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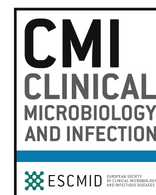

Narrative review

How to interpret viral markers in the management of chronic hepatitis B infection

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ABSTRACT

Background: Hepatitis B virus (HBV) infection is a global public health issue with several unsolved clinical challenges. As multiple new drugs are under development, HBV markers are gaining importance for both diagnostic and prognostic purposes.
Objectives: This review summarizes the most important data on the usefulness of HBV markers in the natural history of this infection, and in predicting clinical and treatment outcomes.
Sources: Selected peer-reviewed publications on HBV markers published between January 2009 and July 2021.
Content: In addition to the classical markers (e.g. HBV-DNA), newer ones, such as quantitative HBsAg, HBcrAg, HBV-RNA and quantitative anti-HBc, have proven useful for predicting events within the natural history of HBV infection, the development of complications (e.g. hepatocellular carcinoma) and the response to antiviral therapy. Most data regarding the response to treatment have been related to nucleos(t)ide analogues, whereas evidence on new therapeutic agents, such as capsid assembly modulators or small interference RNAs, is promising, but still scarce.
Implications: Knowledge on the use of viral markers is a key factor for optimizing the clinical appraisal of HBV infection. The new markers have an enhanced ability to predict clinical outcomes. Further studies should expand the current evidence on the use of markers in relation to antiviral agents currently under evaluation. Wide availability of these markers in regions with a high incidence of HBV infection is of paramount importance. **Mar Riveiro-Barciela, Clin Microbiol Infect 2022;28:355**
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Introduction

Approximately 240 million people worldwide are chronically infected by the hepatitis B virus (HBV) [1]. Chronic HBV is a dynamic infection that progresses through different phases that are strongly associated with the age of the patient and HBV impairment of specific T-cell function [2] (Fig. 1). Untreated individuals with chronic hepatitis B (CHB) have an 8%–20% 5-year risk of developing liver cirrhosis. In patients with decompensation, the 5-year survival rate is 14%–35% [3]. In addition, HBV-infected individuals can develop hepatocellular carcinoma (HCC), although the incidence is much higher in those with liver cirrhosis [4]. However, viral suppression achieved through antiviral treatment often leads to regression of liver fibrosis and low risk of HCC [2].

Despite considerable efforts, HBV infection remains a clinically challenging global health threat that is often undiagnosed or diagnosed late in many regions worldwide. This review focuses on the utility of markers developed during the last two decades to predict the natural history of HBV and support therapeutic decisions in patients with this infection.

Main viral markers of HBV infection

Hepatitis B virus is a partially double-stranded DNA virus and member of the *Hepadnaviridae* family. Although many markers have been described, including inflammatory, genetic and immunological host factors, viral markers are those yielding further

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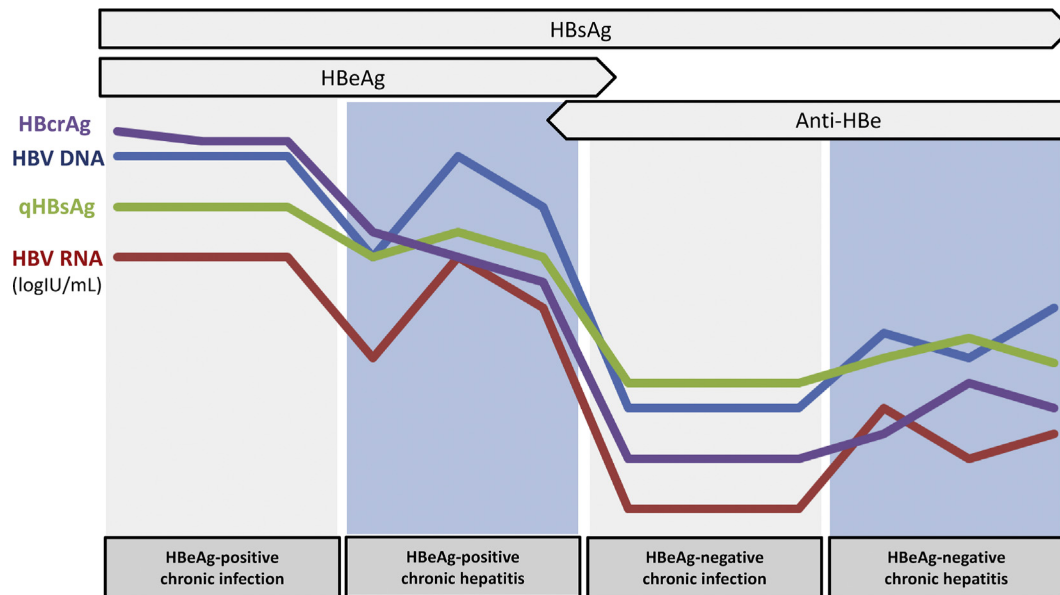

Fig. 1. Evolution of hepatitis B serum viral markers during the natural history of chronic hepatitis B virus (HBV) infection. Levels of viral markers are higher in hepatitis B envelope antigen (HBeAg) -positive individuals than in HBeAg-negative individuals. In addition, their detection and values are more frequent and higher, respectively, in patients with chronic hepatitis compared with chronic infection. During the chronic hepatitis B (CHB) phases, either HBeAg-positive or -negative, the viral markers tend to fluctuate. After HBeAg seroconversion, there is a downturn in their levels, with relative stability during HBeAg-negative infection, and a new upturn and oscillations during HBeAg-negative CHB. Across all phases of HBV infection, HBV-RNA values are usually 2 log lower than HBV-DNA.

evidence and usefulness in the natural history and treatment of HBV infection [5].

Classical HBV markers include HBV-DNA and hepatitis B e antigen (HBeAg). HBV-DNA quantification is central to hepatitis B management, as classification of the infection is mainly based on HBV-DNA values [2] and the association between HBV-DNA and the development of cirrhosis [6] and HCC [7] is well-documented. HBeAg, an HBV protein that enables the virus to establish infection, is linked with high viral replication and infectivity [5]. HBeAg-positive status is associated with poorer prognosis and higher risk of HCC; hence, seroconversion to anti-HBe is a therapeutic end point for HBeAg-positive patients [8]. The lack of commercial tests for HBeAg quantification limits its usefulness in clinical practice, and there are few data on its potential role as a marker for guiding therapy response [5].

Concerning the newer viral markers, the most widely used of these in clinical practice are commercially available tests, such as quantitative hepatitis B surface antigen (qHBsAg), hepatitis B core-related antigen (HBcrAg) and, to a lesser extent, HBV-RNA [5]. HBsAg is the hallmark for diagnosing HBV infection [2]. Although the value of HBsAg quantification was recognized early on, its use has been generalized only in recent years. HBsAg is the glycosylated envelope protein of the mature HBV virion, composed of three HBsAg proteins. Apart from virions, the serum of viraemic patients includes two types of non-infectious particles, which are 1000-fold more numerous than virions and are believed to serve as decoys for humoral immunity [9]. These subviral particles are derived from both covalently closed circular DNA (cccDNA) and integrated DNA, especially in HBeAg-negative patients. Therefore, HBsAg is also produced from HBV-DNA integrated into the host genome [9].

More recently, HBcrAg has been pointed out as a promising viral marker, because the three proteins it includes (hepatitis B core antigen, HBeAg and core-related protein p22-p22cr) derive exclusively from cccDNA transcriptional activity [10], whereas HBsAg is translated from both cccDNA and integrated DNA [9].

Serum HBV-RNA consists of RNA from virion particles in which the pre-genomic RNA has been non- or partially reverse-transcribed [11]. Thus, HBV-RNA quantification would potentially allow monitoring of cccDNA transcriptional activity. Nonetheless, this technique has some shortcomings, such as the limited sensitivity of current assays and the possible interference of HBV-DNA in RNA detection [12]. The latter is especially important in the treatment-naive population. However, as reverse transcription is blocked by antiviral therapy, HBV-RNA quantification could potentially enable monitoring of cccDNA transcriptional activity [13–15] in patients receiving nucleos(t)ide analogues (NAs). A summary of the HBV markers reviewed and their potential uses is shown in Table 1.

Role of markers in the natural history of HBV infection

Identification of HBV inactive carriers

The term HBeAg-negative chronic HBV infection is used to refer to what was formerly known as the inactive carrier (IC) state (Fig. 1). HBeAg-negative infection has a favourable long-term prognosis, with negligible risk of cirrhosis and HCC [2]. Inactive carriers are characterized by persistently normal alanine transaminase levels, HBV-DNA <2000 IU/mL and mild or absent liver fibrosis [2]. Early identification of IC status avoids the close monitoring needed for proper classification of the HBV phase, especially during the first year after diagnosis [2].

Brunetto et al. proposed qHBsAg values < 1000 IU/mL together with HBV-DNA <2000 IU/mL to classify genotype D HBV ICs, with a diagnostic accuracy >94% [16]. However, the impact of the genotype on qHBsAg has made this cut-off less useful in non-D genotypes [17,18]. The composition of HBsAg may also play a role in prompt recognition of ICs, as they show lower proportions of large and middle-sized surface proteins than HBeAg-negative individuals with CHB [19].

A cut-off of HBcrAg 3 logU/mL has also shown good diagnostic performance in single-point identification of ICs, regardless of HBV genotype [20,21].

HBV-RNA was detectable in only 59% of HBeAg-negative individuals in a recent study, dropping to 38% when only ICs were

Table 1
Summary of established and potential usefulness of HBV markers for predicting disease evolution and treatment response

Marker	Identification of inactive carriers	HCC development	HBsAg loss (spontaneous or therapy-induced)	Relapse after NA discontinuation	References
qHBsAg	✓	✓	✓	✓	[16,18,30–32,40,41,43,65]
Large and middle HBsAg proteins	✓		✓		[19,59]
HBcrAg	✓	✓	✓	✓	[18,20,22,33–36,44,45]
HBV-RNA	✓	✓	✓	✓	[11,13,22–24,38,46,54]
qAnti-HBc	✓			✓	[25,26,67]

Abbreviations: HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogues; qAnti-HBc, quantitative hepatitis B core protein antibody; qHBsAg, quantitative HBsAg.

considered [22]. Similarly, some studies have shown significantly lower HBV-RNA levels in HBV ICs [23,24]. Nonetheless, the area under the receiver operating characteristics curve in a cohort including 105 HBeAg-negative naive individuals was only 0.639 [24], and an exact cut-off has yet to be recommended. Data are scarce on quantitative analysis of hepatitis B core protein antibody (qAnti-HBc), although lower levels have been reported in ICs than in individuals with CHB [25,26].

Risk of developing HCC

Infection with HBV is the leading cause of HCC worldwide [27]. In addition to underlying cirrhosis, older age, geographical origin and male sex are well-known risk factors for HBV-related HCC [2]. Concerning specific HBV-associated factors, genotype C, HBV-DNA, core promoter mutations and HBeAg-positive status have been linked with increased HCC rates in Asian populations [8,28,29].

In a large cohort of HBeAg-negative patients with low HBV-DNA levels and no liver cirrhosis, those with baseline qHBsAg at >1000 IU/mL had 14-fold higher risk of HCC [30]. This qHBsAg threshold has also been pinpointed as a predictor of HCC recurrence after surgical resection [31,32]. HBcrAg levels have also been linked with HCC risk. Among naive HBeAg-negative Asian patients, HBcrAg >2.9 logU/mL and >4 logU/mL have been associated with 5-fold and 6.3-fold hazard ratios of incident HCC, respectively [33,34]. This may be useful for selecting naive HBeAg-negative patients not fulfilling treatment criteria but who could benefit from close follow up and HCC surveillance. In patients who have undergone liver transplantation or curative resection for HCC, HBcrAg levels ≥4.8 or 5 logU/mL were predictive of a five-fold and nine-fold risk of recurrence, respectively [35,36].

Detection of pre-genomic RNA and cccDNA in liver tissue of HBV-infected individuals with HCC has been correlated to an absence of tumoral microvascular invasion and to a better prognosis [37]. However, data on these markers as HCC predictors are scarce. Preliminary results suggest an association between higher HBV-RNA levels and an increased risk of HCC development [38].

Spontaneous HBsAg loss

Around 0.5%–1% of individuals with HBV per year achieve HBsAg loss or functional cure of HBV infection [2]. Determinant factors associated with spontaneous HBsAg seroclearance include older age, HBeAg seronegativity and duration of chronic HBV infection, usually >15 years of IC status [39]. Some studies have suggested that qHBsAg levels may help in predicting this outcome. A cohort study including 390 patients reported that HBsAg levels at 1 year following HBeAg seroconversion were inversely associated with HBsAg loss. The HBsAg loss rates were higher in those with HBsAg of 100–999 IU/mL and <100 IU/mL, with hazard ratios of 4.4 and 24.3, respectively [40]. In a Taiwanese study, qHBsAg <100 IU/mL had 75% sensitivity and 91% specificity to predict HBsAg seroclearance after a mean follow up of >7 years [41]. The dynamics of qHBsAg levels have also been linked to the probability of HBsAg loss. In another study, 77% of patients with a qHBsAg drop >1 logIU/mL achieved HBsAg loss [42]. Seto et al. reported similar results, with 0.5 logIU/mL and <200 IU/mL qHBsAg reductions as predictors of HBsAg loss in naive HBeAg-negative individuals [43].

Although most individuals (79%–89%) with spontaneous HBsAg seroclearance have undetectable HBcrAg levels [44,45], longitudinal studies assessing the optimal HBcrAg cut-off for predicting this outcome are lacking. Similar results have been seen with HBV-RNA, with the majority of ICs showing low or undetectable levels [13,46].

Role of serum HBV markers in treated patients with CHB

Current standard therapy

Most treated patients with CHB are currently receiving NAs. Very few are treated with pegylated interferon (pegIFN) because of its considerable limitations. In those receiving NAs, undetectable HBV-DNA is the rule, but HBsAg remains detectable. In recent years, several studies have shown the potential of serum HBV markers for monitoring therapy by identifying patients with a higher likelihood of HBsAg loss and therapy discontinuation.

Monitoring HBV therapy

Levels of HBsAg decline very slowly in virally suppressed patients on NAs, particularly HBeAg-negative patients, as the integrated HBV genomes maintain HBsAg transcription (Fig. 2). A minority of HBeAg-positive individuals show dramatic declines in HBsAg (>1 log at week 24 of NA therapy), which is associated with further HBsAg clearance [47]. HBsAg loss is uncommon, particularly in the HBeAg-negative population [48]. In pegIFN-treated patients, HBsAg level at week 12 or week 24 allows selection of patients for early discontinuation of pegIFN because of the high likelihood of non-response [49]. HBsAg measures the activities of both integrated HBV-DNA and cccDNA, and low HBsAg in patients receiving NAs indicates host immune clearance [50].

Regarding other markers, in a cohort of 222 patients with CHB (90 HBeAg-positive and 132 HBeAg-negative) receiving long-term

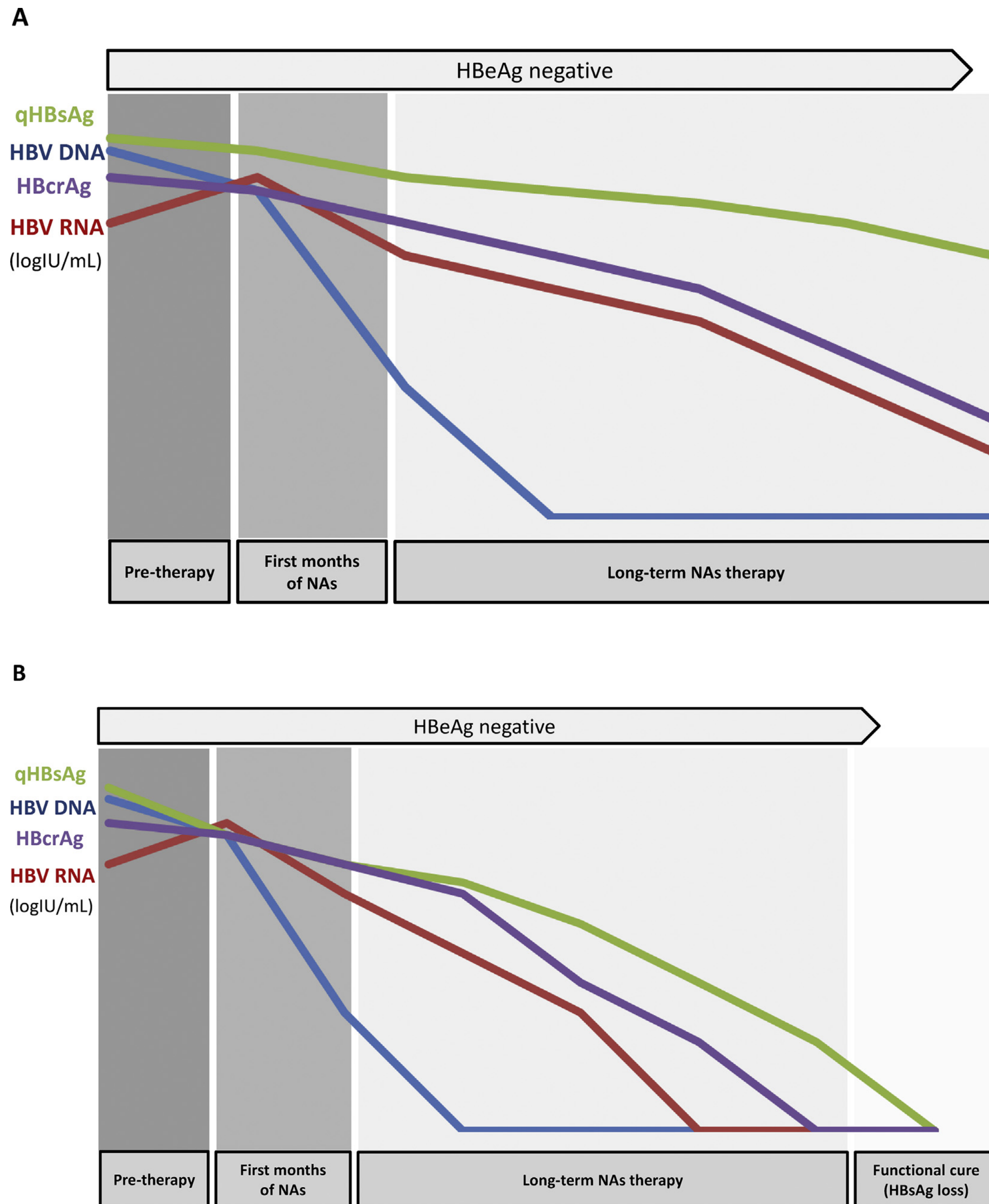

Fig. 2. Evolution of hepatitis B virus (HBV) serum markers during antiviral therapy with nucleos(t)ide analogues (NAs) in hepatitis B envelope antigen (HBeAg) -negative patients with chronic hepatitis B. (a) After the start of NA therapy there is an increase in HBV-RNA as a result of the decrease in production of complete HBV virions [11,73]. The drop in HBV-DNA is usually quick, and most HBeAg-negative patients achieve virological suppression (undetectable HBV-DNA) within the first 2–3 years of treatment [2]. After the initial upturn, HBV-RNA slowly decreases until it becomes undetectable in up to 95% of patients after 10–14 years of therapy [53]. Hepatitis B core-related antigen (HBcrAg) progressively declines over time, and most patients have undetectable levels after 10 years of NAs. After HBV-DNA, HBV-RNA is the next viral marker to become undetectable, followed by HBcrAg [74]. The decline in hepatitis B surface antigen (HBsAg) levels is very slow, as quantitative HBsAg mainly derives from the subviral particles of integrated DNA in HBeAg-negative patients. (b) Estimated evolution of viral markers in the limited number of patients who achieved HBsAg loss after long-term NA therapy.

entecavir, the yearly decline in HBcrAg levels was 0.244 logU/mL [51]. At week 96, this decline was higher in HBeAg-positive patients than in HBeAg-negative patients, with no differences between groups [52]. A retrospective study of 96 HBeAg-negative patients with undetectable HBV-DNA under NAs reported that HBV-RNA was detectable in 32% of patients at year 4 and in 5% at 10–14 years of follow up [53]. In another study, 142 patients receiving NAs showed a 1.46 log and 1.77 log reduction in HBV-RNA at weeks 48 and 96, respectively. At week 96, only 19.1% of tenofovir disoproxil fumarate-treated and 25.7% of entecavir-treated patients had unquantifiable HBV-RNA ($p > 0.05$). Among those with undetectable HBV-DNA, 77.5% and 30% had quantifiable HBV-RNA and HBcrAg, which enabled monitoring of viral activity by serum HBV-RNA analysis [24]. In a study including 66 HBeAg-negative patients, after 5 years of NA therapy, 27% and 14% had detectable HBcrAg and HBV-RNA, whereas in 23 HBeAg-negative virally suppressed patients who stopped NAs before HBsAg loss, all had undetectable HBcrAg and a subset still had detectable HBV-RNA at the time of treatment withdrawal [54]. The latter were more likely to develop severe aminotransferase flares. However, the current HBcrAg assays lack sensitivity to identify this subset of patients. A novel HBcrAg assay with approximately ten-fold higher

sensitivity is still under development [55]. In 279 HBeAg-positive patients with CHB treated with pegIFN regimens, an HBV-RNA decline during therapy was observed in 135 (52%) patients. This decline was associated with higher rates of sustained off-treatment response (HBV-DNA <2000 IU/mL and/or HBsAg loss), showing that an HBV-DNA decline without a concomitant HBsAg decline is associated with low rates of treatment response and HBsAg loss [56].

HBeAg seroconversion

Seroconversion to anti-HBe is an intermediate treatment end point for HBeAg-positive individuals with CHB and a precursor to achieving HBV functional cure [2]. In a multicentre study including 264 HBeAg-positive individuals with CHB, a higher qHBsAg value at baseline and an on-therapy qHBsAg decline were predictors of HBeAg loss at week 96 [57]. In HBeAg-positive patients treated with pegIFN, failure to achieve a qHBsAg decline of at least 20 000 IU/mL at week 24 was found to be a predictor of non-response and a therapy-stopping rule due to the low probability of subsequent HBeAg seroconversion [58]. Interestingly, although HBsAg composition differed between patients who achieved HBsAg loss during pegIFN, no significant differences were reported according to the accomplishment of HBeAg seroconversion [59].

Concerning HBcrAg, in a large European study, HBcrAg >8.35 logU/mL at week 24 identified pegIFN non-responders, but it did not perform better than HBsAg >20 000 IU/mL as a stopping rule [60], results that are in line with a previous study from Asia [61]. HBV-RNA has also been explored as a tool for early identification of HBeAg loss. In a cohort of 50 HBeAg-positive patients treated with NAs, HBV-RNA on-treatment kinetics strongly correlated with the likelihood of achieving HBeAg seroconversion [62]. These results were replicated in an analysis of serum samples from 266 HBeAg-positive pegIFN-treated individuals [63].

What is the optimal marker to guide discontinuation of NA therapy?

There is compelling evidence that HBV-DNA concentrations at completion of NA treatment are not helpful to predict the risk of relapse [64]. NA discontinuation is associated with a relatively high likelihood of HBsAg loss in some HBeAg-negative patients without cirrhosis. It has been suggested that the lower the HBsAg value, the greater the likelihood of HBsAg clearance [65], particularly if HBsAg levels are below 100 IU/mL [66]. However, only a minority of NA-treated patients reach HBsAg concentrations of <2 logIU/mL at the end of treatment. Concerning qAnti-HBc, levels >1000 IU/mL at the end of NA therapy were associated with a seven-fold lower risk of clinical relapse in a prospective cohort of 1000 participants in China [67].

Another potentially useful marker is HBV-RNA. Wang et al. found that HBV-RNA detection in patients discontinuing NAs after at least 3 years of therapy was associated with a significant risk of viral rebound [11]. In addition, persistence of HBV-RNA has been associated with alanine transaminase flares after treatment discontinuation. In patients with high HBV-DNA levels, HBcrAg was undetectable in 29% of cases and HBV-RNA in 17%, even at baseline [54]. This points to an overall lack of sensitivity among these tests, particularly HBcrAg. An optimized HBcrAg assay available for research has shown ten-fold greater sensitivity than the previous technique and may overcome the current limitations of this test [68]. HBV-RNA tests still need to be validated in prospective studies comparing the performance of undetectable HBcrAg, HBV-RNA and HBsAg levels, individually and in combination [13]. Factors associated with a higher likelihood of achieving a sustained response after NA discontinuation are summarized in Table 2.

Table 2
Factors associated with clinical remission[a] or high likelihood of achieving HBsAg loss in non-cirrhotic HBeAg-negative patients who discontinue therapy with nucleos(t)ide analogues

Baseline host factors	HBV markers at the time of therapy discontinuation
Young age Caucasian race	qHBsAg <100 IU/mL HBV-RNA undetectable HBcrAg undetectable qAnti-HBc <1000 IU/mL

Abbreviations: HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; qAnti-HBc, quantitative hepatitis B core protein antibody; qHBsAg, quantitative HBsAg.
[a] Clinical remission was defined as normal alanine transaminase levels and HBV-DNA levels <2000 IU/mL after therapy discontinuation.

New drugs

A reduction in qHBsAg level is used as the main end point in ongoing trials with new HBV drugs, particularly capsid assembly modulators, oligonucleotides, small interfering RNAs and nucleic acid polymers [48]. HBcrAg and HBV-RNA might also be targets of future therapies. Short treatments with a small interfering RNA, JNJ 3989 (three doses for 4 weeks) in virally suppressed patients resulted in a >1 log reduction in qHBsAg during treatment. HBsAg responders display greater reductions in HBcrAg and HBV-RNA [69]. Preliminary results on ARC-520, a small interfering RNA targeting cccDNA-derived transcription, suggest a reduction in antigen production driven by cccDNA in HBeAg-positive patients, with a smaller decline in qHBsAg compared with HBcrAg in HBeAg-negative patients [70]. A capsid assembly modulator, ABI-H0731 (Vebicorvir), administered with NAs in HBeAg-negative patients resulted in undetectable HBV-RNA in all individuals at week 24 [71]. Among naive HBeAg-positive patients with CHB, Vebicorvir plus NAs resulted in deeper on-treatment viral suppression than that achieved with NAs alone. Another capsid assembly modulator, JNJ 6379 (at either 75 mg or 250 mg), combined with NAs for 24 weeks has been evaluated in HBeAg-positive and HBeAg-negative patients [72]. A stronger reduction in HBV-DNA and HBV-RNA was seen at week 24, with a limited effect on mean qHBsAg and HBeAg levels. The additional beneficial HBV-RNA suppression remains to be defined.

In summary, measurement of HBV-RNA and HBcrAg could be useful to monitor the effectiveness of therapy beyond HBV-DNA suppression, particularly in NA-treated patients, as theoretically NAs do not act on cccDNA or inhibit pre-genomic RNA transcription. HBV-RNA can also help to predict which patients are at high risk of HBV relapse after treatment discontinuation, and it has the potential to evaluate the efficacy of new HBV drugs.

In conclusion, enhanced strategies are of the essence to identify those HBV-infected individuals with a greater risk of developing complications, such as liver cirrhosis and HCC, and to monitor antiviral treatment. Viral markers are a promising alternative and have already proved useful in predicting poor outcomes in the natural history and treatment of HBV infection.

Transparency declaration

MB has received research grants from Gilead and has served as an advisor for Gilead, Bristol-Myers Squibb and Novartis. MRB has received research educational and/or travel grants from Gilead and has served as a speaker for Gilead and Grifols. JMP has received educational and/or travel grants from Gilead, Novartis, Pfizer, Accelerate, Astellas, ViiV, Janssen, MSD and Abbvie. Funding: The authors declare that they have received no funding for this study.

Author contributions

MB and MRB contributed to the conception and design of this review. MRB and MB performed the literature search and contributed to writing the first draft of this manuscript. JMP supervised the project. All authors approved the final manuscript version to be submitted.

Access to data

Data that support this study are available from the corresponding author upon reasonable request.

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References

[1] Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 2015;386:1546–55.
[2] EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370–98.
[3] Fattovich G, Olivari N, Pasino M, D'Onofrio M, Martone E, Donato F. Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. Gut 2008;57:84–90.
[4] Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335–52.
[5] Buti M, Riveiro-Barciela M, Rodríguez-Frías F, Tabernero D, Esteban R. Role of biomarkers in guiding cure of viral hepatitis B. Semin Liver Dis 2020;40: 49–60.
[6] Papatheodoridis GV, Manesis EK, Manolakopoulos S, Elefsiniotis IS, Goulis J, Giannousis J, et al. Is there a meaningful serum hepatitis B virus DNA cutoff level for therapeutic decisions in hepatitis B e antigen-negative chronic hepatitis B virus infection? Hepatology (Baltimore, MD) 2008;48:1451–9.
[7] 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.
[8] 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.
[9] Tong S, Revill P. Overview of hepatitis B viral replication and genetic variability. J Hepatol 2016;64:S4–16.
[10] Testoni B, Lebosse F, Scholtes C, Berby F, Miaglia C, Subic M, et al. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. J Hepatol 2019;70:615–25.
[11] Wang J, Shen T, Huang X, Kumar GR, Chen X, Zeng Z, et al. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol 2016;65:700–10.
[12] Liu S, Zhou B, Valdes JD, Sun J, Guo H. Serum hepatitis B virus RNA: a new potential biomarker for chronic hepatitis B virus infection. Hepatology (Baltimore, MD) 2019;69:1816–27.
[13] Butler EK, Gersch J, McNamara A, Luk KC, Holzmayer V, de Medina M, et al. Hepatitis B virus serum DNA and RNA levels in nucleos(t)ide analog-treated or untreated patients during chronic and acute infection. Hepatology (Baltimore, MD) 2018;68:2106–17.
[14] Rydell GE, Prakash K, Larsson SB, Skoglund C, Ringlander J, Andersson M, et al. Quantification of viral RNA in multiple pieces of explant liver tissue shows distinct focal differences of hepatitis B infection. J Infect Dis 2021. epub ahead of print.
[15] Bazinet M, Anderson M, Pântea V, Placinta G, Moscalu I, Cebotarescu V, et al. Analysis of HBsAg immunocomplexes and cccDNA activity during and persisting after NAP-based therapy. Hepatol Comm 2021;5:1873–87. https://doi.org/10.1002/hep4.1767.
[16] 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.
[17] 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.
[18] Riveiro-Barciela M, Bes M, Rodriguez-Frias F, Tabernero D, Ruiz A, Casillas R, et al. Serum hepatitis B core-related antigen is more accurate than hepatitis B surface antigen to identify inactive carriers, regardless of hepatitis B virus genotype. Clin Microbiol Infect 2017;23:860–7.
[19] Pfefferkorn M, Bohm S, Schott T, Deichsel D, Bremer CM, Schroder K, et al. Quantification of large and middle proteins of hepatitis B virus surface antigen (HBsAg) as a novel tool for the identification of inactive HBV carriers. Gut 2018;67:2045–53.
[20] Brunetto MR, Carey I, Maasoumy B, Marcos-Fosch C, Boonstra A, Caviglia GP, et al. Incremental value of HBcrAg to classify 1582 HBeAg-negative individuals in chronic infection without liver disease or hepatitis. Aliment Pharmacol Ther 2021;53:733–44.
[21] Yoshida K, Desbiolles A, Feldman SF, Ahn SH, Alidjinou EK, Atsukawa M, et al. Hepatitis B core-related antigen to indicate high viral load: systematic review and meta-analysis of 10,397 individual participants. Clin Gastroenterol Hepatol 2021;19:46–60.e8.
[22] Ghany MG, King WC, Lisker-Melman M, Lok AS, Terrault N, Janssen HLA, et al. Comparison of novel biomarkers with conventional HBV markers among untreated adults with chronic hepatitis B in North America. Hepatology 2021;74:2395–409. https://doi.org/10.1002/hep.32018.
[23] Wang J, Yu Y, Li G, Shen C, Li J, Chen S, et al. Natural history of serum HBV-RNA in chronic HBV infection. J Viral Hepat 2018;25:1038–47.
[24] Mak LY, Cloherty G, Wong DK, Gersch J, Seto WK, Fung J, et al. HBV RNA profiles in patients with chronic hepatitis B under different disease phases and antiviral therapy. Hepatology (Baltimore, MD) 2021;73:2167–79.
[25] Song LW, Liu PG, Liu CJ, Zhang TY, Cheng XD, Wu HL, et al. Quantitative hepatitis B core antibody levels in the natural history of hepatitis B virus infection. Clin Microbiol Infect 2015;21:197–203.
[26] Yuan Q, Song LW, Cavallone D, Moriconi F, Cherubini B, Colombatto P, et al. Total hepatitis B core antigen antibody, a quantitative non-invasive marker of hepatitis B virus induced liver disease. PloS One 2015;10:e0130209.
[27] EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2021;56:908–43.
[28] 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.
[29] Jung KS, Kim SU, Ahn SH, Park YN, Kim DY, Park JY, et al. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatology (Baltimore, MD) 2011;53:885–94.
[30] 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;142:1140–1149.e3. quiz e13-4.
[31] Liu WR, Tian MX, Jin L, Yang LX, Ding ZB, Shen YH, et al. High levels of hepatitis B surface antigen are associated with poorer survival and early recurrence of hepatocellular carcinoma in patients with low hepatitis B viral loads. Ann Surg Oncol 2015;22:843–50.
[32] Huang G, Lau WY, Zhou WP, Shen F, Pan ZY, Yuan SX, et al. Prediction of hepatocellular carcinoma recurrence in patients with low hepatitis B virus DNA levels and high preoperative hepatitis B surface antigen levels. JAMA Surg 2014;149:519–27.
[33] Tada T, Kumada T, Toyoda H, Kiriyama S, Tanikawa M, Hisanaga Y, et al. HBcrAg predicts hepatocellular carcinoma development: an analysis using time-dependent receiver operating characteristics. J Hepatol 2016;65:48–56.
[34] Tseng TC, Liu CJ, Hsu CY, Hong CM, Su TH, Yang WT, et al. High level of hepatitis B core-related antigen associated with increased risk of hepatocellular carcinoma in patients with chronic HBV infection of intermediate viral load. Gastroenterology 2019;157:1518–1529.e3.
[35] Yu J, Ye Y, Liu J, Xu Y, Lou B, Zhu J, et al. The role of hepatitis B core-related antigen in predicting hepatitis B virus recurrence after liver transplantation. Aliment Pharmacol Ther 2019;50:1025–36.
[36] Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, et al. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int 2010;30:1461–70.
[37] Halgand B, Desterke C, Rivière L, Fallot G, Sebagh M, Calderaro J, et al. Hepatitis B virus pregenomic RNA in hepatocellular carcinoma: a nosological and prognostic determinant. Hepatology (Baltimore, MD) 2018;67:86–96.
[38] Breitkreutz R, Zhang W, Lee M, Hoffmann A, Tokus M, Su Q, et al. Hepatitis B virus nucleic acids circulating in the blood: distinct patterns in HBs carriers with hepatocellular carcinoma. Ann NY Acad Sci 2001;945:195–206.
[39] Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther 2010;15:133–43.
[40] 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;141:525.e1–2.
[41] 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.
[42] 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 (Baltimore, MD) 2010;52:1232–41.
[43] 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 (Baltimore, MD) 2012;56:812–9.
[44] Seto WK, Wong DK, Fung J, Huang FY, Liu KS, Lai CL, et al. Linearized hepatitis B surface antigen and hepatitis B core-related antigen in the natural history of chronic hepatitis B. Clin Microbiol Infect 2014;20:1173–80.

[45] Chan HLY, Yasuda S, Wong GLH, Tada T, Chan CKM, Kumada T, et al. Use of hepatitis B virus core-related antigen to evaluate natural history of chronic hepatitis B. J Gastroenterol Hepatol 2020;35:2202–9.
[46] van Campenhout MJH, van Bommel F, Pfefferkorn M, Fischer J, Deichsel D, Boonstra A, et al. Host and viral factors associated with serum hepatitis B virus RNA levels among patients in need for treatment. Hepatology (Baltimore, MD) 2018;68:839–47.
[47] Marcellin P, Buti M, Krastev Z, de Man RA, Zeuzem S, Lou L, et al. Kinetics of hepatitis B surface antigen loss in patients with HBeAg-positive chronic hepatitis B treated with tenofovir disoproxil fumarate. J Hepatol 2014;61:1228–37.
[48] Buti M, Riveiro-Barciela M, Esteban R. Long-term safety and efficacy of nucleo(t)side analogue therapy in hepatitis B. Liver Int 2018;38:84–9.
[49] Cornberg M, Wong VW, Locarnini S, Brunetto M, Janssen HLA, Chan HL. The role of quantitative hepatitis B surface antigen revisited. J Hepatol 2017;66:398–411.
[50] Chan HL. Letter to the editor: use of pregenomic hepatitis B virus RNA and hepatitis B core-related antigen to predict cure of hepatitis B virus infection. Hepatology (Baltimore, MD) 2021;73:870.
[51] Lam YF, Seto WK, Wong D, Cheung KS, Fung J, Mak LY, et al. Seven-year treatment outcome of entecavir in a real-world cohort: effects on clinical parameters, HBsAg and HBcrAg levels. Clin Transl Gastroenterol 2017;8:e125.
[52] Mak LY, Wong DK, Cheung KS, Seto WK, Fung J, Yuen MF. First-line oral antiviral therapies showed similar efficacies in suppression of serum HBcrAg in chronic hepatitis B patients. BMC Gastroenterol 2021;21:123.
[53] van Bömmel F, Deichsel D, Loglio A, Facchetti F, Pfefferkorn M, Brehm M, et al. HBV RNA can be detected more frequently than HBcrAg but decreases during long term treatment with nucleos(t)ide analogues up to 14 years in patients with HBeAg negative chronic hepatitis B. J Hepatol 2019;70:e487.
[54] Carey I, Gersch J, Wang B, Moigboi C, Kuhns M, Cloherty G, et al. Pregenomic HBV RNA and hepatitis B core-related antigen predict outcomes in hepatitis B e antigen-negative chronic hepatitis B patients suppressed on nucleos(t)ide analogue therapy. Hepatology (Baltimore, MD) 2020;72:42–57.
[55] Inoue T, Tanaka Y. The role of hepatitis B core-related antigen. Genes 2019;10:357–357.
[56] Brakenhoff SM, de Man RA, Boonstra A, van Campenhout MJH, de Knegt RJ, van Bömmel F, et al. Hepatitis B virus RNA decline without concomitant viral antigen decrease is associated with a low probability of sustained response and hepatitis B surface antigen loss. Aliment Pharmacol Ther 2021;53:314–20.
[57] Zoulim F, Carosi G, Greenbloom S, Mazur W, Nguyen T, Jeffers L, et al. Quantification of HBsAg in nucleos(t)ide-naïve patients treated for chronic hepatitis B with entecavir with or without tenofovir in the BE-LOW study. J Hepatol 2015;62:56–63.
[58] Sonneveld MJ, Hansen BE, Piratvisuth T, Jia JD, Zeuzem S, Gane E, et al. Response-guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen levels. Hepatology (Baltimore, MD) 2013;58:872–80.
[59] Pfefferkorn M, Schott T, Böhm S, Deichsel D, Felkel C, Gerlich WH, et al. Composition of HBsAg is predictive of HBsAg loss during treatment in patients with HBeAg-positive chronic hepatitis B. J Hepatol 2021;74:283–92.
[60] Beudeker BJ, Groothuismink ZM, de Man RA, Janssen H, van der Eijk AA, Boonstra A, et al. Hepatitis B core-related antigen levels predict pegylated interferon-α therapy response in HBeAg-positive chronic hepatitis B. Antivir Ther 2020;25:217–22.
[61] Chuaypen N, Posuwan N, Payungporn S, Tanaka Y, Shinkai N, Poovorawan Y, et al. Serum hepatitis B core-related antigen as a treatment predictor of pegylated interferon in patients with HBeAg-positive chronic hepatitis B. Liver Int 2016;36:827–36.
[62] van Bommel F, Bartens A, Mysickova A, Hofmann J, Kruger DH, Berg T, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatology (Baltimore, MD) 2015;61:66–76.
[63] van Campenhout MJH, van Bömmel F, Pfefferkorn M, Fischer J, Deichsel D, Boonstra A, et al. Serum hepatitis B virus RNA predicts response to peginterferon treatment in HBeAg-positive chronic hepatitis B. J Viral Hepat 2020;27:610–9.
[64] Berg T, Lampertico P. The times they are a-changing—a refined proposal for finite HBV nucleos(t)ide analogue therapy. J Hepatol 2021;75:474–80.
[65] 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 (Baltimore, MD) 2012;55:68–76.
[66] Jeng WJ, Chen YC, Chien RN, Sheen IS, Liaw YF. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. Hepatology (Baltimore, MD) 2018;68:425–34.
[67] Chi H, Li Z, Hansen BE, Yu T, Zhang X, Sun J, et al. Serum level of antibodies against hepatitis B core protein is associated with clinical relapse after discontinuation of nucleos(t)ide analogue therapy. Clin Gastroenterol Hepatol 2019;17:182–191.e1.
[68] Inoue T, Kusumoto S, Iio E, Ogawa S, Suzuki T, Yagi S, et al. Clinical efficacy of a novel, high-sensitivity HBcrAg assay in the management of chronic hepatitis B and HBV reactivation. J Hepatol 2021;75:302–10.
[69] Gane E, Locarnini S, Lim T, Strasser S, Sievert W, Cheng W, et al. Short-term treatment with RNA interference therapy, JNJ-3989, results in sustained hepatitis B surface antigen supression in patients with chronic hepatitis B receiving nucleos(t)ide analogue treatment. J Hepatol 2020;73:S20–S20.
[70] Yuen MY, Chan HLY, Liu K, Given BD, Schluep T, Hamilton J, et al. Differential reductions in viral antigens expressed from cccDNA vs integrated DNA in treatment naïve HBeAg positive and negative patients with chronic HBV after RNA interference therapy with ARC-520. J Hepatol 2016;64:S390–1.
[71] Fung S, Sulkowski M, Lalezari J, Schiff ER, Dieterich D, Hassanein T, et al. Antiviral activity and safety of the hepatitis B core inhibitor ABIH0731 administered with a nucleos(t)ide reverse transcriptase inhibitor in patients with HBeAg-negative chronic hepatitis B infection. J Hepatol 2020;73:S51–2.
[72] Janssen H, Hou J, Asselah T, Chan H, Zoulim F, Tanaka Y, et al. Efficacy and safety results of the phase 2 JNJ-56136379 JADE study in patients with chronic hepatitis B: interim week 24 data. J Hepatol 2020;73:S129–30.
[73] Huang YW, Takahashi S, Tsuge M, Chen CL, Wang TC, Abe H, et al. On-treatment low serum HBV RNA level predicts initial virological response in chronic hepatitis B patients receiving nucleoside analogue therapy. Antivir Ther 2015;20:369–75.
[74] Liao H, Liu Y, Li X, Wang J, Chen X, Zou J, et al. Monitoring of serum HBV RNA, HBcrAg, HBsAg and anti-HBc levels in patients during long-term nucleoside/nucleotide analogue therapy. Antivir Ther 2019;24:105–15.