



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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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; IFN γ , interferon-gamma; IL-2, interleukin-2; LOD, the lower limit of detection; NAs, nucleos(t)ide analogues; OBI, occult HBV infection; PegIFN α , pegylated interferon-alpha; TDF, tenofovir dipivoxil; TNF α , 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 (PegIFN α) 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 were 5.3%, 9.3%, and 13.8%. There were no sig-

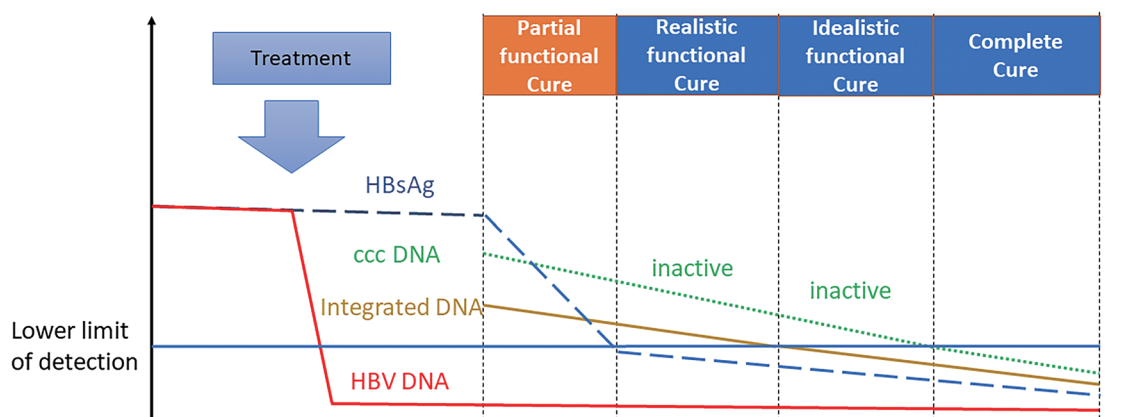


Fig. 1. Characteristics and evolution of the biomarkers defining various hepatitis B cures. Partial functional cure: undetectable HBV DNA with positive HBsAg after completing a short course of therapy. Realistic functional cure: undetectable HBV DNA and HBsAg with detectable cccDNA and integrated HBV 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 PegIFN α -2a in CHB patients who were HBeAg positive. All patients treated with ETV and those with HBV DNA $\leq 1,000$ 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-IFN α 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

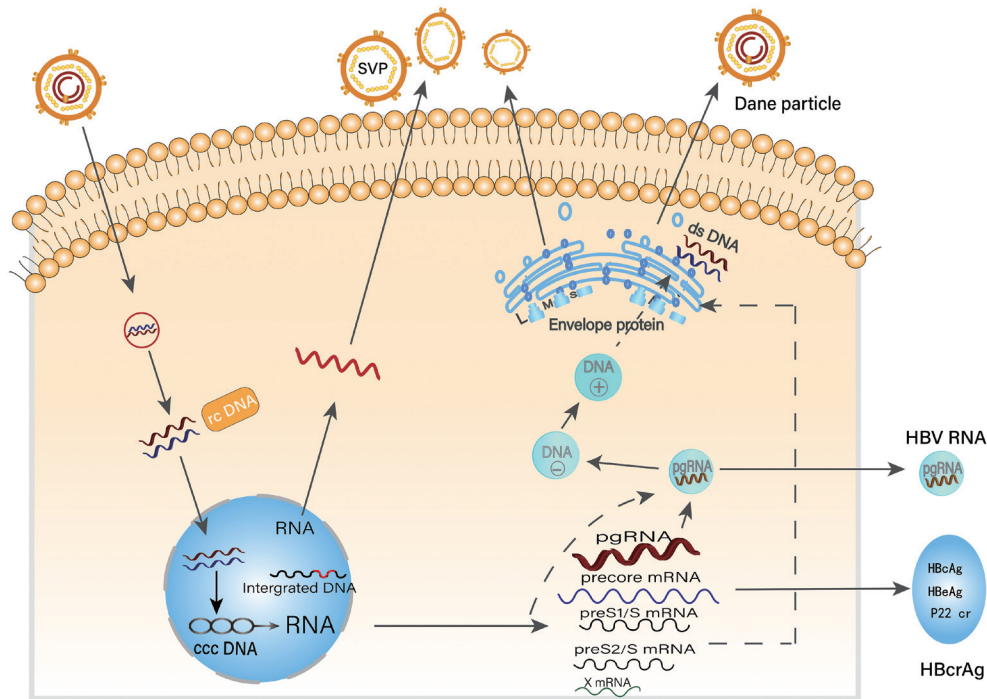


Fig. 2. A marker of complete cure of chronic hepatitis B: HBsAg loss by high-sensitivity assay. Serum HBsAg originates from cccDNA and integrated HBV DNA. cccDNA serves as a transcription template to produce infectious HBV particles and noninfectious SVPs and releases them into the blood. In addition, 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 PegIFNa 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: PegIFNa + TDF, PegIFNa, 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 (PegIFNa + 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 PegIFNa monotherapy.³⁶ HBsAg clearance continued to increase with the prolongation of PegIFNa withdrawal time. Moreover, in inactive HBsAg carriers for whom antiviral therapy was recommended, PegIFNa 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 (IFN γ), and tumor necrosis factor α (TNF α). 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 IFN α 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 PegIFN α 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 IFN α /PegIFN α 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 PegIFN α 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 PegIFN α 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 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 PegIFN α 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 or combined with IFNa	2016	Taiwan, China	Retrospective	110	107 months	NR	0.91%	0.91%	NR
Li MH ⁶⁴	2022	China	Prospective	231	48 weeks	8.2%	0%	0%	NR
Chen J ⁶⁵	2021	China	Prospective	48	24 weeks	6.25%	0%	0%	PegIFNa add on NAs
Wu F ⁶⁶	2021	China	Prospective	68	48 weeks, follow-up 24 weeks	NR	0%	0%	IFNa 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%	IFNa
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; IFNa, interferon α ; PegIFNa, 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 PegIFN α 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 IFN α . IFN α can act on cccDNA and is even regarded as one of the most promising drugs in the elimination of HBV cccDNA.⁸⁵ Additionally, IFN α 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 IFN α 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 detect 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 i2000 _{SR}	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 in-

terpretation 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.

References

- [1] Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* 2018;3(6):383–403. doi:10.1016/S2468-1253(18)30056-6, PMID:29599078.
- [2] WHO global health sector strategy on viral hepatitis 2016–2021. 2016. Available from: https://www.emcdda.europa.eu/drugs-library/who-global-health-sector-strategy-viral-hepatitis-2016-2021_en.
- [3] Regions Dashboard - CDA Foundation. 2020. Available from: <https://cda-found.org/polaris-regions-dashboard/>.
- [4] Schinazi RF, Ehteshami M, Bassit L, Asselah T. Towards HBV curative therapies. *Liver Int* 2018;38(Suppl 1):102–114. doi:10.1111/liv.13656, PMID:29427479.
- [5] Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, *et al*. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018;67(4):1560–1599. doi:10.1002/hep.29800, PMID:29405329.
- [6] European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;67(2):370–398. doi:10.1016/j.jhep.2017.03.021, PMID:28427875.
- [7] Kim SU, Seo YS, Lee HA, Kim MN, Lee EJ, Shin HJ, *et al*. Hepatocellular Carcinoma Risk Steadily Persists over Time Despite Long-Term Antiviral Therapy for Hepatitis B: A Multicenter Study. *Cancer Epidemiol Biomarkers Prev* 2020;29(4):832–837. doi:10.1158/1055-9965.EPI-19-0614, PMID:31988073.
- [8] Ning Q, Han M, Sun Y, Jiang J, Tan D, Hou J, *et al*. Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). *J Hepatol* 2014;61(4):777–784. doi:10.1016/j.jhep.2014.05.044, PMID:24915612.
- [9] Lu J, Zhang S, Liu Y, Du X, Ren S, Zhang H, *et al*. Effect of Peg-interferon α -2a combined with Adefovir in HBV postpartum women with normal levels of ALT and high levels of HBV DNA. *Liver Int* 2015;35(6):1692–1699. doi:10.1111/liv.12753, PMID:25438657.
- [10] Brouwer WP, Xie Q, Sonneveld MJ, Zhang N, Zhang Q, Tabak F, *et al*. Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: A multicenter randomized trial (ARES study). *Hepatology* 2015;61(5):1512–1522. doi:10.1002/hep.27586, PMID:25348661.
- [11] Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical Association, Hou JL, Lai W, Chinese Society of Hepatology, Chinese Medical Association, Chinese Society of Infectious Diseases, Chinese Medical Association. The guideline of prevention and treatment for chronic hepatitis B: a 2015 update. *Zhonghua Gan Zang Bing Za Zhi* 2015;23(12):888–905. doi:10.3760/cma.j.issn.1007-3418.2015.12.002, PMID:26739464.
- [12] Zeisel MB, Lucifora J, Mason WS, Sureau C, Beck J, Levrero M, *et al*. Towards an HBV cure: state-of-the-art and unresolved questions—report of the ANRS workshop on HBV cure. *Gut* 2015;64(8):1314–1326. doi:10.1136/gutjnl-2014-308943, PMID:25670809.
- [13] Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: From discovery to regulatory approval. *J Hepatol* 2017;67(4):847–861. doi:10.1016/j.jhep.2017.05.008, PMID:28778687.
- [14] Cornberg M, Lok AS, Terrault NA, Zoulim F. 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference. *J Hepatol* 2020;72(3):539–557. doi:10.1016/j.jhep.2019.11.003, PMID:31730789.
- [15] Ning Q, Wu D, Wang GQ, Ren H, Gao ZL, Hu P, *et al*. Roadmap to functional cure of chronic hepatitis B: An expert consensus. *J Viral Hepat* 2019;26(10):1146–1155. doi:10.1111/jvh.13126, PMID:31087479.
- [16] Global progress report on HIV vHasti. 2021. Available from: <https://www.who.int/publications/i/item/9789240027077>.
- [17] Kim SW, Yoon JS, Lee M, Cho Y. Toward a complete cure for chronic hepatitis B: Novel therapeutic targets for hepatitis B virus. *Clin Mol Hepatol* 2022;28(1):17–30. doi:10.3350/cmh.2021.0093, PMID:34281294.
- [18] Fung S, Choi HS, Gehring A, Janssen HLA. Getting to HBV cure: The promising paths forward. *Hepatology* 2022;76(1):233–250. doi:10.1002/hep.32314, PMID:34990029.
- [19] Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. Control of cccDNA function in hepatitis B virus infection. *J Hepatol* 2009;51(3):581–592. doi:10.1016/j.jhep.2009.05.022, PMID:19616338.
- [20] Tu T, Budzinska MA, Shackel NA, Urban S. HBV DNA Integration: Molecular Mechanisms and Clinical Implications. *Viruses* 2017;9(4):E75. doi:10.3390/v9040075, PMID:28394272.
- [21] Ishii T, Tamura A, Shibata T, Kuroda K, Kanda T, Sugiyama M, *et al*. Analysis of HBV Genomes Integrated into the Genomes of Human Hepatoma PLC/PRF/5 Cells by HBV Sequence Capture-Based Next-Generation Sequencing. *Genes (Basel)* 2020;11(6):E661. doi:10.3390/genes11060661, PMID:32570699.
- [22] Vaillant A. HBsAg, Subviral Particles, and Their Clearance in Establishing a Functional Cure of Chronic Hepatitis B Virus Infection. *ACS Infect Dis* 2021;

- 7(6):1351–1368. doi:10.1021/acsinfecdis.0c00638, PMID:33302622.
- [23] Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, *et al*. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med* 2017;9(409):eaan0241. doi:10.1126/scitranslmed.aan0241, PMID:28954926.
- [24] Meier MA, Calabrese D, Suslov A, Terracciano LM, Heim MH, Wieland S. Ubiquitous expression of HBsAg from integrated HBV DNA in patients with low viral load. *J Hepatol* 2021;75(4):840–847. doi:10.1016/j.jhep.2021.04.051, PMID:34004216.
- [25] Lazar C, Uta M, Petrescu SM, Branza-Nichita N. Novel function of the endoplasmic reticulum degradation-enhancing α -mannosidase-like proteins in the human hepatitis B virus life cycle, mediated by the middle envelope protein. *Cell Microbiol* 2017;19(2):e12653. doi:10.1111/cmi.12653, PMID:27490136.
- [26] Lin Y, Wu C, Wang X, Kemper T, Squire A, Gunzer M, *et al*. Hepatitis B virus is degraded by autophagosomal-lysosomal fusion mediated by Rab7 and related components. *Protein Cell* 2019;10(1):60–66. doi:10.1007/s13238-018-0555-2, PMID:29876903.
- [27] Zhou K, Contag C, Whitaker E, Terrault N. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analysis. *Lancet Gastroenterol Hepatol* 2019;4(3):227–238. doi:10.1016/s2468-1253(18)30308-x, PMID:30679109.
- [28] 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(5):1187–1192. doi:10.1002/hep.21612, PMID:17465003.
- [29] Terrault NA, Wahed AS, Feld JJ, Cooper SL, Ghany MG, Lisker-Melman M, *et al*. Incidence and prediction of HBsAg seroclearance in a prospective multi-ethnic HBeAg-negative chronic hepatitis B cohort. *Hepatology* 2022;75(3):709–723. doi:10.1002/hep.32231, PMID:34743343.
- [30] Hsu YC, Yeh ML, Wong GL, Chen CH, Peng CY, Buti M, *et al*. Incidences and Determinants of Functional Cure During Entecavir or Tenofovir Disoproxil Fumarate for Chronic Hepatitis B. *J Infect Dis* 2021;224(11):1890–1899. doi:10.1093/infdis/jiab241, PMID:33999179.
- [31] Kuo YH, Wang JH, Hung CH, Lu SN, Hu TH, Chen CH. Combining end-of-treatment HBsAg and baseline hepatitis B core-related antigen reduce HBV relapse rate after tenofovir cessation. *Hepatol Int* 2021;15(2):301–309. doi:10.1007/s12072-021-10159-w, PMID:33665773.
- [32] Broquetas T, Hernandez JJ, Garcia-Retortillo M, Cañillas L, Puigvehí M, Cañete N, *et al*. On-therapy HBsAg kinetics can predict HBsAg loss after nucleos(t)ide analogues interruption in HBeAg-negative patients. The cup is half full and half empty. *Dig Liver Dis* 2022;54(8):1044–1051. doi:10.1016/j.dld.2021.12.017, PMID:35063365.
- [33] Sonneveld MJ, Chiu SM, Park JY, Brakenhoff SM, Kaewdech A, Seto WK, *et al*. Probability of HBsAg loss after nucleos(t)ide analogue withdrawal depends on HBV genotype and viral antigen levels. *J Hepatol* 2022;76(5):1042–1050. doi:10.1016/j.jhep.2022.01.007, PMID:35092743.
- [34] Kao JH, Jeng WJ, Ning Q, Su TH, Tseng TC, Ueno Y, *et al*. APASL guidance on stopping nucleos(t)ide analogues in chronic hepatitis B patients. *Hepatol Int* 2021;15(4):833–851. doi:10.1007/s12072-021-10223-5, PMID:34297329.
- [35] Hu C, Song Y, Tang C, Li M, Liu J, Liu J, *et al*. Effect of Pegylated Interferon Plus Tenofovir Combination on Higher Hepatitis B Surface Antigen Loss in Treatment-naïve Patients With Hepatitis B e Antigen-positive Chronic Hepatitis B: A Real-world Experience. *Clin Ther* 2021;43(3):572–581.e3. doi:10.1016/j.clinthera.2020.12.022, PMID:33516527.
- [36] Liu J, Wang T, Zhang W, Cheng Y, He Q, Wang FS. Effect of combination treatment based on interferon and nucleos(t)ide analogues on functional cure of chronic hepatitis B: a systematic review and meta-analysis. *Hepatol Int* 2020;14(6):958–972. doi:10.1007/s12072-020-10099-x, PMID:33185803.
- [37] Song A, Lin X, Lu J, Ren S, Cao Z, Zheng S, *et al*. Pegylated Interferon Treatment for the Effective Clearance of Hepatitis B Surface Antigen in Inactive HBsAg Carriers: A Meta-Analysis. *Front Immunol* 2021;12:779347. doi:10.3389/fimmu.2021.779347, PMID:34804072.
- [38] Park JJ, Wong DK, Wahed AS, Lee WM, Feld JJ, Terrault N, *et al*. Hepatitis B Virus-Specific and Global T-Cell Dysfunction in Chronic Hepatitis B. *Gastroenterology* 2016;150(3):684–695.e5. doi:10.1053/j.gastro.2015.11.050, PMID:26684441.
- [39] Burton AR, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, Swadling L, *et al*. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. *J Clin Invest* 2018;128(10):4588–4603. doi:10.1172/jci.121960, PMID:30091725.
- [40] Wang Q, Sachse P, Semmo M, Lokhande M, Montani M, Dufour JF, *et al*. T- and B-cell responses and previous exposure to hepatitis B virus in ‘anti-HBc alone’ patients. *J Viral Hepat* 2015;22(12):1068–1078. doi:10.1111/jvh.12428, PMID:26075501.
- [41] Cannizzo ES, Tincati C, Binda F, Ronzi P, Cazzaniga FA, Antinori S, *et al*. Unconventional T cells in chronic hepatitis B patients on long-term suppressive therapy with tenofovir followed by a Peg-IFN add-on strategy: A randomized study. *J Viral Hepat* 2018;25(4):381–390. doi:10.1111/jvh.12820, PMID:29091327.
- [42] Fanning GC, Zoulim F, Hou J, Bertoletti A. Therapeutic strategies for hepatitis B virus infection: towards a cure. *Nat Rev Drug Discov* 2019;18(11):827–844. doi:10.1038/s41573-019-0037-0, PMID:31455905.
- [43] Boni C, Laccabue D, Lampertico P, Giuberti T, Viganò M, Schivazappa S, *et al*. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. *Gastroenterology* 2012;143(4):963–73.e9. doi:10.1053/j.gastro.2012.07.014, PMID:22796241.
- [44] Fumagalli V, Di Lucia P, Venzin V, Bono EB, Jordan R, Frey CR, *et al*. Serum HBsAg clearance has minimal impact on CD8+ T cell responses in mouse models of HBV infection. *J Exp Med* 2020;217(11):e20200298. doi:10.1084/jem.20200298, PMID:32761167.
- [45] Xiong S, Zhu D, Liang B, Li M, Pan W, He J, *et al*. Longitudinal characterization of phenotypic profile of T cells in chronic hepatitis B identifies immune markers associated with HBsAg loss. *EBioMedicine* 2021;69:103464. doi:10.1016/j.ebiom.2021.103464, PMID:34233260.
- [46] Vanwolleghem T, Groothuisink ZMA, Kreeft K, Hung M, Novikov N, Boonstra A. Hepatitis B core-specific memory B cell responses associate with clinical parameters in patients with chronic HBV. *J Hepatol* 2020;73(1):52–61. doi:10.1016/j.jhep.2020.01.024, PMID:32061650.
- [47] Matsushita T, Kobayashi T, Mizumaki K, Kano M, Sawada T, Tennichi M, *et al*. BAFF inhibition attenuates fibrosis in scleroderma by modulating the regulatory and effector B cell balance. *Sci Adv* 2018;4(7):eaas9944. doi:10.1126/sciadv.aas9944, PMID:30009261.
- [48] Mauri C, Menon M. Human regulatory B cells in health and disease: therapeutic potential. *J Clin Invest* 2017;127(3):772–779. doi:10.1172/jci85113, PMID:28248202.
- [49] Gu S, Liu Z, Lin L, Zhong S, Ma Y, Li X, *et al*. Identification and Mapping of HBsAg Loss-Related B-Cell Linear Epitopes in Chronic HBV Patients by Peptide Array. *Front Immunol* 2021;12:767000. doi:10.3389/fimmu.2021.767000, PMID:34721439.
- [50] Cao Z, Meng S, Zheng Y, Wang J, Wang R, Chen X. B cells were related to HBsAg seroconversion in inactive HBsAg carriers following peginterferon therapy. *PLoS One* 2020;15(12):e0242559. doi:10.1371/journal.pone.0242559, PMID:33264330.
- [51] Yip TC, Wong VW, Tse YK, Liang LY, Hui VW, Zhang X, *et al*. Similarly low risk of hepatocellular carcinoma after either spontaneous or nucleos(t)ide analogue-induced hepatitis B surface antigen loss. *Aliment Pharmacol Ther* 2021;53(2):321–331. doi:10.1111/apt.16174, PMID:33222272.
- [52] Park Y, Lee JH, Sinn DH, Park JY, Kim MA, Kim YJ, *et al*. Risk and Risk Score Performance of Hepatocellular Carcinoma Development in Patients With Hepatitis B Surface Antigen Seroclearance. *Clin Transl Gastroenterol* 2021;12(1):e00290. doi:10.14309/ctg.000000000000290, PMID:33433118.
- [53] Choi J, Yoo S, Lim YS. Comparison of Long-Term Clinical Outcomes Between Spontaneous and Therapy-Induced HBsAg Seroclearance. *Hepatology* 2021;73(6):2155–2166. doi:10.1002/hep.31610, PMID:33131063.
- [54] Song C, Zhu J, Ge Z, Yu C, Tian T, Wang H, *et al*. Spontaneous Seroclearance of Hepatitis B Surface Antigen and Risk of Hepatocellular Carcinoma. *Clin Gastroenterol Hepatol* 2019;17(6):1204–1206. doi:10.1016/j.cgh.2018.08.019, PMID:30114488.
- [55] Zhu L, Zhai X, Wang Q, Jiang J, Peng H, Song C, *et al*. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance and seroconversion in hepatitis B e antigen-negative chronic infection patients: A population-based prospective cohort. *J Viral Hepat* 2018;25(12):1588–1598. doi:10.1111/jvh.12978, PMID:30112835.
- [56] Chen YC, Jeng WJ, Chien RN, Chu CM, Liaw YF. Clinical outcomes after spontaneous and nucleos(t)ide analogue-treated HBsAg seroclearance in chronic HBV infection. *Aliment Pharmacol Ther* 2016;43(12):1311–1318. doi:10.1111/apt.13630, PMID:27072504.
- [57] Lim TH, Gane E, Moyes C, Borman B, Cunningham C. HBsAg loss in a New Zealand community study with 28-year follow-up: rates, predictors and long-term outcomes. *Hepatol Int* 2016;10(5):829–837. doi:10.1007/s12072-016-9709-6, PMID:26957439.
- [58] Ari A, Çalik Ş, Tosun S, Özsu Yilmaz S. A persistently low HBV DNA level is a predictor of spontaneous HBsAg clearance in patients with chronic hepatitis B. *Turk J Med Sci* 2016;46(1):48–52. doi:10.3906/sag-1411-156, PMID:27511332.
- [59] Kim MA, Kim SU, Sinn DH, Jang JW, Lim YS, Ahn SH, *et al*. Discontinuation of nucleos(t)ide analogues is not associated with a higher risk of HBsAg seroreversion after antiviral-induced HBsAg seroclearance: a nationwide multicentre study. *Gut* 2020;69(12):2214–2222. doi:10.1136/gutjnl-2019-320015, PMID:32209606.
- [60] Yip TC, Wong GL, Chan HL, Tse YK, Lam KL, Lui GC, *et al*. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. *J Hepatol* 2019;70(3):361–370. doi:10.1016/j.jhep.2018.10.014, PMID:30367899.
- [61] Suárez E, Buti M, Rodríguez M, Prieto M, Pascasio-Acevedo JM, Casanovas T, *et al*. Hepatitis B surface antigen loss after discontinuing nucleos(t)ide analogue for treatment of chronic hepatitis B patients is persistent in White patients. *Eur J Gastroenterol Hepatol* 2019;31(2):267–271. doi:10.1097/meg.0000000000001289, PMID:30576297.
- [62] Sun Y, Zhang Y, Xu Y, Shu M, Bonroy K, Qiu H, *et al*. Real-world study on clinical outcomes of nucleos(t)ide analogues antiviral therapy in patients with chronic hepatitis B. *Epidemiol Infect* 2019;147:e193. doi:10.1017/S0950268819000815, PMID:31364558.
- [63] Chi H, Wong D, Peng J, Cao J, Van Hees S, Vanwolleghem T, *et al*. Durability of Response After Hepatitis B Surface Antigen Seroclearance During Nucleos(t)ide Analogue Treatment in a Multiethnic Cohort of Chronic Hepatitis B Patients: Results After Treatment Cessation. *Clin Infect Dis* 2017;65(4):680–683. doi:10.1093/cid/cix353, PMID:28575292.
- [64] Li M, Sun F, Bi X, Lin Y, Yang L, Lu Y, *et al*. Consolidation treatment needed for sustained HBsAg-negative response induced by interferon-alpha in HBeAg positive chronic hepatitis B patients. *Virology* 2022;37(3):390–397. doi:10.1016/j.virus.2022.03.001, PMID:35257963.
- [65] Chen J, Qi M, Fan XG, Hu XW, Liao CJ, Long LY, *et al*. Efficacy of Peginterferon alpha-2b in Nucleoside Analogue Experienced Patients with Negative HBeAg and Low HBsAg: A Non-Randomized Clinical Trial. *Infect Dis Ther* 2021;10(4):2259–2270. doi:10.1007/s40121-021-00497-5, PMID:34309813.
- [66] Wu F, Lu R, Liu Y, Wang Y, Tian Y, Li Y, *et al*. Efficacy and safety of peginterferon alpha monotherapy in Chinese inactive chronic hepatitis B virus carriers. *Liver Int* 2021;41(9):2032–2045. doi:10.1111/liv.14897, PMID:33896094.

- [67] Pan CQ, Li MH, Yi W, Zhang L, Lu Y, Hao HX, *et al*. Outcome of Chinese patients with hepatitis B at 96 weeks after functional cure with IFN versus combination regimens. *Liver Int* 2021;41(7):1498–1508. doi:10.1111/lu.14801, PMID:33486874.
- [68] Wu Y, Liu Y, Lu J, Cao Z, Jin Y, Ma L, *et al*. Durability of Interferon-induced Hepatitis B Surface Antigen Seroclearance. *Clin Gastroenterol Hepatol* 2020;18(2):514–516.e2. doi:10.1016/j.cgh.2019.04.020, PMID:30981007.
- [69] Choi HSJ, van Campenhout MJH, van Vuuren AJ, Krassenburg LAP, Sonneveld MJ, de Knegt RJ, *et al*. Ultra-Long-term Follow-up of Interferon Alfa Treatment for HBeAg-Positive Chronic Hepatitis B Virus Infection. *Clin Gastroenterol Hepatol* 2021;19(9):1933–1940.e1. doi:10.1016/j.cgh.2020.09.004, PMID:32890755.
- [70] Li MH, Yi W, Zhang L, Lu Y, Lu HH, Shen G, *et al*. Predictors of sustained functional cure in hepatitis B envelope antigen-negative patients achieving hepatitis B surface antigen seroclearance with interferon-alpha-based therapy. *J Viral Hepat* 2019;26(Suppl 1):32–41. doi:10.1111/jvh.13151, PMID:31380582.
- [71] Lok AS, Zoulim F, Dusheiko G, Chan HLY, Buti M, Ghany MG, *et al*. Durability of Hepatitis B Surface Antigen Loss With Nucleotide Analogue and Peginterferon Therapy in Patients With Chronic Hepatitis B. *Hepatology* 2020;71(4):1126–1133. doi:10.1002/hep4.1436, PMID:31909352.
- [72] Liu Y, Li H, Yan X, Wei J. Long-term efficacy and safety of peginterferon in the treatment of children with HBeAg-positive chronic hepatitis B. *J Viral Hepat* 2019;26(Suppl 1):69–76. doi:10.1111/jvh.13154, PMID:31380585.
- [73] Yip TC, Wong GL, Wong VW, Tse YK, Lui GC, Lam KL, *et al*. Durability of hepatitis B surface antigen seroclearance in untreated and nucleos(t)ide analogue-treated patients. *J Hepatol* 2018;68(1):63–72. doi:10.1016/j.jhep.2017.09.018, PMID:28989093.
- [74] Alawad AS, Auh S, Suarez D, Ghany MG. Durability of Spontaneous and Treatment-Related Loss of Hepatitis B s Antigen. *Clin Gastroenterol Hepatol* 2020;18(3):700–709.e3. doi:10.1016/j.cgh.2019.07.018, PMID:31323381.
- [75] Song A, Wang X, Lu J, Jin Y, Ma L, Hu Z, *et al*. Durability of hepatitis B surface antigen seroclearance and subsequent risk for hepatocellular carcinoma: A meta-analysis. *J Viral Hepat* 2021;28(4):601–612. doi:10.1111/jvh.13471, PMID:33455067.
- [76] Yip TC, Chan HL, Wong VW, Tse YK, Lam KL, Wong GL. Impact of age and gender on risk of hepatocellular carcinoma after hepatitis B surface antigen seroclearance. *J Hepatol* 2017;67(5):902–908. doi:10.1016/j.jhep.2017.06.019, PMID:28652084.
- [77] Anderson RT, Choi HSJ, Lenz O, Peters MG, Janssen HLA, Mishra P, *et al*. Association Between Seroclearance of Hepatitis B Surface Antigen and Long-term Clinical Outcomes of Patients With Chronic Hepatitis B Virus Infection: Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2021;19(3):463–472. doi:10.1016/j.cgh.2020.05.041, PMID:32473348.
- [78] Vittal A, Sharma D, Hu A, Majeed NA, Terry N, Auh S, *et al*. Systematic review with meta-analysis: the impact of functional cure on clinical outcomes in patients with chronic hepatitis B. *Aliment Pharmacol Ther* 2022;55(1):8–25. doi:10.1111/apt.16659, PMID:34850415.
- [79] 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(4):700–710. doi:10.1016/j.jhep.2016.05.029, PMID:27245431.
- [80] 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* 2021;73(6):2167–2179. doi:10.1002/hep.31616, PMID:33159329.
- [81] Ghany MG, King WC, Lisker-Melman M, Lok ASF, Terrault N, Janssen HLA, *et al*. Comparison of HBV RNA and Hepatitis B Core Related Antigen With Conventional HBV Markers Among Untreated Adults With Chronic Hepatitis B in North America. *Hepatology* 2021;74(5):2395–2409. doi:10.1002/hep.32018, PMID:34133774.
- [82] Wang X, Chi X, Wu R, Xu H, Gao X, Yu L, *et al*. Serum HBV RNA correlated with intrahepatic cccDNA more strongly than other HBV markers during peg-interferon treatment. *Virology* 2021;18(1):4. doi:10.1186/s12985-020-01471-2, PMID:33407619.
- [83] Testoni B, Lebossé 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(4):615–625. doi:10.1016/j.jhep.2018.11.030, PMID:30529504.
- [84] Lim SG, Phyo WW, Ling JZJ, Cloherty G, Butler EK, Kuhns MC, *et al*. Comparative biomarkers for HBsAg loss with antiviral therapy shows dominant influence of quantitative HBsAg (qHBsAg). *Aliment Pharmacol Ther* 2021;53(1):172–182. doi:10.1111/apt.16149, PMID:33159496.
- [85] Wang G, Guan J, Khan NU, Li G, Shao J, Zhou Q, *et al*. Potential capacity of interferon- α to eliminate covalently closed circular DNA (cccDNA) in hepatocytes infected with hepatitis B virus. *Gut Pathog* 2021;13(1):22. doi:10.1186/s13099-021-00421-9, PMID:33845868.
- [86] Cheng J, Zhao Q, Zhou Y, Tang L, Sheraz M, Chang J, *et al*. Interferon Alpha Induces Multiple Cellular Proteins That Coordinately Suppress Hepadnaviral Covalently Closed Circular DNA Transcription. *J Virol* 2020;94(17):e00442–20. doi:10.1128/jvi.00442-20, PMID:32581092.
- [87] Yang Y, Zhao X, Wang Z, Shu W, Li L, Li Y, *et al*. Nuclear Sensor Interferon-Inducible Protein 16 Inhibits the Function of Hepatitis B Virus Covalently Closed Circular DNA by Integrating Innate Immune Activation and Epigenetic Suppression. *Hepatology* 2020;71(4):1154–1169. doi:10.1002/hep.30897, PMID:31402464.
- [88] Chen Z, Eggerman TL, Bocharov AV, Baranova IN, Vishnyakova TG, Kurlander R, *et al*. Heat shock proteins stimulate APOBEC-3-mediated cytidine deamination in the hepatitis B virus. *J Biol Chem* 2017;292(32):13459–13479. doi:10.1074/jbc.M116.760637, PMID:28637869.
- [89] Kuang XJ, Jia RR, Huo RR, Yu JJ, Wang JJ, Xiang BD, *et al*. Systematic review of risk factors of hepatocellular carcinoma after hepatitis B surface antigen seroclearance. *J Viral Hepat* 2018;25(9):1026–1037. doi:10.1111/jvh.12905, PMID:29624821.
- [90] Kaur SP, Talat A, Karimi-Sari H, Grees A, Chen HW, Lau DTY, *et al*. Hepatocellular Carcinoma in Hepatitis B Virus-Infected Patients and the Role of Hepatitis B Surface Antigen (HBsAg). *J Clin Med* 2022;11(4):1126. doi:10.3390/jcm11041126, PMID:35207397.
- [91] Wong DK, Cheng SCY, Mak LL, To EW, Lo RC, Cheung TT, *et al*. Among Patients with Undetectable Hepatitis B Surface Antigen and Hepatocellular Carcinoma, a High Proportion Has Integration of HBV DNA into Hepatocyte DNA and No Cirrhosis. *Clin Gastroenterol Hepatol* 2020;18(2):449–456. doi:10.1016/j.cgh.2019.06.029, PMID:31252193.
- [92] Yang H, Bae SH, Nam H, Lee HL, Lee SW, Yoo SH, *et al*. A risk prediction model for hepatocellular carcinoma after hepatitis B surface antigen seroclearance. *J Hepatol* 2022;77(3):632–641. doi:10.1016/j.jhep.2022.03.032, PMID:35398462.
- [93] Mak LY, Seto WK, Fung J, Yuen MF. Use of HBsAg quantification in the natural history and treatment of chronic hepatitis B. *Hepatology* 2020;71(1):35–46. doi:10.1007/s12072-019-09998-5, PMID:31745711.
- [94] Ozeki I, Nakajima T, Suii H, Tatsumi R, Yamaguchi M, Kimura M, *et al*. Analysis of hepatitis B surface antigen (HBsAg) using high-sensitivity HBsAg assays in hepatitis B virus carriers in whom HBsAg seroclearance was confirmed by conventional assays. *Hepatology* 2018;48(3):E263–E274. doi:10.1111/hepr.12979, PMID:28884879.
- [95] Wong DK, Chen C, Mak LY, Fung J, Seto WK, Yuen MF. Detection of the Hepatitis B Surface Antigen in Patients with Occult Hepatitis B by Use of an Assay with Enhanced Sensitivity. *J Clin Microbiol* 2022;60(2):e0220421. doi:10.1128/jcm.02204-21, PMID:34936478.
- [96] Shinkai N, Matsuura K, Sugauchi F, Watanabe T, Murakami S, Iio E, *et al*. Application of a newly developed high-sensitivity HBsAg chemiluminescent enzyme immunoassay for hepatitis B patients with HBsAg seroclearance. *J Clin Microbiol* 2013;51(11):3484–3491. doi:10.1128/jcm.00726-13, PMID:23946517.
- [97] Lou S, Taylor R, Pearce S, Kuhns M, Leary T. An ultra-sensitive Abbott ARCHITECT[®] assay for the detection of hepatitis B virus surface antigen (HBsAg). *J Clin Virol* 2018;105:18–25. doi:10.1016/j.jcv.2018.05.009, PMID:29843004.
- [98] Takeda K, Maruki M, Yamagaito T, Muramatsu M, Sakai Y, Tobimatsu H, *et al*. Highly sensitive detection of hepatitis B virus surface antigen by use of a semiautomated immune complex transfer chemiluminescence enzyme immunoassay. *J Clin Microbiol* 2013;51(7):2238–2244. doi:10.1128/jcm.00324-13, PMID:23658266.
- [99] Bazinet M, Anderson M, Pântea V, Placinta G, Moscalu I, Ceborescu V, *et al*. Analysis of HBsAg Immunocomplexes and cccDNA Activity During and Persisting After NAP-Based Therapy. *Hepatology* 2021;73(11):1873–1887. doi:10.1002/hep4.1767, PMID:34558823.
- [100] Suzuki F, Hosaka T, Imaizumi M, Kobayashi M, Ohue C, Suzuki Y, *et al*. Potential of ultra-highly sensitive immunoassays for hepatitis B surface and core-related antigens in patients with or without development of hepatocellular carcinoma after hepatitis B surface antigen seroclearance. *Hepatology* 2021;73(4):426–435. doi:10.1111/hepr.13602, PMID:33270344.
- [101] Revill PA, Chisari FV, Block JM, Dandri M, Gehring AJ, Guo H, *et al*. A global scientific strategy to cure hepatitis B. *Lancet Gastroenterol Hepatol* 2019;4(7):545–558. doi:10.1016/s2468-1253(19)30119-0, PMID:30981686.
- [102] Tsounis EP, Tourkochristou E, Mouzaki A, Triantos C. Toward a new era of hepatitis B virus therapeutics: The pursuit of a functional cure. *World J Gastroenterol* 2021;27(21):2727–2757. doi:10.3748/wjg.v27.i21.2727, PMID:34135551.
- [103] Hosseini SY, Sanaei N, Fattahi MR, Malek-Hosseini SA, Sarvari J. Association of HBsAg mutation patterns with hepatitis B infection outcome: Asymptomatic carriers versus HCC/cirrhotic patients. *Ann Hepatol* 2019;18(4):640–645. doi:10.1016/j.aohp.2018.12.006, PMID:31105017.