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Review Article

An Ideal Hallmark Closest to Complete Cure of Chronic Hepatitis B Patients: High-sensitivity Quantitative HBsAg Loss

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Abstract

In the era of antiviral therapy, the main goal of treatment has shifted from the persistent inhibition of hepatitis B virus (HBV) replication to the pursuit of serological clearance of HBs surface antigen (HBsAg). Based on the life cycle of HBV, HBsAg originates from covalently closed circular DNA (cccDNA) and integrated HBV DNA, thus reflecting their transcriptional activity. Complete HBsAg loss may mean elimination or persistent inactivity of the HBV genome including cccDNA and integrated HBV DNA. HBsAg loss improves the recovery of abnormal immune function, which in turn, may further promote the clearance of residual viruses. Combined with functional cure and the great improvement of clinical outcomes, the continuous seroclearance of high-sensitivity quantitative HBsAg may represent the complete cure of chronic hepatitis B (CHB). For many other risk factors besides HBV itself, patients with HBsAg loss still need regular monitoring. In this review, we summarized the evolution of CHB treatment, the origin of serum HBsAg, the pattern of HBsAg seroclearance, and the effect of HBsAg loss on immune function and disease outcomes. In addition, we discuss the significance of high-sensitivity HBsAg detection and its possibility as a surrogate of complete cure.

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Keywords: Hepatitis B virus; Chronic hepatitis B; HBsAg; Immunoassay; Functional cure; Complete cure; Biomarker.

Abbreviations: cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; ETV, entecavir; HBcrAg, Hepatitis B core-related antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFNγ, interferon-gamma; IL-2, interleukin-2; LLOD, the lower limit of detection; NAs, nucleos(t)ide analogues; OBI, occult HBV infection; PegIFNα, pegylated interferon-alpha; TDF, tenofovir dipivoxil; TNFα, tumor necrosis factor α.

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Introduction

Despite the widespread use of the hepatitis B vaccine and antiviral drugs, chronic hepatitis B virus (HBV) infection remains a threat to human health and is the principal cause of liver cirrhosis and hepatocellular carcinoma (HCC). The latest data show that the global prevalence of HBsAg in the whole population is 3.9% and there are around 290 million HBsAg-positive people worldwide.[1] In 2016, a plan to eliminate viral hepatitis as a public health threat by 2030 was formulated by the World Health Organization (WHO). This plan aimed to reduce the new infection rate of HBV by 90% and the HBV related mortality by 65% in 2030 compared with the same period in 2015.[2] However, according to the current progress rate, with the global cumulative rate of diagnosis and treatment of hepatitis B in 2020 being 12.7% and 8%, respectively, this goal will be met after 2051.[3] To achieve this goal as soon as possible, early diagnosis, active standardization of antiviral treatment, and ultimately, working toward a cure are the most effective strategies. Therefore, it is important to find the ideal biomarker of cure.

Continuous HBsAg seroclearance as a marker of hepatitis B cure

History of anti-HBV regimens

Since the introduction of lamivudine in clinical practice in 1998, effective treatment of chronic hepatitis B (CHB) has been an era of antiviral treatment with other nucleos(t)ide analogues (NAs) that were subsequently developed one after another. In 2005, pegylated interferon-alpha (PegIFNα) was approved for the management of CHB patients, thus forming two categories of anti-HBV drugs.[4] At present, the goal of CHB treatment is to maximize the persistent inhibition of HBV replication, which ultimately lowers the occurrence of liver cirrhosis, decompensation, and HCC.[5,6] Unfortunately, a few patients develop end-stage liver diseases despite treatment. A South Korean study analyzed 3,156 treatment-naïve CHB patients treated with entecavir (ETV) or tenofovir dipivoxil (TDF), and found that 285 patients (9.0%) developed liver cancer during a median follow-up of 58.3 months. The cumulative incidence rates of HCC at 3, 5, and 7 years ere 5.3%, 9.3%, and 13.8%. There were no sig-

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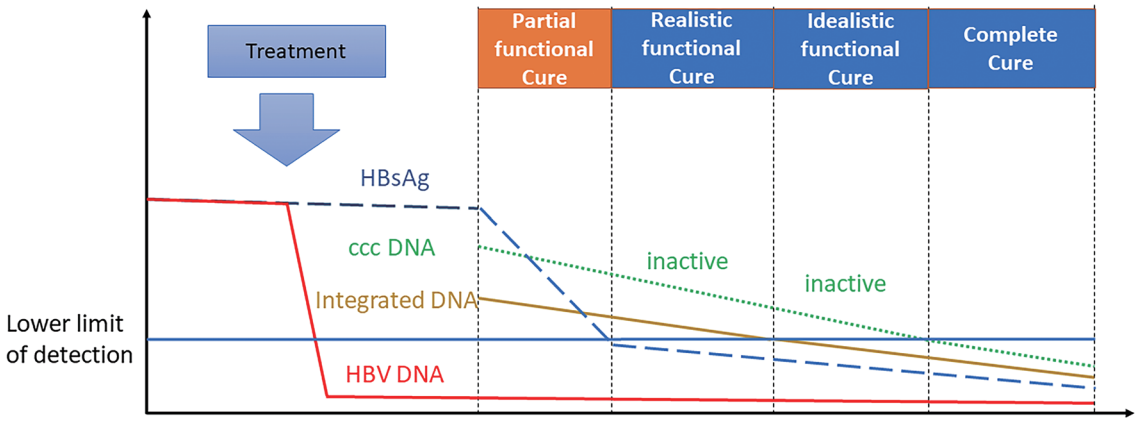

Fig. 1. Characteristics and evolution of the biomarkers defining various hepatitis B cures. Partial functional cure: undetectable HBV DNA with positive HBsAg after completing a short course of therapy. Realistic functional cure: undetectable HBV DNA and HBsAg with detectable cccDNA and integrated HBV DNA. Idealistic functional cure: undetectable HBV DNA and HBsAg with undetectable integrated HBV DNA. Complete cure, sustained serum HBsAg loss with undetectable cccDNA and integrated HBV DNA; HBsAg, HBV surface antigen; cccDNA, covalently closed circular DNA; Inactive cccDNA, transcriptional activity decreases or even disappears; HBV DNA, serum HBV DNA. cccDNA, covalently closed circular DNA; HBsAg, HBV surface antigen.

nificant differences in the incidence of HCC per 100 person-years 5 years before and 5 years after treatment with NAs. No significant differences were observed in the incidence of HCC in high-, middle-, and low-risk groups determined by their modified platelet age gender-HBV (mPAGE-B) scores 5 years before and 5 years after treatment. Prolonging the duration of oral NAs had no significant influence on the overall incidence of HCC after achieving a complete virological response. After long-term NA treatment, the risk of HCC in CHB patients was still present, suggesting that treatment should not be limited to long-term inhibition of the virus, but also to pursue a higher level of efficacy.[7]

Evolution of hepatitis B cure marked by continuous HBsAg seroclearance

In 2009, Ning *et al*.[8] initiated the first clinical study on HBsAg clearance, by transitioning the treatment from ETV to PegIFNα-2a in CHB patients who were HBeAg positive. All patients treated with ETV and those with HBV DNA ≤1,000 copies/mL and HBeAg <100 PEIU/mL were randomly divided to switching to PegIFN or continuing ETV for 48 weeks. HBsAg loss was 8.5% and 0% in the PegIFN and continuous ETV groups, respectively. For patients with negative HBV DNA, negative HBeAg, and HBsAg <1,500 IU/mL, the rate of HBsAg loss was as high as 22.2%. Subsequently, more studies on NAs sequenced or combined with PEG-IFNα were performed to explore and improve the clinical cure rate.[9,10] The definition of clinical cure in the CHB guidelines in China in 2015 was based on those study findings.[11] For some suitable patients, the clinical cure of CHB, that is, the continuous virological response and HBsAg loss after treatment is stopped, should be pursued as far as possible.

In 2014, The French National Agency for Research on AIDS and Viral Hepatitis proposed an HBV cure program.[12] To guide clinical trials of a hepatitis B cure, a workshop was held in 2016 to develop a common perspective on the endpoints of CHB treatment, which was that a functional cure indicated by continuous HBsAg loss could be achieved with currently available treatment regimens. An HBV functional cure was proposed and defined as sustained HBV DNA and HBsAg seroclearance with or without positive hepatitis B surface antibodies (anti-HBs) after a finite course of treatment.[13] The term functional cure was mentioned in both the HBV guidelines of the European Association for the Study of the Liver (EASL) in 2017 and the American Association for the Study of Liver Diseases (AASLD) in 2018.[5,6] In 2019, the EASL and the AASLD jointly organized a conference on HBV treatment endpoint to develop a consensus on HBV cures (Fig. 1).[14] A complete cure for HBV infection is difficult because of the limited treatment options. Thus, the current study focuses on varying degrees of functional cure. Antiviral drugs target HBV reverse transcriptase activity and cannot directly act on cccDNA, which makes them fail to eliminate cccDNA. Therefore, the elimination of cccDNA from infected cells has provided future research directions for drug research and development, which is an idealistic functional cure that we have been long pursuing. Realistic functional cure was defined as undetectable HBV DNA and HBsAg with detectable cccDNA and integrated HBV DNA, as already suggested by its name. That cure is currently an attainable goal for us. An attainable partial functional cure was defined as undetectable HBV DNA with positive HBsAg after completing a short course of therapy. The clearance of HBsAg is the ideal endpoint, thus we need to seek comprehensively inhibition of viral DNA replication.

In the same year, Chinese experts released a consensus on the roadmap to a functional cure for CHB.[15] The WHO highlighted that progress in curing hepatitis B is required to achieve the 2030 objective of eliminating viral hepatitis and continued investment in a functional cure for hepatitis B research is insured as one of priorities in 2021.[16] Of course, complete cure, that is the elimination of integrated HBV DNA and covalently closed circular DNA (cccDNA), is the ultimate goal we pursue, and it may require combination therapy with potent NAs and at least one immunomodulator,[17] or even triple combination therapies of inhibiting HBV replication, reducing antigen levels, and stimulating immune function.[18]

Origin and degradation of serum HBsAg

Serum HBsAg originates from cccDNA and integrated HBV DNA. The former may exist in a condensed state which is transcriptionally inactive or a relaxed state which is transcriptionally active.[19] Infectious HBV particles and noninfectious subviral particles (SVPs) are formed and released into the blood (Fig. 2). The latter is linear HBV DNA that is integrated into different parts of the hepatocyte chromosome. Because of the lack of a normal circular structure, the integrated DNA can only express S- and M-HBsAg, and

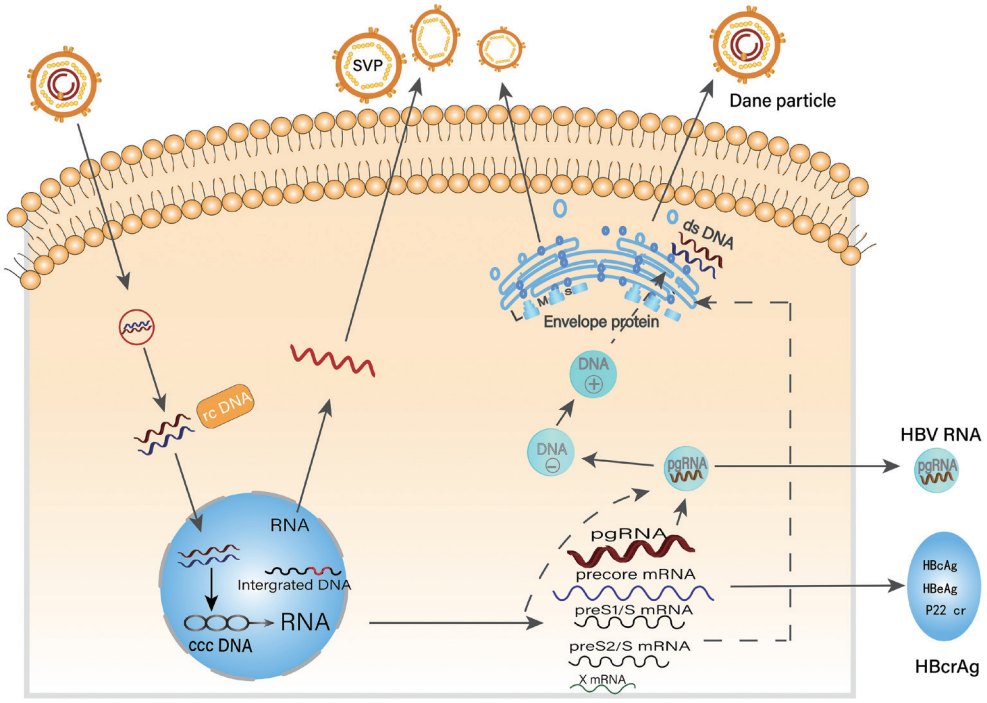

Fig. 2. A marker of complete cure of chronic hepatitis B: HBsAg loss by high-sensitivity assay. Serum HBsAg originates from cccDNA and integrated HBV DNA. cccDNA serves as a transcription template to produce infectious HBV particles and noninfectious SVPs and releases them into the blood. In addition, integrated HBV DNA is also a replication template of S- and M-HBsAg, and only spherical SVPs can release into the blood. cccDNA, covalently closed circular DNA; HBsAg, HBV surface antigen; HBcrAg, hepatitis B core-related antigen; SVP, subviral particle; rcDNA, relaxed circular DNA; ds DNA, double-stranded DNA.

the spherical SVPs that are released into the blood cannot form pregenomic RNA (pgRNA) and other viral proteins.[20,21] Around 99.99% of HBsAg in the blood exists in SVPs.[22] For CHB patients with a complete virological response or negative HBeAg, most of the serum HBsAg comes from integrated HBV DNA rather than cccDNA.[23] Transcriptionally active integrated HBV DNA is present in the entire liver and forms widespread HBsAg independent of HBV replication.[24]

Infected hepatocytes regulate the secretion of HBsAg through a variety of degradation pathways, such as endoplasmic reticulum-mediated proteolysis and autophagy. In addition, the unique proteolytic mechanism of proteasome, ubiquitin, and proteome-independent processes is also involved in the degradation of M- and L-HBsAg.[25,26] The infection of hepatocytes is accompanied by an increase in the activity of the degradation pathways, indicating that HBsAg renewal is involved in the production of SVP and virus.

Patterns and epidemiology of continuous HBsAg seroclearance

Spontaneous HBsAg seroclearance is rarely reported, with a rate of only about 1% per year.[27] A study including 1,076 CHB patients reported cumulative rates of spontaneous HBsAg seroclearance of 8.1% and 44.7% after 10 years and 25 years, respectively.[28] Prospective follow-up of a cohort of 1,240 patients with negative HBeAg and who were not treated for 5.5 years found that the crude incidence rate of HBsAg loss was 1.6 per 100 person-years. HBsAg seroclearance was also found to be highly associated with older age, nonasian race, inactive HBsAg carrier, HBV genotype A, lower HBV DNA, and quantitative HBsAg levels.[29]

NAs suppress HBV DNA rather than directly acting on cccDNA. Consequently, it is very difficult for NAs to prevent the production of HBV particles and their antigens. A recent large multicenter cohort study that enrolled 4,769 CHB patients showed that the 10-year cumulative loss rate of HBsAg was only 2% during 26,614 person-years of ETV or TDF therapy.[30] Although stopping NAs following the withdrawal criteria of the guidelines has the risk of virological relapse, clinical relapse, or exacerbation of the liver disease, it may benefit from HBsAg loss.[31,32] A study including 1,216 CHB patients with undetectable HBV DNA and HBeAg who did not complete long-term NA therapy reported that after an average follow-up of nearly 2 years, 98 patients (8.1%) achieved HBsAg loss. The predictors of HBsAg loss were found to be race, HBV genotype, and viral antigen level at treatment cessation.[33] Based on the findings, the Asian Pacific Association for the Study of the Liver (APASL) developed a guideline for stopping NAs that recommended withdrawal of NAs to obtain HBsAg clearance in patients who had negative HBeAg and relatively low HBsAg levels.[34]

Although the ability of PegIFNα to inhibit HBV DNA is much weaker than that of NAs, the former promotes the decline of HBsAg level more significantly, with a 3–7% clearance rate of HBsAg after 48 weeks of treatment.[6] A real-world study divided 330 CHB patients into three groups: PegIFNα + TDF, PegIFNα, and TDF monotherapy. At 72 weeks, the incidence of HBsAg loss was 11.5%, 5.7%, and 0%, respectively.[35] A meta-analysis indicated that the initial combination (PegIFNα + NA) significantly increased the clearance rate of HBsAg compared with NA monotherapy (relative risk: 15.59, 95% CI: 3.22–75.49). However, there was no significant difference observed between the initial combination and PegIFNα monotherapy.[36] HBsAg clearance continued to increase with the prolongation of PegIFNα withdrawal time. Moreover, in inactive HBsAg carriers for whom antiviral therapy was recommended, PegIFNα induced a high HBsAg clearance rate, especially in patients with low baseline HBsAg levels.[37]

Effect of continuous HBsAg seroclearance on immune function

HBV infection results from the interaction between HBV and the host. Immunological liver injury is the main pathogenesis of hepatitis B. The immune response generated by the host is closely associated with outcomes of the natural history of CHB and acute HBV infection. HBV antigens, especially HBsAg, are major contributors to the immunopathogenesis of CHB, and the chronicity of HBV infection is related to the exhaustion of T and B cell responses.[38,39] Theoretically, the disappearance of HBsAg should improve recovery from abnormal immune function, which is also one of the manifestations of functional cure, and in turn promotes the clearance of residual viruses, including cccDNA and integrated HBV DNA.

Host cellular immune function, especially HBV-specific $CD4^+$ and $CD8^+$ T cells, has a critical impact on the clearance of HBV and the prognosis of hepatitis B infection.[40,41] The continuous loss of HBsAg and the appearance of HBsAb indicate a successful immune response to HBV and mark the complete and sustained control of infection.[42,43] Boni *et al*.[43] compared HBV-specific T cell responses in patients who were given NA with those who experienced other forms of HBV control by measuring intracellular levels of cytokines including interleukin-2 (IL-2), interferon-gamma (IFNγ), and tumor necrosis factor α (TNFα). They found that the T cell response in patients with HBsAg loss was stronger than that in patients with persistent HBsAg. Compared with those of HBsAg-positive patients, $CD4^+$ and $CD8^+$ T cells had a more active phenotype in NA-induced HBsAg clearance and presented higher proliferation 12 weeks after stopping NA treatment[44] A longitudinal study found that patients with negative HBsAg presented definite $CD4^+$ and $CD8^+$ T cell phenotypic characteristics compared with those with persistent HBsAg, and these changes in T cell phenotypes were related to IFNα treatment. Furthermore, the study identified HBsAg quantification combined with CTLA-4, CD95, and CD107a expression on $CD4^+$ T cells, and TIM-3 and HLA-DR expression on $CD8^+$ T cells as potential predictors for HBsAg clearance within 12 months in CHB patients.[45]

A humoral immune response based on neutralizing antibodies to inhibit and eliminate HBV infection has recently attracted more attention.[46] In CHB patients, the differentiation ability of B cells *in vivo* is significantly enhanced, but proliferation is significantly reduced.[47,48] There have been few studies on the effect of HBsAg reduction or loss on B cells. A study that recruited 63 treatment-naïve CHB patients and 46 patients with HBsAg loss induced by antiviral treatment found that compared with HBsAg-positive patients, HBsAg-negative patients had more naïve B cells and plasmablasts and fewer memory B cells. The dominant B cell epitopes (S76 and S78) in patients with negative HBsAg may be significant candidates for treatment to achieve a functional cure.[49] During PegIFNα treatment, the proportion of total B cells and plasma B cells in the HBsAg-negative group was higher than that in the HBsAg-positive group, when other factors including age, sex, and treatment duration were completely matched.[50]

Durability of HBsAg seroclearance

Functional cure is more reflected in the recovery of liver function, especially the specific immune function against HBV, through the maximum long-term suppression of HBV replication, without emphasis on the elimination of integrated HBV DNA and cccDNA. At present, the gap between functional cure and complete cure is still very large. In addition to the detection of integrated HBV DNA and cccDNA, the duration of functional cure after drug withdrawal and the improvement of long-term outcomes are also very important as these can reflect complete cure to some degree (Table 1).[51–70]

Wu *et al*.[68] analyzed 238 cases with HBsAg clearance who were treated with IFNα/PegIFNα alone or combined with NAs. The cumulative recurrence rates at 26, 52, 78, 104, and 597 weeks were 0.84%, 6.29%, 6.88%, 8.18%, and 9.66%, respectively, of which 83% (15/18) recurred within 52 weeks after drug withdrawal. A prospective study enrolled 176 CHB patients who underwent IFN alone or combined NAs treatment and achieved HBsAg clearance. The study found that at 48 weeks of follow-up, 86.63% (149/172) had maintained HBsAg seroclearance.[70] Lok *et al*.[71] followed 55 PegIFNα or NAs treated patients with HBsAg clearance in three clinical studies for an average of 96 weeks. They found that 82% of the patients maintained HBsAg clearance.[71] In another study, 104 HBeAg-positive children 2–16 years of age with CHB who completed at least 36 weeks of PegIFNα and were followed up for 104 weeks. The HBsAg clearance rates were 48.1% at the end of treatment and 53.8% at follow-up. The continuous response incidence of HBsAg clearance was as high as 94%.[72] Long-term follow-up studies in Hong Kong, China and the National Institutes of Health also found that the clinical cure rate of patients with HBsAg clearance can be maintained at more than 95%, whether it is spontaneous clearance or clearance after drug treatment.[73,74] A recent meta-analysis reported that CHB patients had a durable negative HBsAg response after HBsAg seroclearance.[75]

Effect of continuous HBsAg seroclearance on disease outcomes

Outcomes of CHB patients with HBsAg seroclearance are summarized in Table 1. A retrospective cohort study enrolled 4,568 CHB patients with HBsAg clearance, of which 793 had received NAs and 60 had received interferon (IFN) treatment. During a median follow-up of 3.4 years, 54 patients (2.9%) developed liver cancer, including 49 patients with spontaneous clearance of HBsAg and five men over 50 years of age old treated with NA. None of the patients treated with PegIFNα had liver cancer within 5 years. The cumulative incidence rates of HCC were 0.9%, 1.3%, and 1.5% at 1, 3, and 5 years, respectively. In patients with HBsAg clearance, age, and sex were two independent predictors of the risk of HCC. After HBsAg loss, the 5-year cumulative incidence of HCC was 0% in women and 0.7 % in men at ≤50 years of age and 1.0% and 2.5%, respectively in those >50 years of age.[76] The same team also analyzed 7,124 CHB patients with HBsAg loss. Spontaneous clearance and NA-induced clearance occurred in 5,917 and 1,207, respectively. After an average follow-up of 4.3 years, the incidence of HCC was 1.6% and 1.3%, respectively, with no significant difference.[76]

A systematic review included a total of 188,316 CHB patients and showed that the total incidence rate of end-stage liver disease in the HBsAg clearance and HBsAg-persistent groups was 0.19 and 2.45 per 1,000 person-years, respectively. In addition, the incidence of decompensation, HCC, liver transplantation, and all-cause death per 1,000 person-years in the HBsAg clearance and HBsAg-persistent groups was 1.37 and 3.65, 0.14 and 1.81, 1.57 and 12.71, respectively. The combined relative risk of end-stage liver disease, decompensation, HCC, liver transplantation, and all-cause death in the HBsAg clearance group was 0.31, 0.28, 0.30, and 0.22, respectively. The findings suggest that the clinical outcomes of CHB patients after HBsAg seroclearance were significantly improved. Stratified analysis of various treatment regimens (e.g. IFN, NAs, or IFN + NAs) did not find significant differences in the risk of endpoint events among the subgroups.[77] Another meta-analysis also found that the risk of HCC was very low in CHB patients with HBsAg clearance, especially in those treated with IFN.[78] Patients with HBsAg loss had a lower risk of hepatic

Table 1. Summary of durability and outcomes of HBsAg seroclearance

First author	Year	Country or region	Design	Cumulative cases of HBsAg seroclearance	Longest or average follow-up of HBsAg seroclearance	Recurrence of HBsAg, %	Incidence of HCC, %	Deaths or liver transplantation, %	Notes
Spontaneous HBsAg seroclearance									
Yip TC[51]	2021	HK, China	Retrospective	5,917	4.3 (2.2–7.6) years	7.0%	1.64%	NR	NR
Park Y[52]	2021	Korea	Retrospective	984	4.8 years (0.5–17.8)	NR	1.22%	NR	NR
Choi J[53]	2021	Korea	Retrospective	1,624	5.6 (2.8–9.6) years	1.23%	2.16%	2.28%	Risk factors of HCC and clinical events: older age, male sex, and cirrhosis
Song C[54]	2019	China	Prospective	652	NR	NR	1.23%	NR	NR
Zhu L[55]	2018	China	Prospective	348	NR	NR	0.29%	NR	NR
Chen YC[56]	2016	Taiwan, China	Retrospective	312	107 months	NR	1.28%	0%	NR
Lim TH[57]	2016	New Zealand	Prospective	145	72 months (0–300)	NR	0%	0%	NR
Ari A[58]	2016	Turkey	Retrospective	84	NR	NR	0%	NR	NR
NAs induced HBsAg seroclearance									
Yip TC[51]	2021	HK, China	Retrospective	1,207	4.3 (2·2–7·6) years	7.7%	1.32%	NR	NR
Choi J[53]	2021	Korea	Retrospective	348	4.6 (2.4–7.8) years	5.46%	4.02%	5.75%	Risk factors of HCC and clinical events: older age, male sex, and cirrhosis
Kim MA[59]	2020	Korea	Retrospective	276	26.9 (12.2–49.2) months	3.6%	2.9%	NR	NR
Yip TC[60]	2019	HK, China	Retrospective	376	4.8 (2.8–7.0) years	1.4%	0.5%	0%	NR
Suarez E[61]	2019	Spain	Retrospective	69	37.8 (23.8–54.6) months	1.5%	1.5%	0%	NR
Sun Y[62]	2019	China	Retrospective	54	NR	NR	0%	NR	NR
Chi H[63]	2017	Multicenter	Retrospective	70	1.6 (0.5–2.7) years	3.7%	0%	0%	NR
Chen YC[56]	2016	Taiwan, China	Retrospective	110	107 months	NR	0.91%	0.91%	NR
IFNα or combined with NAs induced HBsAg seroclearance									
Li MH[64]	2022	China	Prospective	231	48 weeks	8.2%		0%	NR
Chen J[65]	2021	China	Prospective	48	24 weeks	6.25%	0%	0%	PegIFNα add on NAs
Wu F[66]	2021	China	Prospective	68	48 weeks, follow-up 24 weeks	NR	0%	0%	IFNα monotherapy, Inactive HBV carriers
Pan CQ[67]	2021	China	Prospective	376	96 weeks	17.3%	0.3%	0%	258 IFN monotherapy, 118 IFN add on NA
Wu Y[68]	2020	China	retrospective	238	160 weeks (21–597)	5.88%	0%	0%	IFN monotherapy, or IFN + NA
Choi HS[69]	2020	Canada	Retrospective	65	11.5 (6.6–19.0) years	1.96%	4.44%	8.89%	IFNα
Li MH[70]	2019	China	Prospective	176	48 weeks	13.37%	0.58%	0%	118 IFN monotherapy, 58 IFN add on NAs

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NAs, nucleos(t)ide analogues; IFNα, interferon α; PegIFNα, pegylated interferon α; NR, not reported.

decompensation, incident cirrhosis, overall mortality, and liver-related mortality compared with those with no HBsAg loss.

HBsAg is better than other new biomarkers as an indicator of clinical cure

Serum HBV RNA is mainly derived from pgRNA without initiation of reverse transcription in the nucleocapsid that results in HBV RNA virus-like particles.[79] This is of great significance in drug withdrawal management, optimizing antiviral strategy, and predicting outcomes of CHB patients. However, HBV RNA only indicates the presence and transcriptional activity of cccDNA. It does not reflect the active state of integrated HBV DNA (Fig. 2). Therefore, when studying the association between HBsAg and serum HBV RNA, it was found that the relation between serum HBsAg and serum HBV RNA was significant only in treatment-naïve CHB patients with positive HBeAg.[80,81] In CHB patients with negative HBeAg and those treated with NAs or IFN, the correlation was extensively weakened or was not significant.[80,82] In addition, the detection methods of HBV RNA are not standardized and are easily affected by HBV pgRNA splice variants and HBV DNA.

Hepatitis B core-related antigen (HBcrAg) consists of three antigens encoded by HBV pre-C/C region genes, including HBV core antigen, HBeAg, and precore/core protein with a relative molecular weight of 22 KD (Fig. 2). Its quantitative level is important in guiding the management of chronic HBV infection. As a marker of cccDNA and HBV translation, serum HBcrAg correlates well with the transcriptional activity of intrahepatic cccDNA and thus can monitor the efficacy of the new HBV regimens targeting cccDNA.[83]

Lim *et al*.[84] studied 114 HBeAg-negative patients who were treated with PegIFNα for and evaluated the value of quantitative HBsAg, HBV RNA, and quantitative HBcrAg in predicting HBsAg clearance. Quantitative HBsAg was better than both HBcrAg and HBV RNA whose baseline AUCs were 0.916, 0.649, and 0.542, respectively. Based on the kinetics of these markers, only quantitative HBsAg had a good relationship with HBsAg clearance, HBV RNA had a low correlation, and HBcrAg did not change significantly.

Based on the HBV life cycle and the origin of various markers, HBsAg reveals the transcriptional activity of both integrated HBV DNA and cccDNA, unlike HBV RNA and HBcrAg (Fig. 2). Therefore, the continuous negative HBsAg is closer to complete cure of CHB. Theoretically, the current regimens pursuing clinical cure are mainly based on IFNα. IFNα can act on cccDNA and is even regarded as one of the most promising drugs in the elimination of HBV cccDNA.[85] Additionally, IFNα regulates the transcription of cccDNA by the epigenetic modifications of the histones and indirectly targets cccDNA through APOBEC3 family proteins.[86–88]

Management of patients with continuous HBsAg seroclearance

The incidence of cirrhosis and end-stage liver diseases is significantly reduced. However, the risk of HCC still exists in CHB patients who achieve a functional cure.[88] A meta-analysis showed that 1.86% of patients developed HCC within 19.6 to 336 months after HBsAg clearance compared with 6.56% of patients with positive HBsAg in the control group.[89] Aside from HBV factors, HCC was related to age, sex, family history, liver cirrhosis, treatment regimens (NAs or IFNα induced HBsAg seroclearance), HBV DNA integration, co-infection, obesity, diabetes, and other complications.[90,91] Male sex, a history of cirrhosis, and a family history of HCC are related to a higher incidence of HCC after HBsAg clearance.[90] A recent study from South Korea retrospectively analyzed 831 CHB patients who reached HBsAg loss and found that the age of HBsAg loss, underlying liver cirrhosis, family history of HCC, and excessive drinking were independent predictors of HCC. A prediction model of HCC after HBsAg seroclearance was constructed based on those parameters.[92] Therefore, HCC surveillance should continue even after HBsAg clearance. In particular, patients with a long duration of infection, liver cirrhosis, a first-degree family member with HCC,[5] or other risk factors should be targeted for close HCC surveillance after HBsAg loss.

Necessity of high-sensitivity HBsAg detection and its possible use as a marker of complete cure

Serum HBsAg originates from cccDNA and integrated HBV DNA fragments. Commercially available HBsAg test kits can check out not only total forms of HBsAg such as Dane particles and spherical and filamentous HBsAg, but also detect integrated HBV DNA and cccDNA.[93] Theoretically, continuous HBsAg seroclearance indicates that the activities of cccDNA and the integrated HBV DNA are inhibited.

In the livers of CHB patients with HBsAg seroclearance, integrated HBV DNA and cccDNA can still be detected. HBV DNA can also be found in the blood or liver of patients with occult HBV infection (OBI). Wong *et al*.[91] collected liver tissues from 90 HCC HbsAg-negative patients with HBV DNA, cccDNA, and integrative HBV DNA in DNA of liver cells. They found cccDNA in 29 and integrated HBV DNA in 42 of the 62 HCC patients with concomitant OBI. However, the presence of low-level HBsAg cannot be excluded in those patients. A study enrolled 114 CHB patients with undetectable HBsAg by conventional enzyme immunoassay (EIA) and detected HBsAg in half the patients with a high-sensitivity HBsAg assay with a lower limit of quantitation of 0.005 IU/mL.[94] In another study, the Architect HBsAg Next qualitative assay (Abbott Laboratories, Abbott Park, IL, USA) was used to measure HBsAg in 800 CHB patients who had HBsAg loss by conventional assays. HBsAg was detected in 59/800 (7.3%) patients with HBsAg clearance. At <3, 3–5, 5–8, 8–11, and >11 years after HBsAg seroclearance, HBsAg was detected in 27.8%, 8.2%, 6.9%, 3.8%, and 1.9% samples, respectively.[95] Therefore, in patients with OBI and serum negative HBV DNA, high-sensitivity quantitative HBsAg may detect HBV protein synthesis. The lower limit of detection (LLOD) is between 0.03 and 0.05 IU/mL in conventional commercially available assays. A highly sensitive quantitative HBsAg assay detects serum HBsAg at 0.005 IU/mL with an automated chemiluminescent enzyme immunoassay system (Lumipulse G1200; Fujirebio, Inc., Tokyo, Japan). Moreover, highly sensitive quantitative HBsAg assays detects antigen-antibody complexes in addition to free HBsAg proteins, even mutant HBsAg.[96] Across HBV genotypes A to H and common mutants, the Architect HBsAg Next qualitative assay has a consistent sensitivity.[97] The ultra-sensitive quantitative HBsAg assay has an LLOD of 0.0005 IU/mL which is 100-fold lower than those of conventional HBsAg assays.[98]

After a functional cure, HBsAg may be present at low or very low levels that are related to the recurrence and progression of liver disease after drug withdrawal. Seventeen CHB patients with HBsAg seroclearance confirmed with a conventional assay (Architect HBsAg QT kit; Abbott Laboratories) were tested with an ultra-sensitive assay that had a sensitivity of 0.0005 IU/mL, and three of five patients in the HCC group and 12 in the non-HCC group were found to be HBsAg positive for up to 1 year.[98] Therefore, detection by high-sensitivity or ultra-sensitive quantitative HBsAg is important for the determination of a real cure. Functional cure means the elimination or persistent inactivation of cccDNA. Persistent HBsAg loss can also reflect marked reduction or clearance of integrated HBV DNA.[99] Characteristics of conventional and high-sensitive HBsAg quantitative assays are summarized in Table 2.[95,97,100]

Table 2. Characteristics of HBsAg quantitative and HQ-HBsAg assays

Assay	Supplier	Principle	Technology (tracer)	Pretreatment	Reaction sample volume	Assay duration	Linear range (analytical sensitivity)	On-board dilution	Traceability (NIBSC code)
Abbott Architect HBsAg[97]	Architect $i2000_{SR}$	Sandwich principle, capture mAbs, and polyclonal detection antibodies	CMIA (acridinium)	None	75 µL	29 m	0.05–250 IU/mL (0.05 IU/mL)	1:500 with recalcified negative human plasma	WHO first international standard, subtype ad (80/549)
Roche HBsAg II Quant	Molecular E170	Sandwich principle, two capture mAbs, and a mixture of mAbs and polyclonal antibodies	ECLIA (ruthenium)	None	50 µL	18 m	0.05–130 IU/mL (0.05 IU/mL)	1:400 with buffered negative human serum	WHO second international standard, subtype adw2, genotype A (00/588)
Fujirebio Lumipulse G HBsAg-Quant	Lumipulse G1200	Sandwich principle, two capture mAbs and two detections mAbs	CLEIA (AMPPD)	Yes, to disrupt viral particles and dissociate HBsAg from HBsAg-anti-HBs complexes	100 µL	29 m	0.005–150 IU/mL (0.005 IU/mL)	1:100, 1:200 or 1:1000 with NaCl and Tris buffer	WHO second international standard, subtype adw2, genotype (00/588)
Architect HBsAg Next qualitative assay[95]	Architect i2000SR	Sandwich principle, two monoclonal antibodies solid-phase, and a goat polyclonal antibody conjugate	One-step CMIA	None	75 µL	NR	0.0052– IU/mL (0.0052 IU/mL)	NR	WHO second international standard (00/588) a consistent sensitivity across major HBV genotypes A to H and common mutants
iTACT-HBsAg[100]	LUMIPULSE PRESTO II (Fujirebio, Inc.)	Sandwich principle	ICT-CLEIA	Yes, to inactivate anti-HBs, releases the antigen from the immune complexes, and to disassociate the antigen polymers into monomers	50 µL	20 m	0.0005–113 IU/mL (0.0005 IU/mL)	NR	NR

CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; CLEIA, chemiluminescent enzyme immunoassay; iTACT, immunoassay for total antigen including complex via pretreatment; ICT-CLEIA, immune complex transfer - chemiluminescent enzyme immunoassay; NR, not reported.

In addition, HBsAg loss can likely stimulate and restore HBV-specific immune responses that promote complete resolution of HBV infection.[101] Combined with the persistence of functional cure and the great improvement of clinical outcome of liver disease, the continuous seroclearance of high-sensitivity or ultra-sensitive quantitative HBsAg likely reflects a complete cure of CHB, which is similar to the treatment of chronic hepatitis C virus (HCV) infection. Although the possibility of HCC may still happen, especially in those who have HCV related cirrhosis, it does not hinder the perspective of complete cure.

Conclusions and perspectives

In the last 20 years, significant progress has been made in the antiviral treatment of hepatitis B, which has evolved from persistent inhibition of HBV replication to the pursuit of seroclearance of HBsAg, that is, a functional cure. A complete cure likely means persistent inactivity of cccDNA and integrated HBV DNA rather than complete elimination of the HBV genome. HBsAg reveals the transcriptional activity of both cccDNA and integrated HBV DNA, and to some degree, the continuing seroclearance of high-sensitivity or ultra-sensitive quantitative HBsAg may represent a complete cure of CHB. Alternatively, HBV antigens, especially HBsAg, are involved in the immunopathogenesis of hepatitis B. Thus, HBsAg loss can significantly recover abnormal immune function, which in turn, may further facilitate the clearance of residual viruses conversely, including cccDNA and integrated HBV DNA. Some remaining issues need to be addressed. First, it is unclear whether the continuous HBsAg loss means that cccDNA and integrated HBV DNA are completely inactive, resulting in the inability of HBsAg expression, or most of them are eliminated. Second, more and more new drugs that inhibit HBV and improve host immune response are in the process of clinical trials.[99,102] The effectiveness and safety of these novel drugs, as well as the best treatment strategy in complete cure, still need a lot of exploration. HBsAg cannot reflect the efficacy of some new drugs that inhibit or scavenge cccDNA because the integrated HBV DNA can still express HBsAg. Third, more HBsAg mutants were detected in the patients with HCC/cirrhosis than in the asymptomatic carriers.[103] Although a panel of antibodies has been optimized specifically for HBsAg mutants, qualitative immunoassays may produce false-negative results for HBV with mutant surface antigen. And last, for many risk factors of hepatitis B related HCC, even if HBsAg is serologically cleared, there is still the possibility of end-stage liver disease including HCC. Therefore, regular monitoring is still warranted. The clinical significance of trace amounts of HBsAg needs to be further studied.

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Conflict of interest

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

Author contributions

Study concept and design (BF), acquisition, analysis and interpretation of data, drafting of the manuscript (ZLW, JRZ), critical revision of the manuscript for important intellectual content (RFY, LXH), and study supervision (HSC). All authors have made a significant contribution to this study and have approved the final manuscript.

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