment, the risk of HCC drops further but again persists^[3,4]. Additionally, unlike other etiologies of chronic liver disease, HCC develops in the absence of advanced fibrosis in up to 30% of HBsAg-positive patients^[5]. Taken together, these realities dictate that surveillance for HCC is paramount in nearly all circumstances for patients with CHB.

The surveillance pathway adopted by different international hepatology society groups mostly align, with ultrasound +/- serum alpha-fetoprotein every 6 months recommended in all cirrhotics and some CHB at higher risk, such as Asian men over the age of 40, Asian women over the age of 50 and Africans over the age of 20-40^[6]. With a global average life expectancy of 73 years, one can imagine that the cumulative burden of surveillance on both individuals and healthcare systems over lifetimes is extraordinarily high. Therefore, a key objective has been to develop risk prediction scores to identify CHB groups at high risk for HCC, but more importantly, those in whom risk is low enough that surveillance may not be indicated. These risk



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scores typically stratify on treatment status and include demographic factors (e.g., age, sex), laboratory values reflective of liver inflammation or fibrosis (e.g., serum ALT, albumin, platelet count), and - in some instances - viral factors (e.g., HBV DNA, HBeAg status)^[6]. Despite the development of numerous validated scores with excellent negative predictive values (> 95%), there has not yet been clinical consensus on their use to exclude patients from HCC surveillance. More recently, quantitative hepatitis B surface antigen levels (qHBsAg), hepatitis B core-related antigen levels (HBcrAg), HBV RNA, and markers of HBV evolution (e.g., preS and basal core mutations) have been examined as adjunct tools for HCC risk prediction - with the hope that addition of these viral biomarkers will improve precision of these scores.

Circulating HBsAg comes from either integrated DNA or cccDNA sources, and quantification of HBsAg is a useful tool in the management of CHB, particularly with respect to predicting the likelihood of HBsAg loss, and is, at present, the most accessible biomarker for commercial use. However, a relationship between qHBsAg and the development of HCC has not been consistently demonstrated. Though qHBsAg correlates with cccDNA in both tumor and non-neoplastic tissue^[7], median qHBsAg did not differ between HCC cases and controls in several case-control studies^[8-10] nor did qHBsAg predict HCC risk in several observational studies^[11,12]. A meta-analysis of eight studies of low heterogeneity, however, determined a pooled 2.5-fold (95%CI: 2.2-2.8) increased risk of HCC with qHBsAg \geq 1000 IU/mL^[13]. Further, neither adding qHBsAg nor replacing HBV DNA with qHBsAg in the original REACH-B score (which predicts HCC risk among non-cirrhotics who are treatment-naïve^[14]) resulted in enhanced levels of accuracy^[15]. Use of qHBsAg levels among CHB groups with low HBV DNA levels, such as inactive or indeterminate phenotypes, yield better risk prediction. For example, while the receiver operating curve (ROC) for ALT and HBV DNA both outperformed qHBsAg at predicting long-term HCC risk in a large non-cirrhotic CHB cohort, qHBsAg \geq 1000 IU/mL was strongly associated with HCC among the subgroup of HBeAg-negative with HBV DNA < 2000 IU/mL (HR = 13.7, 4.8-39.3) but notably not for higher viral loads^[16]. Among HBeAg-negative patients with an intermediate viral load (HBV DNA 2000-19,999 IU/mL), the area under ROC for 10-year HCC development was 0.68 for a combination of qHBsAg plus HBV DNA *vs.* only 0.54 for HBV DNA alone^[17]. In fact, the group with the lowest annual incidence rate of HCC were those with either a low viral load plus qHBsAg < 1000 IU/mL or intermediate viral load plus qHBsAg < 100 IU/mL at 0.6 cases per 1000 person-years^[17]. Thus, qHBsAg has limited application in risk prediction scores for HCC, with the exception of HBeAg-negative CHB with low or intermediate levels of HBV DNA.

HBcrAg has emerged as a potentially more promising viral biomarker for HCC risk prediction. HBcrAg components include HBeAg, hepatitis B core antigen, and a precore protein, with production dependent on the level of transcription/translation of the HBV precore/core gene^[18]. Importantly, HBcrAg correlates with intrahepatic cccDNA^[19], even among those with low or undetectable serum HBV DNA^[20]. This characteristic allows for the use of HBcrAg for risk prediction among treated patients, and both pre-treatment and on-treatment HBcrAg values have been associated with HCC^[21-23]. For example, higher HCC risk among HBeAg-negative was observed in those with persistent or on-treatment (at 1 year) HBcrAg > 4.4 log U/mL, whereas those with low pre-treatment or on-treatment decline in HBcrAg were at low risk^[21]. In a small study of patients on antiviral treatment for more than two years, 71% who were negative for HBeAg and HBV DNA were positive for HBcrAg: both on-treatment positivity and delayed loss of HBcrAg were independently associated with the development of HCC^[23]. However, treatment reduces HBcrAg levels in a time-dependent manner^[24], and the impact of antiviral therapy duration has not yet been fully elucidated in current studies. Among treatment-naïve, HBcrAg has been demonstrated to be superior to HBV DNA^[12] and HBsAg^[25] in predicting HCC in ROC analyses. HBcrAg may also specifically be used to stratify risk in treatment-naïve patients of indeterminate phenotype (HBeAg-negative, HBV DNA 2000-20000 IU/mL, and ALT > ULN). In this heterogeneous risk group, at HBcrAg cut-off of 10,000 IU/mL

(= 4 log U/mL), 10-year risk of HCC was 5.3% with high vs. 0.5% with low HBcrAg^[25,26]. An indeterminate CHB patient with HBcrAg < 10,000 IU/mL has, therefore, a comparable HCC risk to the inactive CHB patient^[26]. One pitfall of HBcrAg is that due to the inclusion of HBeAg within HBcrAg, the relationship between HBcrAg and HCC risk is much weaker, or absent, among HBeAg-positive patients, regardless of treatment status^[11,21,25]. Further, in a study of 2666 treatment-naïve adults, nearly half (48%) had an undetectable baseline HBcrAg, limiting its discriminatory ability^[25]. Thus, like qHBsAg, the use of HBcrAg in risk prediction for HCC is best in HBeAg-negative patients, and while it appears quite promising among this subgroup, additional comparative studies are needed to validate the optimal HBcrAg cut-off for HCC risk prediction.

HBV RNA has recently entered the arena as a viral biomarker for treated CHB patients. HBV RNA remains elevated in up to 78% of CHB patients who achieve undetectable HBV DNA on treatment, much higher than the 30% for HBcrAg^[27]. A small case-control study demonstrated that a detectable HBV RNA among treated patients was more common in HCC cases, whereas no difference was seen in qHBsAg levels^[10]. In a prospective cohort study of 2974 treated patients (40% HBeAg-positive, 37% with cirrhosis), the 70% of individuals with baseline detectable HBV RNA were 2.2-fold more likely to develop HCC after 4.4 years of follow-up (cumulative incidence of 4.1% at 5 years if detectable compared to 1.8% if not). The greatest risk was observed for those with markedly elevated baseline HBV RNA of $\geq 100,000$ copies/mL^[28]. The risk of HCC was also 4-fold higher if both HBV RNA and HBV DNA were detectable^[28]. Further studies are needed, but initial results are encouraging.

Lastly, HBV variants, specifically those in the preS, basal core promoter (A1762T and G1764A) and precore (G1896A) regions of the HBV genome, have been linked to HCC risk independent of ALT or HBV DNA levels, with the magnitude of risk modulated by HBV genotype and HBeAg status^[29-31]. Natural history studies highlight that viral mutations accumulate over an individual's lifetime of infection; thus, discerning a causative role of specific mutation beyond that related to the inflammatory-fibrotic process caused by chronic viremia can be challenging. In a recent meta-analysis of 21 studies (all among Asian populations), a 2-3-fold elevated HCC risk was demonstrated with the presence of either preS deletion (all, preS1 or preS2) or preS2 start codon mutation^[32]. A causative role is suggested by a recent translational study showing that intracellular accumulation of a secretion-defective pre-S2 deletion mutant led to increased stress in the endoplasmic reticulum, calcium overload, mitochondrial dysfunction, and enhanced liver fibrosis^[33]. The optimal methods to detect pre-S deletions and pre-S deleted proteins have not been established, and none are available for routine clinical care^[34]. Given the heterogeneity (and infrequent testing) of HBV mutations, few have been incorporated into risk prediction scores, but advances in sequencing and machine learning may pave the way for their inclusion in the future.

While more studies are needed, it is improbable that any single viral biomarker will be a panacea for HCC risk prediction for all CHB phenotypes. Rather, the inclusion of viral biomarkers in risk predictor scores will likely be tailored to the patient context - for example, whether cirrhosis is present, HBeAg positive or negative, on treatment or off, and whether the marker itself is detectable (see [Figure 1](#)). In fact, we would argue that the use or addition of viral biomarkers will be unlikely to achieve the degree of risk stratification necessary to allow clinicians to identify patients who can forgo surveillance for HCC. Rather, the rapidly evolving field of serum tumor biomarkers, such as methylated DNA or single nucleotide polymorphisms, might better stratify risk, or eventually supplant imaging-based surveillance, thereby simplifying the process and cost of surveillance.

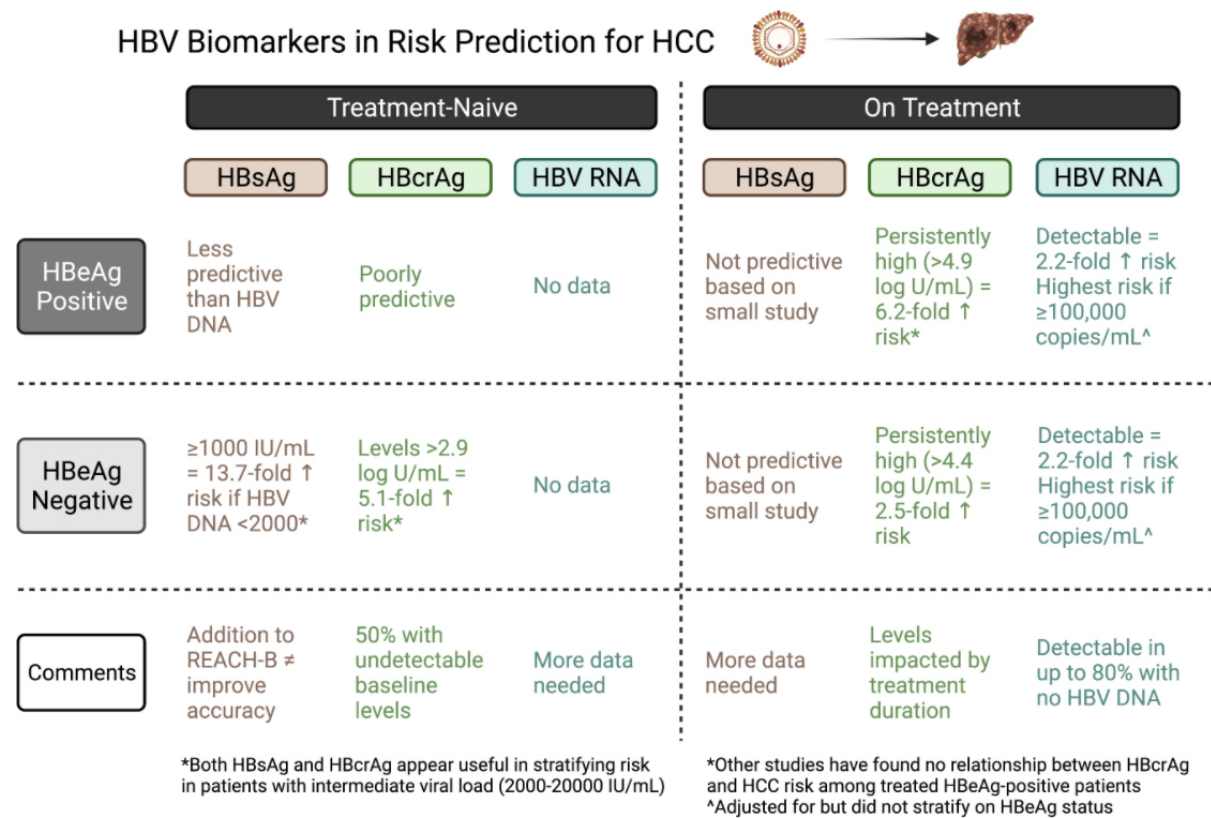


Figure 1. Novel HBV biomarkers in risk prediction for HCC.

However, these data do suggest a role for viral biomarkers as an adjuvant to decisions regarding the need for antiviral therapy to lower HCC risk. This is particularly relevant for patients in the “grey zone”, for whom specific thresholds of qHBsAg, HBcrAg, or HBV RNA may push one towards initiating antiviral therapy, even if ALT and HBV DNA levels are lower than traditional thresholds for treatment. Further, nearly 80% of indeterminate phase patients have at least one high-risk mutation^[35], justifying a potential role in identifying viral variants as part of treatment decision-making. As treatment endpoints shift to HBsAg loss, pre-treatment viral biomarkers might be used to predict HCC risk after seroclearance and potentially influence surveillance strategies. For example, as more patients achieve this favorable outcome at an earlier time point in the duration of their infection (and younger age), the risk of HCC might be so exceedingly low that surveillance after HBsAg loss is not needed. As such, further studies of HBV biomarkers remain paramount, particularly in these special populations and in the face of upcoming new therapies, with the expectation that novel biomarkers will contribute to refinements in HCC prevention and surveillance in the future.

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Authors' contributions

Drafting of the manuscript: Zhou K

Critical revision of the manuscript for important intellectual content: Zhou K, Terrault N

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Conflicts of interest

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Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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