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ORIGINAL ARTICLE

Association Between Elevation of Serum Alanine Aminotransferase and HBsAg Seroclearance After Nucleos(t)ide Analog Withdrawal

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Keywords: acute hepatitis flares | antiviral therapy | finite treatment | HBsAg seroclearance | hepatitis B virus

ABSTRACT

Background: Alanine aminotransferase (ALT) frequently elevates in chronic hepatitis B patients stopping nucleos(t)ide analogs (NAs).

Aims: To clarify the association between ALT elevation and HBsAg seroclearance after NA withdrawal.

Methods: This multicenter cohort study reviewed consecutive patients discontinuing NA between 2004/04/01 and 2022/05/24. Treatment initiation and discontinuation generally followed the Asian-Pacific guidelines. Eligible patients had negative HBeAg and undetectable HBV DNA before treatment cessation, without malignancy, organ transplant or autoimmune disorders. We used competing risk analysis to estimate HBsAg seroclearance incidence and a time-dependent model to investigate post-cessation ALT elevation.

Results: Among 841 patients (74.7% male; median age, 53.2 years; median treatment duration, 34.7 months), 38 patients cleared HBsAg over a median follow-up of 3.7 years, with a 10-year cumulative incidence of 12.4%. The median peak ALT level was significantly lower in patients achieving HBsAg seroclearance versus not (93 vs. 127 U/L; $p < 0.001$). Hepatitis flare after NA cessation (> 5 times upper limit) was inversely associated with HBsAg seroclearance in the univariable analysis (sub-distribution hazard ratio [SHR], 0.31; 95% confidence interval [CI], 0.13–0.73; $p = 0.007$), and the association was not significant (adjusted SHR, 0.42; 95% CI, 0.09–2.01; $p = 0.28$) in the multivariable analysis adjusted for pretreatment HBV DNA. Consistent results were observed in the sensitivity analyses with different ALT cutoffs and subgroup analysis adjusted for HBsAg levels at treatment cessation.

Abbreviations: ALT, alanine transaminase; anti-HBe, hepatitis B e antibody; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CI, confidence interval; EOT, end of treatment; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IQR, interquartile range; NA, nucleos(t)ide analog; SHR, sub-distribution hazard ratio; ULN, upper limit of normal.

Conclusion: ALT elevation after NA cessation is not associated with HBsAg seroclearance following NA withdrawal, suggesting cytolytic pathways are not essential for a functional cure.

1 | Introduction

Chronic hepatitis B virus (HBV) infection influences approximately 257.5 million people globally and stands as the leading cause of liver-related morbidity and mortality [1, 2]. Nucleos(t)ide analog (NA) has been widely used to treat patients with chronic hepatitis B (CHB) [3–5]. By way of inhibiting the viral reverse transcriptase, NA therapy can effectively suppress HBV replication and reduce the risks of clinical complications including hepatocellular carcinoma (HCC) [6–8]. However, the optimal treatment duration is unclear. Seroclearance of hepatitis B surface antigen (HBsAg) is a broadly acceptable treatment endpoint, but it rarely occurs during NA treatment [9–12]. Therefore, the treatment duration is essentially indefinite and can be lifelong in many treated patients.

Finite NA therapy without the prerequisite of HBsAg clearance has been proposed as an alternative treatment strategy for patients with CHB [5, 13, 14]. The finite strategy may increase the chance of HBsAg seroclearance as compared to continuous treatment [15, 16]. Nevertheless, recurrent viremia almost always follows NA cessation and hepatitis flares are common [17, 18], which could progress to acute-on chronic liver failure with fatal consequences [19, 20]. Therefore, the practice of NA therapy relies on accurate risk prediction to distinguish patients with distinct chances of HBsAg seroclearance and at different risk of severe withdrawal flares [21].

The mechanism underlying a higher rate of HBsAg seroclearance following NA cessation has not been elucidated but may involve restoration of HBV-specific T cell immunity that is downregulated with potent viral suppression [22, 23]. The reactivation of HBV-specific T cells following viral suppression can lead to immune-mediated liver damages, manifested as increase in serum alanine aminotransferase (ALT). As a result, elevation of serum ALT after NA cessation was hypothesised to be a 'double-edge sword'. While it indicates hepatic injury, it may conduce HBsAg seroclearance [15, 24]. This hypothesis, however, has not been rigorously examined with empirical data. Thus, we conducted this multicenter cohort study to investigate whether ALT elevation after NA cessation was associated with HBsAg seroclearance.

2 | Methods and Materials

2.1 | Study Design and Setting

This is a retrospective multicenter cohort study based on analysis of the electronic health record (EHR) databases of E-Da Healthcare System in Taiwan. All patients received NA therapy for CHB at E-Da Hospital, E-Da Cancer Hospital and E-Da Dachang Hospital and discontinued the treatment from April 1, 2004 to May 24, 2022 were systematically reviewed. Relevant information encompassing demographic data, laboratory test results, diagnostic codes, medical prescription records and vital statistics were extracted from the EHR database by a dedicated programmer. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of the E-Da Healthcare System (EMRP-111-099).

2.2 | Study Population

Eligible patients were adults (≥18 years) diagnosed with CHB (defined by a specific diagnosis or positive HBsAg serology ≥6 months) who were treatment-naive before starting entecavir, tenofovir disoproxil fumarate or tenofovir alafenamide. The NA therapy was continued for at least 1 year and then discontinued (defined by no drug dispensation for 3 months or longer) with undetectable serum HBV DNA (lower limit of detection, 6 IU/mL) at treatment cessation. Exclusion criteria were seroclearance of HBsAg during NA treatment, HBeAg positivity at treatment cessation, co-infection with hepatitis C virus (HCV), organ transplantation, coagulopathy (international normalised ratio > 1.5), malignancy including HCC or severe comorbidities certified by the registry of catastrophic illness [25].

2.3 | Criteria on the Initiation and Discontinuation of NA Treatment

Most patients were reimbursed for antiviral therapy by the national health insurance in Taiwan, with the indications generally following the Asia-Pacific guidelines [3]. Briefly, treatment was indicated for HBsAg-positive patients presenting with liver insufficiency (jaundice or coagulopathy) regardless of serum ALT or HBV DNA levels. Otherwise, HBV viremia > 2000 IU/mL and ALT elevation > 2 times upper limit of normal (ULN) lasting for at least 3 months are required, with details stratified by HBeAg status [26].

The reimbursement for NA treatment was finite in principle. In the absence of cirrhosis, the reimbursement ended 1 year after HBeAg seroconversion in HBeAg-positive patients. In HBeAg-negative patients without cirrhosis, it ended after three tests (each > 6 months apart) showing HBV DNA undetectable in blood or a maximum of 3 years. According to the Asian-Pacific guidance, treatment discontinuation could be considered for patients with compensated cirrhosis under careful monitoring [14]. Thus, the coverage of NA was indefinite for patients with cirrhosis but histological proof or clinical manifestation of portal hypertension (splenomegaly or oesophagogastric varices) was required.

2.4 | Study Outcomes and Observation After NA Cessation

The primary outcome was seroclearance of HBsAg after NA discontinuation. The observation commenced after the day of treatment cessation, set as the baseline of the study population, and

was censored at death, loss to follow-up or antiviral retreatment. The censoring by death and retreatment were considered to be informative because they could compete against the chance of HBsAg seroclearance.

After the cessation of NA, patients were followed up monthly in principle for the first 3 months and generally every 3–6 months thereafter if the conditions remained stable per the local standards [3]. The reimbursement criteria for retreatment closely resembled those for initial treatment under Taiwan's national health insurance. Accordingly, patients did not immediately resume NA for relapses of viremia or elevations of serum ALT that did not manifest with liver insufficiency (serum total bilirubin > 2 mg/dL or prolonged prothrombin time > 3 s) or persist for 3 months or longer, irrespective of the HBV DNA and ALT levels [7, 26].

2.5 | Statistical Analyses

Descriptive results for categorical and continuous variables were presented as exact numbers with percentages and medians with interquartile ranges (IQRs), respectively. For variables with missing data, multiple imputation was carried out with the assumption of random occurrence. Only pretreatment HBV DNA required imputation and the values were imputed by the fully conditional specification method using gender, pretreatment age, HBeAg, anti-HBe, diabetes mellitus and hypertension [27, 28].

The cumulative incidences of HBsAg seroclearance following NA cessation were estimated by the method developed by Grey to account for death and retreatment as competing risk events [29]. The Fine-Grey sub-distribution hazard model was employed to explore factors associated with HBsAg seroclearance with ALT levels after NA cessation analysed as a time-varying variable. The multivariable model was developed with backward selection of variables based on the Akaike information criterion [30].

As a sensitivity test, retreatment was not considered as a competing event or a censoring event, respectively, for HBsAg seroclearance. The Kaplan–Meier method and the Cox proportional hazard model were thus applied to estimate the incidence of HBsAg seroclearance and its associated risk factors, respectively. Besides, the association between ALT elevation and HBsAg seroclearance was examined using different ALT thresholds (5 times ULN, 2 times ULN and 1 time ULN, with the ULN set at 40 U/L according to the Asian-Pacific guidelines) [3]. Patients with cirrhosis were included in the main analysis, and a sensitivity analysis was additionally performed excluding them. We also conducted a subgroup analysis for patients with quantitative data for serum HBsAg at treatment cessation. Serum HBsAg was quantified by the automated microparticle immunoassay (automatic detection range: 0.05–250 IU/mL), and samples with HBsAg levels higher than the upper limits of automatic detection were manually diluted according to physician's preference. As previous studies have suggested, EOT HBsAg levels could be used as different cutoff values for clinical practice after NA cessation [31, 32]. Thus, the EOT HBsAg levels were analysed as categorical variables (< 10 IU/mL, 10–100 IU/mL, and > 100 IU/mL).

Point estimates were reported along with 95% confidence intervals (CIs). All statistical examinations were two-sided, and p-values < 0.05 were defined as statistically significant. All statistical analyses were performed using the commercially available software SAS (version 9.4, SAS Institute, Cary, NC, USA) and R programming language (version 4.3.1).

3 | Results

3.1 | Characteristics of the Study Population

We screened 6236 CHB patients who had received antiviral therapy between April 1, 2004 and May 24, 2022 and identified a total of 841 eligible patients (Figure 1). This study included 628 male (74.7%) and 213 female (25.3%) patients, with a median age of 53.2 (IQR, 44.3–61.7) years. Most patients ($n = 536$, 63.7%) received entecavir, and only two were treated with tenofovir alafenamide. The median duration of antiviral therapy was 34.7 months (IQR, 31.0–38.0). At treatment initiation, 148 (17.6%) patients had a clinical diagnosis of cirrhosis. The median serum levels of ALT and HBV DNA were 107 (IQR, 54–223) U/L and 5.3 (IQR, 3.6–6.8) log IU/mL, respectively (Table 1). After treatment cessation, 284 patients restarted NA therapy, with a 10-year cumulative incidence of 52.2% (95% CI, 46.1%–58.5%) for retreatment (Figure S1).

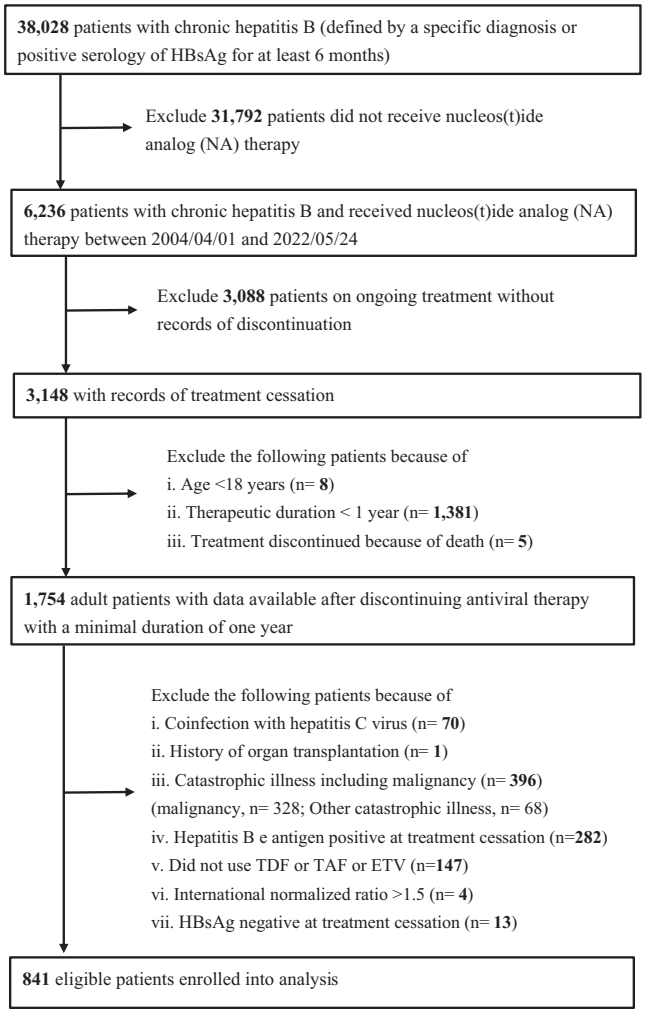

FIGURE 1 | The flow diagram for patient screening and identification of the study cohort.

TABLE 1 | Characteristics of the study cohort.

Characteristics	All ($N=841$)
Biological sex	
Female sex, *n* (%)	213 (25.3)
Male sex, *n* (%)	628 (74.7)
Age, years	53.2 (44.3, 61.7)
Diabetes mellitus, *n* (%)	178 (21.2)
Hypertension, *n* (%)	216 (25.7)
Dyslipidemia, *n* (%)	154 (18.3)
AST, U/L	28 (23, 38)
ALT, U/L	26 (19, 38)
Bilirubin, mg/dL	1.1 (0.8, 1.5)
Creatinine, mg/dL	1.1 (0.9, 1.2)
Prothrombin time, second	10.7 (10.3, 11.4)
International normalised ratio	1.0 (1.0, 1.1)
Antiviral regimen	
Entecavir, *n* (%)	536 (63.7)
Tenofovir disoproxil fumarate, *n* (%)	303 (36.0)
Tenofovir alafenamide, *n* (%)	2 (0.2)
Duration on therapy, months	34.7 (31.0, 38.0)
Pretreatment cirrhosis[a], *n* (%)	148 (17.6)
Pretreatment positive HBeAg, *n* (%)	130 (17.0)
Pretreatment positive anti-HBe, *n* (%)	575 (84.1)
Pretreatment HBV DNA, log IU/mL	5.3 (3.6, 6.8)
Pretreatment AST, U/L	76 (49, 146)
Pretreatment ALT, U/L	107 (54, 223)

Note: The characteristics were summarised at treatment cessation unless the pretreatment status was specified and were expressed as number (percentage) or median (interquartile range).
Abbreviations: ALT, alanine transaminase; anti-HBe, hepatitis B e antibody; AST, aspartate transaminase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.
[a]Cirrhosis was defined mainly by clinical diagnosis based on liver images.

3.2 | HBsAg Seroclearance After NA Cessation in Association With ALT Flares

During a median post-treatment follow-up of 3.7 years (IQR, 1.4–6.3), 38 patients cleared HBsAg in serum. The cumulative incidence was 12.4% (95% CI, 8.2%–16.1%) at 10 years (Figure 2). If observation was not censored by retreatment (Figure S2A), the 10-year cumulative incidence would be 27.8% (95% CI, 19.1%–39.4%). If retreatment was analysed as a non-informative-censoring event (Figure S2B), the estimates would be 21.6% (95% CI, 14.2%–32.1%).

In total, 488 and 314 patients experienced ALT elevation to one time ULN and two times ULN, respectively, after discontinuing NA treatment. ALT flares (serum ALT level above five times ULN) occurred in 175 patients after NA cessation. Among them, 6 patients (3.4%) subsequently cleared HBsAg. In contrast, 32 out of 666 patients (4.8%) without ALT flares achieved HBsAg seroclearance. The peak serum ALT level post NA cessation was significantly lower in patients who achieved HBsAg seroclearance (median, 93 U/L; IQR, 43–136) compared to those who did not (median, 127 U/L; IQR, 47–341; $p<0.001$; Figure 3). Four patients with ALT flares progressed to acute-on-chronic liver failure and one of them subsequently died. No patient underwent liver transplantation.

3.3 | Significant Associated Factors for HBsAg Seroclearance

In the univariable analysis (Table 2), occurrence of ALT flare during follow-up was inversely associated with HBsAg seroclearance. Other univariably significant factors included serum levels of pretreatment HBV DNA, creatinine, bilirubin and antiviral regimen using tenofovir versus entecavir.

In the multivariable-adjusted analysis (Table 2), pretreatment HBV DNA was identified as an independent factor inversely associated with HBsAg seroclearance (adjusted sub-distribution hazard ratio [SHR], 0.69 per log IU/mL; 95% CI, 0.54–0.88; $p=0.003$).

There was no significant association between hepatitis flares after NA cessation and HBsAg seroclearance after adjustment for pretreatment HBV DNA (adjusted SHR, 0.42; 95% CI, 0.09–2.01; $p=0.28$).

3.4 | Sensitivity Analyses With Alternative Approaches for Retreatment, Different Thresholds for ALT Elevation and Patients Without Cirrhosis

In the sensitivity tests not considering retreatment as a competing risk event (Table S1), the results were consistent with the main analysis: ALT flares were not associated with HBsAg seroclearance in either the model (model 1) with retreatment not censoring the observation (adjusted HR, 0.55; 95% CI, 0.12–2.60; $p=0.45$), or the model (model 2) analysing retreatment as a non-informative-censoring event (adjusted HR, 1.15; 95% CI, 0.24–5.48; $p=0.86$), respectively.

Similarly, ALT elevation after NA cessation was not associated with HBsAg seroclearance in the sensitivity tests with ALT cutoff set at two times ULN (adjusted SHR, 0.55; 95% CI, 0.19–1.58; $p=0.27$) or simply the ULN (adjusted SHR, 0.49; 95% CI, 0.19–1.29; $p=0.15$) (Table S2).

In the sensitivity tests of patients without cirrhosis, hepatitis flares after NA cessation were not associated with HBsAg seroclearance (adjusted SHR, 0.51; 95% CI, 0.10–2.68; $p=0.43$) after adjusting for pretreatment HBV DNA (Table S3).

3.5 | Subgroup Analysis for Patients With Data of Serum HBsAg Levels at End of Treatment

Quantitative HBsAg data at EOT were available in 390 patients. Their characteristics were similar to the entire study

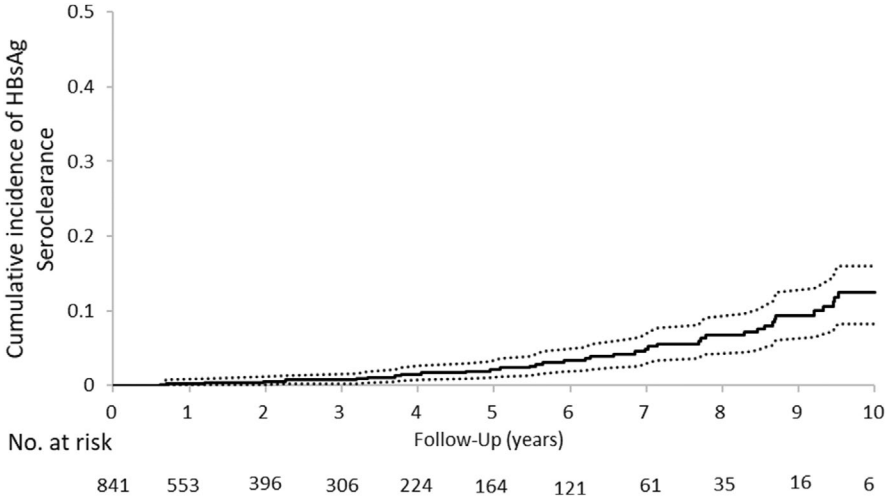

FIGURE 2 | The cumulative incidence of HBsAg seroclearance following discontinuation of nucleos(t)ide analog in the study population. The dotted curves indicated the range of 95% confidence intervals.

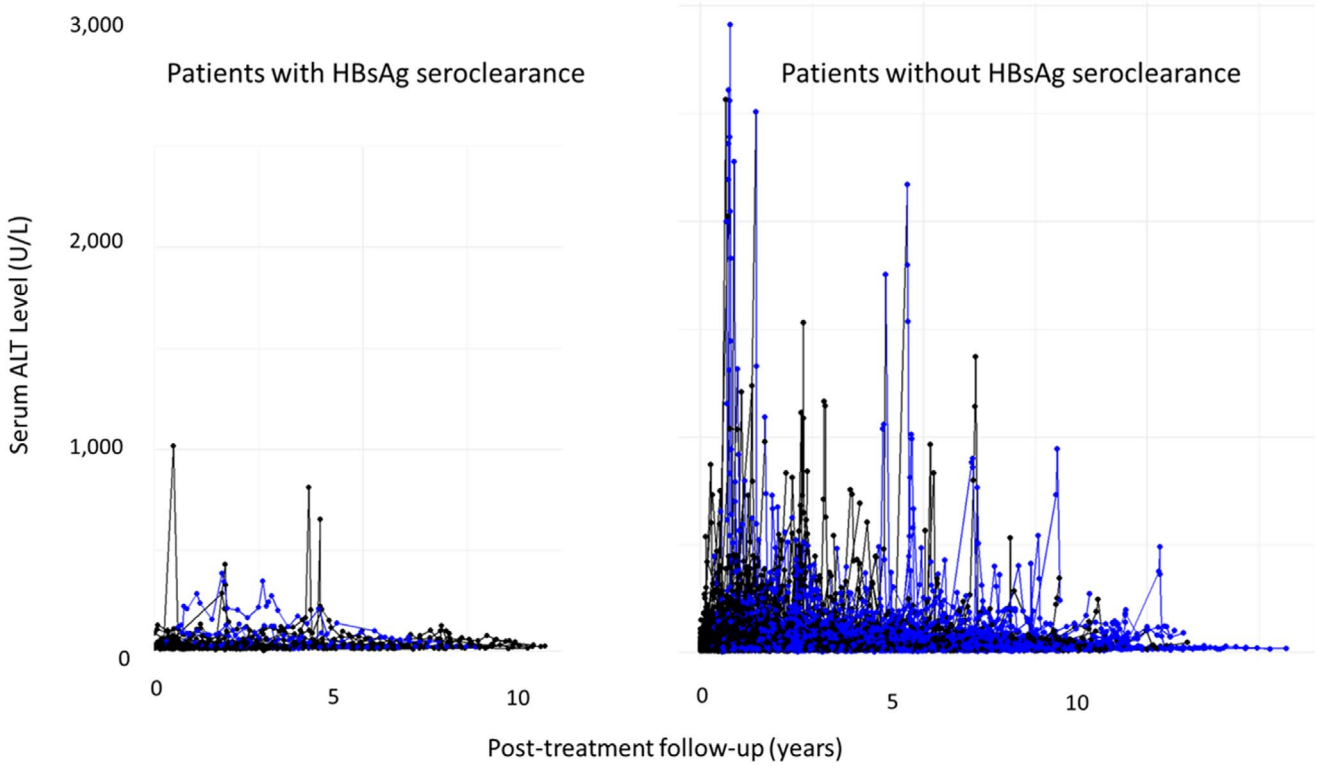

FIGURE 3 | The trajectories of serum ALT levels in patients with and without HBsAg seroclearance following nucleos(t)ide analog cessation (patients who resumed treatment were indicated in blue).

population (Table 3). The 10-year cumulative incidence of HBsAg seroclearance in this subgroup was similarly 12.7% (95% CI, 6.1%–17.9%), with 16 patients clearing HBsAg during follow-up (Figure 4).

Hepatitis flare after NA cessation was not associated with HBsAg seroclearance in the subgroup analysis. The associated factors for HBsAg seroclearance in the univariable analysis included the serum levels of EOT HBsAg, pretreatment HBV DNA and pretreatment aspartate aminotransferase (AST) (Table 4).

The EOT HBsAg level was the only significant factor remaining in the multivariable analysis after variable selection (Table 4), with a higher level associated with a lower incidence of HBsAg seroclearance. Compared to patients with an EOT HBsAg level > 100 IU/mL, those with the HBsAg level between 10 IU/mL and 100 IU/mL (adjusted SHR, 5.39; 95% CI, 1.56–18.59; $p = 0.008$) or HBsAg level < 10 IU/mL (adjusted SHR, 16.21; 95% CI, 4.61–57.10; $p < 0.001$) at EOT had a significantly higher chance of HBsAg seroclearance. There was no association between ALT flare and HBsAg seroclearance (adjusted SHR, 0.90; 95% CI, 0.23–3.57; $p = 0.88$) in the model adjusted for the EOT HBsAg level (Table 4).

TABLE 2 | Time-dependent sub-distribution hazard model for factors associated with HBsAg seroclearance.

	Univariable analysis			Multivariable analysis		
	SHR	**95% CI**	***p***	**Adjusted SHR**	**95% CI**	***p***
Time-varying clinical flare (ALT > 200 U/L)	0.31	0.13–0.73	0.007	0.42	0.09–2.01	0.28
Age, year	0.98	0.95–1.00	0.09			
Male sex	1.34	0.59–3.04	0.49			
Diabetes mellitus	0.48	0.17–1.35	0.17			
Hypertension	0.76	0.34–1.70	0.50			
Dyslipidemia	1.15	0.51–2.60	0.74			
AST, 100 U/L	1.43	0.88–2.33	0.15			
ALT, 100 U/L	1.45	0.75–2.78	0.27			
Bilirubin, mg/dL	1.47	1.15–1.87	0.002			
Creatinine, mg/dL	0.11	0.04–0.34	< 0.001			
Prothrombin time, second	1.69	0.85–3.35	0.14			
Antiviral regimen						
Entecavir (reference)	1.00	—	—			
Tenofovir	1.47	1.14–1.90	0.003			
Duration on therapy, month	1.01	1.00–1.02	0.13			
Pretreatment cirrhosis	1.20	0.53–2.72	0.66			
Pretreatment positive HBeAg	1.09	0.47–2.50	0.84			
Pretreatment positive anti-HBe	0.55	0.24–1.25	0.15			
Pretreatment HBV DNA, log IU/mL	0.66	0.52–0.83	0.001	0.69	0.54–0.88	0.003
Pretreatment AST, 100 U/L	1.01	0.92–1.10	0.82			
Pretreatment ALT, 100 U/L	1.03	0.98–1.09	0.29			

Note: Measured at treatment cessation unless otherwise specified as 'pretreatment'.
Abbreviations: ALT, alanine transaminase; anti-HBe, hepatitis B e antibody; AST, aspartate transaminase; CI, confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; SHR, sub-distributional hazard ratio.

In patients without cirrhosis (Table S4), ALT flares after NA cessation were also not associated with HBsAg seroclearance after adjustment for EOT HBsAg levels (adjusted SHR, 0.91; 95% CI, 0.23–3.65; $p = 0.89$). Similarly, a higher EOT HBsAg level was associated with a significantly lower chance of HBsAg seroclearance.

4 | Discussion

This multicenter cohort study involving 841 consecutively treated patients who stopped entecavir or tenofovir generally in accordance with current Asian-Pacific criteria demonstrated that ALT elevation after NA cessation was not associated with HBsAg seroclearance. Patients who achieved HBsAg seroclearance had significantly lower peak ALT levels during follow-up compared to those who did not achieve seroclearance. In fact, ALT flares were inversely associated with the incidence of HBsAg seroclearance in the univariable analysis. However, after adjusting for pretreatment HBV DNA levels or EOT HBsAg levels, no significant associations were found between ALT elevations after NA cessation and HBsAg seroclearance. These findings were consistently observed in sensitivity tests that employed various analytical approaches to account for the potential influences of retreatment, different ALT cutoffs and study populations with cirrhosis or not. Further analysis of patients with quantitative EOT HBsAg levels confirmed that a lower EOT HBsAg level was significantly associated with a lower incidence of HBsAg seroclearance. Our study suggests that cytolytic pathways are not essential for HBsAg seroclearance, and discontinuing NA treatment with an HBsAg level > 100 IU/mL appears inadvisable for Asian patients. These results may not only guide clinical decision-making regarding the practice of finite NA therapy but also inform the development of novel strategies aimed at achieving a functional cure for CHB.

Current literature presents conflicting findings regarding the association between ALT elevation after NA cessation and subsequent HBsAg seroclearance in patients with CHB. In a large prospective study by Jeng et al. the authors found that untreated HBeAg-negative CHB patients who experienced

TABLE 3 | Characteristics of the patient subgroup with quantitative HBsAg data at treatment cessation.

Characteristics	All ($N = 390$)
Biological sex	
Female sex, *n* (%)	112 (28.7)
Male sex, *n* (%)	278 (71.3)
Age, years	52.2 (43.5, 61.7)
Diabetes mellitus, *n* (%)	71 (18.2)
Hypertension, *n* (%)	93 (23.9)
Dyslipidemia, *n* (%)	72 (18.5)
HBsAg level, IU/mL	
< 10	27 (6.9)
10–100	64 (16.4)
> 100	299 (76.7)
AST, U/L	27 (23, 34)
ALT, U/L	26 (18, 37)
Bilirubin, mg/dL	1.0 (0.8, 1.4)
Creatinine, mg/dL	1.1 (0.9, 1.2)
Prothrombin time, second	10.4 (10.3, 10.8)
International normalised ratio	1.0 (1.0, 1.1)
Antiviral regimen	
Entecavir, *n* (%)	241 (61.8)
Tenofovir, *n* (%)	148 (38.0)
Tenofovir alafenamide, *n* (%)	1 (0.2)
Duration on therapy, month	35.0 (32.0, 39.2)
Pretreatment cirrhosis[a], *n* (%)	47 (12.1)
Pretreatment positive HBeAg, *n* (%)	75 (21.1)
Pretreatment positive anti-HBe, *n* (%)	263 (80.9)
Pretreatment HBV DNA, log IU/mL	5.6 (3.8, 7.0)
Pretreatment AST, U/L	72 (46, 148)
Pretreatment ALT, U/L	109 (55, 230)

Note: The characteristics were summarised at treatment cessation unless the pretreatment status was specified and were expressed as number (percentage) or median (interquartile range).
Abbreviations: ALT, alanine transaminase; anti-HBe, hepatitis B e antibody; AST, aspartate transaminase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.
[a]Cirrhosis was defined mainly by clinical diagnosis based on liver images.

clinical relapse after NA cessation had a higher rate of HBsAg seroclearance compared to those who were retreated [15]. They proposed that transient ALT elevation was beneficial for decreasing viral load and HBsAg levels, possibly due to enhanced immune control. This observation has led to the hypothesis that withdrawal flares could be 'good' and should not be retreated [24]. Nevertheless, the results might be confounded by indication bias, as patients were retrospectively grouped based on whether they were ultimately retreated or not. It is conceivable that patients whose disease severity progressed would not remain untreated. Furthermore, ALT levels after NA cessation tend to fluctuate widely over time, making it difficult to be adequately analysed as a categorical variable fixed at a specific time point.

Our findings corroborated the randomised controlled trial by van Bömmel et al., which observed 10.1% HBsAg loss during 96 weeks after randomised NA cessation, with similar rates in patients with and without ALT flares. Moreover, they found higher ALT elevation in patients who did not clear HBsAg and required retreatment than in those with HBsAg loss [16]. Similarly, Papatheodoridi et al. also reported less frequent ALT flares in patients with HBsAg seroclearance [33]. These previous studies, however, were small in sample size and the statistical power was further limited by few events of HBsAg seroclearance.

In addition to leveraging data from a substantial study population, the time-varying analyses in the current study enabled us to demonstrate that the incidence of HBsAg seroclearance was not related to the occurrence ALT flares. Additionally, it was noted that patients who achieved HBsAg seroclearance had significantly lower peak serum ALT levels during the follow-up after NA cessation. Consistent results from sensitivity analyses further supported the notion that an elevation in serum ALT is not essential for HBsAg seroclearance after NA cessation. Accordingly, treatment cessation is not advisable for patients at risk of acute hepatitis flares, which can rapidly and unpredictably progress into acute-on-chronic liver failure [26, 34].

Previous studies have reported a correlation between HBsAg concentration and HBV DNA level in patients with CHB, both before and during the monitoring of the efficacy of treatment [35–37]. Our study revealed an inverse association between the pretreatment HBV DNA level and HBsAg seroclearance after multivariable adjustment. Therefore, our subgroup analysis validated prior research on the superior performance of EOT HBsAg levels in predicting HBsAg seroclearance [31, 38, 39] and aligned with the known association between HBsAg concentration and HBV DNA level. These findings suggested that risk prediction was achievable at treatment cessation without the need to wait for the occurrence of ALT flares. Notably, it has been proposed that the kinetics of serum HBV DNA and HBsAg levels during withdrawal flares are useful to distinguish candidates for finite NA therapy and patients requiring retreatment [40]. However, the precision of the proposed rule in predicting the outcomes of a flare episode is unclear and has not been vigorously examined by empirical data. Given that the predictive performance of EOT HBsAg level was not affected by changes in serum ALT after NA cessation, our findings do not support reliance on the occurrence of withdrawal flares to select patients who can stop treatment. In fact, our previous studies, together with others, have shown the feasibility of identifying patients both with a higher chance of HBsAg seroclearance and at a lower risk of clinical hepatitis post NA [41, 42].

Our conclusion was further consolidated by different approaches to consider the impact of retreatment on estimating the incidence of HBsAg seroclearance after NA cessation. In our primary

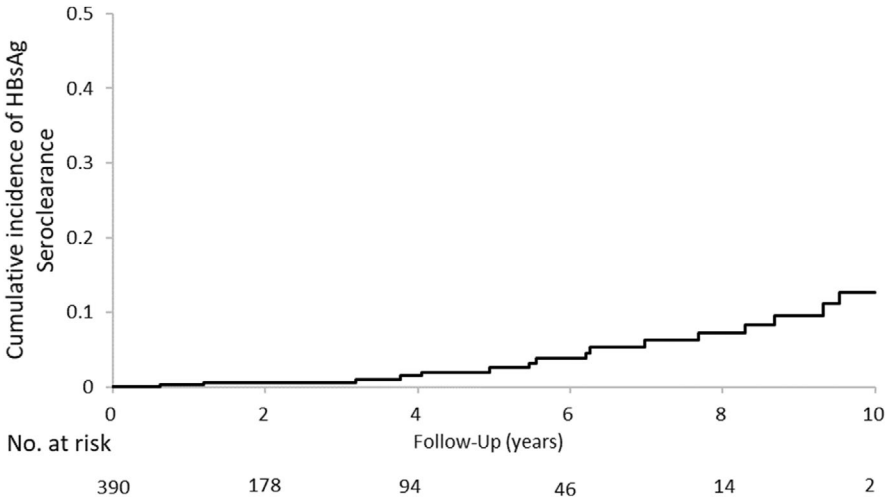

FIGURE 4 | The cumulative incidence of HBsAg seroclearance following discontinuation of nucleos(t)ide analogs in patients with quantitative HBsAg levels at end of treatment.

TABLE 4 | Time-dependent sub-distribution hazard model for factors associated with HBsAg seroclearance in the subgroup of 390 patients with quantitative HBsAg data available at treatment cessation.

	Univariable analysis			Multivariable analysis		
	SHR	**95% CI**	***p***	**Adjusted SHR**	**95% CI**	***p***
Time-varying clinical flare(ALT > 200 U/L)	0.42	0.12–1.50	0.18	0.90	0.23–3.57	0.88
Age, year	0.98	0.95–1.02	0.33			
Male sex	0.94	0.31–2.89	0.91			
Diabetes mellitus	0.82	0.19–3.55	0.79			
Hypertension	0.25	0.03–1.85	0.18			
Dyslipidemia	1.19	0.35–4.11	0.78			
AST, 100 U/L	2.23	0.90–5.54	0.09			
ALT, 100 U/L	3.31	0.80–13.7	0.10			
Bilirubin, mg/dL	1.67	0.86–3.20	0.13			
Creatinine, mg/dL	0.16	0.03–1.04	0.06			
Prothrombin time, second	1.02	0.68–1.52	0.93			
EOT HBsAg level						
HBsAg > 100 IU/mL (Reference)	1.00	—	—	1.00	—	—
10 IU/mL ≤ HBsAg ≤ 100 IU/mL	5.55	1.67–18.43	0.005	5.39	1.56–18.59	0.008
HBsAg < 10 IU/mL	16.8	5.46–51.84	< 0.001	16.21	4.61–57.10	< 0.001
Antiviral regimen						
Entecavir (reference)	1.00	—	—		—	—
Tenofovir	1.45	0.98–2.13	0.06			
Duration on therapy, month	1.01	0.99–1.04	0.18			
Pretreatment cirrhosis	1.27	0.32–5.07	0.73			
Pretreatment liver insufficiency	1.20	0.37–3.88	0.76			
Pretreatment positive HBeAg	0.81	0.23–2.95	0.75			
Pretreatment positive anti-HBe	0.92	0.25–3.41	0.91			
Pretreatment HBV DNA, log IU/mL	0.54	0.38–0.77	< 0.001			
Pretreatment AST, 100 U/L	0.55	0.32–0.97	0.04			
Pretreatment ALT, 100 U/L	0.84	0.68–1.05	0.12			

Note: Measured at treatment cessation unless otherwise specified as 'pretreatment'.
Abbreviations: ALT, alanine transaminase; anti-HBe, hepatitis B e antibody; AST, aspartate transaminase; CI, confidence interval; EOT, end of treatment; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; SHR, sub-distributional hazard ratio.

analysis, the event of retreatment was treated as an informative censoring point. This is because the risk of hepatitis and the probability of HBsAg seroclearance after NA cessation are not mutually independent [38]. Hence, employing non-informative censoring for retreatment events, such as with Kaplan–Meier estimators, could lead to an overestimation of the HBsAg seroclearance incidence [43]. As also shown in the current study, competing risk analysis may offer a more accurate approach to estimate the incidence of HBsAg seroclearance after NA cessation.

This real-world study has some limitations. First, the exploration of the association between novel biomarkers and clinical outcomes was constrained. For instance, quantitative EOT HBsAg data were only available for 390 patients, reflecting its recent integration into routine clinical practice. Although dynamics and precise quantification of serum HBsAg levels would be highly informative, the deficiency of such data was reasonably anticipated in the retrospective study, and further prospective studies are required. Second, variations in patient management could arise due to individual physician preferences or patient decisions. However, the indications for antiviral therapy, including criteria for retreatment, was largely standardised through regulations enforced by the national health insurance system in Taiwan. This policy framework likely reduced the impact of such variability. Finally, only Asian patients were enrolled. Therefore, extrapolating these findings to populations in other regions should be approached with caution, given potential differences in patient ethnicity, viral genotype or modes of transmission.

In summary, this multicenter cohort study involved 841 consecutive patients who stopped entecavir or tenofovir treatment according to the Asian-Pacific standards and found no association between ALT elevation after NA cessation and HBsAg seroclearance, contrary to earlier hypotheses that ALT elevation induced by treatment withdrawal might facilitate HBsAg seroclearance. These findings may inform the risk–benefit assessment for individuals considering finite NA therapy and suggest that patients at risk of withdrawal flares should avoid treatment cessation. Besides, our findings suggest that selecting candidates for finite treatment should not rely on distinguishing ALT flares. These novel findings may contribute to the ongoing discourse on optimal management strategies for CHB, particularly in the context of finite antiviral therapy and the pursuit of functional cure as the treatment endpoint.

Author Contributions

Ying-Nan Tsai: conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, resources, validation, supervision, visualization, writing – original draft, writing – review and editing. **Jia-Ling Wu:** data curation, formal analysis, software. **Cheng-Hao Tseng:** data curation, formal analysis. **Shang-Chen Tseng:** data curation, formal analysis. **Chih-Lung Hung:** data curation. **Mindie H. Nguyen:** writing – review and editing. **Jaw-Town Lin:** data curation. **Yao-Chun Hsu:** data curation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualization, writing – review and editing, writing – original draft.

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Conflicts of Interest

Ying-Nan Tsai reported no conflicts of interest. Jia-Ling Wu reported no conflicts of interests. Cheng-Hao Tseng has received lecture fees from Abbvie, Bristol-Myers Squibb, Gilead Sciences, Bayer, and Roche. Shang-Chen Tseng reported no conflicts of interests. Chih-Lung Hung reported no conflicts of interests. Mindie H. Nguyen received Research support: Pfizer, Enanta, Astra Zeneca, Glycotest, GSK, Delfi, Innogen, Exact Science, CurveBio, Gilead, Helio Health, National Institute of Health, Roche. Consulting and/or Advisory Board: GSK, Exelixis. Jaw-Town Lin reported no conflicts of interests. Yao-Chun Hsu has received research grants from Gilead Sciences, lecture fees from Abbvie, Bristol-Myers Squibb, Gilead Sciences, Grifols, and Roche and has served as an advisory committee member for Gilead Sciences and Sysmex.

Data Availability Statement

The authors have nothing to report.

Authorship

Guarantor of the Article: Ying-Nan Tsai and Yao-Chun Hsu.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.