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Review Article

Evolution of viral biomarkers in predicting outcomes of chronic hepatitis B patients: From DNA to surface antigen

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ABSTRACT

Quantification of hepatitis B virus (HBV) DNA and quantitative hepatitis B surface antigen (HBsAg) have improved our understanding and management of chronic hepatitis B (CHB). Both HBV DNA and HBsAg levels are highest in the immune tolerance phase, start to decline during the immune clearance phase, and further decline after hepatitis B e antigen (HBeAg) seroconversion. These levels are lowest in the inactive carrier state but rise again in patients who develop HBeAg-negative hepatitis. Previous studies have shown that an HBV DNA level ≥2000 IU/mL is associated with high risks of hepatocellular carcinoma, liver cirrhosis, and hepatitis activity, whereas a lower HBV DNA level is associated with a better chance of HBsAg loss, which is very close to a clinical cure for HBV infection. Recent studies further suggested that HBsAg level is not only a better predictor of HBsAg loss compared with the HBV DNA level, but also can complement the HBV DNA level in predicting HBV-related adverse events in patients with an HBV DNA level <2000 IU/mL. In the Asia Pacific region, where HBV genotypes B and C prevail, an HBsAg level ≤100 IU/mL has been shown to serve as a predictor of HBsAg loss over time. 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. European studies also indicated that combining levels of HBsAg <1000 IU/mL and HBV-DNA <2000 IU/mL aids in identifying true inactive carriers in genotype D HBeAg-negative carriers. All this evidence highlights the evolution of viral biomarkers in predicting the prognosis of CHB. Quantitative HBsAg can complement HBV DNA in optimizing the management of CHB patients in clinical practice.

1. Introduction

Although safe and effective vaccines have been available for three decades, hepatitis B virus (HBV) infection remains a major public health problem across the world. The clinical manifestations of HBV infection range from acute or fulminant hepatitis to various forms of chronic infection, including the inactive carrier state, chronic hepatitis, cirrhosis, and even hepatocellular carcinoma (HCC) [1,2].

Hepatitis B surface antigen (HBsAg) is the hallmark of HBV infection and was first discovered by Blumberg et al in 1965 [3]. Since then, the qualitative status of HBsAg has been used to define the HBV infection status. Initially, this marker was not widely used to monitor disease progression in patients with chronic HBV infection. In short, clinical practice has generally relied on qualitative or semiquantitative serological markers, such as HBsAg and the hepatitis B e antigen (HBeAg), to determine HBV disease stages. HBsAg is a marker of chronic HBV infection. HBeAg is a circulating peptide derived from the core gene, then modified and secreted from liver cells. It usually serves as a marker of active viral replication. In addition, liver biochemical markers, such as the alanine aminotransferase (ALT) level, are checked repeatedly to evaluate the severity of liver cell necrosis and to estimate viral activity.

Conflict of interest: none.

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This practice has evolved since 2006, when quantification of the serum HBV DNA level was found to be useful in predicting long-term adverse outcomes [4,5]. Since then, the HBV DNA level has been widely used to evaluate viral activity and the risk of disease progression. The HBV DNA is a more precise predictor of viral activity than the ALT level, but both share the same flaw—they vary with time [6]. Therefore, both require repeated testing to determine viral activity.

Recently, HBsAg quantification has gained much attention because it stratifies the risk of disease progression more accurately than HBV DNA level and may serve as a marker reflecting host immune control over HBV infection [7].

In this review article, the evolutionary role of the HBV DNA and HBsAg levels in predicting the prognosis of chronic hepatitis B (CHB) is summarized and discussed.

2. Life cycle of HBV and synthesis of HBV DNA and HBsAg

With only 3200 bp in its genome, HBV is the smallest DNA virus. Fig. 1 illustrates the pathway of HBV DNA and HBsAg production in the HBV life cycle. The replication template of HBV is the covalently closed circular DNA (cccDNA), which exists in the liver and encodes four overlapping open reading frames (ORF).

S stands for the surface or envelope gene, C for the core gene, P for the polymerase gene, and X for the X gene [2]. The S and C genes have upstream regions designated pre-S and pre-C. The complete infectious virion, or Dane particle, is a 42-nm sphere containing the nucleocapsid and partially double-stranded circular HBV DNA. HBV DNA is synthesized via reverse transcription of the pregenomic RNA, which is also derived from cccDNA. Therefore, cccDNA is the template for both HBV DNA and HBsAg synthesis, although both products are derived from different ORFs of cccDNA (Fig. 1).

HBsAg is a glycosylated envelope protein of the HBV virion. There are three HBsAg proteins—small, medium, and large—and these are produced from three ORFs, called the pre-S1, pre-S2, and S ORF, of cccDNA. In addition to being on the mature virion, there are large numbers of two types of noninfectious HBsAg particles in the sera of HBV carriers, spherical particles and filamentous forms (Fig. 1). These subviral particles do not contain the HBV genome but are secreted at levels in excess (100- to 100,000-fold) of mature virions. In addition, HBsAg can also be derived from viral sequences that are randomly integrated into the host genome. Therefore, whereas serum HBV DNA levels merely reflect viral replication activity, serum HBsAg levels reflect activity from translated messenger RNAs of transcriptionally active cccDNA and from integrated HBV DNA sequences. Thus, the HBsAg level provides complementary information that may improve our understanding of the infection status of patients.

3. Natural history of CHB

In Asia, where HBV infection is hyperendemic, HBV carriers usually acquire the virus perinatally or in early childhood (by the age of 2 years) [8]. Therefore, the age of a given patient can be considered the duration of HBV infection.

Considering virus and host interaction, the natural course of chronic HBV infection in Asian patients can be divided into four chronological phases [9,10] (Fig. 2). The first is the “immune tolerance phase”, where there is active replication of HBV. Hence, this is characterized by positive HBeAg and normal-to-low ALT levels. The second is the “immune clearance phase”, where HBeAg-positive patients have raised ALT and declining HBV DNA levels. In the third “low replication or residual phase”, where HBeAg is lost, anti-HBe is gained, and remission of liver disease occurs, and thus patients are said to be in an “inactive carrier state”. Taken together, HBeAg seroconversion is regarded as a pivotal event in the natural history of HBV infection because it usually confers a favorable clinical outcome [11]. About 20–30% of inactive carriers enter the “reactivation or HBeAg-negative hepatitis phase”, which is a variant form of the immune clearance phase [12–14]. Previous longitudinal studies indicated that HBeAg-negative hepatitis (ENH) is an important risk factor for subsequent cirrhosis and HCC

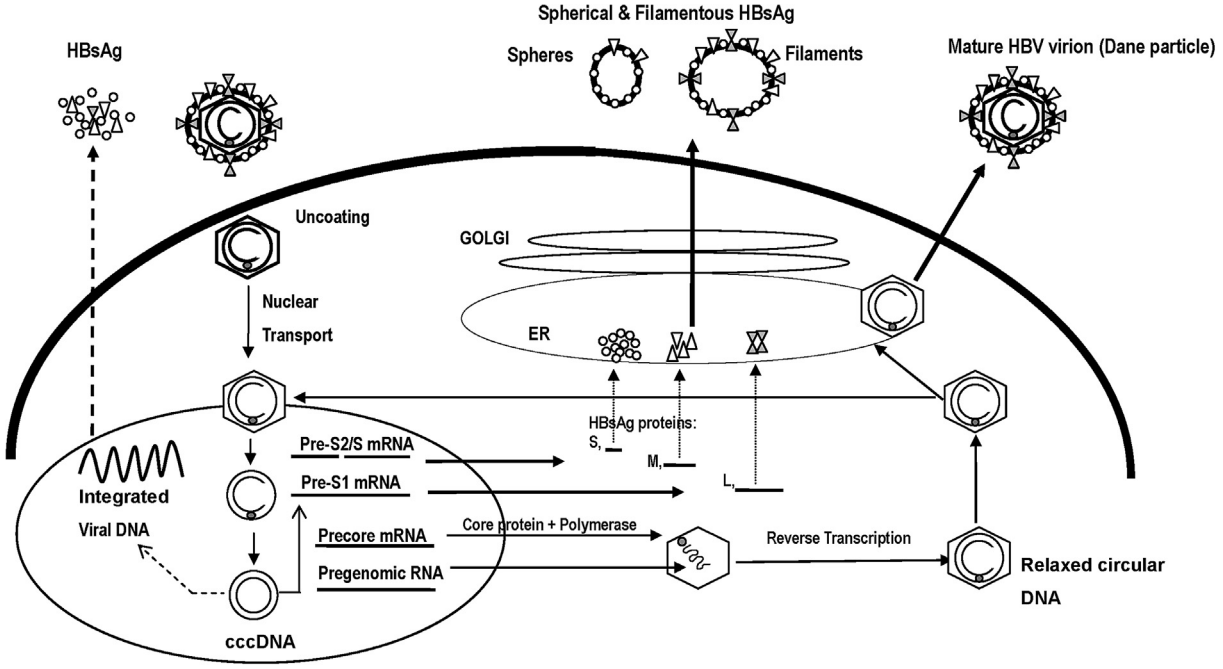

Fig. 1. Pathway of HBsAg production in the life cycle of HBV replication (adapted from J Gastroenterol. 2013;48:13–21). cccDNA = covalently closed circular DNA; HBsAg = hepatitis B surface Antigen; HBV = hepatitis B virus.

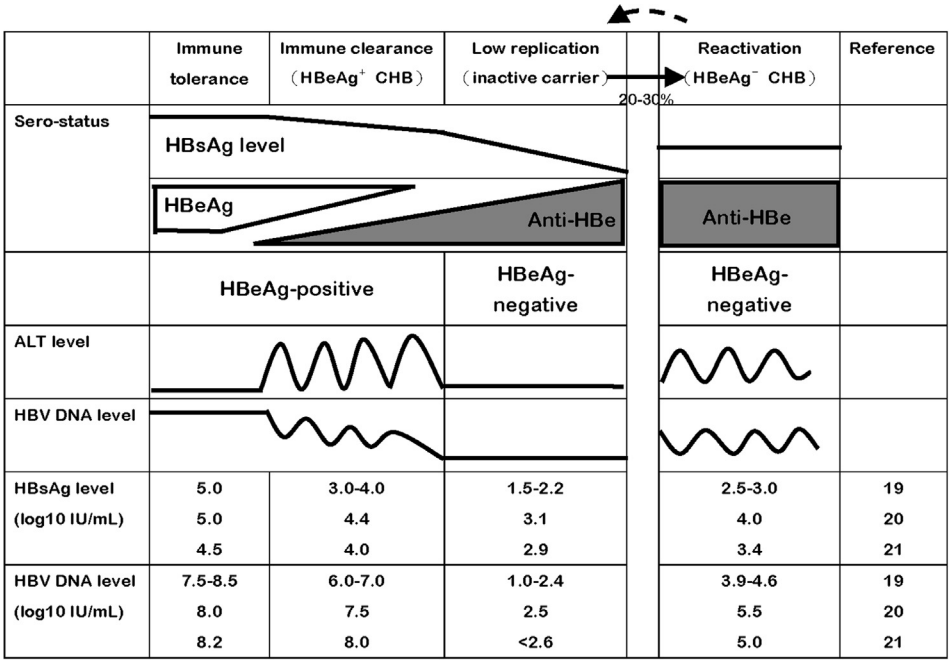

Fig. 2. HBsAg levels in different phases of chronic HBV infection (adapted from J Gastroenterol. 2013;48:13–21). ALT = alanine aminotransferase; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus.

development [12,15–17]. Therefore, early identification of patients at risk of ENH and prompt antiviral treatment are mandatory in preventing or hindering disease progression. However, ENH also provides the opportunity for HBsAg seroclearance or seroconversion, which is a favorable clinical outcome [18].

4. HBsAg and HBV DNA levels in different phases of CHB

Several cross-sectional studies have explored HBV DNA and HBsAg levels in different phases of CHB (Fig. 2) [19–21]. Comparable results have been found irrespective of study population and HBV genotype. Both HBsAg and HBV DNA levels vary in different phases of HBV infection but generally decrease as HBV carriers age. The levels are highest in the initial immune tolerance phase when there is no or only minimal hepatitis activity and hence, serum ALT levels are normal. The levels decline during the immune clearance phase and further decrease in those who maintain normal ALT levels after HBeAg seroconversion. It has been consistently shown that the lowest HBsAg and HBV DNA levels occur in the immune control phase or inactive carrier state but rise again in the reactivation phase or in ENH.

5. Predictive value of HBsAg and HBV DNA in the outcome of CHB

Several previous studies indicated that in HBV carriers, cirrhosis is a consequence of extracellular matrix accumulation from liver cell injury, and HCC may subsequently emerge under this setting [15,16]. Therefore, HBV-related hepatitis, cirrhosis, and HCC are regarded as sequential adverse outcomes. By contrast, HBsAg loss occurs at an annual rate of 0.5–2.3%, which is a marker for disease cure [6,22–27]. Fortunately, in the absence of confounding factors, such as liver cirrhosis, hepatitis C or hepatitis D virus, superinfection or age >50 years at the time of HBsAg loss, the risk for HCC development is minimal [28].

Cohort studies are preferred over cross-sectional studies to investigate the impact of dynamic factors on disease progression, so we will review the role of HBV DNA and HBsAg levels in predicting favorable and unfavorable outcomes by referring to three cohort studies (Table 1). The first was a community-based cohort study 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 patients over a mean follow-up period of 11.4 years. The second study was a hospital-based cohort study, the Study of E Antigen seRoClearance of Hepatitis B (SEARCH-B). It enrolled 390 Taiwanese spontaneous HBeAg seroconverters without liver cirrhosis at enrollment and followed them for an average of 7.4 years. This study is unique in that it enabled investigation of the early HBeAg-negative stage because follow-up started at 1 year after HBeAg seroconversion. The third study was a hospital-based cohort study, the Elucidation of Risk fActors for DIsease Control or Advancement in Taiwanese Hepatitis B Carriers (ERADICATE-B), which enrolled 2688 Taiwanese HBV carriers who did not have evidence of cirrhosis at baseline and were not treated during the follow-up period. The mean follow-up period was 14.7 years.

Table 1
Summary of three Taiwanese HBV cohort studies.

Cohort	Study design	Disease stage	Number of participants	Follow-up (y)	References
REVEAL-HBV	Community -based cohort	Including HBeAg-positive and -negative phases	3653	11.4	[4,5,26,36,48–50,53,54]
SEARCH-B	Hospital-based cohort	Early HBeAg negative phase	390	7.4	[6,14]
ERADICATE-B	Hospital-based cohort	Including HBeAg-positive and -negative phases	2688	14.7	[27,47,51]

ERADICATE-B = Elucidation of Risk fActors for DIsease Control or Advancement in Taiwanese Hepatitis B Carriers; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; REVEAL-HBV = Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus; SEARCH-B = Study of E Antigen seRoClearance of Hepatitis B.

6. Role of HBV DNA and HBsAg levels in predicting HBsAg loss

Spontaneous clearance of HBsAg has been widely accepted as an indicator of disease remission [18,28–31]. Earlier studies suggested that the rate of HBsAg loss is lowest in HBeAg-positive patients, followed by patients with ENH, and is highest in inactive carriers [18]. A Taiwanese longitudinal study of 1965 HBeAg-negative patients with normal ALT levels reported an annual HBsAg loss rate of 1.15% [23]. The authors noted that HBeAg-negative patients with sustained normal ALT levels were more likely to clear HBsAg than those with hepatitis relapse. Later, the REVEAL-HBV study found that a low HBV DNA level could predict HBsAg loss [26], where patients with an undetectable viral load (<60 IU/mL) had an annual HBsAg loss rate of 5.76%. Compared with patients with HBV DNA levels ≥200,000 IU/mL, the hazard ratio (HR) for HBsAg loss was 15.9 [95% confidence interval (CI), 9.3–27.2]. The positive association between limited viral replication and a higher chance of HBsAg loss as well as a low viral load linked to a low risk of hepatitis relapse can explain why HBsAg clearance occurs more frequently in patients with persistently normal ALT levels. In addition, the fact that an undetectable HBV DNA level usually precedes HBsAg loss also stresses the importance of the HBV DNA level in predicting HBsAg loss [26].

The SEARCH-B study revealed another predictor for HBsAg loss, the HBsAg level [6]. In this study, 18 patients cleared HBsAg at an annual rate of 0.6% during a mean follow-up period of 7.4 years. It was noted that both lower HBV DNA and HBsAg levels were associated with a higher likelihood of HBsAg clearance. Evaluation of 6-year HBsAg loss via receiver operating characteristic (ROC) curve analysis showed the HBsAg level was a better predictor than the HBV DNA level (area under the ROC curve: 0.90 vs. 0.69, $p = 0.012$). Even in patients with limited viral replication (HBV DNA level <200 IU/mL), an HBsAg level <100 IU/mL remained an independent predictor for HBsAg loss. Although this study shed much light on the importance of HBsAg levels, it lacked the statistical power to fully establish a relationship between HBsAg levels and HBsAg loss.

The subsequent ERADICATE-B cohort study investigating the relationship between HBsAg loss and both HBV DNA and HBsAg levels in 688 HBeAg-negative patients with HBV DNA levels <2000 IU/mL at baseline showed similar findings [27]. This study had adequate statistical power with 130 patients clearing HBsAg at an annual rate of 1.6% during a mean follow-up of 11.6 years. It also found that the HBsAg level served as a better predictor for HBsAg loss than the HBV DNA level. For example, the HBsAg annual clearance rate was 7% in patients with an HBsAg level <10 IU/mL, and its HR for HBsAg loss was 13.2 (95% CI, 8.1–21.5) compared with an HBsAg level ≥1000 IU/mL. This large-scale study firmly established the importance of HBsAg levels at baseline for subsequent HBsAg loss.

Several additional studies reaffirmed the relationship between the HBsAg level and HBsAg loss (Table 2). [4,27,32–36] A cohort study and a case-control study from Hong Kong showed that a lower HBsAg level was associated with a higher chance of HBsAg loss, and HBsAg levels of 100 and 200 IU/mL were the recommended cutoffs, respectively [32,33]. Other studies from Taiwan, including the REVEAL-HBV cohort study, a pediatric cohort study, and a case-control study, all had similar data [34–36]. In the recent REVEAL-HBV study, the authors proposed a 5- and 10-year HBsAg loss prediction model by combining the HBV DNA and HBsAg levels, and this combination indeed improved the prediction power compared with HBV DNA alone [36]. In summary, multiple lines of evidence support the notion that the HBsAg level serves as a very important predictor for HBsAg loss.

7. Role of HBV DNA and HBsAg levels in predicting HCC risk

Although most longitudinal cohort studies consider chronic hepatitis, cirrhosis, and HCC as sequential complications [12,15–17], HCC is still regarded as the most definitive end point for complications owing to its disastrous prognosis. In 2006, the REVEAL-HBV study reported that the serum HBV DNA level was a major determinant for HCC development [4]. They found that in adult HBV carriers, a higher HBV DNA level was associated with HCC development in a dose response manner [4]. In addition, the risk increased at HBV DNA levels ≥2000 IU/mL and further increased when the HBV DNA level was ≥20,000 IU/mL. These findings assisted in establishing 2000 IU/mL as the HBV DNA threshold required for treatment and defining patients with levels <2000 IU/mL as inactive HBV carriers [37–39]. Several cross-sectional and longitudinal studies in Taiwan, Hong Kong, and China reiterated the impact of the HBV DNA level on HCC development [40–46].

As HBsAg quantification became available, the association between the HBsAg level and HCC was first addressed by the ERADICATE-B cohort [47]. Initially, it demonstrated that elevated HBV DNA and HBsAg levels were both positively correlated with HCC development. When these two biomarkers were compared, the HBV DNA level was found to be a better predictor of 10- and 15-year HCC risk in the overall cohort. However, once the study population was restricted to HBeAg-negative patients with HBV DNA levels <2000 IU/mL, the HBV DNA level had a minimal role in predicting HCC, whereas HBsAg retained its predictive value. More specifically, in HBeAg-negative patients with HBV DNA levels <2000 IU/mL, the HCC risk increased in patients with an HBsAg level ≥1000 IU/mL compared with those with a level <1000 IU/mL (HR of 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 [48]. The

Table 2
Summary of relationship between HBsAg level and HBsAg loss.

Country	Study design	Disease stage	Number of participants	HBsAg cutoff (IU/mL)	Note	References
Taiwan	Cohort	Early HBeAg-negative stage	390	100	SEARCH-B	[4]
Hong Kong	Cohort	HBeAg-negative	103	100		[32]
Taiwan	Cohort	HBeAg-negative with HBV DNA level <2000 IU/mL	688	10	ERADICATE-B (partial)	[27]
Taiwan	Case control	HBeAg-negative	46–46	200		[35]
Hong Kong	Case control	HBeAg-negative	203–203	200		[33]
Taiwan	Cohort	Children	349	1000		[34]
Taiwan	Cohort	Including HBeAg-positive and -negative	3466	100	REVEAL-HBV	[36]

ERADICATE-B = Elucidation of Risk fActors for DIsease Control or Advancement in Taiwanese Hepatitis B Carriers; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; REVEAL-HBV = Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus; SEARCH-B = Study of E Antigen seRoClearance of Hepatitis B.

REVEAL-HBV study similarly found that the HBsAg, but not the HBV DNA level, could further stratify HCC risk in HBeAg-negative patients with an HBV DNA level <2000 IU/mL [49]. This strengthened the role of the HBsAg level in predicting HCC in HBV carriers with low viral loads.

8. Role of HBV DNA and HBsAg levels in predicting liver cirrhosis

It is generally believed that cirrhosis develops with the accumulation of extracellular matrix consequent to liver cell injury, and HCC may subsequently emerge in the setting of cirrhosis [10,12,14–17]. Therefore, it was postulated that if a correlation exists between HCC and HBV DNA as well as HBsAg levels, the relationship between these two biomarkers and cirrhosis development should also hold. In the REVEAL-HBV study, a dose–response relationship between the HBV DNA level and cirrhosis development was indeed noted [50], and the risk started to rise when the HBV DNA level ≥2000 IU/mL. The SEARCH-B study subsequently reaffirmed the HBV DNA level as a major stimulant of cirrhosis development [14].

Based on previous findings, it is logical to extrapolate that a similar relationship should exist between the HBsAg level and cirrhosis development in HBV carriers with low viral loads. The ERADICATE-B cohort study moved on to investigate this issue by analyzing 1068 HBeAg-negative patients with low viral loads. The results showed that an HBsAg level ≥1000 IU/mL was consistently associated with a higher cirrhosis risk compared with an HBsAg level <1000 IU/mL (HR 2.2; 95% CI, 1.1–4.2), suggesting that HBsAg could assist in predicting cirrhosis development [51].

9. Role of HBV DNA and HBsAg levels in predicting HBeAg-negative hepatitis

Hepatitis activity is regarded as the first step in many HBV-related complications. However, unlike cirrhosis and HCC, various definitions exist and it can spontaneously resolve. Therefore, a convincing detection of hepatitis occurrence warrants a cohort study with regular follow-ups and repeated testing of ALT levels. [12,14,27] This issue can only be adequately addressed by hospital-based cohorts that use the following criteria for HBeAg-negative hepatitis: an ALT level ≥2 of the upper limit of normal plus an HBV DNA level ≥2000 IU/mL. These criteria are consistent with the definition of clinical relapse in the guidelines of the Asia Pacific Association for the Study of the Liver [38].

The relationship between the HBV DNA level and ENH was first publicized in the SEARCH-B study [14]. It found that the risk of ENH increased if the HBV DNA level at 1 year after HBeAg seroconversion was >200 IU/mL. Compared with patients with an HBV DNA level <200 IU/mL, the HRs were 2.5 (95% CI, 1.4–4.4) for an HBV DNA level 2000–20,000 IU/mL, 3.8(95% CI, 2.0–7.3) for a level 20,000–200,000 IU/mL, and 6.7 (95% CI, 4.0–11.2) for a level ≥200,000 IU/mL.

The ERADCIATE-B study also explored the role of the HBsAg level in predicting ENH in HBeAg-negative patients with low viral loads. Again, this study used an HBsAg level of 1000 IU/mL as the cutoff and showed that a higher HBsAg level was associated with a higher risk of ENH. Compared with 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) [51]. Furthermore, the low viral load subcohort of the SEARCH-B study yielded consistent findings where the risk of ENH was higher for an HBsAg level ≥1000 IU/mL compared with an HBsAg level <1000 IU/mL (unpublished data).

10. Role of HBsAg in predicting HBV reactivation

Chronic hepatitis, cirrhosis, and HCC are sequential complications of CHB. For patients with low viral loads at baseline, the instigator of these sequential complications is the reactivation of HBV replication. Brunetto et al [51] first investigated whether serum HBsAg levels contribute to define inactive carriers in HBeAg-negative HBV carriers with genotype D infection. They analyzed 209 treatment-naive and asymptomatic carriers in Italy. Defining "inactive carrier state" as an HBV DNA level <2000 IU/mL plus a normal ALT, they found that an HBsAg level <1000 IU/mL at baseline could identify a 3-year inactive state with 94.3% diagnostic accuracy, 91.1% sensitivity, 95.4% specificity, an 87.9% positive predictive value, and 96.7% negative predictive value. In other words, a lower HBsAg level at baseline can predict 3-year sustained viral suppression.

The ERADCATE-B study, which included mostly genotype B and C patients, reported similar findings. We analyzed the HBV DNA level in the 3rd year of follow-up in patients with low viral loads and found that an HBsAg level <1000 IU/mL was associated with a lower rate of HBV DNA reactivation [51].

11. Conclusions and perspectives

Ample evidence confirms that a combination of HBV DNA and HBsAg levels can identify HBV carriers with "minimal viral activity." These specific Asian HBV carriers can thus be regarded at "minimal risk" for cirrhosis and HCC after long-term follow-up if they have minimal liver fibrosis at enrollment. Whether this definition could be extrapolated to HBV carriers with genotypes other than B or C requires further validation in different populations.

Limited disease activity in HBV carriers has long been described as an "inactive carrier state" [37,38]. This relies on patients' maintaining low levels of ALT and HBV DNA indefinitely, and hence, repeated testing of these levels, which is very unlikely in routine daily practice. Snapshot levels of HBV DNA and HBsAg, by contrast, enable the identification of "minimal viral activity," in which multiple Asian and European studies have reported to yield outcomes comparable to "inactive carriers" [47,52]. Physicians may stratify patients into risk levels based on viral activity, providing them with personalized treatment strategies.

From bench to bedside, several issues need to be addressed. First, the appropriate cutoffs of the HBsAg level need further examination. Second, most existing data are from Asian studies examining patients with genotype B or C, and the validity in European populations—where different genotypes predominate and the infection is acquired later in life—needs to be investigated. We hope that, as more lines of evidence accumulate, the HBsAg level can be integrated to improve the risk calculator or nomogram for HBV carriers and treatment guidelines for CHB [53,54]. Third, prospective clinical studies are mandatory to confirm the combined role of HBV DNA and HBsAg for a "minimal viral activity" cutoff and to predict disease progression.

In HBV carriers, HBsAg loss is the treatment goal. However, this is very rarely achieved in patients who acquire the infection perinatally. Therefore, the antiviral treatment response has been defined as an HBV DNA level <2000 IU/mL plus HBeAg seroconversion at 6 months after therapy for HBeAg-positive patients and an HBV DNA level <2000 IU/mL at 6 months after therapy for HBeAg-negative patients. In interferon-based therapy, the HBsAg level during the treatment seems to predict the treatment response [55–58]. However, surging evidence has shown that clinical relapse may occur even if the treatment endpoint is achieved [59]. Similarly, several studies have found a high rate of clinical relapse after achieving treatment endpoints in patients receiving nucleos(t)ide

analogue therapy [60,61]. This is compounded by the fact that the time point of nucleos(t)ide analogue therapy cessation is difficult to determine because most patients achieve undetectable viral loads regardless of future outcome. Because the criteria for "minimal viral activity" has been established in the natural history of HBV, a combination of the HBV DNA and HBsAg levels may be adopted to define new criteria for treatment response.

References

[1] Kao JH, Chen PJ, Chen DS. Recent advances in the research of hepatitis B virus-related hepatocellular carcinoma: epidemiologic and molecular biological aspects. Adv Cancer Res 2010;108:21–72.
[2] Kao JH, Chen DS. Global control of hepatitis B virus infection. Lancet Infect Dis 2002;2:395–403.
[3] Blumberg BS, Sutnick AI, London WT. Hepatitis and leukemia: their relation to Australia antigen. Bull N Y Acad Med 1968;44:1566–86.
[4] Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65–73.
[5] Iloeje UH, Yang HI, Jen CL, Su J, Wang LY, You SL, et al. Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol 2007;5:921–31.
[6] Tseng TC, Liu CJ, Su TH, Wang CC, Chen CL, Chen PJ, et al. Serum hepatitis B surface antigen levels predict surface antigen loss in hepatitis B e antigen seroconverters. Gastroenterology 2011;141:517–25. e2.
[7] Tseng TC, Kao JH. Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic hepatitis B: new trick of old dog. J Gastroenterol 2013;48:13–21.
[8] Hsu HY, Chang MH, Chen DS, Lee CY, Sung JL. Baseline seroepidemiology of hepatitis B virus infection in children in Taipei, 1984: a study just before mass hepatitis B vaccination program in Taiwan. J Med Virol 1986;18:301–7.
[9] Chen DS. From hepatitis to hepatoma: lessons from type B viral hepatitis. Science 1993;262:369–70.
[10] Liaw YF, Chu CM. Hepatitis B virus infection. Lancet 2009;373:582–92.
[11] Liaw YF, Lau GK, Kao JH, Gane E. Hepatitis B e antigen seroconversion: a critical event in chronic hepatitis B virus infection. Dig Dis Sci 2010;55:2727–34.
[12] Chu CM, Liaw YF. Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B. Gastroenterology 2007;133:1458–65.
[13] Chen YC, Chu CM, Liaw YF. Age-specific prognosis following spontaneous hepatitis B e antigen seroconversion in chronic hepatitis B. Hepatology 2010;51:435–44.
[14] Tseng TC, Liu CJ, Chen CL, Wang CC, Su TH, Kuo SF, et al. Serum hepatitis B virus-DNA levels correlate with long-term adverse outcomes in spontaneous hepatitis B e antigen seroconverters. J Infect Dis 2012;205:54–63.
[15] Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002;35:1522–7.
[16] Tai DI, Lin SM, Sheen IS, Chu CM, Lin DY, Liaw YF. Long-term outcome of hepatitis B e antigen-negative hepatitis B surface antigen carriers in relation to changes of alanine aminotransferase levels over time. Hepatology 2009;49:1859–67.
[17] Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. Am J Med 2004;116:829–34.
[18] Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther 2010;15:133–43.
[19] Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology 2010;52:1232–41.
[20] Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol 2010;52:508–13.
[21] Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol 2010;52:514–22.
[22] Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. Hepatology 1991;13:627–31.
[23] Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology 2007;45:1187–92.
[24] McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med 2001;135:759–68.
[25] Kim JH, Lee JH, Park SJ, Bae MH, Kim do Y, Kim JK, et al. Factors associated with natural seroclearance of hepatitis B surface antigen and prognosis after seroclearance: a prospective follow-up study. Hepatogastroenterology 2008;55:578–81.
[26] Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology 2010;139:474–82.
[27] Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. Determinants of spontaneous surface antigen loss in hepatitis B e antigen-negative patients with a low viral load. Hepatology 2012;55:68–76.
[28] Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology 2002;123:1084–9.
[29] Fattovich G, Giustina G, Sanchez-Tapias J, Quero C, Mas A, Olivotto PG, et al. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). Am J Gastroenterol 1998;93:896–900.
[30] Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, et al. Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology 1998;28:231–6.
[31] Yuen MF, Wong DK, Sablon E, Tse E, Ng IO, Yuan HJ, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology 2004;39:1694–701.
[32] Chan HL, Wong GL, Tse CH, Chan HY, Wong VW. Viral determinants of hepatitis B surface antigen seroclearance in hepatitis B e antigen-negative chronic hepatitis B patients. J Infect Dis 2011;204:408–14.
[33] Seto WK, Wong DK, Fung J, Hung IF, Fong DY, Yuen JC, et al. A large case-control study on the predictability of hepatitis B surface antigen levels three years before hepatitis B surface antigen seroclearance. Hepatology 2012;56:812–9.
[34] Chang MH, Chiu YC, Wu JF, Lin CY, Ni YH, Chen HL, et al. Spontaneous clearance of hepatitis B surface antigen during the natural history of chronic hepatitis B virus infection. Hepatology 2011;54. Abstract 731.
[35] Chen YC, Jeng WJ, Chu CM, Liaw YF. Decreasing levels of HBsAg predict HBsAg seroclearance in patients with inactive chronic hepatitis B virus infection. Clin Gastroenterol Hepatol 2012;10:297–302.
[36] Liu J, Lee MH, Batrla-Utermann R, Jan CL, Iloeje UH, Lu SN, et al. A predictive scoring system for the seroclearance of HBsAg in HBeAg-seronegative chronic hepatitis B patients with genotype B or C infection. J Hepatol 2012 Dec 13. [Epub ahead of print].
[37] Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661–2.
[38] Liaw YF, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 2012;6:531–61.
[39] European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 2012;57:167–85.
[40] Wong VW, Chan SL, Mo F, Chan TC, Loong HH, Wong GL, et al. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. J Clin Oncol 2010;28:1660–5.
[41] Yuen MF, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol 2009;50:80–8.
[42] Chen CH, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, et al. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. Gastroenterology 2007;133:1466–74.
[43] Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, et al. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. J Infect Dis 2006;193:1258–65.
[44] Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, et al. Role of hepatitis B virus precore/core promoter mutations and serum viral load on noncirrhotic hepatocellular carcinoma: a case-control study. J Infect Dis 2006;194:594–9.
[45] Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. Am J Gastroenterol 2006;101:1797–803.
[46] Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005;97:265–72.
[47] Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012;142:1140–9. e3.
[48] Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology 2010;138:1747–54.
[49] Chen CJ, Lee MH, Jessica L, Ricard B-U, Chin-Lan J, Uchenna I, et al. Quantitative serum levels of hepatitis B virus DNA and surface antigen are independent risk predictors of hepatocellular carcinoma. Hepatology 2011;54. Abstract 1095.
[50] Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678–86.
[51] Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low HBV loads. Hepatology 2013;57:441–50.
[52] Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, et al. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology 2010;139:483–90.

[53] Yang HI, Sherman M, Su J, Chen PJ, Liaw YF, Iloeje UH, et al. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Clin Oncol 2010;28:2437–44.
[54] Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011;12:568–74.
[55] Liaw YF, Jia JD, Chan HL, Han KH, Tanwandee T, Chuang WL, et al. Shorter durations and lower doses of peginterferon alfa-2a are associated with inferior hepatitis B e antigen seroconversion rates in hepatitis B virus genotypes B or C. Hepatology 2011;54:1591–9.
[56] Sonneveld MJ, Rijckborst V, Boucher CA, Hansen BE, Janssen HL. Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline. Hepatology 2010;52:1251–7.
[57] Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 2009;49:1141–50.
[58] Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009;49:1151–7.
[59] Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology 2009;136:2169–79. e1–4.
[60] Tseng TC, Liu CJ, Su TH, Yang HC, Wang CC, Chen CL, et al. Young chronic hepatitis B patients with nucleos(t)ide analogue-induced hepatitis B e antigen seroconversion have a higher risk of HBV reactivation. J Infect Dis 2012;206:1521–31.
[61] Reijnders JG, Perquin MJ, Zhang N, Hansen BE, Janssen HL. Nucleos(t)ide analogues only induce temporary hepatitis B e antigen seroconversion in most patients with chronic hepatitis B. Gastroenterology 2010;139:491–8.