Virology Journal

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Nucleos(t)ide analogs continuation is not associated with a lower risk of HBsAg seroreversion following PEG-IFN-induced HBsAg loss

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Abstract

Background/Aims It is unclear whether nucleos(t)ide analogs (NUCs) continuation provides clinical benefits following HBsAg seroclearance with pegylated interferon (PEG-IFN)-based therapy. This study aims to investigate the role of NUCs continuation in HBsAg seroreversion.

Methods Patients who experienced serum HBsAg loss after PEG-IFN-based therapy were enrolled and followed up for 96 weeks. Propensity score matching (PSM) was performed using a 1:1 ratio to adjust for the associated factors. A multivariate logistic regression analysis was used to determine the factors associated with HBsAg seroreversion.

Results In total, 220 patients with HBsAg seroclearance were divided into NUCs ($n=54$) and non-NUCs ($n=166$) consolidation therapy groups. At week 96, the HBsAg seroreversion (12/54 vs. 31/166, $P=0.709$) and virological relapse (2/54 vs. 10/166, $P=0.759$) rates were similar in the NUCs and non-NUCs groups. After PSM, HBsAg seroreversion (12/53 vs. 13/53; $P=1.000$) and virological relapse (2/53 vs. 4/53; $P=0.674$) rates were not significantly different between the two groups. Serum hepatitis B surface antibody titer (odds ratio, 0.388; 95% confidence interval, 0.245–0.616; $P<0.001$) was found to be associated with HBsAg seroreversion, while NUCs continuation was not related to HBsAg seroreversion.

Conclusions NUCs continuation is not associated with a lower risk of HBsAg seroreversion in patients with serum HBsAg loss following PEG-IFN-based therapy.

Keywords Chronic hepatitis B, Nucleos(t)ide analogs, Seroreversion, Functional cure

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Introduction

Hepatitis B surface antigen (HBsAg) seroclearance, aimed at reducing the risk of cirrhosis and hepatocellular carcinoma, is a well-accepted chronic hepatitis B treatment endpoint worldwide [1–4]. Current antiviral therapies, including pegylated-interferon (PEG-IFN) and nucleos(t)ide analogs (NUCs) therapies, are more likely to result in transcriptional silencing rather than elimination of covalently closed circular DNA (cccDNA) and hepatitis B virus (HBV) integration. Although PEG-IFN-based therapy provides a higher chance of HBsAg seroclearance than NUCs therapy, more than one-fifth of patients who experience functional cure after PEG-IFN-based therapy may experience HBsAg seroreversion owing to residual trace transcription of HBV integration and/or cccDNA [5–7]. Whether additional antiviral regimens after PEG-IFN-induced HBsAg loss could further attenuate intrahepatic HBV replication and reduce the risk of relapse remains unclear.

High titers of hepatitis B surface antibody (HBsAb) promote the durability of serum HBsAg loss; moreover, PEG-IFN consolidation therapy promotes HBsAb production in patients with serum HBsAg loss and negative HBsAb, which may help reduce the risk of HBsAg seroreversion [8, 9]. However, no studies have reported whether NUCs consolidation therapy can help prevent HBV infection relapse in patients with PEG-IFN-induced HBsAg loss. Therefore, a better understanding of the benefits of NUCs consolidation therapy will help determine the ideal time for discontinuation of NUCs therapy in patients who achieved serum HBsAg loss following PEG-IFN-based therapy. Here, we aimed to explore the factors related to HBsAg seroreversion in patients with HBsAg seroclearance after PEG-IFN-based therapy and investigate the role of NUCs consolidation therapy in HBsAg seroreversion.

Methods

Figure 1 shows a flowchart of the patients enrolled in this study. In this retrospective study, we included 220 patients with HBsAg seroclearance after PEG-IFN-based therapy between January 2017 and July 2021 at the Third Affiliated Hospital of Sun Yat-sen University. The inclusion criterion was HBsAg seroclearance after

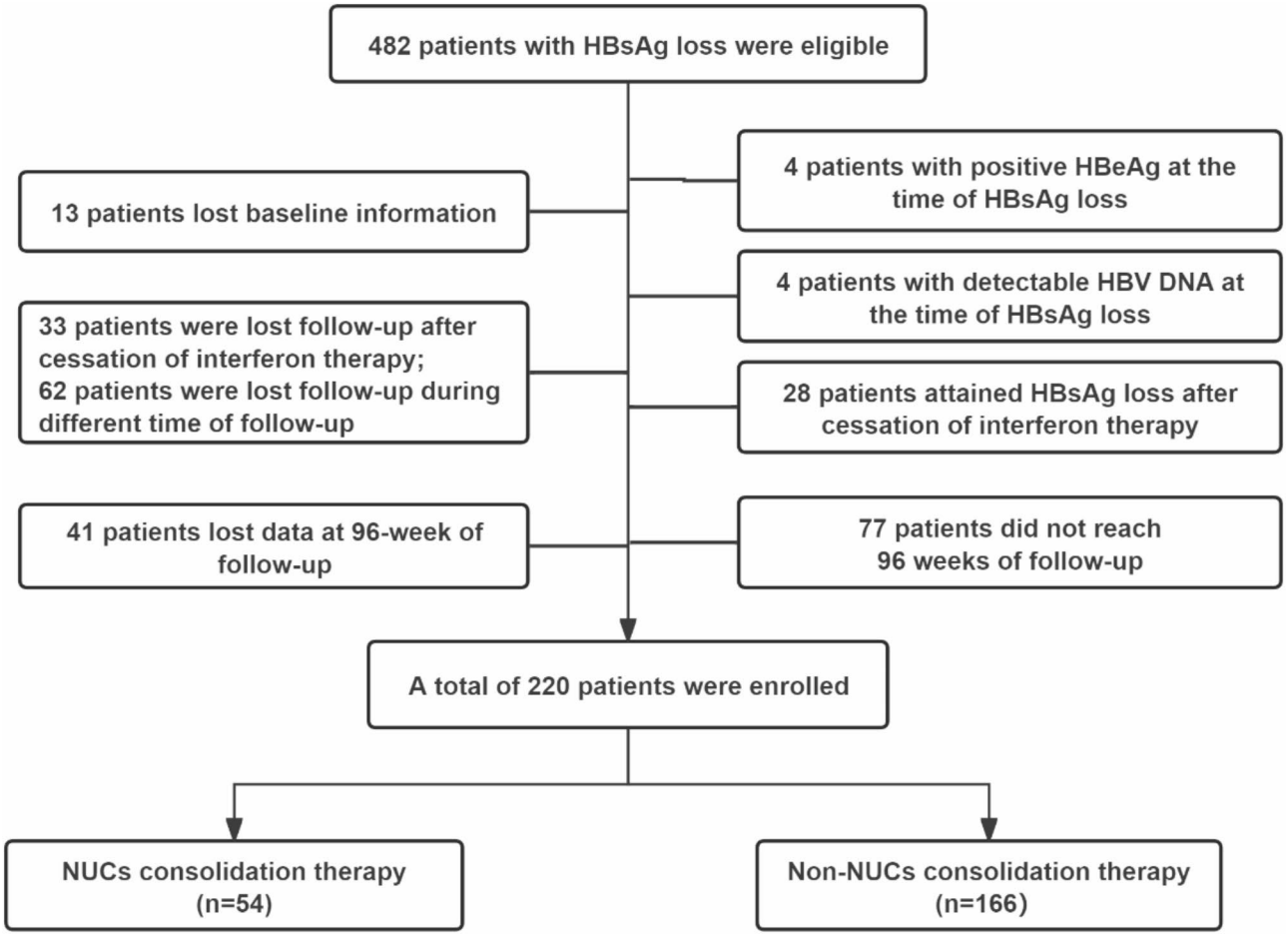

Fig. 1 Flow chart of patients with HBsAg seroclearance during the follow-up. HBsAg, Hepatitis B surface antigen

PEG-IFN-based therapy, defined as a serum HBsAg level of < 0.05 IU/mL or cutoff index of 1. Patients with serum HBsAg loss completed at least 96 weeks of follow-up. The exclusion criteria were co-infection with hepatitis C virus or human immunodeficiency virus, evidence of hepatocellular cancer or decompensated cirrhosis, and receipt of immunosuppressive and biological therapy. Clinical and laboratory data after PEG-IFN therapy were collected as baseline data.

Propensity score matching (PSM) was performed using a ratio of 1:1 for the NUCs and non-NUCs consolidation therapy groups to adjust for serum HBsAg levels before PEG-IFN-based treatment, age, sex, compensated cirrhosis, PEG-IFN therapy duration, PEG-IFN therapy duration required to achieve HBsAg loss, PEG-IFN consolidation duration, antiviral regimens, and HBsAb titer at cessation of PEG-IFN therapy. After PSM, 53 patients were assigned to each of the NUCs and non-NUCs groups.

Ethics approval statement

All clinical investigations were conducted following the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008). Informed consent was obtained from all patients for being included in the study. The protocol of this study was approved by the Research Ethical Committee of the Third Affiliated Hospital of Sun Yat-Sen University, China (ethnics number: [2016]2-129).

Serum viral parameters

HBsAg was quantified (lower limit of quantification [LLOQ], 0.05 IU/mL) using Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, Indianapolis, IN, USA). HBsAg (LLOQ, cutoff index of 1) and HBsAb (LLOQ, 2 mIU/mL) were detected using Elecsys HBsAg II (Roche Diagnostics) and Elecsys anti-HBs II kits (Roche Diagnostics), respectively. Serum HBV DNA (LLOQ, 20 IU/mL) was tested using the COBAS AmpliPrep and COBAS TaqMan HBV test (version 2.0; Roche Diagnostics). A liver function test was performed using a Hitachi 7600 automatic analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan), and the normal upper limit of alanine aminotransferase (ALT) was set to 40 U/L.

Intrahepatic HBsAg, HBcAg and CccDNA detection

Fourteen of 220 patients completed liver biopsy. Formalin-fixed liver biopsy tissues were used for the immunohistochemical staining of HBsAg and hepatitis B core antigen (HBcAg) for diagnostic purposes. DNA was extracted from 1.5-cm segments of liver tissue that were stored in liquid nitrogen. Primers and probes targeting the gap region of the HBV genome were designed to quantify covalently closed circular DNA (cccDNA) using quantitative real-time PCR (qPCR), as previously described [6].

Follow-up and primary outcome measures

Data regarding HBsAg seroreversion and HBV DNA relapse were collected. The primary outcome was HBsAg seroreversion (HBsAg relapse to ≥0.05 IU/mL or cutoff index of 1). The secondary outcome was virological relapse (HBV DNA relapse to ≥20 IU/mL).

Statistical analysis

Descriptive statistics were used to summarize the baseline demographics of patients with HBsAg seroclearance after PEG-IFN-based therapy. The χ^2 and Fisher's exact tests were performed to compare categorical variables, whereas Mann–Whitney's U and Student's t-tests were performed to compare continuous variables. A logistic regression analysis with backward selection was performed to assess the association between the variables and HBsAg seroreversion. Statistical significance was set at $P < 0.05$ (two-tailed). All data were analyzed using SPSS software (version 24.0; IBM Corp., Armonk, NY, USA). PSM was performed using R (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics of patients with PEG-IFN-induced HBsAg loss

Among 220 patients with chronic hepatitis B (CHB) who experienced HBsAg seroclearance during PEG-IFN-based therapy included in this study, 166 (75.5%) and 54 (24.5%) were in the non-NUCs and NUCs consolidation therapy groups, respectively (Fig. 1). The total PEG-IFN-based treatment duration (median [quartile 1–quartile 3]) was 48.00 weeks (36.00–60.00 weeks) in 220 patients. Patients in the NUCs consolidation therapy group underwent 46.00 weeks (12.75–92.5 weeks) of NUCs treatment following PEG-IFN. The distribution of NUCs was as follows: tenofovir disoproxil fumarate (TDF, 48.1%), entecavir (ETV, 42.6%), tenofovir alafenamide (TAF, 7.4%), and tenofovir amibufenamide (TMF, 1.9%) (Supplementary Fig. 1). The baseline characteristics of patients in the NUCs and non-NUCs consolidation therapy groups before and after PSM are presented in Table 1. Patients in the NUCs consolidation therapy group were older (44.44 ± 8.21 years versus [vs.] 40.12 ± 8.22 years; $P = 0.001$) than those in the non-NUCs consolidation therapy group (Table 1). Regarding different antiviral regimens before HBsAg loss, NUCs and PEG-IFN combination therapy (88.9%) was more commonly used for patients in the NUCs consolidation therapy group, whereas the proportion of PEG-IFN

Table 1 Characteristics of patients with HBsAg seroclearance in NUCs consolidation and none-NUCs consolidation group

	Before matching		*P* Value	After matching		*P* Value
	NUCs consolidation group (*n* = 54)	Non-NUCs consolidation group (*n* = 166)		NUCs consolidation group (*n* = 53)	Non-NUCs consolidation group (*n* = 53)	
Age	44.44 ± 8.21	40.12 ± 8.22	0.001	44.30 ± 8.22	44.45 ± 8.21	0.882
Sex, male (n%)	45 (83.3%)	135 (81.3%)	0.897	44 (83.02%)	46 (86.79%)	0.786
Serum ALT (IU/ml)	43.00 (25.25, 61.75)	38.50 (27.25,57.00)	0.827	43.00 (25.00, 62.00)	37.00 (25.00, 52.00)	0.417
Serum HBsAb (Log mIU/ml)[a]	1.63 (0.99, 2.40)	1.93 (1.18, 2.46)	0.415	1.63 (0.99, 2.40)	1.76 (1.11, 2.17)	0.762
Serum HBsAg before treatment (Log IU/ml)	2.16 (1.43, 2.62)	2.18 (1.60, 2.62)	0.795	2.16 (1.53, 2.63)	2.18 (1.65, 2.64)	0.762
Compensated cirrhosis (n%)[b]	6 (11.1%)	6 (3.6%)	0.080	5 (9.43%)	4 (7.55%)	1.000
PEG-IFN therapy duration required to achieve HBsAg loss (weeks)	27.50 (16.00, 48.00)	29.00 (24.00, 41.75)	0.884	28.00 (16.00, 48.00)	36.00 (24.00, 48.00)	0.365
PEF-IFN consolidation treatment duration (weeks)	12.00 (9.25, 21.50)	12.50 (8.00, 24.00)	0.731	12.00 (9.00, 22.00)	14.00 (10.00, 24.00)	0.517
Total PEG-IFN duration (weeks)	48.00 (36.00, 57.50)	48.00 (36.00, 60.00)	0.750	48.00 (36.00, 58.00)	50.00 (46.00, 64.00)	0.210
Antiviral regimens			< 0.001			0.774
Peg-IFN monotherapy (n%)	6 (11.1%)	76 (45.8%)		6 (11.3%)	8 (15.1%)	
Combination NAs and Peg-IFN(n%)	48 (88.9%)	90 (54.2%)		47 (88.7%)	45 (84.9%)	

[a] HBsAb detection was missing for one patient in the NUCs consolidation group

[b] B ultrasound finding was missing for one patient in the non-NUCs consolidation group

HBsAb, hepatitis B surface antibody; HBsAg, Hepatitis B surface antigen; NUCs, nucleos(t)ide analogs; PEG-IFN, pegylated-interferon; HBV, hepatitis B virus

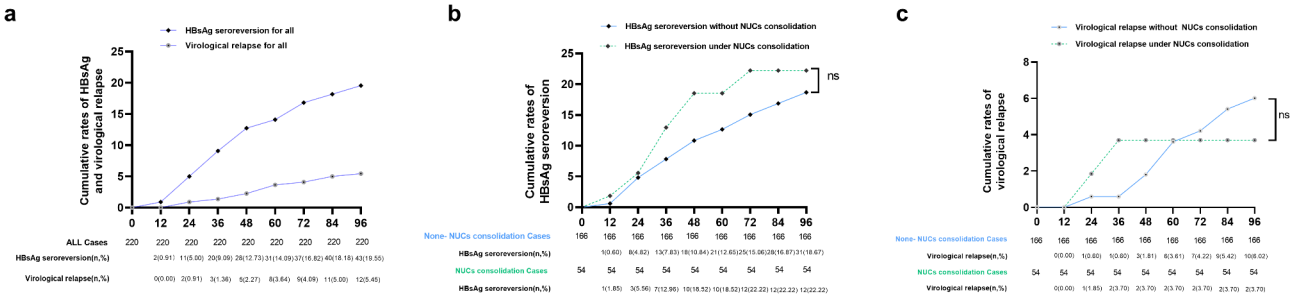

Fig. 2 Cumulative rates of HBsAg seroreversion and virological relapse during the 96-week of follow-up patients in the NUCs and non-NUCs consolidation therapy groups. The dark blue line with lozenge indicates the rates of HBsAg seroreversion, and the dark blue line with circle stands for virological relapse at every follow-up point in all patients with HBsAg seroclearance. The light blue line indicates the non-NUCs consolidation therapy group, and the green dotted line represents the NUCs consolidation therapy group. Comparison of the cumulative rates of HBsAg seroreversion and virological relapse were made between the NUCs and non-NUCs groups at the 96-week follow-up. NUCs, nucleos(t)ide analogs; HBsAg, hepatitis B surface antigen

monotherapy (45.8%) was higher in the non-NUCs consolidation therapy group than NUCs consolidation group ($\chi^2 = 19.49$; $P < 0.001$) (Table 1). No significant differences were observed in sex, serum ALT levels, HBsAb titer, HBsAg levels before treatment, presence of compensated cirrhosis, PEG-IFN therapy duration required to achieve HBsAg loss, PEG-IFN consolidation treatment duration, and total PEG-IFN therapy duration between the two groups (Table 1). After PSM, the differences in all variables between the NUCs ($n = 53$) and non-NUCs consolidation therapy groups ($n = 53$) were reduced and not statistically significant (Table 1).

Patients in the NUCs and non-NUCs consolidation therapy groups have similar risks of HBsAg seroreversion and virological relapse

Pre-PSM analysis indicated that for all study participants, the cumulative rates of HBsAg seroreversion at 12, 24, 36, 48, 60, 72, 84, and 96 weeks were 0.91%, 5.00%, 9.09%, 12.73%, 14.09%, 16.82%, 18.18%, and 19.55%, respectively (Fig. 2a). Furthermore, their virological relapse rates were 0%, 0.91%, 1.36%, 2.27%, 3.64%, 4.09%, 5.00%%, and 5.45% at 12, 24, 36, 48, 60, 72, 84, and 96 weeks, respectively (Fig. 2a). No significant differences were observed in the cumulative rates of HBsAg seroreversion (12/54 vs. 31/166; $\chi^2 = 0.14$; $P = 0.709$) and virological relapse (2/54 vs. 10/166; $\chi^2 = 0.094$; $P = 0.759$) between the NUCs and non-NUCs consolidation therapy groups at 96 follow-up weeks after PEG-IFN therapy (Fig. 2b and c). One patient

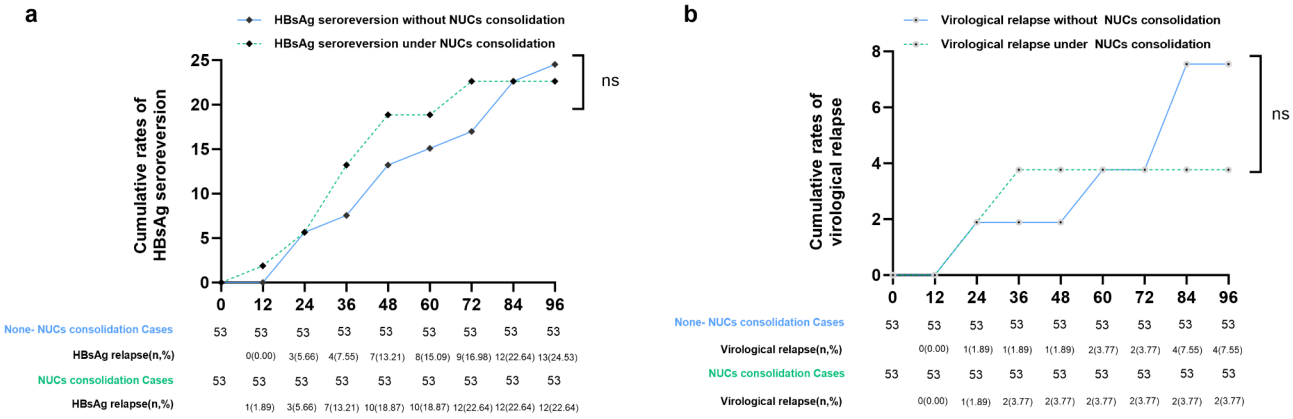

Fig. 3 Cumulative rates of HBsAg seroreversion and virological relapse during the 96-week follow-up of patients in the NUCs and non-NUCs consolidation therapy groups after propensity score matching. The light blue line indicates the non-NUCs consolidation therapy group, and the green dotted line represents the NUCs consolidation therapy group. Comparison of the cumulative rates of HBsAg seroreversion and virological relapse were made between the NUCs and non-NUCs groups at the 96-week follow-up after propensity score matching. NUCs, nucleos(t)ide analogs; HBsAg, hepatitis B surface antigen

Table 2 Multivariable logistic analysis for predictors of HBsAg seroreversion

	Univariable analysis			Multivariable logistic analysis		
	HBsAg seroreversion group (*n* = 43)	Non-HBsAg seroreversion group (*n* = 177)	*P*-value	OR	95% CI	*P*-value
Age (years)	40.49 ± 7.37	41.34 ± 8.65	0.514	0.985	0.938–1.034	0.540
Sex, male (n, %)	36 (83.7%)	144 (81.4%)	0.888	0.648	0.231–1.812	0.408
Serum ALT level (IU/mL)	52.00 (35.00, 70.500)	36.00 (25.00, 56.00)	0.001	1.007	0.998–1.015	0.127
Serum HBsAb level (Log mIU/mL)[a]	1.39 (0.68, 1.69)	2.05 (1.25, 2.51)	< 0.001	0.388	0.245–0.616	< 0.001
NUCs consolidation (n%)	12 (27.9%)	42 (23.7%)	0.709	1.210	0.513–2.855	0.664
Serum HBsAg level before treatment (Log IU/mL)	2.36 (1.82, 2.70)	2.14 (1.39, 2.62)	0.126	1.442	0.785–2.650	0.238
PEG-IFN therapy duration required to achieve HBsAg loss (weeks)	36.00 (28.00, 42.00)	26.00(22.00, 46.00)	0.017	1.012	0.990–1.034	0.294
PEG-IFN consolidation treatment duration (weeks)	12.00 (5.00, 19.00)	12.00 (10.00, 24.00)	0.097	0.999	0.963–1.036	0.946
Compensated cirrhosis (n, %)[b]	2 (4.7%)	10 (5.7%)	1.000	0.820	0.151–4.456	0.819

[a] HBsAb detection was missing for one patient in the non-HBsAg seroreversion group

[b] B ultrasound finding was missing for one patient in the non-HBsAg seroreversion group

HBsAb, hepatitis B surface antibody; HBsAg, Hepatitis B surface antigen; NUCs, nucleos(t)ide analogs; PEG-IFN, pegylated-interferon; HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase

who did not receive antiviral consolidation therapy after achieving HBsAg loss and undetectable HBV DNA experienced virological relapse (HBV DNA value, 31 IU/mL) without HBsAg seroreversion at week 96, which may also indicate trace transcriptional activity of cccDNA at that time.

Similar to the pre-PSM analysis, the post-PSM analysis revealed no significant differences in HBsAg seroreversion (12/53 vs. 13/53; $P = 1.000$) and virological relapse rates (2/53 vs. 4/53; $\chi^2 = 0.177$; $P = 0.674$) between the NUCs and non-NUCs consolidation therapy groups at week 96 after PEG-IFN (Fig. 3a and b).

Serum HBsAb titer is a predictor of HBsAg seroreversion

To investigate factors associated with HBsAg seroreversion, we conducted a multivariate analysis among patients with PEG-IFN-induced HBsAg loss. The serum HBsAb titer was less abundant in the HBsAg seroreversion group than in the non-HBsAg seroreversion group (1.39 [0.68–1.69] vs. 2.05 [1.25–2.51]; $P < 0.001$) (Table 2). Additionally, ALT levels were higher in the HBsAg seroreversion group than in the non-HBsAg seroreversion group (52.00 [35.00–70.50] vs. 36.00 [25.00–56.00]; $P = 0.001$) (Table 2). No significant differences were observed in age, sex, NUCs consolidation, serum HBsAg levels before treatment, PEG-IFN therapy duration required to achieve HBsAg loss, PEG-IFN consolidation treatment duration, and presence of compensated cirrhosis between the two groups (Table 2). The multivariate analysis showed that the serum HBsAb titer was a predictor of HBsAg seroreversion (odds ratio [OR], 0.388; 95% confidence interval [CI], 0.245–0.616; $P < 0.001$)

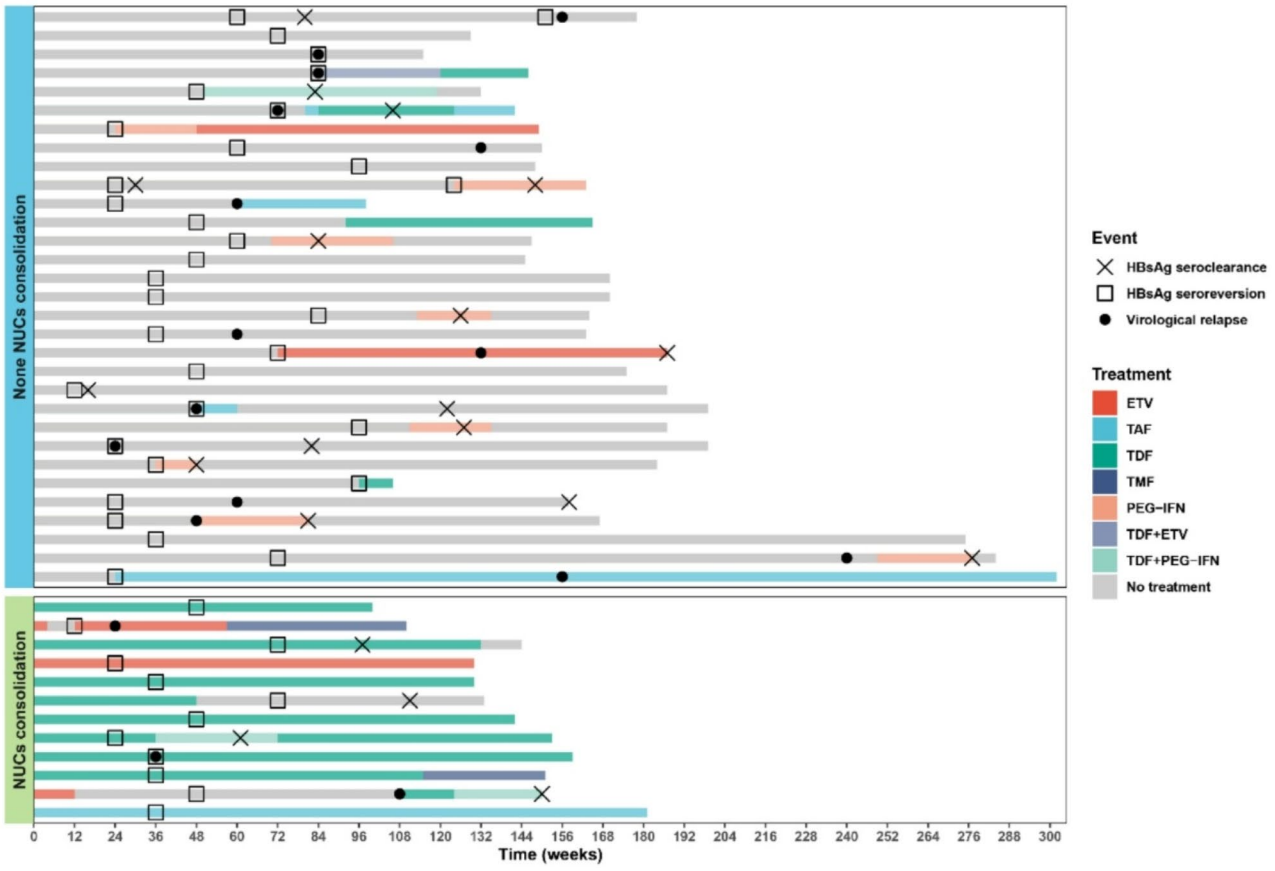

Fig. 4 Timeline of HBsAg seroreversion, virological relapse, and re-HBsAg seroclearance in patients with HBsAg seroreversion. Each line represents a patient with HBsAg seroreversion. The length of the bar indicates the follow-up duration in weeks. The colors of the line represent the different antiviral treatments. The symbols represent HBsAg seroreversion, virological relapse, and re-HBsAg seroclearance. HBsAg, hepatitis B surface antigen

(Table 2). However, NUCs consolidation was not an independent risk factor for HBsAg seroreversion.

Among 220 patients, 14 completed liver biopsies after discontinuing PEG-IFN and had at least 96 weeks of follow-up (Supplementary Table 1). Notably, patient L-08 achieved HBsAg seroconversion with a cccDNA level of 2.757 copies per 1000 cells and had positive intrahepatic HBsAg. This patient received 48 weeks of TDF consolidation therapy following the cessation of PEG-IFN. However, serum HBsAg relapse was observed at 48-week follow-up (Supplementary Table 1). The other patients remained serum HBsAg loss throughout the 96-week follow-up period with or without NUCs consolidation therapy (Supplementary Table 1). These findings suggest that residual intrahepatic HBV replication might not be fully inhibited through NUCs consolidation therapy.

Predictors for virological relapse

A multivariate analysis among patients was conducted to find predictors for virological relapse. We found that the serum HBsAb titer after PEG-IFN therapy was lower in the virological relapse group than in the non-virological group (1.28 [0.99–1.45] vs. 1.94 [1.17–2.48]; $P<0.001$) (Supplementary Table 2). This indicated that a higher serum HBsAb titer, an indication of restored host immunity against HBV, played an important role in preventing both HBsAg seroreversion and virological relapse. There were no significant differences in age, sex, serum ALT, NUCs consolidation, serum HBsAg levels before treatment, PEG-IFN therapy duration required to achieve HBsAg loss, and PEG-IFN consolidation treatment duration between the two groups (Supplementary Table 2). Multivariate analysis showed that the serum HBsAb titer (OR, 0.346; 95% CI, 0.154–0.778; $P=0.010$) is the only predictor of virological relapse (Supplementary Table 2). Additionally, NUCs consolidation was not a protective factor for virological relapse in multivariate analysis.

Re-HBsAg seroclearance in patients with HBsAg seroreversion

We included 43 patients with HBsAg seroreversion who completed 153 weeks (142–178 weeks) of follow-up in this study. HBsAg seroreversion, virological relapse, re-HBsAg seroclearance, and antiviral treatment times are shown in Fig. 4. Among the patients, 17 with HBsAg seroreversion experienced virological relapse during

the follow-up. Of 11 (25.6%) patients who underwent repeat PEG-IFN-based therapy, 10 re-experienced serum HBsAg loss. Furthermore, of 17 patients (39.5%) who were retreated with NUCs, 3 experienced HBsAg seroclearance during NUCs treatment and 1 experienced HBsAg loss after cessation of NUCs; conversely, of 15 (34.9%) patients who underwent close surveillance without antiviral treatment, 5 experienced serum HBsAg loss.

Discussion

Here, we found that 19.55% of patients with HBsAg loss after PEG-IFN-based therapy experienced HBsAg seroreversion within a 96-week follow-up period. Furthermore, we found that NUCs consolidation was not associated with a lower risk of HBsAg seroreversion, and the serum HBsAb titer (OR, 0.388) was the only predictive factor of HBsAg seroreversion.

HBsAg seroreversion rates of 24.83% and 23.08% at 96 weeks for patients who received IFN and NUC-IFN, respectively, have been previously reported [7]. Furthermore, residual transcriptional cccDNA persists after HBsAg seroclearance, resulting in HBV reactivation in patients with resolved HBV [6, 10]. Given this, whether NUCs consolidation contributes to decreasing HBV relapses in patients with HBsAg loss after PEG-IFN-based therapy is unclear. Our results showed that NUCs consolidation was not associated with a lower risk of HBsAg seroreversion in patients with PEG-IFN-induced HBsAg loss; this finding is consistent with the observation that NUCs discontinuation was safe following NUCs-induced HBsAg loss [11]. Although NUCs inhibit reverse transcription of pregenomic RNA to HBV DNA, they do not directly target cccDNA; hence, eliminating cccDNA via NUCs monotherapy is difficult [12, 13]. Continuous HBsAg production during NUCs therapy is common, primarily due to incomplete suppression of cccDNA transcriptional activity [14, 15]. This is in line with patient L-08 in our study, HBsAg seroreversion occurred despite consolidation therapy with TDF. Multivariate analysis in this study showed that NUCs consolidation following discontinuation of PEG-IFN is not associated to HBsAg seroreversion.

However, our findings indicated that a higher HBsAb titer was a protective factor for HBsAg seroreversion. This supports previous studies which have shown that an HBsAb titer of ≥100 mIU/mL can identify sustained HBsAg loss after PEG-IFN-based therapy [9, 16]. Moreover, a higher HBsAb titer correlates with a lower likelihood of HBsAb loss during follow-up [9]. Notably, stable and broad HBsAb diversity also play critical roles in sustainable HBsAg loss [17]. Additionally, we previously reported that residual transcriptional integrated HBV DNA and cccDNA are also important influencing factors for HBsAg seroreversion [6].

Intrahepatic HBsAg can continue to be transcribed from cccDNA and/or integrated HBV DNA, even when serum HBsAg levels are below 0.05 IU/mL. This phenomenon highlights the importance of considering different scenarios that can lead to undetectable serum HBsAg. The first scenario involves serum anti-HBs immune complexes, which could sequester circulating HBsAg from detection, potentially leading to a transient and unstable serum "HBsAg loss". Additionally, trace serum HBsAg below 0.05 IU/mL should be noticed. Recently developed HBsAg assays have LLOQs that are 10- to 100-fold lower, enabling the screening of extremely low titers of HBsAg [10, 18, 19]. Moreover, further studies are required to determine whether ultrasensitive HBsAg assays in combination with pregenomic RNA or HBV core-related antigens, can help detect trace transcription from cccDNA and/or HBV integration [16, 20]; this approach may also aid in identifying pre-S/S variants that induce undetectable HBsAg [20].

In 97.7% (42/43) of patients experiencing HBsAg seroreversion, the relapsed HBsAg level was below 20 IU/mL or 5 COI (data not shown). Previous studies have reported that patients with baseline HBsAg levels < 100 IU/mL achieved an HBsAg clearance rate of 81.1% following Peg-IFN therapy, indicating a strong association between lower baseline HBsAg levels and a higher rate of HBsAg clearance [21]. Consistent with these findings, our study shows that patients with low relapsed HBsAg levels responded well to retreatment and experienced serum HBsAg loss again. Furthermore, We found that five patients with HBsAg seroreversion who received no antiviral treatment and underwent close surveillance experienced serum HBsAg loss again. This may be attributable to the fluctuating levels of anti-HBs immune complexes; however, further studies are necessary to confirm this. Additionally, we observed that 26 of 43 patients with HBsAg antigenemia did not have viremia during the 96 weeks of follow-up, suggesting that HBsAg antigenemia might mainly originate from transcriptionally active HBV integration instead of cccDNA. Moreover, the majority of patients with serum HBsAg loss sustain HBsAg loss during the 96 weeks of follow-up, indicating a stable and sustained response to HBV.

Based on our findings, we propose that NUCs consolidation after cessation of PEG-IFN is not beneficial for decreasing HBsAg seroreversion. Notably, higher HBsAb titer was a protective factor against HBsAg seroreversion, which indicated that the restoration of host immunity plays an important role in preventing HBsAg reversion.

This study has some limitations. First, this was a retrospective study that did not account for the history of antiviral resistance associated with HBsAg seroreversion, which requires more attention. Prospective studies with larger sample sizes and more information are necessary.

Second, HBsAg assays with sensitive LLOQs can help define overt CHB; however, they are not widely used in real-world settings. In this study, HBsAg assays with higher sensitivity were not available because the collected blood samples were limited. Finally, the study patients were recruited from southern China; therefore, multicenter studies involving different ethnic populations are needed.

Conclusions

NUCs consolidation therapy is not associated with a lower HBsAg seroreversion in patients with serum HBsAg loss following PEG-IFN-based therapy. Furthermore, the serum HBsAb titer after PEG-IFN is a protective predictor of HBsAg seroreversion.

Abbreviations

ALT	Alanine aminotransferase
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
LLOQ	Lower limit of quantification
NUCs	Nucleos(t)ide analogs
TDF	Tenofovir disoproxil fumarate
ETV	Entecavir
TAF	Tenofovir alafenamide
TMF	Tenofovir amibufenamide
OR	Odds ratio
PEG-IFN	Pegylated-interferon
PSM	Propensity score-matching
vs.	Versus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02700-2.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

We would like to thank all workers on the Everest project.

Author contributions

Na Gao designed the study and wrote the manuscript; Haishi Wu followed up with patients and performed part of the analysis. Bin Li wrote the R scripts for propensity score matching and performed part of the statistical analysis. Huiying Yu and Lili Wu collected some of the patient data and assisted in report preparation. Jing Zhang and Bingliang Lin followed up with patients and offered suggestions. Nan Zhang helped collect the data and participated in care coordination. Qiyi Zhao helped design and offered important suggestions. Zhiliang Gao was responsible for the quality of the study and guided the other authors. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82170612, 81672701, 82173064, and 81971481), Guangdong Basic and Applied Basic Research Foundation (2023A1515110584 and 2022B1515020023), Guangzhou Science and Technology Program Key Projects (2023B01J1007), Science and Technology Program of Guangzhou (202201020467), Fundamental Research Funds for the Central Universities (22ykqb01), and Postdoctoral Fellowship Program of CPSF (GZC20233249).

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All clinical investigations were conducted following the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008). Informed consent was obtained from all patients for being included in the study. The protocol of this study was approved by the Research Ethical Committee of the Third Affiliated Hospital of Sun Yat-Sen University, China (ethnics number: [2016]2-129).

Consent for publication

All participants provided written informed consent, including specific consent for the publication of their anonymized data. No identifying details or images of individuals are included in this manuscript.

Competing interests

The authors declare no competing interests.

Received: 11 January 2025 / Accepted: 9 March 2025
Published online: 19 March 2025

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