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停止類核苷(酸)藥物治療後急性肝炎發作與 B 型肝炎表面抗原血清清除發生率的關聯

Association of Acute Hepatitis Flares with Incidence of HBsAg Seroclearance after Cessation of Nucleos(t)ide Analogues

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Association of Acute Hepatitis Flares with Incidence of HBsAg Seroclearance after Cessation of Nucleos(t)ide Analogues

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中文摘要

背景：患有慢性 B 型肝炎病毒感染的病人在停止使用核苷(酸)類似物治療後經常會經歷急性肝炎發作。這些肝炎發作可能會導致肝功能衰竭，但也被認為可以促進 B 型肝炎表面抗原(HBsAg)的血清清除，這是停止使用核苷(酸)類似物的主要理由之一。我們的目的是評估和釐清在慢性 B 型肝炎患者中，核苷(酸)類似物停藥後急性肝炎發作與隨後的 HBsAg 血清清除之間的關聯。

目的：釐清慢性 B 型肝炎患者停止類核苷(酸)藥物治療後若發作急性肝炎，是否會影響 B 型肝炎表面抗原血清清除發生率

方法：這是一項多中心的回溯世代研究，我們將系統性分析義大醫療體系中接受核苷(酸)類似物治療的所有慢性 B 型肝炎患者。我們將納入在 2004 年 4 月 1 日至 2022 年 5 月 24 日之間停止核苷(酸)類似物藥物，且在停藥前至少已經連續治療一年的成人，惡性腫瘤、肝功能不全(經由黃疸和凝血功能障礙定義)或其他病毒共感染的患者將被排除。急性肝炎發作的定義是血清丙氨酸轉氨酶(ALT)上升超過正常上限(ULN; 40 U/L)5 倍，主要研究結果事件是核苷(酸)類似物停藥期間的 HBsAg 血清清除發生率。我們將使用競爭風險分析估計 HBsAg 血清清除的發生率，並考慮死亡或再次治療作為非隨機的資料設限，對結果估計的影響，並且在多變項調整的次分佈風險回歸(sub-distribution hazard)模型中，將急性 ALT 發

作作為一個隨時間而變動的變數。

結果：本研究納入了 850 位患者（中位年齡 53.2 歲；男性 74.5%；使用 entecavir 63.7%），這些患者接受治療的中位期為 34.7 個月。在中位隨訪期 3.7 年內，有 47 位患者出現 HBsAg 清除。年度清除率為 1.37%，10 年累積發生率為 13.2%。在 175 位經歷急性肝炎發作的患者中，定義為血清 ALT > 正常上限 5 倍，其中 6 位（3.43%）隨後清除了 HBsAg。相反地，675 位沒有急性肝炎發作的患者中有 41 位（6.07%）達到了 HBsAg 清除（P = 0.0014，修正的對數秩檢驗）。然而，在根據治療結束時的血清 HBsAg 濃度而調整的隨時變化多變數次群分析中，血清 ALT 升高並不獨立於 HBsAg 清除相關。這些結果在將血清 ALT 升高水平設定為正常上限值兩倍或一倍的敏感性分析中保持一致。

結論：B 肝抗病毒治療停藥後 ALT 升高與隨後的 HBsAg 清除無關，根據此發現，對於具有停藥後急性肝炎發作風險的患者，不應停止抗病毒治療。

關鍵詞：慢性 B 性肝炎；核苷(酸)類似物抗病毒治療；有限療程；B 型肝炎血清表面抗原清除

ABSTRACT

Background & Aims: Patients with chronic hepatitis B (CHB) frequently experience hepatitis after nucleos(t)ide analog (NA) cessation. We aimed to clarify the association between aminotransferase (ALT) elevation and HBsAg seroclearance following withdrawal of NA.

Methods: This retrospective, multi-center cohort study systematically reviewed patients who discontinued entecavir or tenofovir between April 01, 2004 and May 24, 2022 in the E-Da healthcare system. We estimated the incidence of HBsAg seroclearance using a competing risk analysis to account for the informative censoring by retreatment and developed time-dependent models to investigate the association between posttreatment ALT elevation and HBsAg seroclearance.

Results: We included 850 patients (median age of 53.2 years; 74.5% male; 63.7% entecavir) who had been treated for a median duration of 34.7 months. During a median follow-up of 3.7 years, HBsAg seroclearance occurred in 47 patients. The annual rate was 1.37%, with a cumulative incidence of 13.2% at 10 years. Of the 175 patients experienced ALT flares, defined by serum ALT > five times upper limit of normal (ULN), 6 (3.43%) subsequently cleared HBsAg. Conversely, 41 of 675 patients

(6.07%) without ALT flares achieved HBsAg seroclearance (P = 0.0014, modified log-rank test). In the time-varying multivariable analysis adjusted for serum HBsAg level at the end of treatment, however, the elevation of serum ALT was not independently associated with HBsAg seroclearance. The results were consistent in sensitivity analyses with the level of serum ALT elevation set at two times ULN or ULN.

Conclusions: ALT elevation following NA withdrawal is not associated with subsequent HBsAg seroclearance. Our findings suggest NA cessation inadvisable for patients at risk of posttreatment flares.

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 analogs (NAs) have 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. Currently, 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 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 variable 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, posttreatment elevation of serum ALT was hypothesized 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 systematically reviewed all patients who had stopped NA regimens in this multicenter cohort study to investigate whether posttreatment ALT elevation was associated with HBsAg seroclearance.

MATERIALS AND METHODS

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. 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).

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 one 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 normalized ratio >1.5), malignancy including HCC, or severe comorbidities certified by the registry of catastrophic illness.(25)

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 one 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. The coverage was indefinite for patients with cirrhosis but histological proof or clinical manifestation of portal hypertension (splenomegaly or esophagogastric varices) was required.

Study outcomes and posttreatment observation

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.

The reimbursement criteria for retreatment essentially resembled those for treatment initiation. 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 seconds) or persist for 3 months or longer, irrespective of the HBV DNA and ALT levels.

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 Gray to account for death and retreatment as competing risk events.(29) The Fine-Gray sub-distribution hazard model was employed to explore factors associated with HBsAg seroclearance with posttreatment ALT levels analyzed 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 were 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) We also conducted a subgroup analysis for patients with quantitative data for serum HBsAg at treatment cessation.

Point estimates were reported along with 95% confidence intervals (CIs). All statistical examinations were two sided and P-values less than 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).

RESULTS

Characteristics of the study population

We screened 6,236 CHB patients who had received antiviral therapy between April 1, 2004 and May 24, 2022 and identified a total of 850 eligible patients (Figure 1). This study included 633 male (74.5%) and 217 female (25.5%) patients, with a median age of 53.2 (IQR, 44.2-61.7) years. Most patients (n=541, 63.7%) received entecavir and only two were treated with tenofovir alafenamide. The median duration of antiviral therapy was 34.7 months (IQR, 30.9-38.4). At treatment initiation, 149 (17.5%) and 152 (17.9%) patients had a clinical diagnosis of cirrhosis and presentations suggesting hepatic insufficiency, respectively. The median serum levels of ALT and HBV DNA were 107 (IQR, 54-226) U/L and 5.3 (IQR, 3.6-6.8) IU/mL, respectively (Table 1). After treatment cessation, 284 patients restarted NA therapy, with a 10-year cumulative incidence of 73.7 % (95% CI, 67.4-79.6%) for retreatment (Supplementary Figure 1).

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), 47 patients cleared HBsAg in serum. The average annual rate was 1.37% (95% confidence interval

[CI], 1.01-1.83%), with a cumulative incidence of 13.2% (95% CI, 9.59-17.39%) at 10 years (Figure 2). If observation was not censored by retreatment (Supplementary Figure 2; left panel), the average annual rate would be 1.37% (95% CI, 1.01-1.83%) and the 10 year-cumulative incidence 29.28% (95% CI, 20.60-40.57%). If retreatment was analyzed as a non-informative-censoring event (Supplementary Figure 2; middle panel), the estimates would be 2.05% (95% CI, 1.51-2.73%) and 22.44 % (95% CI, 15.00-32.77%), respectively.

ALT flares (serum ALT level above five times ULN) occurred in 175 patients. Among them, 6 patients (3.43%) subsequently cleared HBsAg. In contrast, 41 out of 675 patients (6.07%) without ALT flares achieved HBsAg seroclearance. The peak serum ALT level post NA cessation was significantly lower in patients who achieved HBsAg seroclearance (median, 77 U/L; IQR, 42-136) compared to those who did not (median, 127 U/L; IQR, 47-341; $P < 0.0001$; Figure 3).

Factors associated with HBsAg seroclearance in the multivariable model

In the univariable analysis (Table 2), factors associated with HBsAg seroclearance were bilirubin level, thrombocytopenia (defined as a platelet count of less than 100,000/μL), tenofovir as compared to entecavir, and manifestation of liver insufficiency at treatment

initiation. Additionally, ALT flare during follow-up, creatinine level, pretreatment anti-HBe antibody positivity, and pretreatment HBV level were inversely associated with HBsAg seroclearance.

In the multivariable-adjusted analysis (Table 2), ALT flare during follow-up (sub-distribution hazard ratio [SHR], 0.28; 95% CI, 0.12-0.66; P=0.003) and age (adjusted SHR, 0.98 per year; 95% CI, 0.96-1.00; P=0.05) were identified as the independent risk factors for HBsAg seroclearance.

Sensitivity tests with alternative approaches for retreatment and different thresholds for ALT elevation

In the sensitivity tests not considering retreatment as a competing risk event (Supplementary Table 1), ALT flares were consistently associated with a significantly lower incidence of HBsAg seroclearance in the model with retreatment not censoring the observation (adjusted SHR, 0.29; 95% CI, 0.12-0.68; P=0.005). The association was not statistically significant when retreatment was analyzed as a non-informative-censoring event (adjusted SHR, 0.69; 95% CI, 0.29-1.62; P=0.39).

Similarly, posttreatment ALT elevation was inversely associated with HBsAg

seroclearance in the sensitivity tests with ALT cutoff set at 2 times ULN (adjusted SHR, 0.37; 95% CI, 0.21-0.67; P=0.001) and 1 time ULN (adjusted SHR, 0.51; 95% CI, 0.29-0.87; P=0.014) (Supplementary Table 2).

Subgroup analysis for patients with data of serum HBsAg levels at end of treatment

Quantitative HBsAg data at end of treatment (EOT) were available in 399 patients. The characteristics of this subgroup were similar to the entire study population (Table 3). During a median post-treatment follow-up of 3.3 years (IQR, 1.29-5.63), 25 patients cleared HBsAg in serum. The average annual rate was 1.73% (95% CI, 1.12-2.56%), with a cumulative incidence of 15.91% (95% CI, 8.94-21.20%) at 10 years (Figure 4).

In the univariable analysis, HBsAg seroclearance was associated with white blood cell count, EOT HBsAg, use of tenofovir, ALT flare, creatinine level, and pretreatment HBV DNA level (Table 4). EOT HBsAg level was the only significant factor remaining in the multivariable analysis after variable selection, with a higher level associated with a lower incidence of HBsAg seroclearance. The association between ALT flare and HBsAg seroclearance was not significant (adjusted SHR, 0.83; 95% CI, 0.25-2.70; P=0.752) after adjustment for the EOT HBsAg level (Table 4).

DISCUSSION

This multicenter cohort study revealed an inverse association between posttreatment ALT elevation and HBsAg seroclearance in 850 consecutively treated patients who stopped entecavir or tenofovir according to current Asian-Pacific criteria. The peak serum ALT level during the follow-up was significantly lower in patients who achieved HBsAg seroclearance compared to those who did not. Moreover, posttreatment ALT elevation was associated with a lower incidence of HBsAg seroclearance, a finding consistent across various analytical approaches accounting for potential influence of retreatment and different ALT cutoffs. Additionally, the subgroup analysis showed that EOT HBsAg level was deterministic of HBsAg seroclearance, irrespective of serum ALT elevation post treatment cessation. Our findings indicate that posttreatment ALT elevation is not conducive to HBsAg seroclearance. Therefore, stopping treatment cannot be advisable for patients at risk of withdrawal flares.

Current literature presents conflicting findings regarding the association between posttreatment ALT elevation 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 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 some withdrawal flares are “good” and should not be retreated.(24) Nevertheless, the results could 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, posttreatment ALT levels tend to fluctuate widely over time, making it difficult to be adequately analyzed as a categorical variable fixed at a specific time point.

Our findings corroborated the randomized controlled trial by van Bömmel et al., which observed 10.1% HBsAg loss during 96 weeks after randomized NA cessation, with similar rates in patients with and without ALT flares. Moreover, they found higher ALT elevation in patients without HBsAg loss requiring retreatment than in those with HBsAg loss. (16) Similarly, Papatheodoridi et al. also reported less frequent ALT flares in patients with HBsAg seroclearance.(31) 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, time-varying analyses

in the current study enabled us to demonstrate that the incidence of HBsAg seroclearance significantly decreased after ALT flares occurred. Additionally, it was noted that patients who achieved HBsAg seroclearance had significantly lower peak serum ALT levels during the posttreatment follow-up. Consistent results from sensitivity analyses further supported the notion that an elevation in serum ALT is not essential for HBsAg seroclearance posttreatment. Indeed, individuals experiencing acute flares post-NA cessation were found to have a reduced likelihood of HBsAg seroclearance in comparison to those who remained in clinical remission. 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, 32)

Our subgroup analysis not only validated prior research on the performance of EOT HBsAg levels in predicting HBsAg seroclearance,(33-35) but also suggested that risk prediction was achievable at treatment cessation without the need to wait for the occurrence of ALT flares. 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.(36) However, the precision of the proposed rule in predicting the outcomes of a flare episode is unclear, and any inaccurate prediction in the face of acute flares can mean the difference between life and death.

Given that the predictive performance of EOT HBsAg level was not affected by post-treatment changes in serum ALT, our findings suggest no reason to rely 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.(37, 38)

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 analysis, the event of retreatment was treated as an informative censoring point. This is because the risk of posttreatment hepatitis and the probability of HBsAg seroclearance are not mutually independent. (39) Hence, employing non-informative censoring for retreatment events, such as with Kaplan-Meier estimators, may lead to an overestimation of the HBsAg seroclearance incidence. (40) 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 was only available for 399 patients, , reflecting its recent integration into routine clinical practice. 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 standardized 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 850 patients who stopped entecavir or tenofovir treatment according to the Asian-Pacific standards and found an inverse association between posttreatment ALT elevation 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. Our subgroup analysis further demonstrated that the predictive performance of EOT quantitative HBsAg was not affected by ALT flares during follow-up, suggesting that the selection of candidates for finite treatment should

not rely on distinguishing between ALT flares. Additionally, our findings advocate for the use of competing risk analysis over Kaplan-Meier estimators to prevent overestimation of HBsAg seroclearance post-NA treatment. In light of these novel findings, our study may contribute to the ongoing discourse on optimal management strategies for CHB, particularly in the context of finite antiviral therapy and the pursuit of HBsAg seroclearance as the treatment endpoint.

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TABLES

Table 1. Characteristics of the study cohort

Characteristics	All (*N* = 850)
Biological sex	
Female sex, *n* (%)	217 (25.5)
Male sex, *n* (%)	633 (74.5)
Age, years	53.2 (44.2, 61.7)
Diabetes mellitus, *n* (%)	179 (21.0)
Hypertension, *n* (%)	216 (25.4)
Dyslipidemia, *n* (%)	154 (18.1)
AST, U/L	28 (23, 38)
ALT, U/L	26 (19, 38)
Bilirubin, mg/dL	1.05 (0.74, 1.46)
Creatinine, mg/dL	1.1 (0.9, 1.2)
Prothrombin time, second	10.7 (10.3, 11.4)
International normalized ratio	1.04 (1.01, 1.12)
White blood cell count, 10^3/μl	5.59 (4.48, 6.90)
Hemoglobin, g/dL	14.2 (12.4, 15.4)
Thrombocytopenia†, *n* (%)	42 (4.9)
Antiviral regimen	
Entecavir, *n* (%)	541 (63.7)
Tenofovir disoproxil fumarate, *n* (%)	307 (36.1)
Tenofovir alafenamide, *n* (%)	2 (0.2)
Duration on therapy, months	34.7 (30.9, 38.4)
Pretreatment cirrhosis#, *n* (%)	149 (17.5)
Pretreatment hepatic insufficiency*, *n* (%)	152 (17.9)
Pretreatment positive HBeAg, *n* (%)	133 (17.2)
Pretreatment positive anti-HBe, *n* (%)	579 (83.8)
Pretreatment HBV DNA, log IU/ml	5.3 (3.6, 6.8)
Pretreatment AST, U/L	76.5 (49.0, 148.0)
Pretreatment ALT, U/L	107.0 (54.0, 226.0)

Notes: expressed as number (percentage) or median (interquartile range). anti-HBe, hepatitis B e antibody; ALT, Alanine transaminase; AST, Aspartate transaminase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus. †platelet count of less than 100,000/μL. # mainly by clinical diagnosis based on liver images. *serum bilirubin >2mg/dL.

488 **Table 2.** Time-dependent sub-distribution hazard model for factors associated with HBsAg seroclearance and retreatment as a competing risk

	Univariable analysis			Multivariable analysis		
	SHR	95 % CI	*P*	Adjusted SHR	95 % CI	*P*
Time-varying clinical flare (ALT > 200 U/L)	0.26	0.11--0.60	0.002	0.28	0.12--0.66	0.003
Age, year	0.98	0.96--1.00	0.062	0.98	0.96--1.00	0.05
Male sex	1.00	0.51--1.97	0.998			
Diabetes mellitus	0.49	0.20--1.23	0.129			
Hypertension	0.58	0.26--1.29	0.180			
Dyslipidemia	0.87	0.39--1.94	0.738			
AST, 100 U/L	1.23	0.71--2.14	0.465			
ALT, 100 U/L	1.15	0.55--2.41	0.713			
Bilirubin, mg/dL	1.37	1.06--1.76	0.015			
Creatinine, mg/dL	0.09	0.03--0.31	0.0001			
Prothrombin time, second	1.63	0.95--2.79	0.077			
White blood cell count, $10^3/\mu l$	1.06	0.72--1.56	0.785			
Hemoglobin, g/dL	0.98	0.80--1.21	0.883			
Thrombocytopenia	2.85	1.09--7.42	0.032	2.57	0.96--6.88	0.060
Antiviral regimen						
Entecavir (reference)	1.00	-	-			
Tenofovir	1.50	1.17--1.92	0.002			
Duration on therapy, month	1.01	1.00--1.02	0.190			
Pretreatment cirrhosis	1.09	0.51--2.31	0.828			

Pretreatment liver insufficiency	1.90	1.02--3.55	0.045
Pretreatment positive HBeAg	1.36	0.67--2.79	0.396
Pretreatment positive anti-HBe	0.48	0.24--0.98	0.043
Pretreatment HBV DNA, log IU/ml	0.73	0.58--0.91	0.006
Pretreatment AST, 100 U/L	1.00	0.97--1.10	0.327
Pretreatment ALT, 100 U/L	1.03	0.98--1.08	0.269

Note: measured at treatment cessation unless otherwise specified as “pretreatment”

*Abbreviations: anti-HBe, hepatitis B e antibody; ALT, Alanine transaminase; AST, Aspartate transaminase; CI, confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; SHR,sub-distributional hazard ratio

494 **Table 3** Characteristics of the subgroup

Characteristics	**All (*N* = 399)**
Biological sex	
Female sex, *n* (%)	116 (29.1)
Male sex, *n* (%)	283 (70.9)
Age, years	52.2 (43.4, 61.7)
Diabetes mellitus, *n* (%)	72 (18.1)
HBsAg level, IU/mL	
< 10	36 (9.0)
10 - 100	60 (15.0)
> 100	303 (75.9)
Hypertension, *n* (%)	93 (23.3)
Dyslipidemia, *n* (%)	72 (18.1)
AST, U/L	27 (22, 34)
ALT, U/L	26 (18, 37)
Bilirubin, mg/dL	1.00 (0.74, 1.35)
Creatinine, mg/dL	1.1 (0.9, 1.2)
Prothrombin time, second	10.4 (10.3, 10.8)
International normalized ratio	1.02 (1.00, 1.06)
White blood cell count, 10^3/μl	5.62 (4.75, 6.85)
Hemoglobin, g/dL	14.5 (13.0, 15.8)
Thrombocytopenia†	12 (3.0)
Antiviral regimen	
Entecavir, *n* (%)	246 (61.7)
Tenofovir, *n* (%)	152 (38.1)
Tenofovir alafenamide, *n* (%)	1(0.3)
Duration on therapy, month	35.0 (31.9, 39.2)
Pretreatment cirrhosis#, *n* (%)	48 (12.0)
Pretreatment hepatic insufficiency*, *n* (%)	60 (15.0)
Pretreatment positive HBeAg, *n* (%)	78 (21.5)
Pretreatment positive anti-HBe, *n* (%)	267 (80.4)
Pretreatment HBV DNA, log IU/ml	5.55 (3.77, 7.01)
Pretreatment AST, U/L	74.5 (46.0, 150.5)
Pretreatment ALT, U/L	109 (55, 248)

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Table 4. Time-dependent sub-distribution hazard model for factors associated with HBsAg seroclearance (subgroup analysis)

	Univariable analysis			Multivariable analysis		
	SHR	95 % CI	*P*	Adjusted SHR	95 % CI	*P*
Time-varying clinical flare (ALT>200 U/L)	0.29	0.09--0.96	0.042	0.83	0.25--2.70	0.752
Age, year	0.98	0.95--1.01	0.282			
Male sex	0.72	0.31--1.65	0.432			
Diabetes mellitus	0.76	0.23--2.52	0.654			
Hypertension	0.16	0.02--1.13	0.065			
Dyslipidemia	0.68	0.21--2.23	0.527			
AST, 100 U/L	1.39	0.44--4.38	0.573			
ALT, 100 U/L	1.40	0.34--5.68	0.642			
Bilirubin, mg/dL	1.20	0.56--2.57	0.636			
Creatinine, mg/dL	0.12	0.02--0.82	0.030			
Prothrombin time, second	1.39	0.82--2.36	0.227			
White blood cell count, 10^3/μl	1.35	1.07--1.72	0.012			
Hemoglobin, g/dL	0.98	0.73--1.31	0.878			
Thrombocytopenia	1.85	0.23--14.57	0.560			
EOT HBsAg level						
HBsAg > 100 IU/mL (Reference)				1.00	-	-
10 IU/mL ≤ HBsAg ≤ 100 IU/mL	5.10	1.52--17.04	0.008	4.85	1.44--16.35	0.011
HBsAg < 10 IU/mL	34.0	13.18--87.80	<0.001	32.06	11.83--86.92	<0.001

Antiviral regimen					
Entecavir (reference)	1.00	-	-	-	-
Tenofovir	1.49	1.03--2.16	0.036		
Duration on therapy, month	1.01	0.99--1.03	0.337		
Pretreatment cirrhosis	1.20	0.38--3.75	0.760		
Pretreatment liver insufficiency	2.01	0.87--4.62	0.101		
Pretreatment positive HBeAg	1.21	0.47--3.14	0.690		
Pretreatment positive anti-HBe	0.65	0.25--1.70	0.382		
Pretreatment HBV DNA, log IU/ml	0.71	0.52--0.97	0.033		
Pretreatment AST, 100 U/L	1.00	0.91--1.09	0.913		
Pretreatment ALT, 100 U/L	0.95	0.87--1.02	0.169		

Note: measured at treatment cessation unless otherwise specified as “pretreatment”

*Abbreviations: anti-HBe, hepatitis B e antibody; ALT, Alanine transaminase; 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

FIGURE LEGENDS:

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

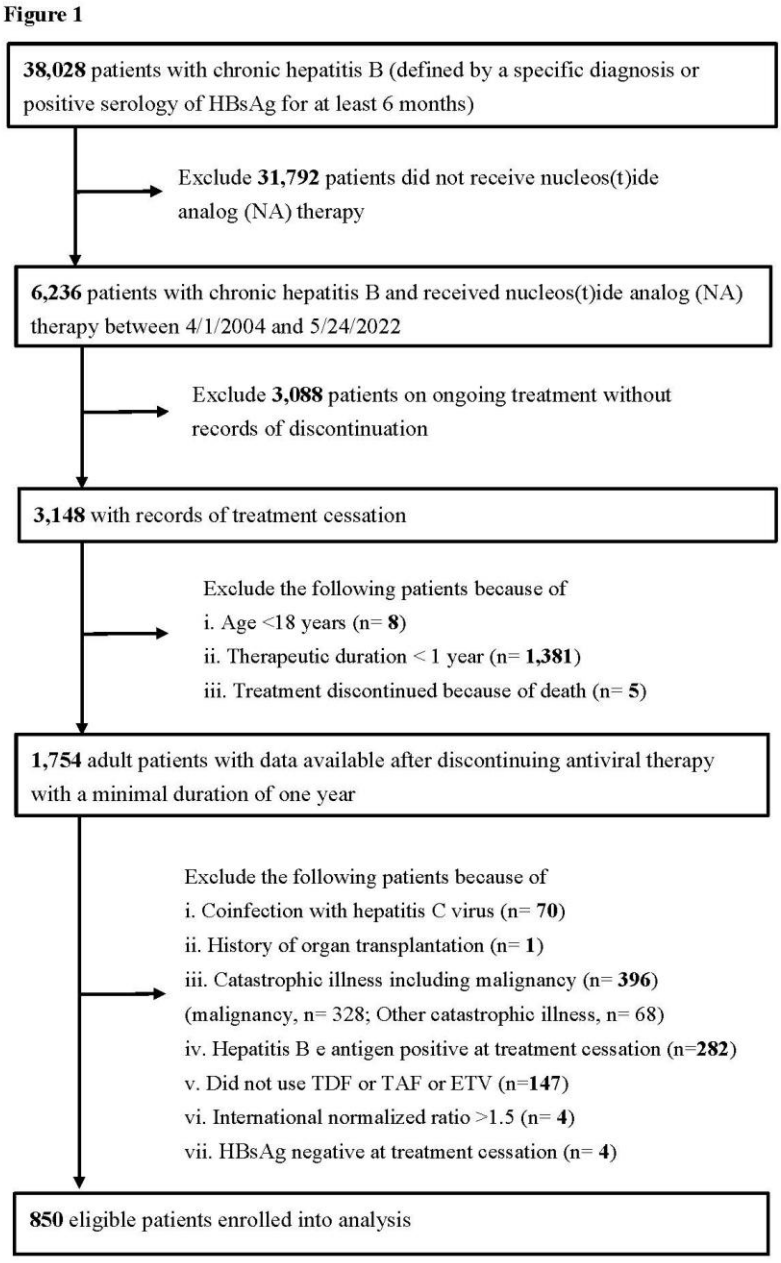
Figure 2. The cumulative incidence of HBsAg seroclearance following discontinuation of nucleos(t)ide analogs in the study population.

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)

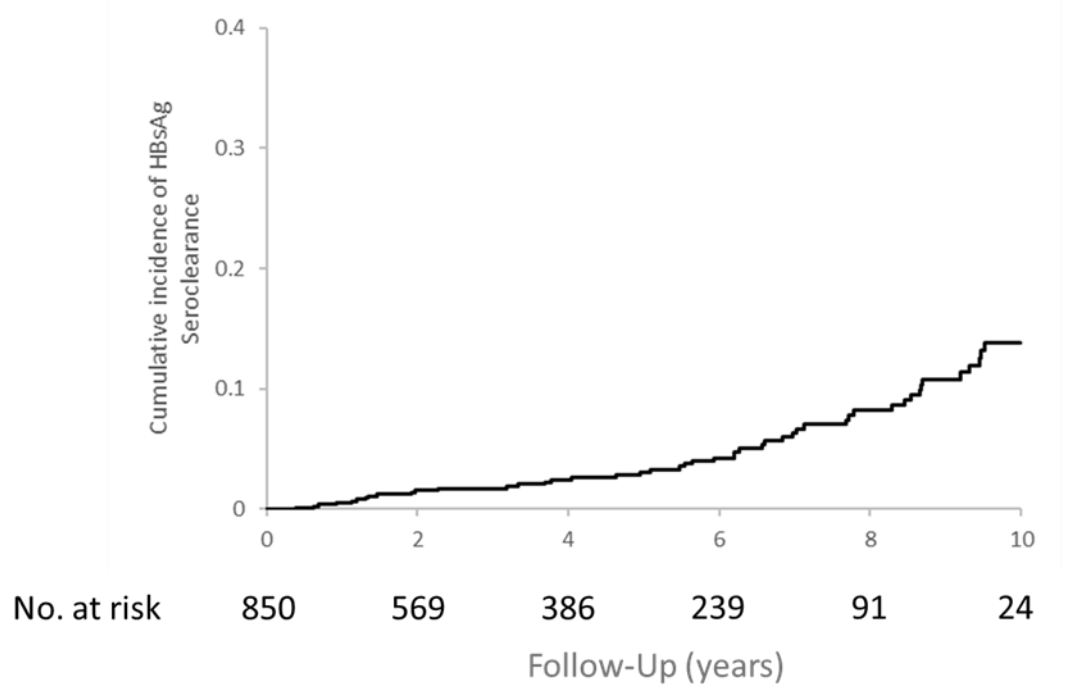
Figure 4. The cumulative incidence of HBsAg seroclearance following discontinuation of nucleos(t)ide analogs in patients with quantitative HBsAg levels at end of treatment.

FIGURES

Figure 1.



520 Figure 2.



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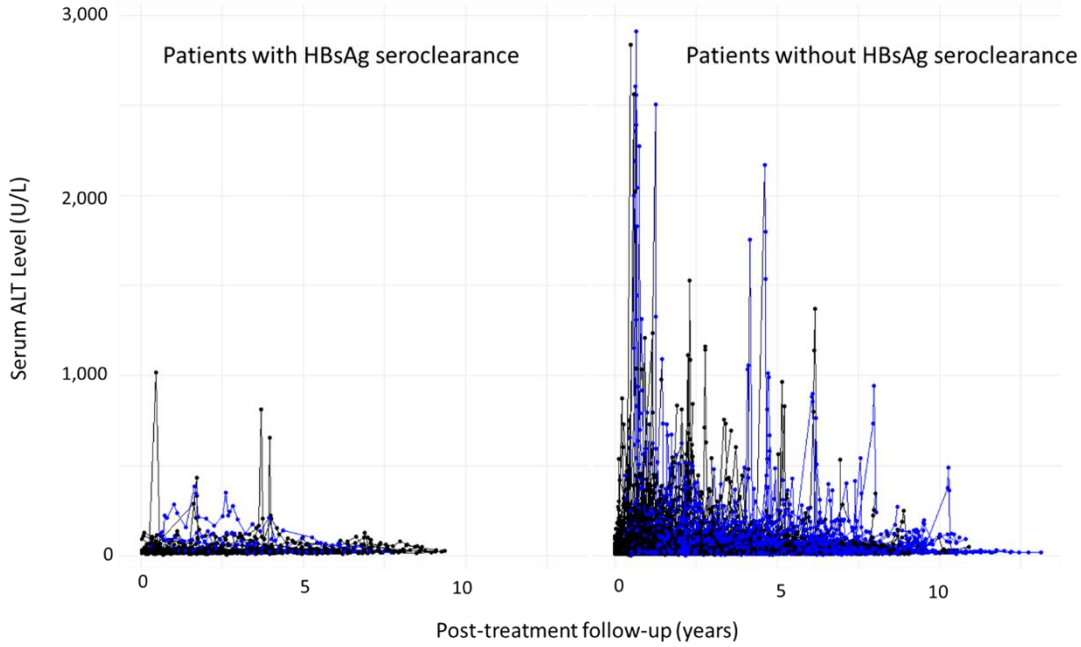
523 Figure 3.



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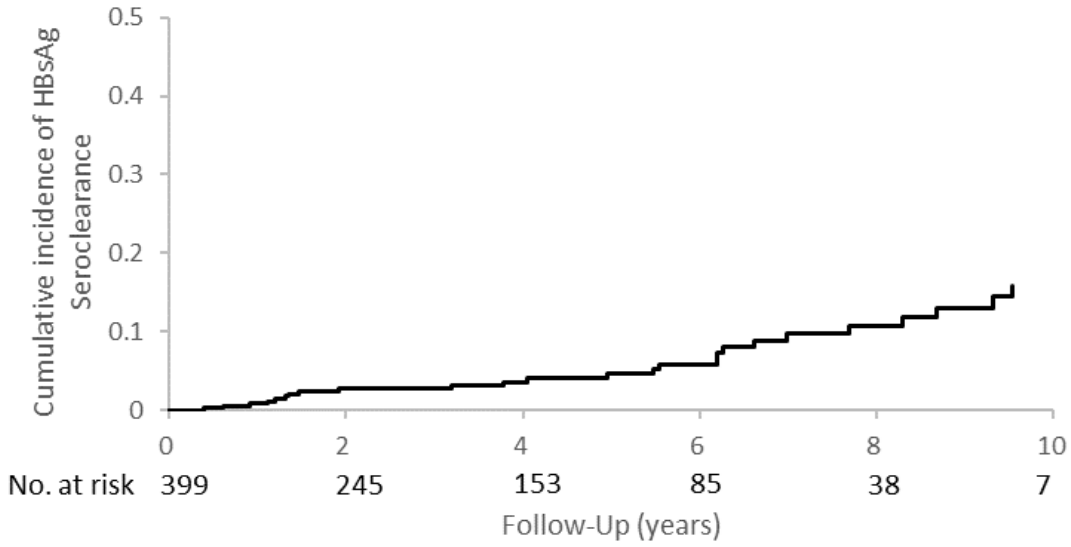
527 Figure 4.



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Supplementary Tables

Supplementary Table 1. Multivariable regression model to explore factors associated with HBsAg seroclearance

	Model 1			Model 2			Model 3		
	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*
Time-varying clinical flare (ALT>200 U/L)	0.29	0.12--0.68	0.005	0.69	0.29--1.62	0.389	0.28	0.12--0.66	0.003
Age, year	-	-	-	-	-	-	0.98	0.96--1.00	0.050
Thrombocytopenia	3.48	1.36--8.89	0.009	3.25	1.27--8.32	0.014	2.57	0.96--6.88	0.060

Note: measured at treatment cessation unless otherwise specified as “pretreatment”

*Abbreviations: ALT, Alanine transaminase; CI, confidence interval; SHR,sub-distributional hazard ratio

Supplementary Table 2. Multivariable competing risks regression to explore factors associated with HBsAg seroclearance

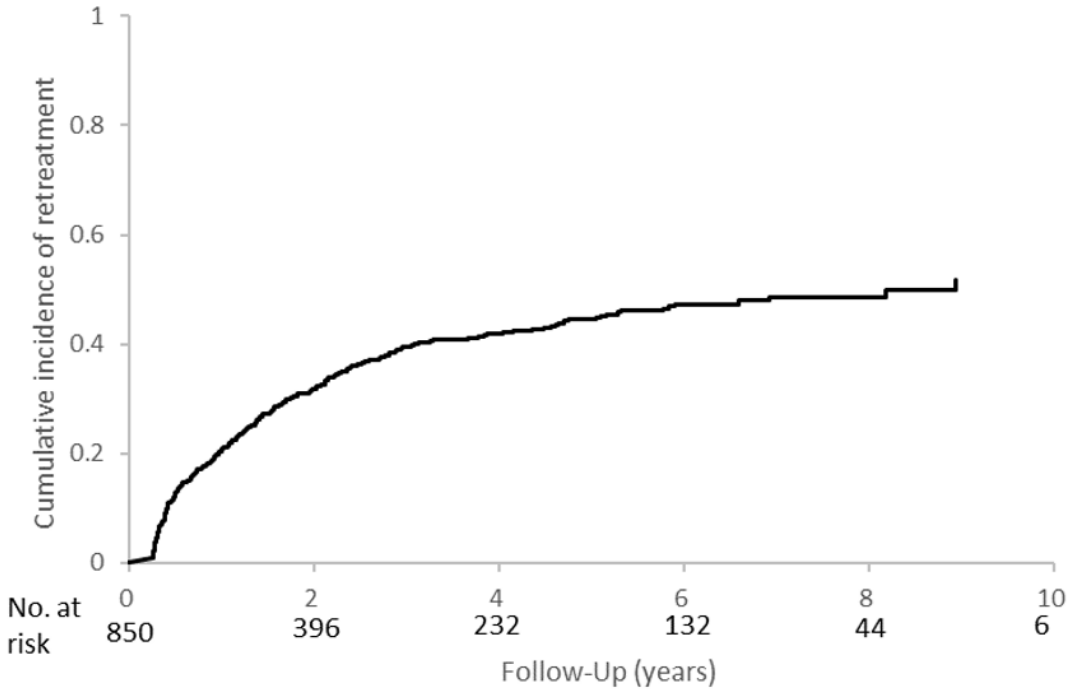
	ALT > 80 U/L			ALT > 40 U/L		
	Adjusted SHR	95 % CI	*P*	Adjusted SHR	95 % CI	*P*
Time-varying ALT elevation	0.37	0.21--0.67	0.001	0.51	0.29--0.87	0.014
Age, year	0.97	0.95--1.00	0.022	0.97	0.95--1.00	0.024
Thrombocytopenia	2.97	1.14--7.58	0.026	3.02	1.15--7.90	0.024

Note: measured at treatment cessation unless otherwise specified as “pretreatment”

*Abbreviations: ALT, Alanine transaminase; CI, confidence interval; SHR,sub-distributional hazard ratio.

Supplementary Figures

Supp Fig. 1



Supp Fig. 2