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TITLE: Serum Levels of Hepatitis B Surface Antigen at Cessation of Entecavir Treatment Stratify Relapse Risk in End-of-therapy Hepatitis B E Antigen-negative Patients

Short title: HBsAg stratifies relapse risk off entecavir

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Keywords: chronic hepatitis B; antiviral therapy; nucleos(t)ide analogues; hepatitis B surface antigen; off-therapy durability

ABSTRACT

Background and Aims: We aimed to explore if serum hepatitis B surface antigen (HBsAg) level at the end of therapy (EOT) predicted relapse risk off entecavir treatment.

Methods: This prospective multicenter research enrolled 161 consecutive chronic hepatitis B patients who achieved viral undetectability after receiving entecavir for 3 years or longer. Following treatment cessation between July 1, 2011 and July 1, 2015, participants were monitored for clinical relapse (viral DNA >2,000IU/mL plus ALT >2 folds upper normal limit), and virological relapse (solely viral DNA >2,000 IU/mL). Outcome incidences were calculated by the Kaplan Meier method and risk factors determined by the Cox proportional hazard modelling.

Results: During follow-up until October 1, 2015, clinical and virological relapses occurred with a 2-year cumulative incidence of 49.2% (95% CI, 40.9-58.1%) and 81.7% (95% CI, 74.3-88.0%), respectively, in the entire cohort; and 39.2% (95% CI, 30.3-49.6%) and 77.4% (95% CI, 68.6-85.2%), respectively, in 124 EOT HBeAg-negative patients. There was a dose-response association between serum level of EOT HBsAg and relapse risk among EOT HBeAg-negative patients (P_{trend}=0.006 for clinical and 0.0001 for virological relapse). Multivariate Cox regression analysis revealed an adjusted hazard ratio (per log IU/mL increment) of 2.47 (95% CI,

1.45-4.23) for clinical, and 1.80 (95% CI, 1.33-2.45) for virological relapse. In 11 (9%) patients with EOT HBsAg <10 IU/mL, clinical remission was sustained throughout observation.

Conclusions: Serum level of EOT HBsAg is associated with relapse risk off entecavir treatment in EOT HBeAg-negative patients. A low titer predicts a durable off-therapy remission.

INTRODUCTION

Hepatitis B virus (HBV) infection remains a serious threat to global public health, chronically infecting 250 million people worldwide.[1] Chronic hepatitis B (CHB) may persist for decades even lifetime and lead to substantial morbidity and mortality.[2] Patients with CHB can be managed with nucleos(t)ide analogue (NUC),[3, 4] which potently inhibits the viral polymerase. Through sustained viral suppression, NUC is able to ameliorate hepatitis, reverse liver fibrosis, and may attenuate hepatocellular carcinogenesis.[5-7] However, cessation of treatment usually results in loss of viral remission. Clinical hepatitis may follow viral reactivation in some patients.[8-12] It remains controversial whether or when NUC can be discontinued.[13-16]

Serum level of hepatitis B surface antigen (HBsAg) is believed to correlate with host immune control of the virus.[17] Low level of the antigen helps to define inactive carrier state,[18] predict subsequent HBsAg loss,[19] and stratify the risk of HCC.[20] Moreover, quantification of HBsAg is pivotal in the response-guided therapy using interferons.[21] Whether it may help to identify patients who can safely discontinue NUC, however, has not been clarified.[11-13, 22-24]

This prospective multicenter cohort study aimed to elucidate if HBsAg at the end of therapy could stratify the risk of off-therapy relapse. Because viral reactivation does not always progress to clinical event,[13] we investigated both virological and clinical

relapses as the study outcomes.

METHODS

Study design, setting, and participants

This open cohort study prospectively recruited CHB patients under NUC therapy in E-Da Hospital (Kaohsiung, Taiwan), Lotung Poh-Ai Hospital (Yilan, Taiwan), and National Taiwan University Hospital Yun-Lin Branch (Yunlin, Taiwan). Institutional review board in each site approved the study protocol (EMRP100-049) prior to patient enrollment. All participants provided written informed consent.

NUC therapy for CHB has been covered by Taiwan national health insurance since October, 2003. Indications for and duration of reimbursement have been detailed.[6] Generally, the regimen was continuous for up to 3 years unless patients had special conditions such as liver cirrhosis, organ transplantation, or malignancy requiring cytotoxic chemotherapy. Hepatitis B e antigen (HBeAg)-positive patients were entitled for additional one-year consolidation after loss of HBeAg on treatment. When the reimbursement expired, those who could not bear the expense had to stop the regimen.

From July 1, 2011 to July 1, 2015, consecutive CHB patients who were about to discontinue NUC therapy were screened for eligibility: age >20 years, a diagnosis of

CHB for longer than 6 months, entecavir monotherapy for a minimum of 3 years, and undetectable viral DNA in serum at treatment cessation. Patients with human immunodeficiency virus or hepatitis C virus co-infection, malignancy, cirrhosis, hepatic encephalopathy, variceal hemorrhage, organ transplantation, exposure to interferon alpha for one month or longer, and use of cytotoxic or immunosuppressive agent were excluded. Cirrhosis was defined either histopathologically or clinically. In addition to sonographic criteria, splenomegaly or esophagogastric varices must be documented for a clinical diagnosis of liver cirrhosis.

Methods of measurement

Enrolled patients were evaluated for demographic information, biochemical tests, serological markers (HBsAg, HBeAg, anti-HBs, anti-HBe), and viral DNA. Study baseline was set at the day when patients discontinued entecavir. Thereafter, they were followed up every 3 months.

At each visit, serum specimens were collected and sent to the central laboratory in the Taipei Pathology Institutes (Taipei, Taiwan) for HBsAg and viral DNA quantification. HBsAg was quantified by the automated micro-particle immunoassay (Abbott Architect i2000, Abbott Park, IL, USA; automatic range 0.05~250 IU/mL), in conjunction with manual dilution for samples with a concentration >250 IU/mL. HBV DNA was quantified by the commercialized polymerase chain reaction method

(COBAS TaqMan HBV Test, version 2.0, Roche Molecular Systems, Inc., USA) with a detection range from 20 to 1.7 x 10^8 IU/mL.

Definition of end points

Primary endpoint was clinical relapse, defined as serum HBV DNA >2,000 IU/mL and serum alanine transaminase (ALT) >2 folds the upper limit of normal. Secondary endpoint was virological relapse, defined solely as serum HBV DNA >2,000 IU/mL regardless of ALT abnormality. According to the insurance regulations,[6] clinical relapse was at first closely observed without immediate retreatment. Patients would reuse NUC only if hepatitis unremitted for at least 3 months, unless there was a concern of hepatic decompensation (serum bilirubin >2 mg/dL or prothrombin time prolonged >3 seconds). Outcomes were compared according to end-of-therapy (EOT) HBsAg. We observed participants for outcome occurrence until they reused antiviral therapy or the last hospital visit. The observation period ended on October 1, 2015.

Data analysis and statistical methods

Continuous variables were summarized by median and interquartile range (IQR). Between-group difference was examined by the Mann-Whitney test. Categorical variables were expressed with proportion and exact number, and the difference was compared by the Fisher's exact test. Cumulative incidences of the outcomes were estimated and plotted by the Kaplan Meier method with right censoring. We used the

log rank test to examine difference in the failure time among groups of patients, and the Cox proportional hazard model to identify predictors of off-therapy relapse. Irrespective of results in the univariate analyses, the multivariate modelling appraised all potential factors with a stepwise approach that retained only statistically significant variables in the final model. The hazard ratio (HR) along with its 95% confidence interval (CI) was reported. Data was managed and analyzed with a commercial software (Stata, version 13.0; Stata Corp, College Station, TX, USA). All statistical analyses were two-sided with a *P* value <0.05 defined as significant.

RESULTS

Baseline characteristics of the study participants

This study screened a total of 260 patients scheduled to discontinue NUC and finally enrolled 161 eligible individuals (Supplementary Figure 1), whose baseline features were summarized in Table 1. EOT HBeAg was negative in 124 patients, among whom 34 were HBeAg-positive on treatment commencement.

Clinical and virological relapses after NUC discontinuation

Clinical and virological relapses occurred in 68 and 115 participants, respectively, during a median follow-up of 17.0 (IQR, 9.4-24.8) months. The corresponding 2-year cumulative incidences among all participants were 49.2% (95% CI, 40.9-58.1%) and

81.7% (95% CI, 74.3-88.0%), respectively (Table 2). Besides, 30 patients suffered hepatitis flare (defined as ALT>200 IU/mL),[25] with a 2-year cumulative incidence of 27.5% (95% CI, 19.8~37.4%).

The risk of relapse significantly differed according to EOT HBeAg status (supplementary Figure 2). Almost all EOT-HBeAg-positive patients encountered virological relapse (Table 2), and most (27 of 32, 84.4%) of them went on to develop clinical hepatitis within a short interval (median, 3; IQR, 0-6 months). Among EOT HBeAg-negative patients, 77.4% (95% CI, 68.6-85.2%) would experience virological relapse at 2 years (Table 2), and half (49.4%, 41 out of 83) of them would then suffer clinical relapse.

Off-therapy outcomes according to HBsAg levels

The association between EOT HBsAg and off-therapy relapse depended on EOT HBeAg status. Relapse rates in those who remained HBeAg-positive were strikingly high regardless of HBsAg (Supplementary Figure 3). Significant association between EOT HBsAg levels and relapse risk was noted in EOT HBeAg-negative patients (Figure 1), among whom clinical hepatitis relapsed in no one with EOT HBsAg <10 IU/mL (N=11, 9%), but in 26.4% (95% CI, 9.1-62.4%), 41.1% (95% CI, 28.4-56.9%), and 49.7% (95% CI, 34.7-67.0%) of patients with HBsAg 10~100, 100~1,000 IU/mL and ≧1,000 IU/mL, respectively (P_{trend}=0.006; Figure 1A). Similarly, virological

relapse rose from 9.5% (95% CI, 1.4-50.9%), 63.2% (95% CI, 37.9-87.7%), 81.1% (95% CI, 68.8-90.9%), to 93.1% (95% CI, 79.8-98.9%) with HBsAg <10 IU/mL, 10~100 IU/mL, 100~1,000 IU/mL, and ≧1,000 IU/mL, respectively (P_{trend}=0.0001; Figure 1B). There were still relapses after 2 years; clinical hepatitis took place only in patients with HBsAg>1,000 IU/mL and virological event only in HBsAg>100 IU/mL.

Eight EOT HBeAg-negative patients lost HBsAg 3-18 months after NUC cessation. EOT HBsAg was lower than 10 IU/mL in 7 and 10.49 IU/mL in one of these patients. In other words, 63.6% (7 out of 11) of those with EOT HBsAg <10 IU/mL would lose HBsAg during follow-up (P<0.0001 by log rank test, as compared with >10 IU/mL).

Multivariate regression and stratified analyses for the association between HBsAg and off-therapy relapse in EOT HBeAg-negative patients

Unadjusted analyses found that HBsAg, AST, and ALT were associated with clinical relapse, whereas HBsAg, ALT, and pretreatment viral load with virological relapse. In the multivariate-adjusted analysis, age, HBsAg, and ALT were risk factors for both clinical and virological relapses (Table 3). The discrepancy between unadjusted and adjusted analyses for age was explained by an inverse correlation between age and HBsAg (Spearman's rho= -0.31, P=0.0004; Supplementary Figure 4).

The association between HBsAg gradient and off-therapy relapse was consistent

across patient subgroups stratified by age, gender, pretreatment HBeAg, pretreatment and EOT anti-HBe status, and the treating hospital (Figure 2). In particular, it was compatible whether HBeAg was positive or negative at NUC commencement. Formal statistical test ascertained no interaction between pretreatment HBeAg status and HBsAg in the association with off-therapy relapse (*P* value for the interaction term was 0.63 for clinical and 0.7 for virological), despite an insignificant result for clinical relapse in the pretreatment HBeAg-positive subgroup, probably as a result of its sample size. In fact, relapse patterns were similar in EOT HBeAg-negative patients irrespective of their pretreatment HBeAg status (Supplementary Figure 5).

HBsAg cutoff to distinguish patients with durable off-therapy remission

The cutoffs were set at 10 and 100 IU/mL to illustrate how HBsAg level might help to distinguish off-therapy outcomes in EOT HBeAg-negative patients (Figure 3). All individuals with HBsAg <10 IU/mL (N=11, 9%) maintained clinical remission through follow-up (Figure 3A). One male patient faced recurrence of viremia up to 2,949 IU/mL at 9 months (Figure 3B), with serum ALT peaked at 33 IU/L. HBV DNA was undetected thereafter.

With the cutoff set at 100 IU/mL (N=25, 20%), 3 patients would develop clinical relapse (cumulative incidence of 15.3% at 2 years; Figure 3C). Virological relapse would reach 39.6% (95% CI, 22.7-62.7%) at 2 years in these patients (Figure 3D).

DISCUSSION

This prospective research uncovers a significant association between level of EOT HBsAg and risk of relapse following entecavir cessation in EOT HBeAg-negative CHB patients. One log IU/mL increment of HBsAg is associated with an adjusted HR of 2.47 (95% CI, 1.45-4.23) for clinical relapse, and 1.80 (95% CI, 1.33-2.45) for virological relapse. The association is consistent regardless of pretreatment HBeAg and anti-HBe status. These findings suggest a low HBsAg titer (e.g. <10 IU/mL) indicates durable off-therapy remission.

When to stop NUC remains a controversial issue in the management of CHB.[8, 14-16] For pretreatment HBeAg-positive patients, emerging data has questioned HBeAg/anti-HBe seroconversion as a reliable endpoint.[9, 10, 24] Our study corroboratively unraveled a high relapse rate despite HBeAg loss with treatment consolidation, and indicated that NUC-induced HBeAg seroconversion was essential but insufficient for a durable remission off therapy. In patients who start NUC on negative HBeAg, this issue is even more debatable. HBsAg loss has been shown to foresee off-therapy durability,[26] but it is unfortunately remote in most NUC-treated patients,[27] and hardly realistic as a national policy in countries prevalent with CHB.[28] Besides, indefinite drug exposure raises concern of potential harms that may evade

short-term surveillance.

The ability of HBsAg to predict relapse risk probably results from its correlation with intranuclear covalently closed circular DNA, which in turn gauges immune control of the virus.[29] Nonetheless, results from existing literature has been conflicting.[11-13, 22-24] Compatible with our research, Chan et al., found that EOT HBsAg $\leqq$100 IU/mL in conjunction with on-therapy decline >1 log predicted viral remission (HBV DNA $\leq$200 IU/ml) 12 months off lamivudine.[23] Hadziyannis et al., also observed a significant association of EOT HBsAg with virological and biochemical relapses following cessation of adefovir.[13] Chen and colleagues recently demonstrated in retrospective analyses that EOT HBsAg level was associated with subsequent HBsAg loss and viral remission in lamivudine[22] and entecavir users.[24] In agreement with our study, they also found older age was a risk factor for off-therapy relapse.[22, 24]

Contradictory to aforementioned supportive data, Jeng et al., reported HBsAg, measured either prior to or at the end of treatment, was unrelated to clinical relapse after discontinuing entecavir.[11] In Jeng's study, however, only 8 patients had EOT HBsAg below 100 IU/mL. A multicenter study from Hong Kong also revealed a negative result for viral reactivation in 184 patients who stopped entecavir,[12] but it could not elucidate the association with clinical event because participants reused

NUC for virological relapse. Discrepancy among these studies may result from differences in patient number with low EOT HBsAg, prospective or retrospective design, outcome definition, treatment duration, length of observation, and retreatment policy.

What event defines a "meaningful" relapse that indicates resumption of NUC is another source of controversy. Some studies regarded resurgence of viral DNA as the endpoint,[12, 22, 23] whilst others considered elevation of ALT necessary to define a clinically relevant event.[11, 13, 24] Moreover, a spot measurement was all it needed in some studies,[12, 23] while a certain duration of persistent abnormality was required in others.[13, 22] Therefore, our study covered both virological and clinical relapses. As what has been shown by Hadziyannis and colleagues,[13] we also noted that clinical hepatitis did not always follow virological relapse in EOT HBeAg-negative patients. In fact, half of (49.4%) virological episodes were clinically unremarkable. Furthermore, clinical relapse might resolve spontaneously and we found not all (n=27, 65.9%) events result in retreatment. On the other hand, some experts believe retreatment justified as long as viral DNA resurges above 2,000 IU/mL, which is a recognized risk factor for long-term complications.[30] Clearly, it warrants further research to elucidate these controversies.

While we explicitly showcased the dose-response relationship of EOT HBsAg

with off-therapy relapse, the present study did not intend to endorse any specific cutoff point. Apparently, there is a tradeoff between less risk of relapse and more patients allowed to stop medication safely. A titer <10 IU/mL forecasts a negligible risk, but this threshold permits only 11 from 124 (9%) EOT HBeAg-negative patients to discontinue entecavir. With the cutoff elevated to 100 IU/mL, 25 patients (20%) were qualified to stop therapy, but some would encounter clinical hepatitis again, with an estimated incidence of 15.3% at 2 years. In our opinion, a healthcare system as well as a treating physician needs to thoroughly appraise prevalence of the disease, cost-effectiveness evaluation, resource allocation priority, patient expectation, and accessibility to medical attention, when deciding the cut-point.

Consecutive enrollment with prospective follow-up in multiple centers is an obvious strength of our study. This minimizes the concerns of selection bias and permits extrapolation to the real-world practice. Besides, our study is informative in various off-therapy outcomes since participants were closely observed without immediate retreatment on occasion of relapse. In order to unbiasedly assess the relationship of HBsAg and off-therapy outcomes, we meticulously analyzed the data from different angles and deliberately avoided the “optimal point” approach that dichotomized HBsAg in regression analysis.[31] Despite its popularity, this approach may adversely inflate the type I error as a result of multiple testing.[32] Furthermore, our

findings were ascertained by multivariate-adjusted analysis that also took interaction among covariates into account.

Several caveats warrant attention. First, the virus could not be genotyped since HBV DNA was undetectable when patients were enrolled and blood specimen was not routinely collected prior to enrollment. Chen et al., did report a positive link between off-therapy relapse and genotype C in pretreatment HBeAg-positive entecavir users, but not in the HBeAg-negative counterparts.[24] Most previous studies found no association with viral genotype.[8, 11, 22] Therefore, we believe adjustment for genotype would not change our result although we do acknowledge this limitation. Second, this study was not purposefully designed to clarify how treatment or consolidation duration might affect the relapse risk, in that the health insurance tightly regulated the antiviral regimen.[6] The strict regulation might limit capability to detect influence of therapeutic length, but meanwhile it should control its potential confounding influence. After all, the entire cohort did not differ much in therapeutic duration and all pretreatment HBeAg-positive patients consolidated treatment for a minimum of one year after losing HBeAg. Finally, we understood it would evoke concern to enroll EOT HBeAg-positive patients who had to stop treatment. Against guideline recommendation, this situation happens in the real-world, especially in resource-constrained countries.[28] In view of the complete paucity of data from these

patients, we believed their results worthy of report.

In summary, this study reveals a higher level of EOT HBsAg is associated with a greater risk of relapse following cessation of entecavir in EOT HBeAg-negative patients. This association is independent to multiple covariates including pretreatment HBeAg status. A low titer such as <10 IU/mL may indicate durable off-therapy remission, although it warrants further research to determine the cut point in clinical practice. These findings add to current knowledge on how to utilize HBsAg in guiding NUC cessation.

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Table 1. Characteristics of the study cohort

Characteristics	All (*n* =161)
Status at NUC discontinuation	
Age, years	48.08 [39.71-56.10]
Male gender, *n* (%)	122 (75.8%)
Positive HBeAg, *n* (%)	37 (23.0%)
Positive anti-HBe, *n* (%)	118 (73.3%)
HBsAg, log IU/mL	2.89 [2.31-3.28]
HBsAg <100 IU/mL	28 (17.4%)
HBsAg <10 IU/mL	11 (6.8%)
AST, IU/L	25 [21-32]
ALT, IU/L	23 [16-32]
Alpha-fetoprotein, ng/ml	2.68 [1.98-3.65]
Treatment duration, months	36.6 [36.4-37.0]
Consolidation duration*, months	20.6 [12.2-28.5]
Recruiting hospital	
E-Da Hospital	100 (62.1%)
Lotung Poh-Ai Hospital	35 (21.7%)
NTUH Yun-Lin Branch	26 (16.2%)
Status prior to NUC therapy	
Positive HBeAg, *n* (%)	71 (44.1%)
Positive anti-HBe, *n* (%)	92 (57.1%)
HBV DNA, log IU/ml	6.60 [5.03-9.04]
HBsAg <100 IU/ml, *n* (%)	13 (8.3%)
HBsAg <10 IU/ml, *n* (%)	2 (1.2%)
AST, IU/L	102 [61-229]
ALT, IU/L	164 [99-451]

Notes. *Consolidation indicates antiviral therapy following HBeAg seroconversion in pretreatment HBeAg-positive patients. ALT, Alanine transaminase; AST, Aspartate transaminase HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NUC, nucleos(t)ide analogue; NTUH, National Taiwan University Hospital

Table 2. Clinical and virological outcomes in all participants, and according to HBeAg status at the end of therapy

	All study participants (n=161)	
	First year	Second year
Clinical relapse	37.8% (95% CI, 30.3-46.4%)	49.2% (95% CI, 40.9-58.1%)
Virological relapse	66.2% (95% CI, 58.4-73.8%)	81.7% (95% CI, 74.3-88.0%)
	End-of-therapy HBeAg-negative patients (n=124)	
	First year	Second year
Clinical relapse	25.8% (95% CI, 18.6-35.2%)	39.2% (95% CI, 30.3-49.6%)
Virological relapse	59.8% (95% CI, 50.8-69.0%)	77.4% (95% CI, 68.6-85.2%)
	End-of-therapy HBeAg-positive patients (n=37)	
	First year	Second year
Clinical relapse	68.6% (95% CI, 53.6-82.6%)	81.7% (95% CI, 67.1-92.5%)
Virological relapse	87.4% (95% CI, 73.9-95.9%)	95.8% (95% CI, 83.1-99.7%)

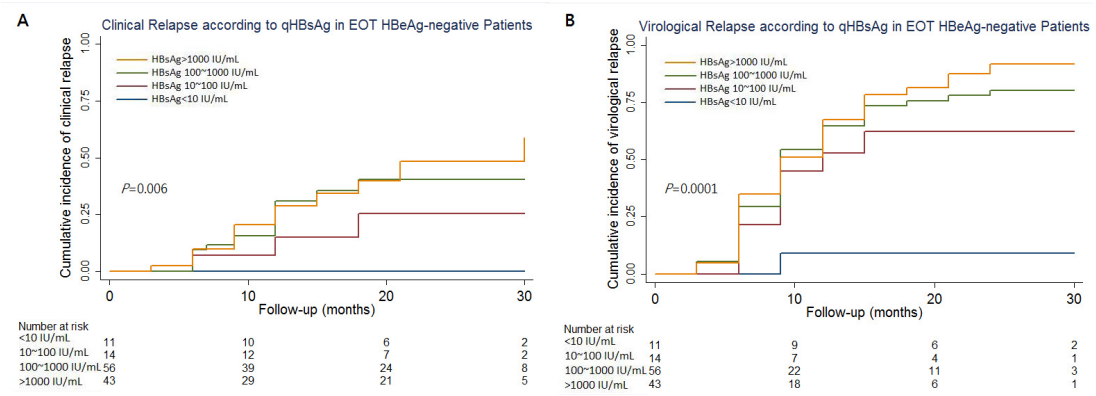
Note: CI, confidence interval; NA indicated that no patients remained free of the event at that time point

Table 3: Cox proportional hazard analysis for clinical and virological relapses in end-of-therapy HBeAg-negative patients

Clinical Relapse	Univariate analysis			Multivariate modelling		
	HR	95 % CI	*P*	Adjusted HR	95% CI	*P*
Age, year	1.01	0.99-1.04	0.26	1.04	1.01-1.07	0.002
Female gender	0.63	0.28-1.42	0.26			
HBsAg, log IU/mL	1.89	1.21-2.97	0.006	2.47	1.45-4.23	0.001
Anti-HBe positivity	0.73	0.22-2.35	0.59			
AST, IU/L	1.03	1.01-1.04	<0.001			
ALT, IU/L	1.02	1.01-1.03	<0.001	1.03	1.02-1.04	<0.001
Alpha-fetoprotein, ng/ml	1.08	0.99-1.17	0.08			
Treatment duration, months	1.02	0.97-1.08	0.36			
Consolidation duration*, months	0.98	0.92-1.03	0.4			
Pretreatment HBeAg positivity	0.76	0.38-1.51	0.43			
Pretreatment anti-HBe positivity	1.17	0.59-2.29	0.65			
Pretreatment viral load, log IU/ml	1.14	0.96-1.36	0.13			
Pretreatment AST, IU/L	1.0	0.998-1.0	0.22			
Pretreatment ALT, IU/L	1.0	0.999-1.0	0.22			
Virological Relapse	**Univariate analysis**			**Multivariate modelling**		
	HR	95 % CI	*P*	Adjusted HR	95% CI	*P*
Age, years	1.01	0.99-1.03	0.24	1.03	1.01-1.05	0.004
Female gender	0.98	0.58-1.63	0.92			
HBsAg, log IU/mL	1.59	1.23-2.05	<0.001	1.80	1.33-2.45	<0.001
Anti-HBe positivity	0.74	0.30-1.83	0.51			
AST, IU/L	1.01	0.998-1.02	0.10			
ALT, IU/L	1.01	1.0-1.02	0.03	1.01	1.0-1.02	0.003
Alpha-fetoprotein, ng/ml	1.03	0.95-1.12	0.46			
Treatment duration, months	1.02	0.99-1.06	0.26			
Consolidation duration*, months	1.0	0.96-1.04	0.9			
Pretreatment HBeAg positivity	0.90	0.56-1.44	0.66			
Pretreatment anti-HBe positivity	1.18	0.73-1.89	0.51			
Pretreatment viral load, log IU/ml	1.16	1.03-1.31	0.02			
Pretreatment AST, IU/L	1.0	0.999-1.0	0.74			
Pretreatment ALT, IU/L	1.0	0.999-1.0	0.37			

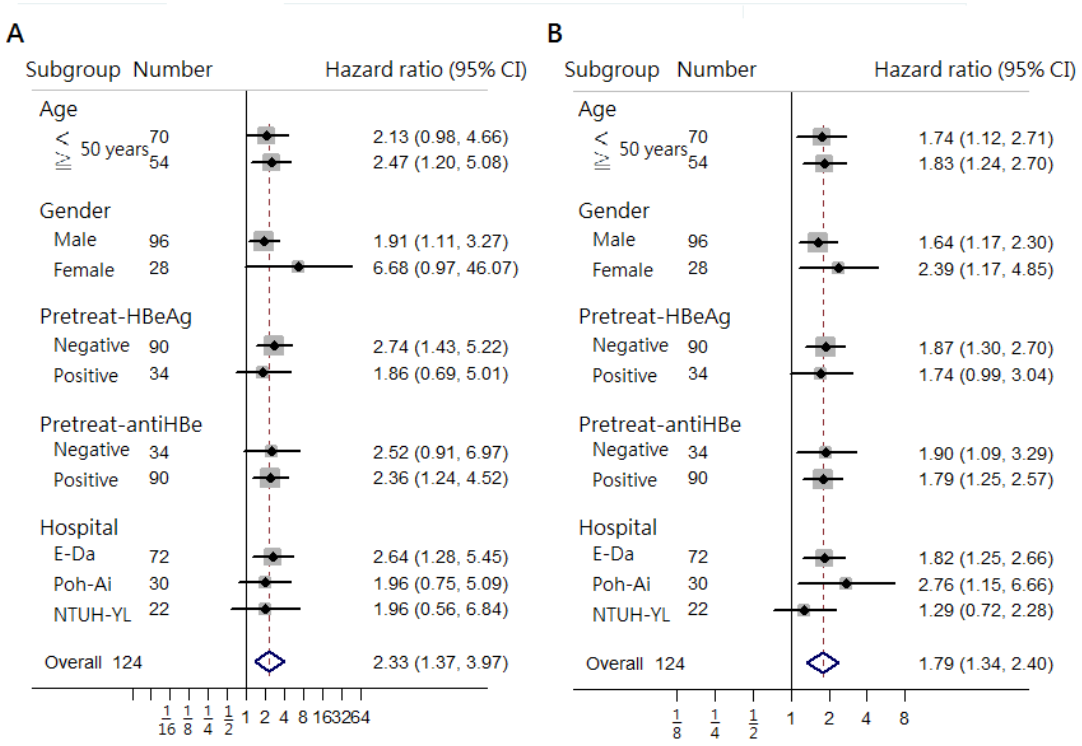
Note. *Consolidation indicates antiviral therapy following HBeAg seroconversion in pretreatment HBeAg-positive patients, and is only examined in this subgroup. Explanatory variables were measured at treatment cessation, unless specified with "pretreatment" description

Figure 1



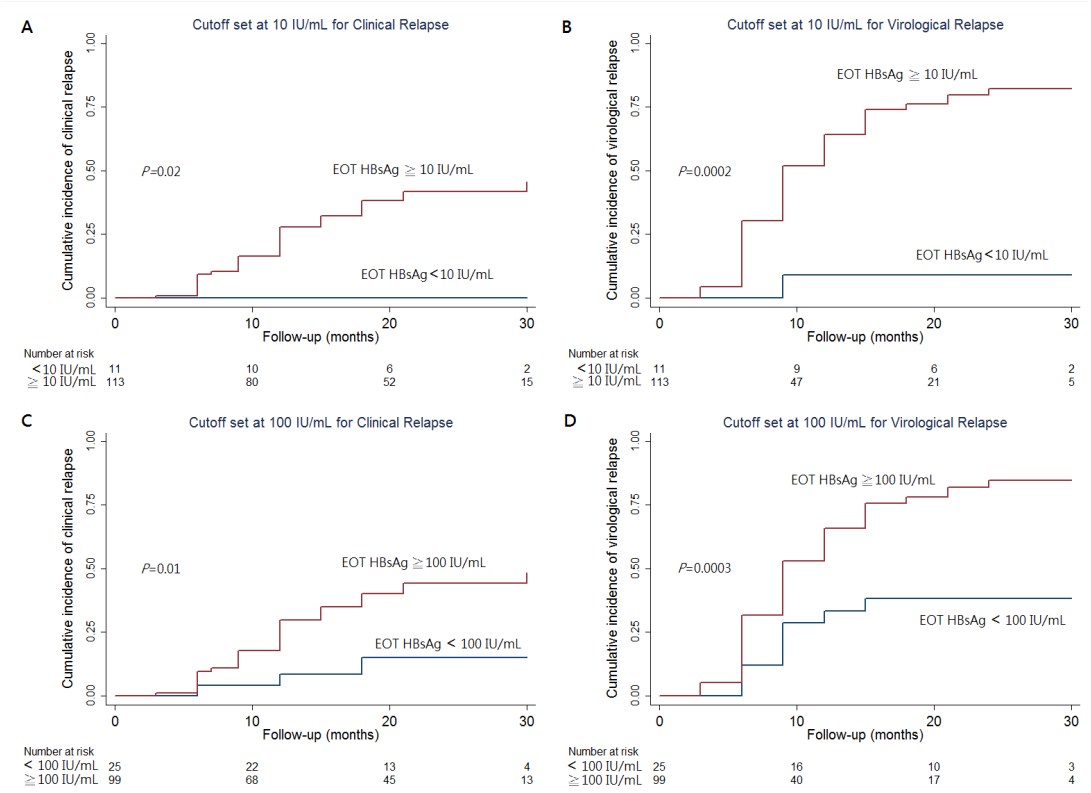
Legends. Significant dose-response association between end-of-therapy HBsAg level and clinical (panel A) as well as virological (panel B) relapse in patients with negative HBeAg at the end of treatment. EOT, end-of-therapy.

Figure 2



Legengds: Age-adjusted stratified analyses for the association of end-of-therapy HBsAg with clinical (panel A) and virological (panel B) relapses.

Figure 3



Legends: HBsAg cutoff set at 10 IU/mL (upper panels: A for clinical, B for virological) and 100 IU/mL (lower panels: C for clinical, D for virological) to stratify relapse risks.

Supplementary Figures

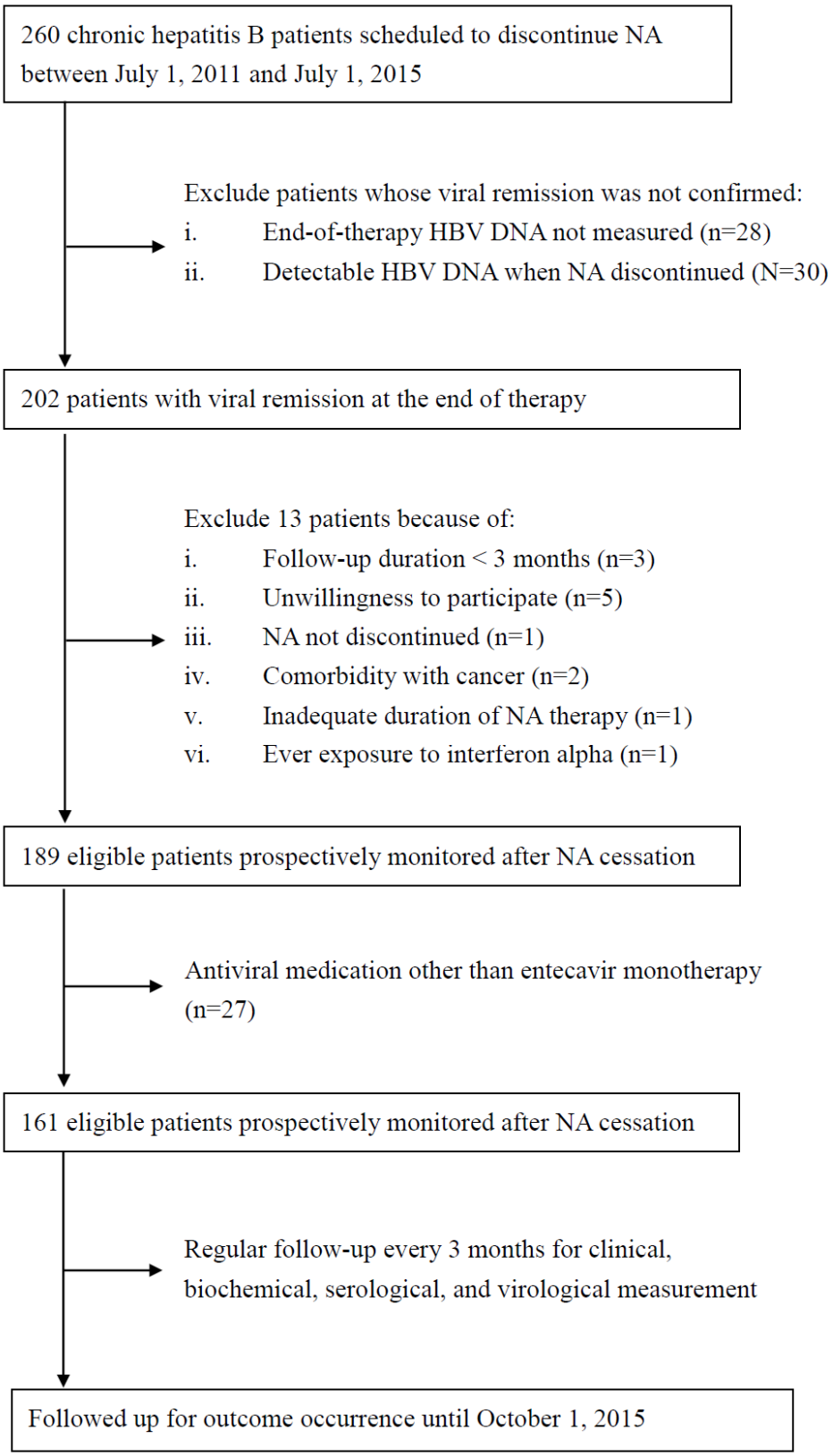
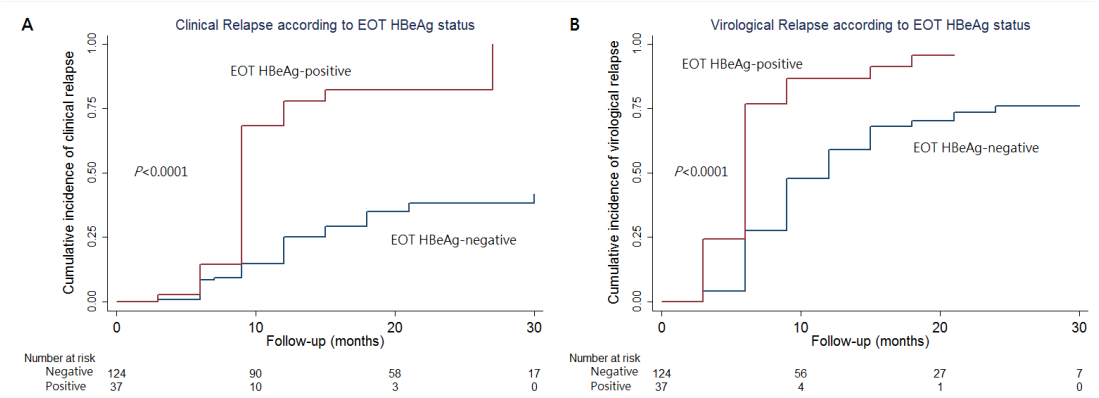
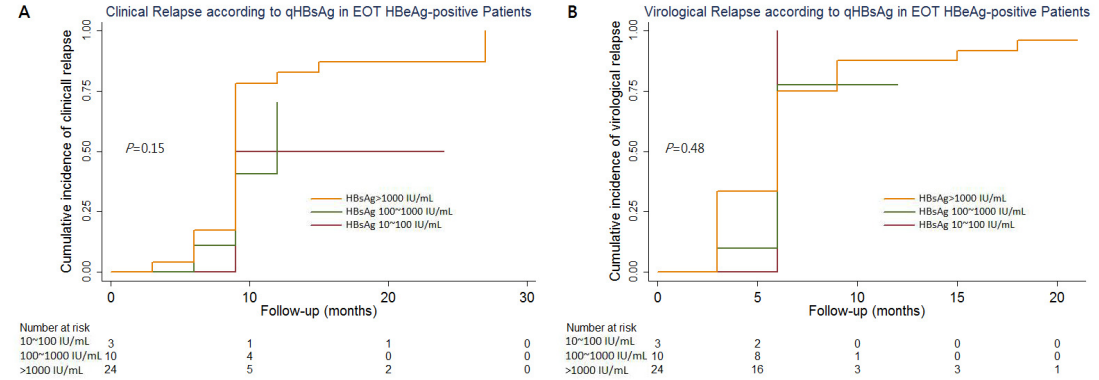


Figure 1. Flowchart illustrating identification and enrollment of participants

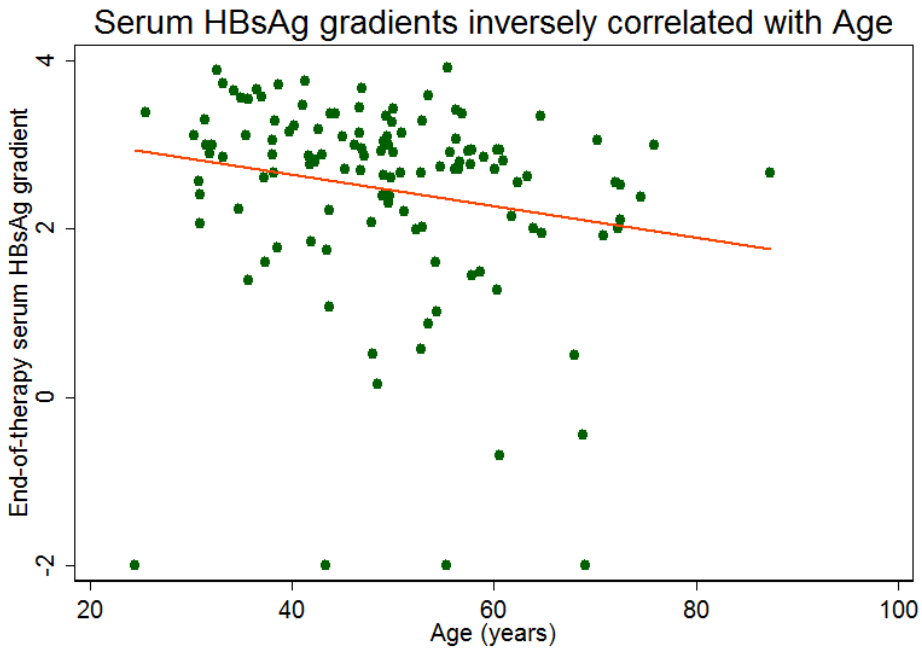
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5