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HBsAg Seroclearance and Seroconversion: Incidence and Patient Profiles at the Centre Hospitalier Universitaire Yalgado Ouedraogo

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ABSTRACT

Introduction: *HBsAg seroclearance is a rare event in the natural history of chronic hepatitis B virus (HBV) infection. Data on HBsAg seroclearance and seroconversion are scarce in Burkina Faso. This study assessed the incidence and determined the profile of patients with HBsAg clearance and conversion at the Yalgado Ouédraogo University Hospital.*

Patients and Methods: *This was a retrospective descriptive cross-sectional study conducted from 1 January 2006 to 31 July 2023, i.e. 17 years and 6 months, at the Yalgado Ouédraogo University Hospital. Patients were included who were chronic HBV carriers who had lost HBsAg spontaneously or after the introduction of nucleoside analogue therapy, had a viral load of HBV on admission and were negative for antibodies to hepatitis C virus (HCV), hepatitis D virus (HDV) and human immunodeficiency virus (HIV). HBsAg seroclearance was defined as serum HBsAg negativity, checked at least once, and seroconversion as the disappearance of serum HBsAg and the appearance of anti-HBs antibodies > 10 IU/ml.*

Results: *Of the 1507 patients in our cohort, 27 lost their HBsAg during the study period. The overall incidence of HBsAg loss was 1.8% and 55.5% of patients seroconverted to HBsAg within 12 months of HBsAg loss. The mean age of patients on admission was 31.3 ± 8.9 years, with a sex ratio of 2. The mean age of patients at the time of HBsAg loss was 39.9 ± 10.2 years. The mean duration of HBsAg carriage was 9 ± 5 years. All patients were inactive carriers. Untreated patients represented 74.1% of the cohort. Quantitative HBsAg was less than 100 IU/mL in 81.8% of patients, and 88% of patients had undetectable DNA before loss of HBsAg.*

Conclusion: *Although HBsAg seroclearance is associated with a good long-term prognosis, it remains a rare event. Patients at risk of developing hepatocellular carcinoma (HCC) after HBs seroclearance should be monitored at intervals that can be determined on the basis of further studies.*

Keywords
HBsAg, HBs seroclearance, HBs seroconversion, Ouagadougou.

Introduction
HBV infection is one of the major public health problems, despite the existence of a safe and highly effective vaccine [1]. In 2019, 296 million people were living chronically with the virus worldwide, with 1.1 million deaths by 2022, mainly attributable to liver cancer and cirrhosis [2]. In the African region, hepatitis B affects between 5% and 8% of the population, mainly in West and Central Africa [3]. Burkina Faso is a highly endemic country, with a national prevalence rate of 9.1% [4]. Loss of hepatitis B surface antigen (HBsAg) and the development of hepatitis B surface antibodies (HBsAb) is the therapeutic goal in patients chronically infected with the hepatitis B virus. Studies have shown that those who achieve HBsAg loss have significantly improved clinical outcomes, such as better overall survival, reduced risk of hepatocellular carcinoma (HCC) and liver-related mortality [5]. However, HBsAg seroclearance is a rare event in the natural history of chronic hepatitis B infection [6]. It may occur spontaneously or following treatment. The annual incidence of spontaneous HBsAg seroclearance varies between 0.15% and 3.02% depending on the cohort studied [7]. The annual rate of HBs seroclearance after treatment with nucleoside analogues has been estimated at 0.3% in one study [8]. For the management of patients with chronic hepatitis B, it seems important to identify the profile of patients achieving HBsAg seroclearance and seroconversion. Data on HBsAg seroclearance and seroconversion are scarce in Burkina Faso. The aim of this study was therefore to assess the incidence and determine the profile of patients with HBsAg clearance and conversion at the Yalgado Ouédraogo University Hospital.

Patients and Methods
This was a retrospective descriptive cross-sectional study which took place from 1 January 2006 to 31 July 2023, i.e. 17 years and 6 months, at the Yalgado Ouédraogo University Hospital. We proceeded to an exhaustive recruitment of all chronic HBV carriers of the cohort who had obtained HBs seroclearance. Patients were included in the study:

- Cchronic HBV carriers who have lost HBsAg spontaneously or after initiation of nucleosi(ti)dik analogue (NUC) therapy;
- with HBV DNA available on admission;
- without pre-existing hepatocellular carcinoma at the time of diagnosis;
- negative for anti-hepatitis C virus (anti-HCV), anti-hepatitis D virus (HDV) and anti-human immunodeficiency virus (HIV) antibodies.

Serological analyses (HBsAg and anti-HBsAb) were performed using the MiniVidasBioMérieux ELISA technique. Quantitative HBsAg was performed by Siemens Attelica IM/Roche Cobas 6000). HBV viral load (DNA) was determined by real-time PCR (Roche CobasTaqMan with a detection threshold of 10 IU/mL and a linearity range of 10 - 1,000,000,000 IU/mL (1-9 log).

Our data were collected using a data collection form designed to record socio-demographic aspects, lifestyle, history, clinical, paraclinical, therapeutic and evolutionary aspects.

The data were analysed using R software version 3.3. Descriptive analysis was performed for qualitative variables with absolute and relative frequencies, and for quantitative variables with the median or mean, depending on the distribution of the variables. A p value < 0.05 was considered statistically significant.

- Hepatitis B surface antigen seroclearance was defined as persistent serum HBsAg negativity monitored at least once.
- HBsAg seroconversion was defined as disappearance of serum HBsAg and development of anti-HBs >10 IU/ml.
- Due to variable detection limits, undetectable HBV DNA was defined as <10 IU/mL.

Confidentiality of the information collected and patient anonymity were respected in our study.

Results
Our cohort consisted of one thousand five hundred and seven (1507) outpatients. Twenty-seven (27) of these patients were HBsAg negative. Our study focused exclusively on these patients.

The overall incidence of HBsAg loss was 1.8%. The specific incidence of HBs seroclearance in treated and untreated patients was 1.4% and 2% respectively. Of the patients who lost HBsAg, 18 (66.6%) were tested for anti-HBsAb. Ten (10) developed anti-HBs antibodies (55.5%) in the year following the loss of HBsAg. The mean anti-HBs level was 105.1 IU/l with a minimum of 1 IU/l and a maximum of 1000 IU/l. It was 97.6 IU/l in treated patients and 109.8 IU/l in untreated patients.

The mean age of our patients on admission was 31.3 ± 8.9 years, with extremes of 18 and 57 years. The 20-30 and 30-40 age groups each accounted for 44.4%. The mean age of patients at the time of HBsAg loss was 39.9 ± 10.2 years, with a minimum of 23 years and a maximum of 64 years. The mean age of patients who seroconverted was 32.2 ± 5.13 years, with a minimum of 24 years and a maximum of 39 years. Our cohort was predominantly male, with 18 men (66.7%), giving a sex ratio of 2. The duration of HBsAg carriage varied from 2 to 21 years, with an average of 9 ± 5 years. The mean carriage time was shorter (7.1 ± 3.6 years) in treated patients than in untreated patients (9.8 ± 5.3 years), with a $p = 0.2$. Mean ALT was 76 with a minimum of 8 and a maximum of 960. Mean AST was 101.22 with a minimum of 13 and a maximum of 66.

All patients who lost HBsAg were HBeAg-negative and had positive anti-HBeAb. Untreated patients represented 74.1% of patients. Of the seven (07) patients treated, 03 (42.9%) and 04 (57.1%) were on Lamivudine and Tenofovir respectively. The mean duration of treatment was 3.6 ± 2.9 years, with a minimum of 0.4 years and a maximum of 7 years. At inclusion, HBsAg titration was performed in 17 patients. The mean titre was 685.7 IU/ml with extremes of 0.3 and 5787 IU/ml. Eight patients (47.1%) had an initial quantitative HBsAg of less than 100 IU/ml (Figure 1).

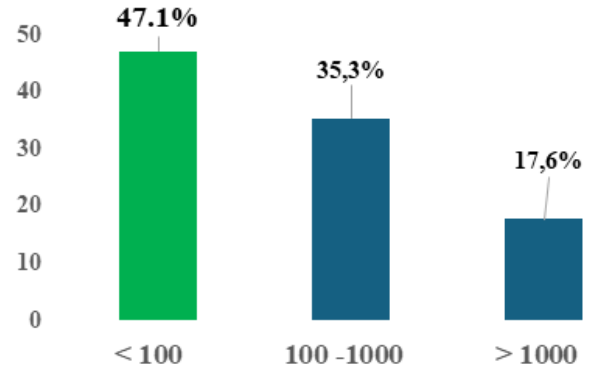

Figure 1: Distribution of patients according to quantitative HBsAg at inclusion.

Prior to HBs seroclearance, 11 patients had quantitative HBsAg. Of these, 81.8% had a quantitative HBsAg of less than 100 IU/ml (Figure 2).

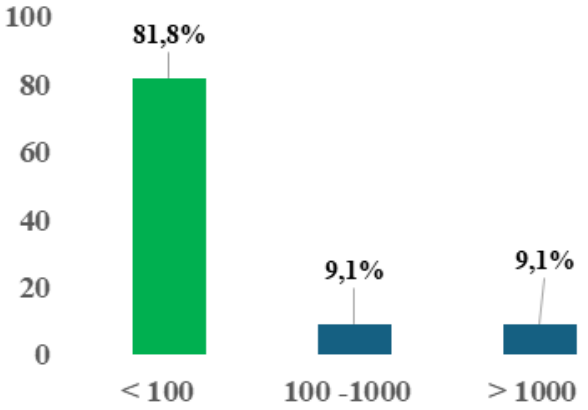

Figure 2: Distribution of patients according to last quantitative HBsAg.

- The kinetics of the evolution of quantitative HBsAg over time are decreasing (Figure 3).

- At inclusion, HBV DNA performed by all patients noted that 65.7% of them had a viral load between 20 and 2000 IU/l (Figure 4).

- Prior to HBs seroclearance, twenty-two (22) patients performed HBV DNA and 88% of them had undetectable DNA as shown in Figure 5. Twelve (12) patients or 44.4% of our cohort had consistently undetectable HBV DNA throughout follow-up.

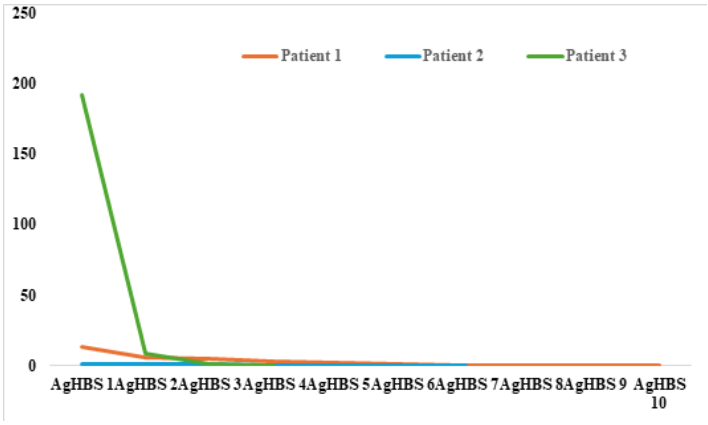

Figure 3: Kinetics of changes in quantitative HBsAg over time.

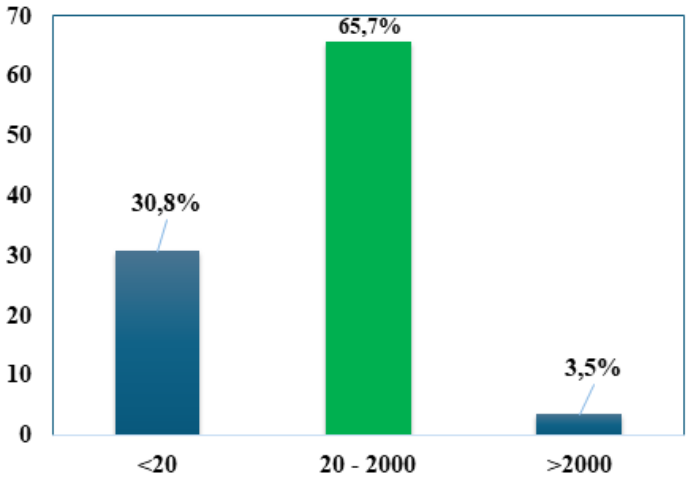

Figure 4: Distribution of patients according to HBV DNA at inclusion.

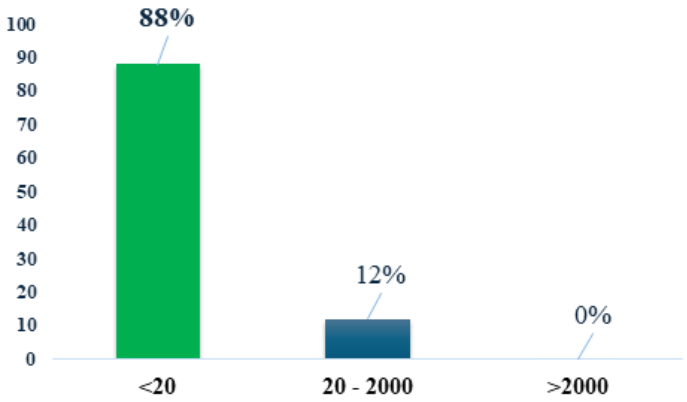

Figure 5: Distribution of patients according to latest HBV DNA.

	Results	Numbers (%)		Results	Numbers (%)
Abdominal ultrasound	Normal	**20 (74)**	Fibroscan	F0 – F1	**12 (85,7)**
	Hepatic steatosis	**05 (19)**		F1 – F2	**02 (14,3)**
	Cirrhosis	**02 (7)**			
Total		**27 (100)**	**Total**		**14 (100)**

Table 1: Distribution of patients according to abdominal ultrasound and fibroscan results.

Discussion

The limitations of this study were: the small size of our sample, which affected the power of the statistical tests, the high cost of the various follow-up examinations, entirely borne by the patients, which limited the regularity in the follow-up and performance of certain paraclinical explorations. Despite these limitations, the study is relevant, providing guidance on the incidence and profile of patients who achieved HBs seroclearance and seroconversion.

HBs seroclearance is a rare event and is associated with improvements in liver histology, decreased risk of hepatocellular carcinoma (HCC), and prolonged survival [5]. Its overall incidence was 1.8% in our cohort. It varied between 0.15 and 3.02% depending on the cohort studied [7]. In our cohort, patients were 31.3 ± 8.9 years old with a range of 18 to 57 years. The mean age at which patients lost HBsAg was 39.9 ± 10.2 years with a minimum of 23 years and a maximum of 64 years. The mean age of those who achieved seroconversion was 32.2 ± 5.1 years with a minimum of 24 years and a maximum of 39 years. The male gender was predominant with a sex ratio of 2. Chu et al. [9] suggested that HBsAg seroclearance was significantly more likely to occur in patients who were older at study entry and in those with prolonged remission of hepatitis. Additionally, HBsAg seroclearance was more likely to occur in men than women. In the series of Ferreira et al. [10], although the difference was not statistically significant, there was a predominance of men (55%) with a mean age of 46.0 ± 14.4 years at the time of HBsAg seroconversion. Liu J et al. [11] demonstrated a significantly higher probability of spontaneous HBsAg seroclearance in men. Tseng et al. [12] have indicated in their study that male gender, older age, were associated with higher rates of HBsAg loss. These results suggest that age at baseline is a major prognostic factor for HBsAg clearance. Older participants were more likely to clear HBsAg. This makes sense because older individuals are more likely to have remained in the inactive stage of HBV for a longer period of time than younger individuals. In our context, most of the included patients were expected to have a long duration of infection by the vertical mode of transmission. Infection at an early age being associated with a significantly lower risk of HBe seroconversion or HBs seroclearance [12-14]. The mean age at which patients lost HBsAg was 39.9 ± 10.2 years with a minimum of 23 years and a maximum of 64 years. He was from 47.8 ± 9.6 years with extremes of 41 and 53 years in the series of Chu et al. [9]. In the series by Ferreira et al. [10], the peak of spontaneous seroclearance of HBsAg was observed between 40 and 60 years of age, occurring after 40 years of age in 67.5% of cases. These data could be in agreement with the epidemiological characteristics of chronic HBV infection in Burkina Faso, according to which the prevalence rate of HBsAg in the general population decreased remarkably after 45 years [4].

The incidence of HBsAg loss in the untreated and treated was 2% and 1.4%, respectively. Jaroszewiczet et al. [15] showed that patients who received no treatment had similar HBsAg kinetics to those treated with NUC during the natural course of HBV infection. Habersetzer et al. [16] reported that there was no statistical link between antiviral therapy and loss of HBsAg. This suggests that therapy NUC does not have only a limited impact on the suppression of HBsAg [17]. However, the long-term NUC therapy has proven effective in preventing hepatic necro-inflammation and fibrosis and could also increase cumulative HBsAg seroclearance rates above 10% [18-20]. It can be concluded that combined NUC therapy with active host immune responses may increase the likelihood of obtaining HBs seroclearance in patients with chronic HBV carriers.

Our results regarding HBs seroconversion in the 12 months following loss of HBsAg (55.5%) are significantly higher than those in the literature. In fact, the data from the literature on this subject are heterogeneous but most often less than 20% in the 12 months following the loss of HBsAg [7]. Roushan [21], Yuen [14] and Chen [22] respectively reported 8.7%, 11.4% and 16.7% of HBs seroconverts within 12 months following HBsAg seroclearance. In their series, Arase [23] and Ferreira [10] reported an HBs seroconversion rate of 50.2% over five years and 72.5% over seven years, respectively i.e. an annual rate of around 10%. Advanced age at the time of HBs seroclearance would be associated with earlier development of anti-HBs [21]. Furthermore, unlike cases of acute hepatitis B, in which anti-HBs develops within 6 months following the start of infection, in people with chronic HBV infection, seroconversion to anti-HBs may develop several years after clearance of HBsAg.

In our study all patients were inactive carriers. In our series as in the literature this predominance of inactive carriers is reported. Indeed, Ferreira SC et al. [22] reported a higher proportion of inactive carriers (62.5%) among patients who obtained HBs seroclearance. In their series, Habersetzer et al. [16] reported an annual incidence rate of HBsAg clearance of 2.3% in inactive carriers, which was higher than that of the other groups. The impact of inactive carriage in HBs seroclearance is explained by the initial immunological control of HBV infection. Spontaneous control of HBV infection would therefore be essential for HBV elimination. Studies to further characterize the immunological and genetic determinants associated with HBsAg clearance could be of interest.

In our series, the mean titer of quantitative HBsAg was 685.7 IU/ml with extremes of 0.3 and 5787 IU/ml and 47.1% of patients had a quantitative HBsAg less than 100 IU/ml at admission. The last quantitative HBsAg performed before HBs seroclearance was less than 100 IU/ml in 81.8% of patients. Kim et al. [8] showed significantly lower levels and a greater drop in HBsAg titers in patients with HBsAg seroclearance than those without HBsAg seroclearance ($p<0.01$). Tseng et al. also indicated that lower HBsAg levels were associated with higher rates of HBsAg loss. Compared to patients with HBsAg levels greater than or equal to 1000 IU/mL, rates of HBsAg loss were higher in those with HBsAg levels 100 to 999, 10 to 99, and < 10 IU/mL [12].

More than half of our patients (65.7%) had DNA between 20 and 2000 IU/l and 30.8% had undetectable DNA on admission. Before HBs seroclearance, 88% of patients had undetectable DNA. In the

study by Zhu et al. [24], 82.2% of patients having lost HBsAg in this study had undetectable HBV DNA. Ferreira SC team reported 62.5% of patients with DNA below 2000 UI/mL [10]. Tseng TC et al. [12], reported in their study that the prevalence of HBsAg level < 10 IU/mL was significantly higher in patients with HBV DNA level < 15 IU/mL compared to HBV DNA levels between 15 and 199 IU/ mL (28.7% vs. 12.5%, P.¼ 0.001) and HBV DNA levels between 200 and 1999 (28.7% vs. 4.4%, P < 0.001). Shimakawa et al. [25] in their study reported that low HBV DNA levels at baseline were associated with early HBsAg seroclearance. These authors observed that most patients who experienced spontaneous HBsAg seroclearance had undetectable HBV DNA levels before seroconversion. Thus, these data support the idea that low viral load is an important factor for spontaneous HBsAg seroclearance. Among the patients who obtained HBs seroclearance in our series, two (02) had cirrhosis diagnosed by ultrasound. After the loss of HBsAg, they benefited from annual follow-up. In current guidelines, there is no consensus on the follow-up interval for those with HBs seroclearance [8,14]. Cirrhosis, co-infection with HCV or HDV, male gender and age ≥ 50 years have been reported as independent risk factors for HCC development after HBs seroclearance [14,26]. These patients & may also present a reappearance of HBsAg. Guidelines concerning these patients should be established to facilitate their monitoring while taking into account cost-effectiveness.

Conclusion

Spontaneous HBsAg seroclearance is associated in the long term, in patients with chronic HBV infection, with a good prognosis, including improved lesions, liver functions and increased long-term survival. However, spontaneous seroclearance occurs in a small proportion of patients during the natural history of chronic HBV infection. In our series, the overall incidence of HBsAg loss was 1.8%; that of HBs seroconversion within 12 months following loss of HBsAg was 55.5%. At the end of our study, it appears that advanced age, inactive HBsAg carrier status, low HBV DNA level and titer of HBsAg could be predictive factors of HBsAg clearance. Further follow-up studies involving a larger numbers of patients may be needed to confirm these results.

References

1. Regional Strategic Plan for Vaccination 2014-2020. https://iris.who.int/bitstream/handle/10665/192560/9789290312109.pdf;sequence=1
2. World Health Organization. Global hepatitis report 2024: action for access in low- and middle-income countries. 2024. https://www.who.int/publications-detail-redirect/9789240091672
3. Schweitzer A, Horn J, Mikolajczyk R, et al. Estimates of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. Lancet. 2015; 386: 1546-1555.
4. Meda N, Tuaillon E, Kania D, et al. Hepatitis B and C virus seroprevalence, Burkina Faso: a cross-sectional study. Bull World Health Organ. 2018; 96: 750-759.
5. Lok ASF, McMahon BJ, Brown RS, et al. Antiviral therapy for chronic hepatitis B viral infection in adults: A systematic review and meta-analysis. Hepatology. 2016; 63: 284-306.
6. Liaw YF, Chu CM. Hepatitis B virus infection. The Lancet. 2009; 373: 582-592.
7. Yeo YH, Ho HJ, Yang HI, et al. Factors Associated with rates of HBsAg Seroclearance in Adults with Chronic HBV Infection: A Systematic Review and Meta-analysis. Gastroenterology. 2019; 156: 635-646.
8. Kim GA, Lim YS, An J, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut. 2014; 63: 1325-1332.
9. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007; 45: 1187-1192.
10. Ferreira SC, Chachá SGF, Souza FF, et al. Factors associated with spontaneous HBsAg clearance in chronic hepatitis B patients followed at a university hospital. Ann Hepatol. 2014; 13: 762-770.
11. Liu J, Yang HI, Lee MH, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010; 139: 474-482.
12. Tseng TC, Liu CJ, Yang HC, et al. Determinants of spontaneous surface antigen loss in hepatitis B e antigen-negative patients with a low viral load. Hepatology. 2012; 55: 68-76.
13. Yuen MF, Wong DKH, Sablon E, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology. 2004; 39: 1694-1701.
14. Yuen MF, Wong DKH, Fung J, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008; 135: 1192-1199.
15. Jaroszewicz J, Ho H, Markova A, et al. Hepatitis B surface antigen (HBsAg) decrease and serum interferon-inducible protein-10 levels as predictive markers for HBsAg loss during treatment with nucleoside/nucleotide analogues. Antivir Ther. 2011; 16: 915-924.
16. Habersetzer F, Moenne-Loccoz R, Meyer N, et al. Loss of hepatitis B surface antigen in a real-life clinical cohort of patients with chronic hepatitis B virus infection. Liver Int. 2015; 35: 130-139.
17. Chevaliez S, Hézode C, Bahrami S, et al. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol. 2013; 58: 676-683.
18. Gish RG, Chang TT, Lai CL, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naïve HBeAg-positive patients with chronic hepatitis B. J Viral Hepat. 2010; 17: 16-22.
19. Hadziyannis SJ, Sevastianos V, Rapti I, et al. Sustained responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop long-term treatment with adefovir. Gastroenterology. 2012; 143: 629-636.

20. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013; 381: 468-475.
21. Roushan MRH, Mohammadpour M, Baiany M, et al. Time to seroconversion of HBsAg to anti-HBs in individuals who lost HBsAg during follow-up. Epidemiol Infect. 2016; 144: 2648-2653.
22. Chen YC, Jeng WJ, Chu CM, et al. Decreasing levels of HBsAg predict HBsAg seroclearance in patients with inactive chronic hepatitis B virus infection. Clin Gastroenterol Hepatol. 2012; 10: 297-302.
23. Arase Y, Ikeda K, Suzuki F, et al. Long-term outcome after hepatitis B surface antigen seroclearance in patients with chronic hepatitis B. Am J Med. 2006; 119: 71.e9-16.
24. Zhu L, Zhai X, Wang Q, 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: 1588-1598.
25. Shimakawa Y, Lemoine M, Njai HF, et al. Natural history of chronic HBV infection in West Africa: a longitudinal population-based study from The Gambia. Gut. 2016; 65: 2007-2016.
26. Simonetti J, Bulkow L, McMahon BJ, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010; 51: 1531-1537.