Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

REVIEW ARTICLE

What can we learn from hepatitis B virus clinical cohorts?

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Keywords
chronic hepatitis B – HBsAg – HBV DNA – hepatocellular carcinoma – risk calculator

Abbreviations
AUROC, areas under receiver operating characteristic; BCP, basal core promoter; CHB, chronic hepatitis B; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratios; OR, odds ratio.

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DOI:10.1111/liv.12716

Abstract

Chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) are considered to be sequential adverse outcomes in patients with persistent hepatitis B virus (HBV) infection. HBV infection is endemic in Taiwan and most HBV carriers acquire the virus early in life. The impact of HBV factors on the natural course of patients with chronic HBV infection has been investigated in three cohort studies. The first Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) cohort study revealed that HBV viral load is a strong predictive factor for the risk of cirrhosis and HCC and baseline serum HBV DNA levels >2000 IU/ml may increase the risk of cirrhosis and HCC in adult HBV carriers. In the second Study of E Antigen seRoClearance of Hepatitis B (SEARCH-B), HBsAg level <100 IU/ml at 1-year post HBeAg seroconversion was shown to be a predictor of HBsAg seroclearance over time. Recently, the third Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B Carriers (ERADICATE-B) cohort study also suggested that HBsAg levels were a complementary predictive risk factor to HBV DNA levels for predicting HBV-related adverse events in patients with low viral load (HBV DNA level <2000 IU/ml). An HBsAg level >1000 IU/ml in HBeAg-negative patients with low viral load, is associated with higher risks of HCC, cirrhosis, and HBeAg-negative hepatitis. Based on results of the REVEAL-HBV cohort study, a risk calculator to predict HCC in non-cirrhotic patients was developed and validated by independent international cohorts (REACH-B). In the recent update of the REVEAL-HBV study, HBsAg level was incorporated into the HCC risk prediction model with excellent accuracy. In conclusion, evidence from these HBV clinical cohorts confirms the progression and integration of viral biomarkers for the prediction of the prognosis of Asian chronic hepatitis B (CHB) patients. If the predictive power of the HCC risk calculator could be validated in non-Asian populations, it could be used in clinical practice to individualize the management of HBV carriers with different levels of HCC risk.

Key points

- The REVEAL-HBV cohort study showed that HBV viral load is a strong predictive factor of the risk of cirrhosis and HCC. Baseline serum HBV DNA level >2000 IU/ml may increase the risk of cirrhosis and HCC in adult HBV carriers.
- In the SEARCH-B cohort study, HBsAg level <100 IU/mL at 1 year post HBeAg seroconversion predicted HBsAg seroclearance within 6 years in spontaneous HBeAg seroconverters with HBV DNA level <200 IU/ml.
- The ERADICATE-B cohort study showed that HBsAg level >1000 IU/ml is associated with higher

risks of HBeAg-negative hepatitis, cirrhosis, and HCC in HBeAg-negative patients with HBV DNA level <2000 IU/ml.
- The predictive factors of HBV-related HCC include age, sex, family history of HCC, HBeAg serostatus, serum HBV DNA, ALT, HBsAg levels, HBV genotype and variant. The risk calculator can help individualize the management of Asian HBV carriers with different HCC risk levels.

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. More than 75% of HCC cases occur in the Far East and Southeast Asia. Marked geographical and ethnic variations have been found in the incidence of HCC, with the lowest rate of 3.8 per 100 000 in Caucasian men in the USA to the highest rate of more than 25 per 100 000 in Asian men in the Far East and Southeast Asia (1–3). The risk factors associated with the development of HCC include chronic infection with either hepatitis B virus (HBV) or hepatitis C virus, the presence of cirrhosis, exposure to carcinogens especially aflatoxin B1, cigarette smoking, alcohol abuse, obesity, ageing and male gender. Chronic HBV infection is the most common of these risk factors, especially in Asia and Africa (4, 5). Numerous results suggest an aetiological association between persistent HBV infection and HCC, including the geographical correlation between the prevalence of chronic HBV infection and the incidence of HCC, the high prevalence of hepatitis B surface antigen (HBsAg) in HCC patients, the increased relative risk of HCC in HBsAg carriers, the presence of integrated HBV DNA in HCC tissue, the reduced incidence of childhood HCC after HBV vaccination, and the association of chronic hepadnavirus infection with HCC in animal models (4, 5). Thus, HBV-related chronic hepatitis, cirrhosis and HCC are recognized as sequential adverse outcomes in HBV carriers (6, 7).

In Taiwan, a high annual incidence of HCC has been reported, ranging from 15/100 000 in the 1980s to approximately 30/100 000 in the 2000s (8). Before 1990, approximately 80% of patients had HBV related HCC (8). In addition, the prevalence of HBsAg in the general population was approximately 15–20% before the implementation of universal hepatitis B vaccination for newborns (9). The unique epidemiological and clinical features of HBV infection in Taiwan provide local investigators more opportunities to explore the impact of hepatitis B viral factors on the natural course of HBV infection through large cohort studies (Table 1). The first study was a community-based cohort known as the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV), which followed 3653 adult Taiwanese HBsAg seropositive subjects over a mean follow-up period of 11.4 years (10). The second study was a hospital-based cohort, the Study of E Antigen seRoClearance of Hepatitis B (SEARCH-B) (11). It enrolled 390 Taiwanese spontaneous hepatitis B e antigen (HBeAg) seroconverters without cirrhosis at enrolment and followed them for an average of 7.4 years. This unique study allowed investigation of the early HBeAg-negative stage because follow-up began 1 year after HBeAg seroconversion. The third study was a hospital-based cohort, the Elucidation of Risk fActors for DIsease Control or Advancement in Taiwanese Hepatitis B Carriers (ERADICATE-B) that enrolled 2688 Taiwanese HBV carriers with no evidence of clinical cirrhosis at baseline who remained treatment-naive during follow-up. The mean follow-up period was 14.7 years (12). In this review, recent advances made in relation to the impact of HBV factors on disease progression are evaluated, and a model for the prediction of the risk of developing HCC based on the HBV clinical cohorts in Taiwan is summarized and discussed.

Evolution of hepatitis B viral biomarkers

HBV is the smallest human DNA virus with 3200 bp in a partially double-stranded circular DNA. After HBV infection, the partially double stranded DNA transforms into covalently closed circular DNA (cccDNA), a transcriptionally active template in the nucleus of

Table 1. Summary of three HBV cohort studies from Taiwan

Cohort	REVEAL-HBV	SEARCH-B	ERADICATE-B
Study design	Community-based cohort	Hospital-based cohort	Hospital-based cohort
Disease stage	Including HBeAg-positive and -negative phases	Early HBeAg-negative phase	Including HBeAg-positive and -negative phases
Number of patients	3653	390	2688
Follow-up (years)	11.4	7.4	14.7
Main results	Baseline serum HBV DNA level >2000 IU/ml start to increase risk of cirrhosis and HCC	A lower serum HBsAg level at early HBeAg-negative phase is associated a higher HBsAg loss rate in spontaneous HBeAg seroconverters	In HBeAg-negative patients with an HBV DNA level <2000 IU/ml, an HBsAg level >1000 IU/ml is associated with higher risks of hepatocellular carcinoma, cirrhosis and HBeAg-negative hepatitis.

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hepatocytes. During the HBV life cycle, pregenomic RNA can be transcribed from cccDNA to serve as the replication template of negative-strand DNA through reverse transcription, and then fully double-stranded DNA through DNA polymerase within the nucleocapsid, with, finally, the assembly of the envelope protein to form mature HBV virions (13, 14). The HBsAg is presumably responsible for receptor binding and is composed of large, middle and major (or small) proteins that are synthesized by beginning transcription with the pre-S1, pre-S2 or S gene of cccDNA. Serum HBsAg can be produced by three pathways: (i) translation of cccDNA molecules to form the envelope of an HBV virion; (ii) a spherical or filamentous form of non-infectious subviral particles and (iii) small HBsAg and truncated Pre-S protein generated from HBV DNA integrated to the host genome (15, 16). HBV DNA is the only biomarker related to the HBV lifecycle from infectious particles, and its level reflects viral replication. Thus a decrease in HBV DNA is a sign of reduced HBV replication. In contrast, circulating HBsAg can be derived from both mature virions and defective subviral particles. Recent studies also suggest that restoration of host immunity against HBV infection may reduce serum HBsAg levels (16, 17). Therefore, serum HBsAg levels not only reflect cccDNA transcription or mRNA translation but also host immune control over HBV infection (16–19). It is generally believed that the combination of HBsAg and HBV DNA levels provides the best differentiation of the clinical phases of the natural history of HBV infection. For example, levels are highest during the initial immune tolerance phase. The levels then decrease during the immune clearance phase and further decrease to their lowest levels during the immune control phase or inactive carrier state. During the reactivation or HBeAg-negative hepatitis phase, both HBsAg and HBV DNA levels rise again (20). The role of HBV DNA and HBsAg levels in predicting favourable and poor clinical outcomes of chronic hepatitis B were investigated in three Taiwanese HBV clinical cohorts based on the dynamics of HBV DNA and HBsAg during the natural history of HBV infection.

REVEAL-HBV cohort: The impact of viral load on cirrhosis and HCC prediction

Detection and quantification of HBV DNA is recommended to diagnose HBV infection, determine the indication for treatment, and monitor antiviral treatment response and emergence of drug resistance (21–23). HBV DNA levels also provide valuable prognostic information. In 2006, the impact of viral load on the risk of HCC was first assessed in the REVEAL-HBV cohort study. Eighty-five per cent of 3653 adult HBV carriers were HBeAg-negative and were followed for a mean 11 years. The cumulative incidence of HCC increased with serum HBV DNA levels in a dose dependent manner. The incidence ranged from 1.3% to 14.9% in patients with an HBV DNA level of less than 300 copies/ml (~60 IU/mL) and 10^6 copies/ml (~200 000 IU/mL) or more respectively ($P < 0.001$). After adjustment for HBeAg status and serum ALT levels among other variables, hepatitis B viral load was the strongest predictor of the development of HCC. The relative risk began to increase at an entry HBV DNA level of 2000 IU/ml (HR: 2.3; 95% CI: 1.1–4.9; $P = 0.02$). Patients with HBV DNA levels of 200 000 IU/ml or more had the greatest risk (HR: 6.1; 95% CI: 2.9–12.1; $P < 0.001$). In particular, the dose-dependent relationship was most marked in HBeAg seronegative patients with normal serum ALT levels and no cirrhosis at study entry (10, 24). Several cross sectional and longitudinal cohort studies in Taiwan, Hong Kong and China confirmed the impact of HBV DNA levels on the development of HCC (25–31).

In the REVEAL-HBV cohort study, a dose-dependent relationship between HBV DNA levels and the development of cirrhosis was also noted (32), and the risk began to increase when the HBV DNA level ≥2000 IU/mL. In addition, liver-related mortality rate also increased with serum HBV DNA levels. HCC mortality ranged from 72.8 to 815.6 per 100 000 person-years and chronic liver disease/cirrhosis deaths ranged from 9.1 to 267.4 per 100 000 person-years for subjects with HBV DNA levels below 60 IU/ml and 200 000 IU/ml or more respectively (33). These findings support the general notion that cirrhosis develops with the accumulation of extracellular matrix as a result of liver cell injury, and HCC or mortality may subsequently emerge in the setting of cirrhosis (6, 7, 34, 35).

In summary, the REVEAL-HBV cohort study showed that HBV viral load is a strong predictive risk factor of cirrhosis, HCC and mortality in HBV carriers, independent of HBeAg status, ALT level and other risk factors. Baseline serum HBV DNA level >2000 IU/mL may increase the risk of cirrhosis, HCC and mortality in HBV carriers 30–65 years old after >10 years of follow-up. In addition, patients with persistently high HBV DNA levels have the highest risk (36). Thus, HBV viral load measurements can help define which HBV carriers over the age of 30 are at a high risk of cirrhosis and HCC.

SEARCH-B cohort study: importance of HBsAg levels in prediction of HBsAg clearance

Although the annual rate of HBsAg loss in Asian HBV carriers who acquire the virus early in life is extremely low (~1–2% per year in HBeAg (−) patients with normal ALT levels) (37), spontaneous HBsAg seroclearance has been widely accepted as an indicator of disease remission and favourable clinical outcome (38–40). However, little is known about the predictors of HBsAg seroclearance. The REVEAL-HBV cohort study found that a low HBV DNA level (<200 IU/ml) was significantly associated with HBsAg seroclearance [adjusted odds ratio (OR) of 4.17; 95% CI: 2.55–6.82]. Among those with HBsAg seroclearance, 95.8% had undetectable HBV

DNA levels before seroclearance. In addition, patients with an undetectable viral load (<60 IU/mL) had an annual HBsAg seroclearance rate of 5.76% (41).

Later, the SEARCH-B cohort study investigated the role of HBsAg levels in predicting HBsAg seroclearance. This cohort study enrolled 390 non-cirrhotic chronic hepatitis B (CHB) patients with spontaneous HBeAg seroconversion with an average follow-up of 7.4 years. Both lower HBV DNA and HBsAg levels were associated with a greater probability of HBsAg seroclearance. Areas under receiver operating characteristic (AUROC) curves for HBV DNA and HBsAg levels were compared to predict 6-year HBsAg seroclearance. HBsAg level was shown to be a better predictor than HBV DNA level (AUROC curve: 0.90 vs. 0.69, $P = 0.012$). Even in patients with a very low viral load (HBV DNA level <200 IU/ml), the HBsAg level <100 IU/ml remained an independent predictor of HBsAg seroclearance (11).

The investigators of the SEARCH-B cohort then examined the impact of viral load on long-term outcomes after spontaneous HBeAg seroconversion. Compared to patients with HBV DNA levels <2000 IU/ml, the adjusted hazard ratios of HBeAg-negative hepatitis, a precursor of cirrhosis and HCC, were 2.4 (95% CI: 1.3–4.4), 3.6 (95% CI: 1.8–7.2) and 5.3 (95% CI: 2.8–10.0), respectively, for serum HBV DNA levels of 2000 -2×10^4, 2×10^4 -2×10^5 and $\geq 2 \times 10^5$ IU/mL after a mean follow-up of 6.8 years (34), suggesting that serum HBV DNA levels ≥2000 IU/ml at 1 year post HBeAg seroconversion were correlated to an increased risk of HBeAg-negative hepatitis.

In addition to viral load and HBsAg levels, previous case-control studies and the REVEAL-HBV cohort study reported that with the presence of HBV basal core promoter (BCP) A1762T/G1764A variants was significantly associated with the development of HCC (28, 29, 42–45). Recently, in a substudy of the SEARCH-B cohort, BCP A1762T/G1764A variants were determined qualitatively and correlated quantitatively with cirrhosis. The data showed that BCP A1762T/G1764A variants served as an independent risk factor for the development of cirrhosis (HR: 4.26; 95% CI: 1.32–13.77). Quantitative analysis using pyrosequencing revealed that the risk of cirrhosis was higher in patients with BCP A1762T/G1764A variants ≥45% compared to <45% (adjusted OR: 2.81; 95% CI: 1.40–5.67; $P = 0.004$) (46).

In summary, lower serum HBsAg levels during the early HBeAg-negative phase in spontaneous HBeAg seroconverters is associated with a higher HBsAg seroclearance rate. HBsAg level <100 IU/ml is predictive of HBsAg seroclearance within 6 years in spontaneous HBeAg seroconverters with HBV DNA level <200 IU/ml.

ERADICATE-B cohort study: Use of HBsAg quantification and HBV DNA to predict HCC

The association between HBsAg levels and HCC was first addressed by the ERADICATE-B cohort study as HBsAg quantification became available in clinical practice (12). This cohort showed that elevated HBV DNA and HBsAg levels were both positively correlated with the development of HCC in dose-dependent manner. The risk of HCC also increased when HBV DNA levels were higher than 2000 IU/ml, which is consistent with the findings in the REVEAL-HBV study. When we compared the predictive power of serum HBV DNA and HBsAg levels for HCC, it was shown that HBV DNA levels were a better predictor than HBsAg. However, the predictive value of the two biomarkers reversed if we focused on 1068 HBeAg-negative patients with HBV DNA levels <2000 IU/ml, for whom HBV DNA levels played a minimal role in predicting HCC. The risk of HCC was significantly increased in these patients, with an HBsAg level ≥1000 IU/ml compared to those with a level <1000 IU/ml (HR: 5.4; 95% CI: 2.1–14.2). The 10-year cumulative incidence rate of HCC was 0.2% for HBeAg-negative patients with an HBV DNA level <2000 IU/ml and an HBsAg level <1000 IU/ml, similar to the rate of non-HBV and non-HCV infected individuals (47). Multivariate analysis showed that HBsAg level ≥1000 IU/mL was an independent risk factor for the development of HCC (HR: 13.7; 95% CI: 4.8–39.3). Furthermore, the relationship between the risk of HCC and dynamic changes in serum HBV DNA, HBsAg and ALT levels were evaluated in the ERADICATE-B study. Patients with persistently high HBV DNA, HBsAg or ALT levels were at a higher risk of HCC than those with persistently low levels.

In addition to low viral load, we also explored the predictive role of HBsAg levels in HBeAg-negative patients with intermediate viral loads (HBV DNA levels between 2000 and 20 000 IU/ml) and high viral loads (HBV DNA >20 000 IU/ml) in the ERADICATE-B cohort study (48). It was shown that HBsAg could stratify HCC risk in patients with intermediate viral loads but not in those with high viral loads. Based on HBsAg levels of 100 IU/ml and 1000 IU/ml, the risk of HCC could be stratified into three groups with an annual incidence of 0.09%, 0.34% and 0.46% respectively.

The recent update of the REVEAL-HBV cohort consistently showed that HBsAg and HBV DNA levels were independent predictors of the development of HCC. In particular, HBsAg levels were shown to complement HBV DNA levels in stratifying the risk of HCC, especially in patients with HBV DNA levels <200 000 IU/mL (49). Although the HBV DNA cut-off level is slightly different from the ERADICATE-B cohort study (20 000 IU/ml), both data suggest that the predictive role of HBsAg level is the most effective in patients with HBV DNA levels <2000 IU/ml.

In summary, data from both the REVEAL-HBV community-based cohort study and the ERADICATE-B hospital-based cohort study showed that serum HBsAg and HBV DNA levels were complementary markers for predicting HCC in Taiwanese adult HBV carriers.

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On the basis of previous findings, it is reasonable to hypothesize that a similar relationship should exist between HBsAg levels and the development of cirrhosis in HBV carriers with low viral loads. Not surprisingly, the ERADICATE-B cohort examined this issue by analysing 1068 HBeAg (−) patients with low viral loads (<2000 IU/ml). The results showed that an HBsAg level ≥1000 IU/ml was consistently associated with a higher risk of cirrhosis than HBsAg levels <1000 IU/ml (HR: 2.2; 95% CI: 1.1–4.2), suggesting that HBsAg levels could help predict the development of cirrhosis (50). The ERADICATE-B cohort also explored the role of HBsAg levels in predicting HBeAg-negative hepatitis in HBeAg-negative patients with low viral loads. An HBsAg level of 1000 IU/ml was chosen as the cut-off and the results showed that higher HBsAg levels were associated with a higher risk of HBeAg-negative hepatitis. Compared to patients with an HBsAg level <1000 IU/ml, an HBsAg level ≥1000 IU/ml had an HR of 1.4 (95% CI: 1.1–1.8) (50). This study further analysed the third year of follow-up in a subcohort of patients with low viral loads plus normal baseline ALT levels and found that an HBsAg level ≥1000 IU/ml was associated with a higher rate of HBV DNA reactivation (17.2% vs. 9.9%, $P = 0.002$) (50).

In contrast, Martinot-Peignoux *et al.* recently reported that HBeAg-positive patients with advanced fibrosis had significantly lower serum HBsAg and HBV DNA levels compared to those with no or mild hepatic fibrosis. Of particular note is that HBeAg-positive patients with genotype B or C infection could be correctly classified as the presence of advanced fibrosis by using a lower serum HBsAg level (51). The biological mechanisms leading to discrepant results of serum HBsAg level for the assessment of disease severity between HBeAg-positive and -negative patients await further investigations.

Finally, the ERADICATE-B cohort explored the association of HBsAg seroclearance with both HBV DNA and HBsAg levels in 688 HBeAg-negative patients with low baseline viral loads. Results were similar to the SEARCH-B cohort study. The annual HBsAg clearance rate was 7% in patients with an HBsAg level <10 IU/ml, and the HR for HBsAg seroclearance was 13.2 (95% CI: 8.1–21.5) compared to an HBsAg level ≥1000 IU/ml (52). This large-scale study confirmed the importance of baseline HBsAg levels for the prediction of HBsAg seroclearance over time.

In summary, the ERADICATE-B cohort study showed that elevated HBV DNA and HBsAg levels were positively correlated with the development of HCC. HBV DNA level played a minimal role in predicting HCC in HBeAg-negative patients with HBV DNA levels <2000 IU/ml, whereas HBsAg level retained its predictive power. HBsAg level <1000 IU/ml can serve as an indicator for a lower risk of HBeAg-negative hepatitis, cirrhosis and HCC. In our clinical practice, combining HBsAg level <1000 IU/ml with low or normal levels of HBV DNA and ALT at baseline may help identify HBV carriers with a minimal risk of HBV-related adverse outcomes (Fig. 1).

Risk calculator for HBV-related HCC in treatment-naïve CHB patients

Because HCC is the most common cause of death in CHB patients, a simple formula with different weights for different clinical and virological variables is urgently needed to predict the risk of HCC in a few years. An easy-to-use formula for HCC risk estimation is usually derived from existing longitudinal cohorts with long-term

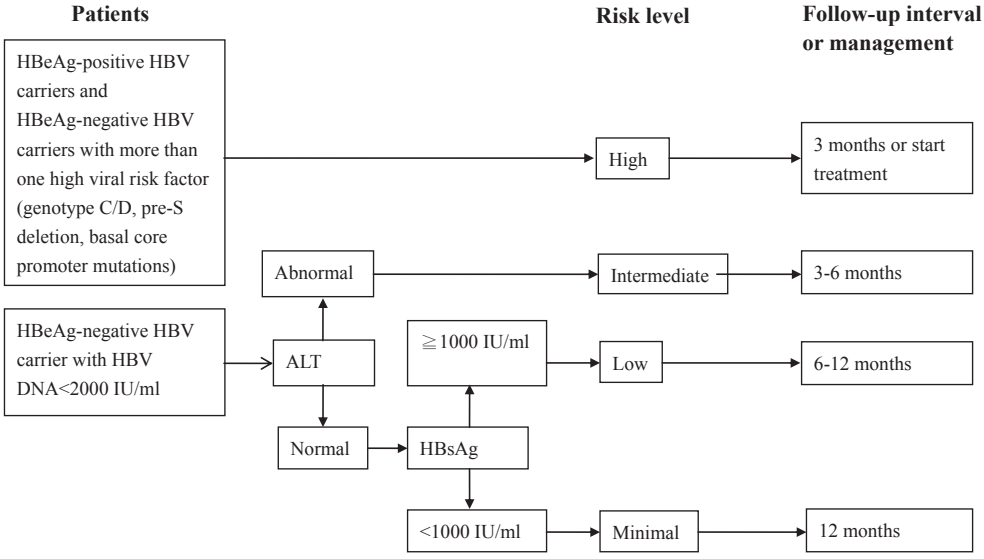

Fig. 1. A hypothetical algorithm to categorize risk levels of disease progression and hepatocellular carcinoma with corresponding management in Asian patients with chronic hepatitis B virus infection.

Table 2. Hepatocellular carcinoma risk score and risk levels stratification for chronic hepatitis B patients: an upgraded version from the REVEAL-HBV cohort study

Baseline predictor	Risk score
Age (year)	
30–34	0
35–39	1
40–44	2
45–49	3
50–54	4
55–59	5
60–65	6
Sex	
Female	0
Male	2
Levels of ALT (IU/L)	
<15	0
15–44	1
≥45	2
Family history of hepatocellular carcinoma	
No	0
Yes	2
HBeAg/HBV DNA(copies/ml)/HBsAg(IU/ml)/genotype	
Negative/<10^4/<100/any type	0
Negative/<10^4/100–999/any type	2
Negative/<10^4/≥1000/any type	2
Negative/10^4–10^6/<100/any type	3
Negative/10^4–10^6/100–999/any type	3
Negative/10^4–10^6/≥1000/any type	4
Negative/≥10^6/any level/B or B + C	5
Negative/≥10^6/any level/C	7
Positive/any level/any level/B or B + C	6
Positive/any level/any level/C	7
Risk levels of developing hepatocellular carcinoma	Sum of score
Low risk	<9
Medium risk	9–12
High risk	≥13

Modified from Lee *et al.* Hepatology 2013 and Yang *et al.* World J Gastroenterol 2014.

follow-up. Although several calculators to predict the risk of HBV-related HCC have been reported, they must be externally validated (25, 26, 53, 54).

Through international collaboration the REACH-B (risk estimation for hepatocellular carcinoma in chronic hepatitis B) study developed and validated a predictive score for the risk of development of HBV-related HCC (55). This study included a risk score development cohort with 3584 non-cirrhotic, CHB patients without antiviral treatment (REVEAL-HBV cohort) and a validation cohort with 1050 patients from three independent hospitals in Hong Kong and South Korea. The 17-point risk score included 5 predictors of HCC, including sex, age, serum ALT level, HBeAg status and serum HBV DNA levels (SALED). The risk score precisely estimated the risk of developing HCC at 3, 5 and 10 years of follow-up in the validation cohort. Additional ROC curves and calibration charts also confirmed the predictive value of this risk score in non-cirrhotic patients. The AUROCs for predicting 3-, 5- and 10-year HCC risk were 0.811 (95% CI: 0.790–0.831), 0.796 (95% CI: 0.775–0.816) and 0.769 (95% CI: 0.747–0.790) respectively (55).

Although the REACH-B risk calculator of HCC in non-cirrhotic CHB patients has been externally validated, this scoring system may underestimate the risk in patients with very low viral load at baseline. Based on the ERADICATE-B cohort, HBsAg level is known to be a complementary marker for the risk of HCC in the low viral load group (12). In the ERADICATE-B subcohort study, the predictive power of HBsAg level for HCC was analysed for different viral loads. A total of 2165 Taiwanese HBeAg-negative non-cirrhotic patients were followed for 14.9 years. There was no association between HBsAg level and HCC in patients with higher viral loads (≥20 000 IU/ml). HBsAg level was then included to stratify the risk of HCC in patients with low (<2000 IU/ml) and intermediate viral loads (2000–19 999 IU/ml). ROC curve analysis showed that combining HBV DNA and HBsAg level improved the prediction of the development of HCC at 10 years compared to the HBV DNA level alone in patients with low and intermediate viral loads as well as the overall cohort ($P = 0.004$ and 0.028 respectively) (48). In the recently updated REVEAL-HBV study, HBsAg level was incorporated into the HCC risk prediction model (49). Predictive factors of risk included age, sex, family history of HCC, HBeAg serostatus, serum HBV DNA, ALT, HBsAg levels and HBV genotype. The sum risk scores ranged from 0 to 19

Table 3. Comparison of various versions of risk calculator models for hepatitis B virus-related hepatocellular carcinoma

Model	REACH-B	REACH-B IIa	REACH-B IIb
Risk factors	Basic predictors plus HBV DNA	Basic predictors plus HBV DNA plus qHBsAg	Basic predictors plus qHBsAg
Discriminatory capability	Worst	Best	Good
Cost	Fairly expensive	Priciest	Cheapest
Potential usage	Should be replaced by newer versions	Used by hepatologist for the management of chronic hepatitis B patients	First-line risk calculator for general practitioners, community surveys and countries with constrained medical resources

Basic predictors: sex, age, serum ALT level, HBeAg status
REACH-B, risk estimation for hepatocellular carcinoma in chronic hepatitis B; qHBsAg, quantitative HBsAg.

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in the HCC prediction model (Table 2). Patients were categorized by their sum risk scores into low-risk (risk score <9), medium-risk (risk score 9–12) and high-risk (risk score ≥13) groups. The observed cumulative risk of HCC for the high-risk group was significantly higher than in the low-risk and medium-risk groups ($P < 0.001$) (49). Although the predictive accuracy of the upgraded REVEAL-HBV risk calculator for HCC was excellent in non-cirrhotic CHB patients, its cost-effectiveness must be confirmed. Testing serum HBV DNA levels for the REACH-B or upgraded REVEAL-HBV risk calculator is relatively costly. Thus new models need to be generated for target populations. With the recent progress in HBsAg quantification, serum HBsAg levels have been included in the REACH-B IIa model and replaced serum HBV DNA levels in the REACH-B IIb model (Table 3). The REACH-B IIa model could be used in clinical practice by hepatologists for the management of CHB patients, while the REACH-B IIb model could be a first-line risk calculator for general practitioners, community surveys and countries with limited medical resources (56).

Conclusions and perspectives

CHB is a complex disease with various clinical outcomes. Over the past decade, extensive research has identified several hepatitis B viral factors such as serum HBsAg levels, viral load, genotype and mutants that contribute to disease progression in chronic hepatitis B patients. Through the evaluation of the natural history of HBV in cohorts from Taiwan, HBV factors affecting the remission or progression of liver disease and the predictive value of each factor have been identified. Serum HBV DNA level is the most powerful predictive factor of an adverse outcome in Asian HBV carriers with high viral load (≥2000 IU/ml), while HBsAg levels can complement HBV DNA levels to predict HBV-related adverse events in patients with a low viral load (<2000 IU/ml). On the other hand, both lower HBV DNA and HBsAg levels were strongly associated with HBsAg seroclearance and disease remission. If these findings are externally validated in other ethnic populations, practicing physicians could combine serum HBV DNA and HBsAg levels to identify genuine inactive carriers and stratify the risk of disease progression or HCC in Asian HBV carriers.

Although integrating HBsAg level into risk calculators can help classify patients whose disease will or will not progress, the potential interactive effects of known hepatitis B viral factors on the development of HCC are not incorporated into these models. Several studies have reported that the combination of HBV genotype C, HBV mutants of the pre-S, precore and basal core promoter regions and high HBV viral loads were significantly associated with the risk of HCC (27–29, 31, 57, 58). In the future, qualitative and quantitative hepatitis B viral factors must be incorporated into risk calculation models to develop more comprehensive models that will be clinically applicable to various forms of chronic liver disease, including inactive carrier state, chronic hepatitis and cirrhosis (59). In addition, the management of CHB with antivirals has markedly improved the long-term outcomes of CHB patients. Risk modification through antiviral therapy may prevent disease progression and reduce the risk of development of HCC. HBV-related HCC risk calculators should be associated with current HBV treatment guidelines to establish personalized management strategies for HBV carriers with different levels of risk of HCC.

Acknowledgements

Conflict of interest: The authors do not have any disclosures to report.

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