財團法人明日醫學基金會補助專題研究計畫

☑成果報告 ☐期中進度報告

計畫名稱：

慢性 B 型肝炎患者停止類核苷（酸）藥物後再接受抗病毒治療與表面抗原血清清除之間的關聯

Association Between Retreatment and Hepatitis B Surface Antigen Seroclearance in Patients with Clinical Relapses Following Withdrawal of Nucleos(t)ide Analogues

計畫類別：☑ 個別型計畫　　☐ 整合型計畫

執行期間： 113 年 1 月 1 日起 至 113 年 12 月 31 日止

計畫主持人： **許耀峻**
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計畫參與人員：

成果報告類型：☐精簡報告☑完整報告

處理方式：☐可公開查詢

執行單位：義守大學醫學研究所及義大醫院醫學研究部

Association Between Retreatment and Hepatitis B Surface Antigen Seroclearance in Patients with Clinical Relapses Following Withdrawal of Nucleos(t)ide Analogues

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中文摘要

背景：使用核苷（酸）類似物治療慢性 B 型肝炎的最佳療程仍存在爭議，其中停藥策略被認為可以誘導 B 型肝炎表面抗原（HBsAg）的血清清除。然而，停藥常常導致臨床肝炎復發，而臨床復發通常意味著需要再治療。目前尚不清楚再治療對於 B 型肝炎表面抗原血清清除的影響。

目的：本研究目的在釐清停止核苷（酸）類似物治療後出現臨床復發的患者中，再治療與 B 型肝炎表面抗原血清清除之間的關聯

方法：這是一項回溯性、多中心世代研究，我們將系統地檢視義大醫療體系中，所有接受第一線核苷（酸）類似物，亦即恩替卡韋(entecavir)或替諾福韋(tenofovir)，治療的慢性 B 型肝炎患者，並且找出隨後停止治療的人。符合條件的患者在停止治療前必須確認 B 型肝炎 e 抗原（HBeAg）為陰性，並且具有檢測不到 B 型肝炎病毒 DNA 的紀錄。我們將排除患有惡性腫瘤、接受器官移植或病毒合併感染的患者。臨床肝炎復發的定義是停止治療後血清丙氨酸氨基轉移酶(ALT)升高到正常上限的兩倍以上。主要結果是臨床復發後 B 型肝炎表面抗原(HBsAg)的血清清除。恢復核苷（酸）類似物治療被分析為一個時變變量，並對潛在的干擾因素進行調整，以闡明其與 B 型肝炎表面抗原血清清除的關聯。

結果：在 841 位停止核苷類似物治療且符合納入標準的病人中（治療中位時間為 35.1 個月），共有 320 位病人出現臨床復發，其 10 年累積發生率為 60.6%（95% 信賴區間，43.5–72.5）。在這 320 位出現 CR 的病人中（79.4%為男性，中位年齡為 51.6 歲），有 188 人重新接受治療，而 132 人則在後續觀察期間未再接受治療。臨床復發後，共 15 位病人達到 HBsAg 清除，10 年累積發生率為 12.1%（95%信賴區間，5.2–18.6）。其中 5 位是在重新治療後發生 HBsAg 清除，另有 10 位則是在未接受治療的情況下自然清除。在單變量分析中，重新治療與較低的 HBsAg 清除率相關（風險比 [HR]，0.30；95%信賴區間，0.10–0.93；p=0.03）。但在調整包括 HBsAg 變動濃度等多個共變項後，重新治療與 HBsAg 清除之間就無顯著關聯（調整後 HR，0.41；95%信賴區間，0.13–1.29；p=0.13）。無論是否重新接受治療，最近一次 HBsAg 濃度低於 100 IU/mL 的病人，其 HBsAg 清除率明顯較高（調整後 HR，7.99；95%信賴區間，2.48–25.69；p=0.0005）

結論：慢性 B 型肝炎病人停用核苷類似物後，發生臨床肝炎復發後，再治療與否和 HBsAg 清除不存在獨立相關性。我們的研究結果顯示，病人在停藥後若發生臨床肝炎發作，不需擔心在治療會降低 HBsAg 清除的機會。

關鍵詞：B 性肝炎病毒感染；抗病毒藥物治療；有限療程；B 型肝炎表面抗原清除

ABSTRACT

Background: Withdrawal of nucleos(t)ide analogue (NA) therapy has been proposed for patients with chronic hepatitis B (CHB) to induce seroclearance of hepatitis B surface antigen (HBsAg). However, this approach is frequently followed by clinical relapse (CR) that often indicates treatment resumption. The impact of retreatment on HBsAg seroclearance in patients experiencing CR is unclear.

Methods: This retrospective, multi-center cohort study systematically reviewed all consecutive patients who received entecavir or tenofovir therapy for CHB and subsequently discontinued the NA in a healthcare group in Taiwan. Eligible patients were documented with negative HBeAg and undetectable HBV DNA before stopping treatment. Patients with a malignancy, organ transplant, or viral coinfection were excluded. CR was defined as an elevation of serum alanine aminotransferase (ALT) to more than twice the upper limit of normal after treatment cessation. The primary outcome was seroclearance of HBsAg following the occurrence of CR. Resumption of NA therapy was analyzed as a time-varying variable and adjusted for potential confounding factors to elucidate its association with HBsAg seroclearance.

Results: Among the 841 patients who stopped NA treatment and met the eligibility

criteria (median duration of treatment, 35.1 months), 320 patients encountered CR with a cumulative incidence of 60.6% (95% CI, 43.5-72.5) at 10 years. In these 320 patients with CR (79.4% male, median age of 51.6 years), 188 patients resumed NA therapy whereas 132 patients remained un-retreated through the posttreatment observation. Following CR, 15 patients cleared HBsAg with a 10-year cumulative incidence of 12.1 % (95% CI, 5.2-18.6). HBsAg seroclearance occurred in 5 patients after they resumed NA therapy and in 10 patients who remained un-retreated. In univariable analysis, retreatment was associated with a lower incidence of HBsAg loss (hazard ration [HR], 0.30; 95% CI, 0.10-0.93; p=0.03). However, after adjustment for covariates including time-varying levels of serum HBsAg, there was no significant association between retreatment and HBsAg seroclearance (adjusted HR, 0.41; 95% CI, 0.13-1.29; p=0.13). Regardless of retreatment or not, the incidence of HBsAg seroclearance was significantly higher with a most recent HBsAg level <100 IU/mL (adjusted HR, 7.99; 95% CI, 2.48-25.69; p=0.0005).

Conclusions: No independent association was found between retreatment and HBsAg seroclearance in CHB patients encountering CR after NA withdrawal. Our findings suggest that CHB patients who stop NA therapy can resume antiviral treatment for CR without the concern of reducing the chance of HBsAg seroclearance.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a primary contributor to liver-related morbidity and mortality globally, especially in Asian regions including Taiwan. The care of individuals with chronic hepatitis B (CHB) has evolved to include antiviral treatments, primarily interferon alpha and nucleos(t)ide analogs (NAs). By effectively suppressing viral replication, NAs not only decrease viremia and mitigate liver inflammation but also hold potential in halting or even reversing liver scarring. Numerous studies validate the efficacy of NAs in enhancing patient outcomes. Yet, maintaining the therapeutic benefits often proves challenging after discontinuation of NA therapy.

Because of high off-therapy relapse rates, major international guidelines currently recommend an indefinite prolongation of NA therapy, possibly until loss of hepatitis B surface antigen (HBsAg) with or without appearance of accompanying antibodies. However, this strategy entails life-long treatment for most treated patients. Recently, intense research has been carried out to clarify predictors of off-therapy relapse and identify patients who can maintain remission without resuming medication. One of the unresolved issues is the impact of resuming antiviral treatment on HBsAg seroclearance in patients who experience relapse after discontinuing NA therapy.

In this study, we aim to investigate whether resumption of antiviral treatment is associated with seroclearance of hepatitis B surface antigen in patients with chronic hepatitis B who experience relapse after discontinuation of nucleos(t)ide analog therapy. This research will provide valuable insights into the long-term management of CHB patients and potentially inform decisions regarding retreatment strategies

MATERIALS AND METHODS

Design and setting

This retrospective study will analyze electronic health records (EHRs) of patients who received nucleos(t)ide analog (NA) treatment for chronic hepatitis B (CHB) at E-Da Hospital, E-Da Cancer Hospital, and E-Da Dachang Hospital in Kaohsiung, Taiwan. Data extraction will include demographic information, laboratory results, diagnostic codes, pharmacy prescription claims, and vital statistics from the EDA Healthcare System EHR database, supplemented by manual chart review when necessary. The study adheres to the Declaration of Helsinki and has been approved by the E-Da Healthcare System's institutional review board.

Study patients

Eligible participants will be adults (≥20 years) with CHB, defined by a specific diagnosis

code or positive HBsAg for at least 6 months. Inclusion criteria include: 1) Previously untreated patients, 2) initiated on NA regimen (lamivudine, adefovir, telbivudine, entecavir, or tenofovir), 3) minimum one year of NA treatment prior to cessation. Patients are excluded for any of the following criteria: seroclearance of HBsAg during the NA treatment, positive serology of HBeAg at treatment cessation, co-infection with hepatitis C virus (HCV), organ transplantation, malignant disease including hepatocellular carcinoma (HCC), or any severe comorbidity that was certified in the registry of catastrophic illness patient database. The study baseline was set at the day of treatment cessation, as confirmed by pharmacy records. The definition of cirrhosis was based upon a clinical diagnosis, usually made according to clinical assessment including liver images mainly by sonography. Hepatic insufficiency was defined by laboratory measurements of serum bilirubin >2mg/dL or prolongation of prothrombin time >3 seconds because this operational definition indicated reimbursement for antiviral treatment according to the national health insurance in Taiwan.

Criteria for initiation and discontinuation of antiviral treatment

NA treatment initiation followed Taiwan's national health insurance guidelines, which generally required high viral load (>2,000 IU/mL) and persistent ALT elevation (>2x ULN for ≥3 months), with specific criteria based on HBeAg status. For cirrhotic patients,

HBV DNA >2,000 IU/mL was required.

Treatment duration was typically limited to 3 years, with exceptions for certain cirrhotic patients meeting specific criteria after July 1, 2010. The indefinite coverage for cirrhosis required portal hypertension (i.e., splenomegaly or esophagogastric varices) in addition to imaging characteristics of cirrhosis if pathological proof is absent. Moreover, HBV DNA >2,000 IU/mL is a prerequisite. Thus, throughout the study period, patients with a clinical diagnosis of cirrhosis but without viremia of at least 2,000 IU/mL or without overt features of portal hypertension were ineligible for indefinite treatment.

Criteria for retreatment, off-therapy observation, and outcome measurement

The criteria for antiviral retreatment also followed the national health insurance in general. Retreatment was indicated for hyperbilirubinemia (serum bilirubin >2mg/dL), coagulopathy (prolonged prothrombin time >3 seconds), and persistent hepatitis flares (ALT >2x ULN for >3 months). Retreatment was not indicated for virological relapse alone or transient (<3 months) ALT elevation.

Study outcomes

The primary outcome is seroclearance of HBsAg that occurred during the off-therapy

period. HBV reactivation is defined with reoccurrence of HBV DNA >2000 IU/mL, and clinical relapse is defined by elevation of serum ALT above two times the upper limit of normal. The observation for occurrence of study outcome starts after the day of treatment cessation and is censored at death, the last EHR encounter, or 6 months following antiviral re-treatment.

Statistical analysis

Descriptive statistics will be used to summarize the data. Continuous variables will be presented as medians with interquartile ranges (IQRs), while categorical variables will be expressed as numbers and percentages. The cumulative incidence rates of clinical events will be estimated using the Kaplan-Meier method. Differences between patient subgroups will be examined using the log-rank test. Incidence rates of study outcomes will be calculated as events per person-time, and Poisson regression will be used to assess chronological trends for statistical significance.

For study outcomes with more than 10 event occurrences, we will employ the Cox proportional hazard model to explore risk factors. Multivariable analysis will be conducted in a stepwise manner, removing variables that are not statistically significant. Point estimates will be reported with 95% confidence intervals (CIs). A sensitivity

analysis will be performed, including only patients who received either entecavir or tenofovir. All statistical tests will be two-sided, with statistical significance set at a P value <0.05. Analyses will be conducted using SAS software (version 9.4, SAS Institute, Cary, NC, USA).

RESULTS

Characteristics of the study population

The study population comprised 841 patients (median age 53 years [IQR 44–62]), of whom 25.3% were female; common comorbid conditions included diabetes mellitus in 21.2%, hypertension in 25.7%, and dyslipidemia in 18.3% (**Table 1**). At the time of NA withdrawal (study entry), liver function was well preserved, with median AST and ALT levels of 28 U/L and 26 U/L, respectively, and a median total bilirubin of 1.1 mg/dL; coagulation parameters were within normal limits (median prothrombin time 10.7 seconds, INR 1.0). Renal function was also preserved, with a median serum creatinine of 1.1 mg/dL. All patients were treated with NA therapy for a median of 34.7 months (IQR 31.0–38.0) before withdrawal, with entecavir being the most common agent (63.7%) followed by tenofovir (36.3%). Pre-treatment disease characteristics reflected active chronic hepatitis B, with 17.6% of patients having cirrhosis and 17.0% being hepatitis B e antigen (HBeAg) positive prior to therapy; the median HBV DNA

level before treatment was 5.3 log10 IU/mL and the median ALT was 107 U/L, indicating significant viral replication and hepatic inflammation at treatment initiation.

Table 1. Baseline characteristics

Characteristics	All (N = 841)
Female sex, n (%)	213 (25.3)
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 normalized ratio	1.0 (1.0, 1.1)
Antiviral regimen	
Entecavir, n (%)	536 (63.7)
Tenofovir*, n (%)	305 (36.3)
Duration on therapy, months	34.7 (31.0, 38.0)
Pretreatment cirrhosis[#], n (%)	148 (17.6)
Pretreatment positive HBeAg, n (%)	130 (17.0)
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)

The median follow-up after treatment cessation was 3.6 years (IQR 1.4–6.2). During the study period, HBsAg loss in 38 patients (**Figure 1**), with a 10-year cumulative incidence

of 12.3% (95% CI, 7.4-16.9%). Besides, antiviral treatment was resumed in 309 patients whereas acute flares progressed to acute-on-chronic liver failure in four patients and one of them died.

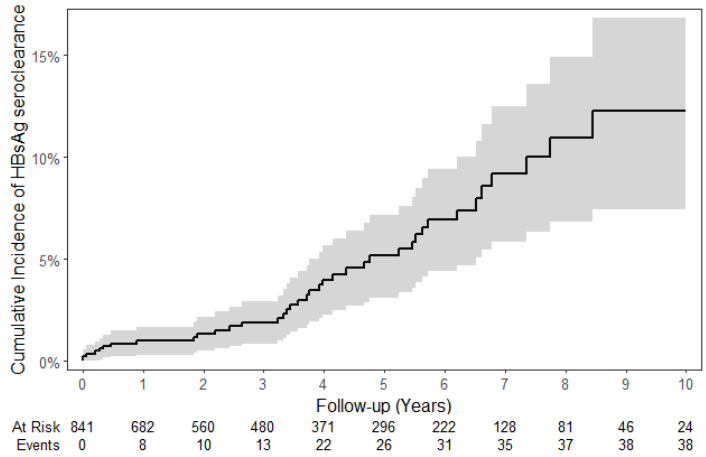

Figure 1. Cumulative incidence of HBsAg clearance during the 10-year study period

Retreatment linked to a lower incidence of HBsAg loss in unadjusted analysis without consideration of immortal time bias

In the conventional unadjusted analysis beginning at NA treatment cessation, patients who did not undergo retreatment experienced a significantly higher rate of HBsAg loss over 10 years than those who did receive retreatment (**Figure 2**). The 10-year cumulative incidence of HBsAg loss was 18.7% (95% confidence interval [CI], 9.3–27.1%) in the non-retreatment group compared to 5.8% (95% CI, 1.5–9.9%) in the retreatment group (log-rank test, p = 0.002). Correspondingly, retreatment was associated with a substantially lower likelihood of achieving HBsAg loss, with an unadjusted hazard ratio (HR) of 0.31 (95% CI, 0.14–0.67; p = 0.003).

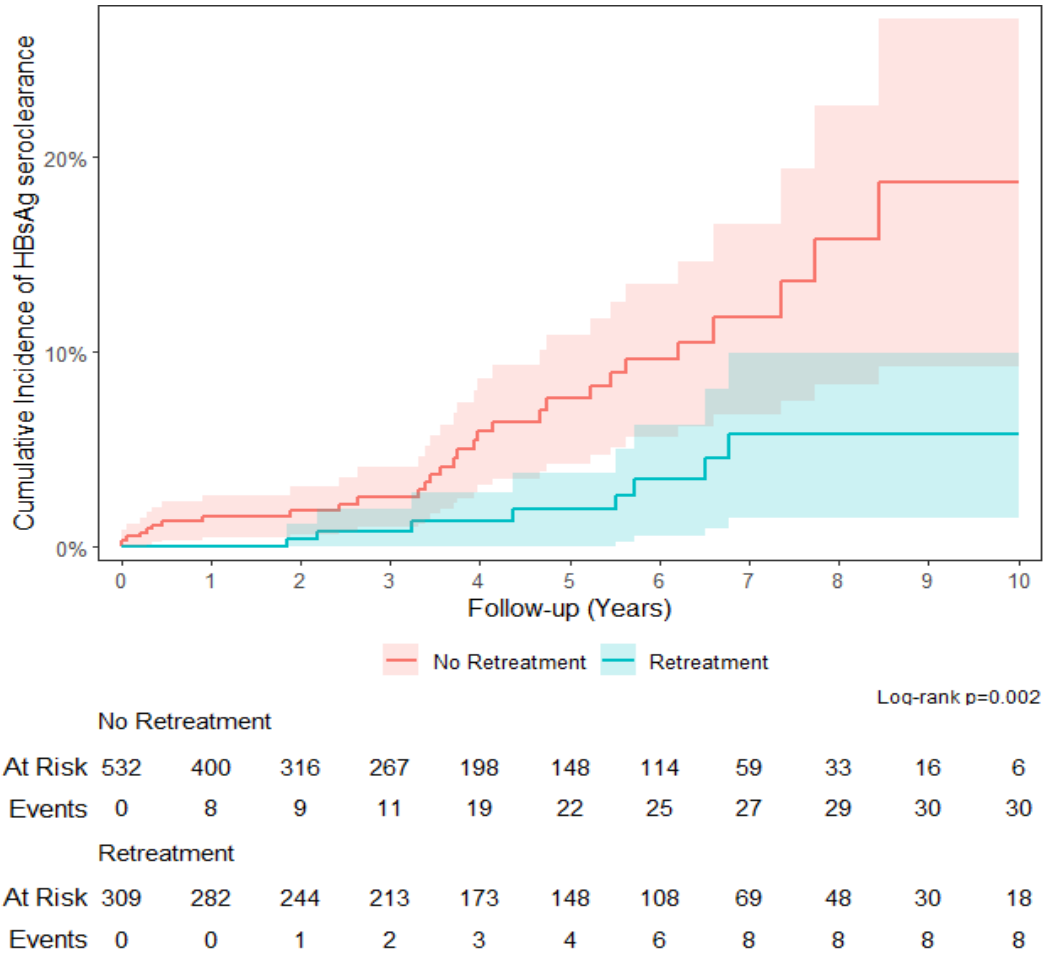

Figure 2. Retreatment associated with a lower incidence of HBsAg clearance if the analysis was not adjusted for immortal time and confounding factors

Association between retreatment and HBsAg loss after adjustment for immortal time bias and confounding factors

If retreatment was analyzed as a time-dependent covariate to account for immortal time bias, it was not significantly associated with HBsAg seroclearance. The hazard ratio for HBsAg loss with retreatment was 0.46 (95% confidence interval 0.20–1.03; p = 0.06), indicating no statistically significant difference in HBsAg clearance rates with or without retreatment.

In the multivariable analysis adjusted for potential confounders (**Table 2**), time-varying retreatment was not significantly associated with HBsAg loss (adjusted aHR, 0.56, 95% confidence interval [CI] 0.25–1.26, p = 0.16). In contrast, a low serum HBsAg level at the time of treatment cessation was a strong independent predictor of HBsAg loss (aHR 5.76, 95% CI 3.01–11.02, p < 0.0001). The duration of the first retreatment episode showed a trend toward significance, with longer retreatment tending to correlate with a reduced likelihood of HBsAg clearance (per additional month of retreatment: aHR 0.98, 95% CI 0.96–1.00, p = 0.06). Other baseline covariates, including older age, male sex, baseline HBeAg positivity, presence of cirrhosis, and longer duration of initial antiviral therapy, were not associated with HBsAg loss in the multivariable analysis.

Table 2. Multivariable analysis adjusted for confounding factors

Variables	Univariable Analysis			Multivariable Analysis		
	N	Hazard Ratio	95%CI	Adjusted Hazard Ratio	95%CI	p-value
Age, years	841	0.98	0.95 - 1.01			
Male sex	841	1.41	0.62 - 3.19			
Tenofovir use (vs. Entecavir)	841	0.87	0.41 - 1.85			
First retreatment duration, months	841	1.02	1.00 - 1.04	1.02	1.00 - 1.04	0.06
EOT diabetes mellitus	841	0.53	0.19 - 1.49			
EOT hypertension	841	0.78	0.34 - 1.78			
EOT dyslipidemia	841	1.09	0.48 - 2.47			
EOT ALT, U/L	780	1.00	1.00 - 1.01			
HBsAg before treatment cessation low (vs. High)	834	6.22	3.27 - 11.85	5.76	3.01 - 11.02	< 0.0001
Pre-treatment cirrhosis	841	1.82	0.79 - 4.18			
Pre-treatment positive HBeAg	767	0.98	0.42 - 2.27			
Pre-treatment ALT, U/L	829	1.00	1.00 - 1.00			
Pre-treatment HBVDNA (interp)	841	0.83	0.71 - 0.98			
Time-varying retreatment	841	0.46	0.20 - 1.03	0.56	0.25 - 1.26	0.16
Time-varying HBsAg low (vs. High)	840	14.18	6.23 - 32.26			

* HBsAg low: <=100 IU/mL, high: > 100 IU/mL (N=834)

Subgroup analysis for patients with quantitative data of HBsAg at treatment cessation

In the subgroup of 392 patients with available quantitative HBsAg measurements at treatment cessation (**Table 3**), both univariable and multivariable Cox regression analyses identified low end-of-treatment HBsAg levels as a key determinant of HBsAg loss. On univariable analysis, patients who did not undergo retreatment had a significantly higher likelihood of achieving HBsAg loss compared to those who received retreatment, and notably a low HBsAg level (≤100 IU/mL) at the end of therapy was strongly associated with subsequent HBsAg clearance. In the multivariable model, after adjustment for potential confounders, an end-of-treatment HBsAg level ≤100 IU/mL emerged as a robust independent predictor of HBsAg loss (adjusted hazard ratio [aHR] 7.75, 95% confidence interval [CI] 2.71–22.16; p = 0.0001). By contrast, the effect of retreatment was attenuated after adjustment: the time-varying retreatment status was not significantly associated with HBsAg loss (aHR 0.54, 95% CI 0.14–2.06; p = 0.37) in this subgroup. The duration of the first retreatment course showed a trend toward a positive association with HBsAg loss (aHR 1.02, 95% CI 1.00–1.05; p = 0.09), but this did not reach statistical significance. Overall, in patients with available HBsAg quantification at treatment stop, a low HBsAg level at the end of treatment was the strongest factor associated with eventual HBsAg loss, whereas retreatment (and its duration) had limited impact on the likelihood of HBsAg clearance after treatment

discontinuation.

Table 3. Subgroup analysis for patients with quantitative HBsAg data at treatment cessation

Variables	Univariable Analysis			Multivariable Analysis		
	N	Hazard Ratio	95%CI	Adjusted Hazard Ratio	95%CI	p-value
Age, years	392	0.99	0.94 - 1.03			
Male sex	392	0.99	0.32 - 3.10			
Tenofovir use (vs. Entecavir)	392	0.53	0.15 - 1.90			
First retreatment duration, months	392	1.03	1.00 - 1.05	1.02	1.00 - 1.05	0.09
EOT diabetes mellitus	392	0.86	0.19 - 3.81			
EOT hypertension	392	0.27	0.04 - 2.04			
EOT dyslipidemia	392	1.07	0.30 - 3.77			
EOT ALT, U/L	367	1.00	1.00 - 1.02			
EOT HBsAg low (vs. High)	392	8.53	3.01 - 24.15	7.75	2.71 - 22.16	0.0001
Pre-treatment cirrhosis	392	2.12	0.47 - 9.63			
Pre-treatment positive HBeAg	358	0.78	0.21 - 2.86			
Pre-treatment ALT, U/L	388	1.00	1.00 - 1.00			
Pre-treatment HBVDNA (interp)	392	0.74	0.58 - 0.95			
Time-varying retreatment	392	0.56	0.15 - 2.11	0.54	0.14 - 2.06	0.37
Time-varying HBsAg low (vs. High)	392	13.45	3.81 - 47.22			

* HBsAg low: <=100 IU/mL, high: > 100 IU/mL (N=392)

In fact, serum levels of HBsAg at treatment cessation can effectively distinguish the incidence of HBsAg loss regardless of retreatment (**Figure 3**). Patients with low EOT HBsAg levels had a higher rate of HBsAg loss (21.8%) compared to those with high HBsAg (21.8 vs. 7.6% at 10 years of follow-up, $p<0.001$). Moreover, EOT HBsAg levels also predicted clinical relapses and antiviral retreatment, with lower HBsAg levels associated with lower rates of clinical relapse (37.1% vs. 59.1%) and lower retreatment rates (24.2% vs. 55.2%).

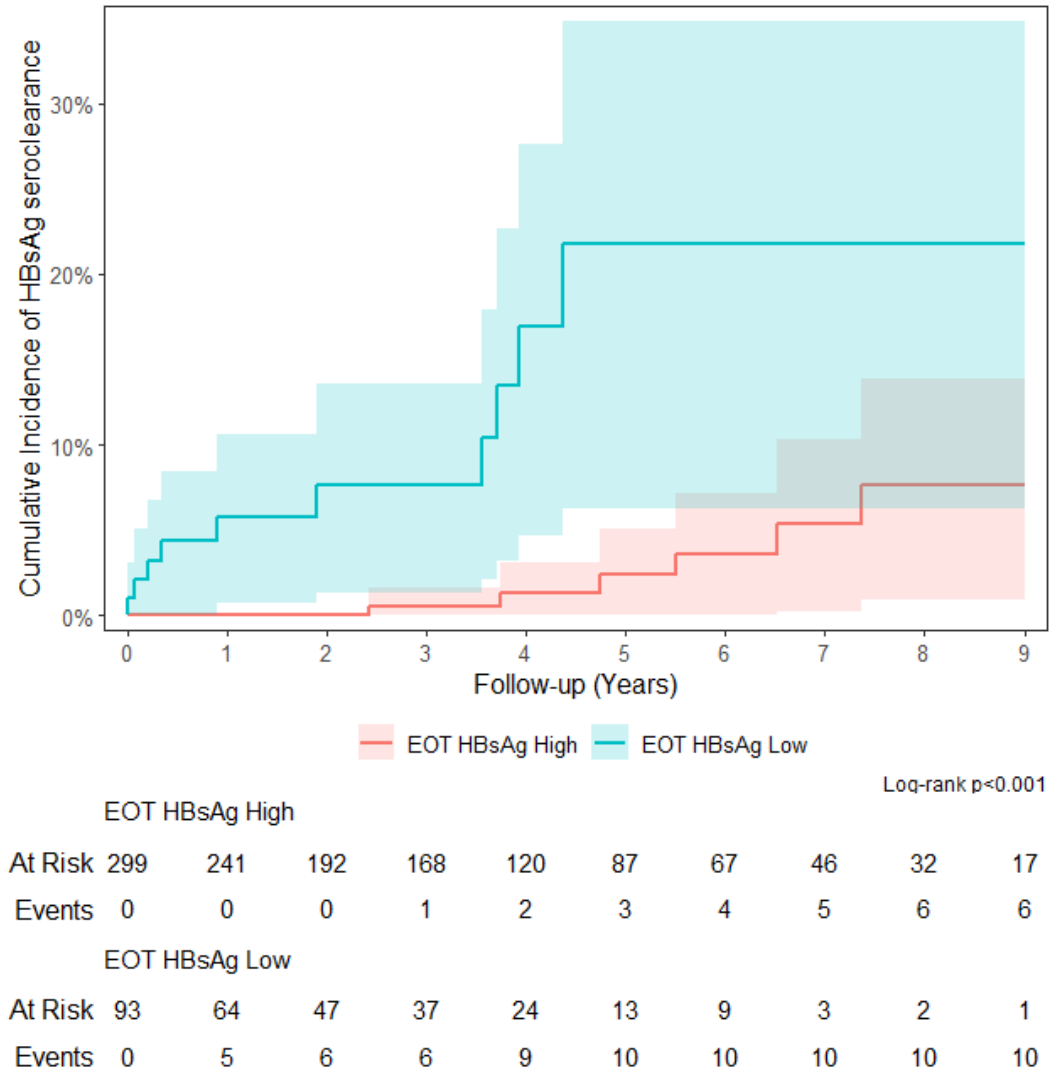

Figure 3. Serum HBsAg levels at treatment cessation effectively stratify the incidences of HBsAg loss following treatment cessation

DISCUSSION

In this large cohort of chronic hepatitis B patients who discontinued long-term nucleos(t)ide analogue (NA) therapy, we found that retreatment for post-NA relapse was not significantly associated with HBsAg seroclearance after appropriate adjustment for immortal time bias and confounding factors. Initial unadjusted analyses suggested that

patients who underwent retreatment had a lower cumulative incidence of HBsAg loss than those who remained off therapy. However, this apparent association disappeared when we treated retreatment as a time-dependent variable in survival analyses, indicating that the earlier finding was likely due to **immortal time bias** (i.e. patients who eventually required retreatment had to remain HBsAg-positive during the lead-up to retreatment). After accounting for differences in baseline risk profiles and timing, **prompt antiviral retreatment did not independently prevent nor promote HBsAg loss**. In practical terms, this means that whether or not a patient was retreated did not alter their ultimate chance of clearing HBsAg.

Instead, the **strongest predictor of HBsAg seroclearance** in our study was the serum HBsAg level at the time of NA withdrawal. Patients with a low EOT HBsAg (≤100 IU/mL) had a markedly higher likelihood of achieving HBsAg loss during off-treatment follow-up. These individuals accounted for a disproportionate share of all observed HBsAg clearance events and exhibited significantly greater cumulative HBsAg loss rates over time, whereas patients with higher EOT HBsAg levels rarely cleared the antigen. This finding aligns with a growing body of evidence that **low quantitative HBsAg at therapy cessation is the key determinant of off-therapy functional cure**. For example, a recent international cohort study (RETRACT-B) reported that having

296 HBsAg <100 IU/mL at NA discontinuation was associated with a >20-fold higher rate
297 of HBsAg loss. Similarly, a meta-analysis found an HBsAg loss rate of ~42% among
298 patients with EOT HBsAg <100 IU/mL, compared to only ~5% in those above this
299 threshold. Our results corroborate these figures, underscoring that patients with very low
300 residual HBsAg are the most likely to achieve HBsAg seroclearance after stopping
301 therapy.
302
303 Furthermore, we observed that **patients with low EOT HBsAg not only had higher**
304 **HBsAg loss rates but also lower rates of relapse and retreatment**. In our cohort, those
305 with HBsAg ≤100 IU/mL at treatment cessation experienced fewer virological
306 breakthroughs and clinical flares, resulting in a reduced need for NA resumption, relative
307 to patients with higher HBsAg levels. This suggests that low HBsAg levels reflect a state
308 of better immune control, which translates into both a higher chance of viral clearance
309 and a more benign off-therapy course. Notably, the aforementioned meta-analysis
310 demonstrated the same pattern: patients with EOT HBsAg <100 IU/mL had dramatically
311 lower risks of virologic relapse (~33% vs 72%) and biochemical relapse (~17% vs 35%)
312 compared to those with EOT HBsAg ≥100 IU/mL. Thus, **a low HBsAg level at**
313 **treatment withdrawal appears to identify an optimal subset of patients** who are
314 more likely to remain in remission and eventually clear the virus, whereas higher levels

portend frequent relapse requiring retreatment.

Overall, our findings are consistent with and extend prior research on finite NA therapy. The cumulative HBsAg loss rate in our total population (approximately 12% at 10 years) falls within the wide range reported by previous studies (≈2%–27% over 1–8 years) that included heterogeneous patient cohorts. Notably, some landmark studies in highly selected patients have reported very high functional cure rates – for instance, one HBeAg-negative cohort showed ~37% HBsAg clearance after NA discontinuation, highlighting that outcomes vary greatly depending on patient selection. Our data help clarify these discrepancies by confirming that **stringent selection criteria, especially using HBsAg levels, largely explain the differences in reported outcomes**. Indeed, the **prognostic value of low EOT HBsAg has been well documented** across studies. An international multicenter study found that HBsAg <100 IU/mL at cessation was associated with an adjusted hazard ratio of ~22 for HBsAg loss. Likewise, a recent meta-analysis of 24 studies identified EOT HBsAg ≤100 IU/mL (in Asian patients) as the optimal cutoff for stopping therapy, with an associated HBsAg loss rate of ~42%. Our results strongly reinforce these observations in a real-world setting, underscoring that HBsAg quantification is an indispensable tool for comparing and interpreting NA cessation studies.

In contrast, **few prior studies have explicitly examined the role of retreatment** in influencing HBsAg clearance, likely due to analytical complexity. Some observational reports noted lower HBsAg loss in patients who restarted therapy, but those analyses were subject to bias. By employing time-dependent models, our study provides novel insight that **retreatment itself does not alter the likelihood of HBsAg loss**. This finding makes intuitive sense given the virology of chronic HBV: HBsAg clearance is exceedingly rare during ongoing NA treatment (estimated <1% per year in Asian patients on therapy), so patients who relapse and resume NAs would not be expected to clear HBsAg unless they were predisposed to do so from the outset. In other words, the **act of continuing therapy (or not)** was not the determining factor for cure in our cohort; rather, the patient's immunovirological status at the end of therapy was. Our results therefore address an important gap in the literature by disentangling the effect of retreatment from the underlying risk factors. They suggest that the lower functional cure rates observed in retreated patients are attributable to those patients' inherently unfavorable profiles (e.g. higher HBsAg, indicating ongoing infection activity), rather than a truly suppressive effect of retreatment on the chance of clearance.

When placed in context of existing **clinical guidelines**, these findings support a more individualized approach to NA discontinuation. Major liver society guidelines have

353 historically differed on if and when to stop antivirals. For HBeAg-positive patients who
354 seroconvert, all guidelines endorse a finite consolidation therapy period before stopping
355 . However, for the more common HBeAg-negative patients, recommendations diverge.
356 The Asian Pacific Association for the Study of the Liver (APASL) suggests that
357 treatment can be stopped after at least 2 years of virological suppression in non-cirrhotic
358 patients, and the European Association for the Study of the Liver (EASL) allows
359 consideration of cessation after ≥3 years of suppression in select non-cirrhotic cases. In
360 contrast, the American Association for the Study of Liver Diseases (AASLD) has
361 traditionally recommended **continuing NA therapy indefinitely in HBeAg-negative**
362 **patients unless HBsAg loss is achieved**. Our findings lend support to the more
363 **permissive strategy of finite therapy** advocated by APASL and EASL – provided
364 patients are carefully chosen. We demonstrate that it is feasible to attain HBsAg
365 clearance off-treatment in a substantial subset of patients, which aligns with the
366 emerging view that *functional cure* can be pursued by stopping NAs under the right
367 circumstances. Importantly, our data also highlight the need for **clear retreatment**
368 **criteria and close monitoring**, which is reflected in the cautious tone of current
369 guidelines. The variability in retreatment practices across studies has been noted as a
370 factor impacting outcomes. By showing that low-HBsAg patients can maintain
371 remission with fewer interventions, our study provides evidence to refine future

guidelines – for instance, by incorporating **HBsAg threshold criteria into decisions about NA cessation** and by outlining which relapses truly warrant re-initiation of therapy.

Our study carries several practical implications for optimizing patient selection and management when stopping NAs. **First**, it underscores that **quantitative HBsAg is a critical metric for patient selection**. Clinicians should consider measuring HBsAg levels at the end of therapy as part of the decision-making process. Patients with EOT HBsAg levels ≤100 IU/mL are excellent candidates for a trial of NA discontinuation, as they have the highest probability of achieving HBsAg seroclearance and are relatively less prone to severe relapses. Using such a threshold could dramatically improve outcomes – one meta-analysis found that stopping therapy under this criterion yielded a functional cure in ~42% of cases. In our cohort, these patients not only cleared infection more often but also avoided retreatment in the majority of cases. In contrast, patients with high HBsAg levels (e.g. ≥1000 IU/mL) at the end of therapy have a very low chance of HBsAg loss (<5% in published studies) and a high risk of viral relapse. For these individuals, **NA continuation remains the safer strategy** unless additional interventions are employed. Thus, tailoring the timing of NA withdrawal to a patient's virological profile – especially their HBsAg titer – can improve the balance between

391 achieving cure and avoiding unnecessary risk. This approach is in line with recent
392 proposals to incorporate HBsAg thresholds (e.g. ≤100 IU/mL for Asians, ≤1000 IU/mL
393 for non-Asians) into stopping criteria, which our data strongly support.
394
395 Our findings suggest that the **timing of retreatment should be individualized**, and that
396 immediate retreatment after any sign of relapse may not always be necessary from the
397 standpoint of HBsAg clearance. Given that retreatment did not influence ultimate
398 HBsAg loss, it may be reasonable in certain low-risk patients to observe them through
399 modest virological relapses or ALT flares to allow the immune system a chance to clear
400 the virus. In patients with low EOT HBsAg (and no cirrhosis), a controlled off-therapy
401 observation period – with vigilant monitoring – could maximize the opportunity for
402 spontaneous HBsAg seroclearance. This concept is supported by the observation that
403 **HBsAg loss often follows an ALT flare** in those who successfully clear the infection
404 off-treatment. However, it is critical to emphasize that such an approach must be coupled
405 with careful safety measures. Our study recorded four patients (0.5% of the cohort) who
406 progressed to acute-on-chronic liver failure during a relapse (with one hepatitic flare
407 resulting in death), underscoring the potential dangers of untreated reactivation in
408 susceptible individuals. Therefore, **stringent criteria for retreatment are essential**.
409 Patients should be restarted on therapy promptly if they meet established safety

endpoints – for example, persistent or symptomatic ALT elevations, jaundice, or any evidence of incipient hepatic decompensation. Current practice generally advocates retreatment if a patient's ALT exceeds 5–10× the upper limit of normal or if clinical symptoms arise during relapse. Adhering to such criteria in our cohort likely prevented more severe outcomes, as the overall incidence of liver failure was low. In summary, while **delaying retreatment** may be considered in select cases to facilitate HBsAg clearance, this must be done within a robust monitoring framework. Regular follow-up (often every 1–3 months initially) with HBV DNA and liver chemistry testing is mandatory, and patients should be educated about reporting symptoms early. By combining judicious patient selection (favoring those with low HBsAg and good liver reserve) with a proactive monitoring and retreatment plan, clinicians can safely navigate the balance between achieving a cure and preventing adverse events.

In conclusion, this study provides new insights into the outcomes of NA discontinuation in chronic hepatitis B and the factors influencing functional cure. We demonstrated that after accounting for time-dependent biases, **retreatment for clinical relapse was not an independent determinant of HBsAg seroclearance** – instead, the chance of achieving HBsAg loss was overwhelmingly driven by baseline virological factors, particularly the patient's HBsAg level at the end of therapy. Our findings reinforce the

concept that **low EOT HBsAg is the paramount predictor of HBsAg clearance**, and such patients can achieve off-therapy outcomes that rival those reported in controlled trials. From a clinical perspective, our study supports the selective cessation of NA therapy in carefully chosen patients and provides evidence to refine guidelines on when to stop and when to restart treatment. We also highlight the need for vigilant monitoring, as rare but serious flares can occur, reminding us that safety should remain the top priority. Key limitations of our study include its retrospective design and the relatively small number of patients who achieved HBsAg loss (38 cases), which may limit the granularity of subgroup analyses. Additionally, our cohort was drawn predominantly from a single ethnic population and management setting, which may affect generalizability to other regions where host genetics, HBV genotypes, or retreatment practices differ. These limitations notwithstanding, our study contributes substantially to the understanding of NA discontinuation strategies by using robust time-to-event analysis to control for biases and by focusing on clinically relevant outcomes of relapse and retreatment. **Future research** should build on these findings by conducting prospective trials or registries that apply HBsAg-guided stopping rules, to confirm the safety and efficacy of this approach. Further investigation into complementary biomarkers (such as HBV RNA and HBcrAg) and immune correlates may improve risk stratification for relapse and cure. Moreover, studies on adjunctive therapies (for

example, therapeutic vaccines or immune modulators given at the point of NA withdrawal) could explore ways to increase the HBsAg loss rate in patients who do not meet the ideal criteria. In sum, our study helps refine the paradigm of finite NA therapy, suggesting that with the right patient selection and timing of retreatment, **functional cure of chronic hepatitis B is an attainable goal** beyond the traditional confines of indefinite antiviral therapy.

REFERENCES

1. Hsu YC, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. Nat Rev Gastroenterol Hepatol. 2023 Aug;20(8):524-537.
2. Polaris Observatory Collaborators. Global prevalence, cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modelling study. Lancet Gastroenterol Hepatol. 2023 Oct;8(10):879-907.
3. Hsu YC. Finite therapy of chronic hepatitis B infection: Cons. Clin Liver Dis (Hoboken). 2024 May 3;23(1):e0146.
4. Tseng CH, Chen TH, Wu JL, Lee TY, Borghi JA, Lin JT, Nguyen MH, Hsu YC. Serious adverse events after cessation of nucleos(t)ide analogues in individuals with chronic hepatitis B: A systematic review and meta-analysis. JHEP Rep. 2022 Oct

28;5(1):100617.

5. Liaw YF. Hepatitis B flare: the good, the bad and the ugly. Expert Rev Gastroenterol Hepatol. 2022 Nov-Dec;16(11-12):1043-1051.

6. Hirode G, Choi HSJ, Chen CH, Su TH, Seto WK, Van Hees S, Papatheodoridi M, Lens S, Wong G, Brakenhoff SM, Chien RN, Feld J, Sonneveld MJ, Chan HLY, Forns X, Papatheodoridis GV, Vanwolleghem T, Yuen MF, Hsu YC, Kao JH, Cornberg M, Hansen BE, Jeng WJ, Janssen HLA; RETRACT-B Study Group. Off-Therapy Response After Nucleos(t)ide Analogue Withdrawal in Patients With Chronic Hepatitis B: An International, Multicenter, Multiethnic Cohort (RETRACT-B Study). Gastroenterology. 2022 Mar;162(3):757-771.e4.

7. Tsai YN, Wu JL, Tseng CH, Chen TH, Wu YL, Chen CC, Fang YJ, Yang TH, Nguyen MH, Lin JT, Hsu YC. Hepatitis B core-related antigen dynamics and risk of subsequent clinical relapses after nucleos(t)ide analog cessation. Clin Mol Hepatol. 2024 Jan;30(1):98-108.

8. Hsu YC, Yeh ML, Wong GL, Chen CH, Peng CY, Buti M, Enomoto M, Xie Q, Trinh H, Preda C, Liu L, Cheung KS, Yeo YH, Hoang J, Huang CF, Riveiro-Barciela M, Kozuka R, Istratescu D, Tsai PC, Accarino EV, Lee DH, Wu JL, Huang JF, Dai CY, Cheung R, Chuang WL, Yuen MF, Wong VW, Yu ML, Nguyen MH. Incidences and Determinants of Functional Cure During Entecavir or Tenofovir Disoproxil

Fumarate for Chronic Hepatitis B. J Infect Dis. 2021 Dec 1;224(11):1890-1899.

9. Hsu YC, Nguyen MH, Mo LR, Wu MS, Yang TH, Chen CC, Tseng CH, Tai CM, Wu CY, Lin JT, Tanaka Y, Chang CY. Combining hepatitis B core-related and surface antigens at end of nucleos(t)ide analogue treatment to predict off-therapy relapse risk. Aliment Pharmacol Ther. 2019 Jan;49(1):107-115.

10. Liaw YF. Hepatitis B Flare After Cessation of Nucleos(t)ide Analogue Therapy in HBeAg-Negative Chronic Hepatitis B: To Retreat or Not to Retreat. Hepatology. 2021 Feb;73(2):843-852.

11. Liu YC, Jeng WJ, Peng CW, Chien RN, Liaw YF. Higher end-of-treatment HBsAg levels is associated with later onset but not severe relapse in HBeAg-negative chronic hepatitis B patients stopping antivirals. Aliment Pharmacol Ther. 2024 Mar;59(6):762-773.

12. Liaw YF, Chien RN. Finite nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B: From an "option" to an "active recommendation". Kaohsiung J Med Sci. 2022 Apr;38(4):295-301.

13. Tsai YN, Wu JL, Tseng CH, Tseng SC, Hung CL, Nguyen MH, Lin JT, Hsu YC. Association Between Elevation of Serum Alanine Aminotransferase and HBsAg Seroclearance After Nucleos(t)ide Analog Withdrawal. Aliment Pharmacol Ther. 2025 Apr;61(7):1208-1217.