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Incidence and Predictors of Hepatitis B Surface Antigen Seroclearance After Cessation of Nucleos(t)ide Analogue Therapy in Hepatitis B e Antigen–Negative Chronic Hepatitis B

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Hepatitis B surface antigen (HBsAg) loss is a rare event during nucleos(t)ide analogue (Nuc) therapy. Limited data suggest that stopping Nuc therapy may increase HBsAg loss rate in hepatitis B e antigen–negative patients. A large study was conducted to investigate this issue in more detail. Of the 1,075 hepatitis B e antigen–negative patients treated with Nuc for a median of 156 (61-430) weeks, 5 showed HBsAg seroclearance during treatment at an estimated annual incidence of 0.15%. Of the patients who remained HBsAg-seropositive, 691 (52.3 years old, 86% male, 44.6% cirrhosis) had stopped Nuc therapy by the Asian-Pacific Association for the Study of the Liver stopping rule and then were prospectively followed up. Baseline and on-treatment clinical and viral features, treatment duration, consolidation duration, time to undetectable hepatitis B virus DNA, time to normal alanine aminotransferase, end-of-treatment HBsAg, and HBsAg log reduction were compared between patients with and without HBsAg seroclearance after end of treatment. During a median off-therapy follow-up period of 155 (2-614) weeks, HBsAg seroclearance was confirmed in 42 patients. The 6-year cumulative incidence was 13% with an estimated annual incidence of 1.78%. Cox regression analysis showed that shorter time to undetectable hepatitis B virus DNA (<12 weeks), greater HBsAg reduction during therapy (>1 $\log_{10}$), lower end-of-treatment HBsAg level (<100 IU/mL), patients with sustained response, and relapsers not retreated were factors for off-therapy HBsAg seroclearance. *Conclusion:* The incidence of HBsAg seroclearance after stopping Nuc was much higher than that during therapy and highest in patients without virologic and clinical relapse; patients with clinical relapse who remained untreated had a 7.34 times higher incidence of HBsAg clearance than those who received retreatment, suggesting that transient untreated clinical relapse may drive sufficient immune control to functional cure. (HEPATOLOGY 2017; 00:000-000).

Chronic hepatitis B virus (HBV) infection is difficult to eradicate by current therapy with nucleos(t)ide analogue (Nuc) or interferon. All guidelines of major liver associations have considered hepatitis B surface antigen (HBsAg) seroclearance as a functional cure of HBV infection and the ideal endpoint of HBV treatment.[1-3] Unfortunately, clinical studies have shown that HBsAg seroclearance during long-term Nuc therapy is a rare event.[4,5] A large study involving 5,409 patients with hepatitis B e antigen (HBeAg)–negative chronic hepatitis B (CHB) even showed an annual incidence as low as 0.33%

Abbreviations: ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; CHB, chronic hepatitis B; EOT, end of treatment; ETV, entecavir; HBeAg, hepatitis B e antigen/antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; Nuc, nucleoside(t)ide analogue; qHBsAg, HBsAg quantification level; TDF, tenofovir disoproxil fumarate.

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during 6-year Nuc therapy.[6] As calculated, one to several decades of Nuc therapy are required to achieve HBsAg seroclearance.[7,8] Obviously, HBsAg seroclearance is an ideal but a remote and unrealistic endpoint of Nuc therapy. In addition, there are problems of indefinite Nuc therapy such as cost, reimbursement, adherence, and unknown safety over 10 years. For these reasons, the Asian Pacific Association for the Study of the Liver (APASL) has set up a stopping rule since year 2008 that cessation of Nuc therapy can be considered in HBeAg-negative patients with chronic HBV infection if undetectable HBV DNA has been documented on three separate occasions, each at least 6 months apart.[3,9] Since then more and more studies on the cessation of Nuc therapy in HBeAg-negative hepatitis with CHB have been reported, not only from Asia but also from non-Asian countries.[10,11]

Because a pivotal study in 33 HBeAg-negative patients showed that cessation of a course of 4-5 years of adefovir therapy was followed by a 5-year cumulative HBsAg loss rate of 39%,[12] cessation of Nuc therapy has been attempted as a strategy to increase HBsAg seroclearance during off-Nuc follow-up in small studies.[13,14] However, the issues surrounding HBsAg seroclearance after cessation of Nuc therapy in HBeAg-negative CHB have not been well addressed or elucidated by studies involving large enough numbers of patients. We therefore conducted this prospective follow-up study after cessation of Nuc therapy in 691 HBeAg-negative patients with chronic HBV infection.

Patients and Methods

PATIENTS

This real-world prospective study with retrospective assays is part of a study project on posttreatment relapse in CHB patients receiving NUC therapy at Chang Gung Memorial Hospital, Linkou Medical Center, approved by the institutional review board of our hospital (104-9716B).

In October 2003, health authorities of Taiwan started to reimburse antiviral therapy for (1) patients who had cirrhosis with HBV DNA levels $\geq$2,000 IU/mL regardless of alanine aminotransferase (ALT) level or (2) viremic CHB patients with serum bilirubin levels >2 mg/dL or whose ALT remained >2 times the upper limit of normal for 3-6 months. The maximal duration of reimbursement for CHB patients has been extended from 18 months to 3 years since November 2009, with no more time limit of reimbursement for patients with cirrhosis, diagnosed by pathology or presence of both ultrasonographic features of coarse liver parenchyma plus splenomegaly/endoscopic varices since July 2010.

This study included all HBeAg-negative, antibody to HBeAg-positive patients who had been treated with Nuc monotherapy and discontinued therapy after demonstration of undetectable HBV DNA on three occasions, each at least 6 months apart, which is consistent with the APASL stopping rule.[9] Patients whose Nuc therapy was switched from one Nuc to another and patients with other liver disease or concurrent hepatitis virus(es) infection were excluded.

MONITORING DURING AND AFTER CESSATION OF Nuc THERAPY

In addition to conventional tests, HBV DNA was assayed at baseline, every 3-6 months during Nuc therapy, and at the end of treatment (EOT). Serum HBsAg quantification level (qHBsAg) was assayed at baseline and EOT, with additional assays when appropriate.

After cessation of Nuc therapy, serum ALT level was monitored every 1-1.5 months in the first 3 months and then at least every 3 months in addition to

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a serum HBV DNA assay every 3 months in the first year and every 3-6 months afterward during off-therapy follow-up evaluation. Hepatocellular carcinoma (HCC) surveillance using α-fetoprotein and ultrasonography was performed every 3-6 months. If the serum HBV DNA level was >2,000 IU/mL or an ALT increase was detected, the HBV DNA and/or ALT levels were measured more frequently. If the ALT level was increasing or more than 5 times the upper limit of normal, serum ALT, bilirubin, and prothrombin time were measured every 1-2 weeks before stabilization or subsidence, as recommended by the APASL guidelines.[9] At the discretion of their treating physician, patients with relapse were retreated if eligible for reimbursements.

LABORATORY METHODS

Biochemical tests were performed using routine automated techniques at the clinical pathology laboratories of the hospital. Serum hepatitis markers including HBsAg, antibody to HBsAg, HBeAg, antibody to HBeAg, anti–hepatitis D virus, and anti–hepatitis C virus were assayed using the Enzyme Immunoassay kit (Abbott Diagnostics, North Chicago, IL). HBV genotype was determined using polymerase chain reaction–restriction fragment length polymorphism of the surface gene of HBV. Serum HBV DNA was assayed using the Roche Cobas Amplicor TaqMan HBV Monitor test (detection limit, 20 IU/mL; Roche Diagnostics, Pleasanton, CA), and the serum qHBsAg level was measured using the Roche Elecsys HBsAg II quant assay (range, 0.05-52,000 IU/mL; Roche Diagnostics, Mannheim, Germany).

DEFINITIONS

The duration of consolidation therapy was calculated from the first demonstration of undetectable HBV DNA level to the EOT. According to APASL guidelines, virologic relapse was defined as a serum HBV DNA level increase of >2,000 IU/mL, and clinical relapse was defined as a virologic relapse plus a serum ALT level >2 times the upper limit of normal.[3] Hepatic decompensation was defined as a severe clinical syndrome with jaundice and prolonged prothrombin time (international normalized ratio ≥1.5) and/or occurrence of ascites/encephalopathy in patients with or without cirrhosis.[15,16] Cirrhosis was diagnosed by histologic findings (144 patients) or repeated ultrasonography consistent with cirrhosis, supplemented with the presence of varices (29 patients), splenomegaly (47 patients), and/or thrombocytopenia, as described.[17]

STATISTICAL ANALYSIS

Age, sex, presence of cirrhosis, prior treatment, baseline biochemical data and viral features, serum HBV DNA and ALT levels at the end of 3 and 6 months on therapy, serum HBsAg, HBV DNA, and ALT levels at baseline and at EOT, as well as treatment duration and consolidation duration, were compared between patients with clinical relapse (*relapsers*) and those without clinical relapse (*nonrelapsers*). Statistical analysis was performed with the chi-squared test or the Fisher exact test and an independent Student *t* test for the categorical and continuous variables, respectively, between relevant groups. The Mann-Whitney U test and the Wilcoxon test were used for nonparametric analysis. Continuous variables are shown as mean ± SD or median (range) depending on whether there was a normal distribution. Cox regression analysis was performed to find the predictor(s) for clinical relapse. The Kaplan-Meier method with the log-rank test was used to compare cumulative HBsAg loss rates. Statistical procedures were performed with SAS 9.4. $P < 0.05$ was considered significant. Receiver operating characteristic curves and the Youden index were applied for summary measures of optimal discriminative levels of pretreatment/EOT HBsAg, baseline HBV DNA level, age at entry, optimal treatment time to DNA undetectable, and consolidation duration.

Results

Of the original cohort of 1,075 HBeAg-negative patients with chronic HBV infection who had discontinued Nuc therapy, 5 (0.47%) showed HBsAg loss during 156 (38.4-568) weeks of Nuc therapy. The 6-year cumulative incidence and calculated annual incidence of HBsAg seroclearance during Nuc therapy were 3% and 0.15%, respectively.

Of the remaining patients still seropositive for HBsAg, 691 who had stopped Nuc therapy by the APASL stopping rule and had adequate HBV data were included for analysis (Fig. 1). Of these 691 patients, 308 (44.6%) had evidence of cirrhosis at pretherapy baseline. Comparing patients with and without cirrhosis, patients with cirrhosis were significantly older, were more frequently infected with genotype C

HBV, more frequently presented with decompensation, and had lower baseline HBV DNA (Supporting Table S1). Entecavir (ETV) was used in 537 (83.8%), tenofovir disoproxil fumarate (TDF) in 104, adefovir in 10, lamivudine in 10, and telbivudine in 40 patients. ALT became normal and HBV DNA reduced to an undetectable level at a median time of 13 (0-157) and 27 (4-166) weeks, respectively. The median treatment duration of 156 (61-430) weeks included consolidation therapy of 111 (48-385) weeks.

During a median follow-up period of 155 (2-614) weeks after cessation of Nuc therapy, virologic relapse and clinical relapse were documented in 547 patients (79.2%) and 419 patients (60.6%), respectively.

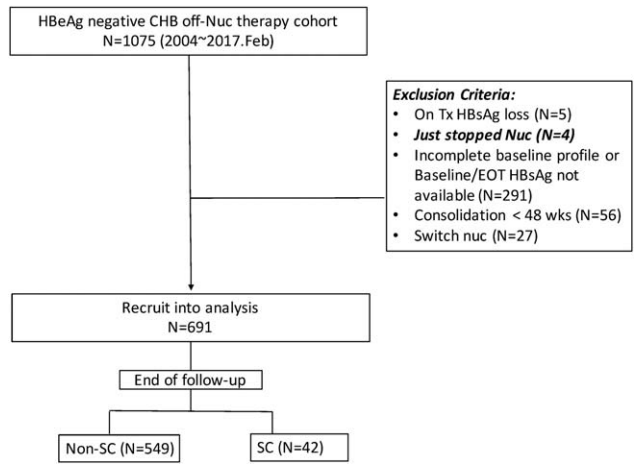

FIG. 1. Flowchart of patient recruitment in the HBeAg negative off-therapy cohort. Abbreviations: SC, HBsAg seroclearance; Tx, treatment.

CHANGES OF qHBsAG DURING OFF-THERAPY FOLLOW-UP

Overall, the EOT qHBsAg was 2.6 (–1.2 to 4.4) $\log_{10}$IU/mL, and the median qHBsAg reduction from EOT to end of follow-up was –0.3 (–3.4 to 3.1) $\log_{10}$IU/mL. Patients with sustained response (no virologic relapse) showed significantly greater qHBsAg reduction (median [range], –0.41 [–3 to 3]) than those with virologic relapse and clinical relapse (Fig. 2).

EOT qHBsAg <100 IU/mL was documented in 114 patients. During off-therapy follow-up, HBsAg seroclearance was confirmed in 42 patients, and 29 of them seroconverted to antibody to HBsAg–seropositive. The median time to HBsAg seroclearance was 166 (12.9-519) weeks. The overall 6-year cumulative incidence was 13%, with an estimated annual incidence of 1.78%. The 6-year cumulative and estimated annual incidence rates of HBsAg seroclearance were 9% and 1.69%, respectively, in patients with cirrhosis and 16% and 1.87%, respectively, in patients without cirrhosis (log-rank test, $P = 0.531$).

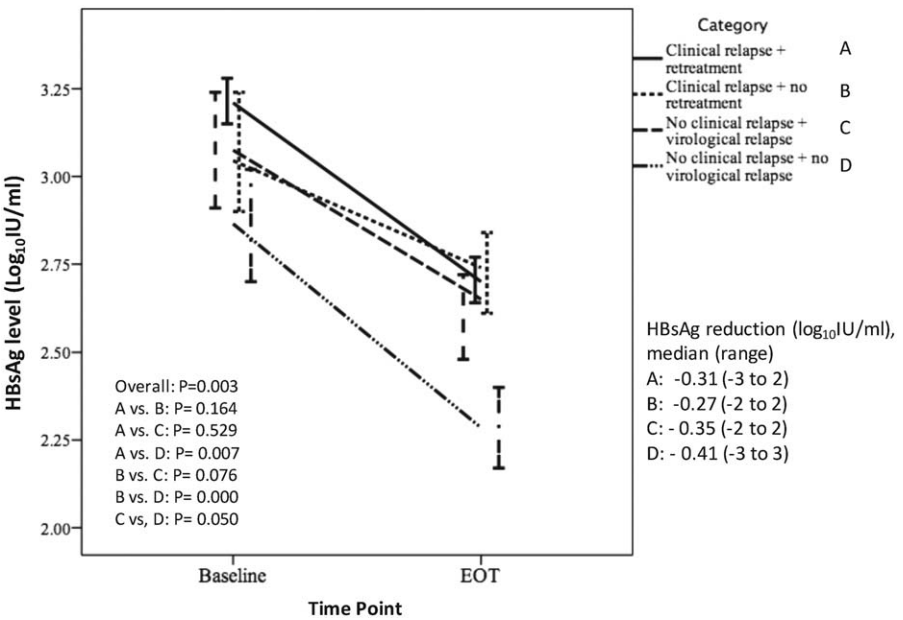

FIG. 2. On-treatment HBsAg kinetics (from baseline to EOT) in patient groups with different off-therapy events; patients with both clinical and virologic remission showed the greatest decline ($P < 0.0001$). Data expressed as median (95% confidence interval).

COMPARISONS OF PATIENTS WITH AND WITHOUT HBsAg LOSS

The features at the pretherapy baseline of patients with HBsAg seroclearance and those who remained HBsAg-seropositive are compared in Table 1. Lower baseline qHBsAg is the only statistically significant baseline factor (median, 2.7 versus 3.1 $\log_{10}$IU/mL, $P = 0.0004$) for off-therapy HBsAg seroclearance.

Of the on-treatment features, patients with HBsAg loss had a significantly shorter time to achieve undetectable HBV DNA (median, 23.9 versus 27.1 weeks, $P = 0.0016$) and related shorter treatment duration (median, 129.6 versus 156.1 weeks, $P = 0.0005$), greater qHBsAg reduction from baseline to EOT (median, –0.8 versus –0.3 $\log_{10}$IU/mL, $P = 0.0016$), and lower EOT qHBsAg (median, 1.9 versus 2.7 $\log_{10}$IU/mL, $P < 0.0001$) compared to those without HBsAg loss (Table 2). HBsAg seroclearance rate was 3 times higher in ETV-treated patients than in TDF-treated patients, but the difference was not statistically significant (33/537, 6.1% versus 2/104, 1.9%, $P = 0.0987$).

During follow-up after cessation of Nuc therapy, 269 of the 419 clinical relapsers were retreated with Nuc, and 12 of the 272 nonrelapsers also received retreatment for virologic relapse at the discretion of their physicians. The follow-up duration was shorter in patients who remained HBsAg-seropositive compared to those with HBsAg seroclearance (median, 150 versus 269 weeks; $P < 0.0001$). The 6-year cumulative incidence and estimated annual incidence were 36% and 6.3%, respectively, in patients with sustained response (no virologic relapse); 13% and 2.4%, respectively, in patients with virologic relapse but no clinical relapse; 19% and 1.7%, respectively, in clinical relapsers without retreatment; and 1% and 0.18%, respectively, in relapsers who were retreated ($P < 0.001$). The cumulative HBsAg seroclearance incidence rates in these groups of patients are displayed in Fig. 3. The 12 nonrelapsers who received retreatment (4 with cirrhosis) had higher EOT qHBsAg (median, 2.95 versus 2.42 $\log_{10}$IU/mL; $P = 0.003$) compared to nonrelapsers, and none showed HBsAg seroclearance during a follow-up period of 213 (79-307) weeks (Table 2).

PREDICTORS FOR HBsAg SEROCLEARANCE AFTER CESSATION OF Nuc THERAPY

Because the off-therapy follow-up duration was significantly longer in patients with HBsAg seroclearance, the Cox regression model was applied in the analysis of the predictors for HBsAg seroclearance using variables already available at EOT but excluding pretherapy baseline variables (Table 3). In univariate analysis, time to undetectable HBV DNA, qHBsAg reduction from baseline to EOT, EOT qHBsAg, clinical relapse not retreated, virologic relapse alone, and no virologic

TABLE 1. Comparisons of Baseline Features of the Patients With and Without HBsAg Seroclearance

		HBsAg Loss		
Baseline*	All (n = 691)	No (n = 649)	Yes (n = 42)	*P*
Age	52.3 (27.3-93.0)	52.4 (27.3 -93.0)	51.0 (34.4 -73.6)	0.1684
Male	594 (85.96%)	557 (85.8%)	37 (88.1%)	0.6814
Cirrhosis	308 (44.57%)	289 (44.5%)	19 (45.2%)	0.9287
Prior decompensation Tx	66 (9.55%)	61 (9.41%)	5 (11.63%)	0.5924
Prior Nuc†	330 (47.76%)	308 (47.53%)	22 (51.16%)	0.5369
Prior IFN†	87 (12.59%)	81 (12.48%)	6 (14.29%)	0.7326
Decompensation at Tx	17 (2.46%)	17 (2.62%)	0 (0%)	0.6166
Genotype B	490 (80.46%)	462 (81.2%)	28 (70%)	0.0843
Genotype C	119 (19.54%)	107 (18.84%)	12 (29.27%)	
ALT	147 (16-5,470)	146 (16 -5,470)	184 (28 -1,393)	0.1229
ALT ≥5× ULN	280 (40.52%)	259 (39.97%)	21(48.84%)	0.1966
Total bilirubin >2mg/dl	72 (10.43%)	65 (10.03%)	7 (16.28%)	0.1715
INR >1.5	16 (2.32%)	16 (2.47%)	0 (0%)	0.6158
HBV DNA ($\log_{10}$ IU/mL)	6.2 (2.0-9.7)	6.3 (2.0 -9.7)	6.1 (3.7 -8.7)	0.3511
HBsAg (Log_{10}IU/mL)	3.1 (−0.8-6.4)	3.1 (−0.8 -6.4)	2.7 (0.9 -4.6)	0.0004

*Baseline stands for start of treatment.
†Prior Nuc or Prior IFN stands for Nuc or IFN experienced in the past.
Abbreviations: IFN, interferon; INR, international normalized ratio; Tx, treatment; ULN, upper limit of normal.

TABLE 2. Comparisons of the On-Treatment and Off-Treatment Features of the Patients With and Without HBsAg Seroclearance

		HBsAg Loss		
	All (n = 691)	No (n = 649)	Yes (n = 42)	
On treatment				
Nucleoside	587 (84.95%)	547 (84.3%)	40 (95.2%)	0.0712
Nucleotide	104 (15.05%)	102 (15.7%)	2 (4.8%)	
ETV	537 (83.78%)	504 (83.17%)	33 (94.29%)	0.0987
TDF	104 (16.22%)	102 (16.83%)	2 (5.71%)	
Time to normal ALT (weeks)	13.0 (0-157.0)	13.0 (0-157.0)	12.1 (2.0-124.7)	0.3032
Time to ud (weeks)	27.0 (4.0-165.9)	27.1 (4.0-165.9)	23.9 (4.0-56.0)	0.0016
Consolidation (weeks)	111.0 (48.0-385.4)	111.4 (48.0-385.4)	104.3 (51.1-308.0)	0.2483
Tx duration (weeks)	156.0 (60.9-429.6)	156.1 (60.9-429.6)	129.6 (75.0-312.7)	0.0005
HBsAg reduction (log)	−0.3 (−3.4 to 3.1)	−0.3 (−3.3 to 3.1)	−0.8 (−3.4 to 1.5)	0.0016
>1 log reduction	142 (20.55%)	124 (19.14%)	18 (41.86%)	0.0002
EOT age	55.2 (30.1-97.7)	55.3 (30.1-97.7)	53.9 (36.4-75.2)	0.1298
EOT HBsAg (IU/mL)	429.0 (0.1-28,109.0)	456.6 (0.7-28,109.0)	80.4 (0.1-1,913.6)	<0.0001
EOT HBsAg (Log_{10}IU/mL)	2.6 (−1.2 to 4.4)	2.7 (−0.2 to 4.4)	1.9 (−1.2 to 3.3)	<0.0001
Off therapy				
Follow-up duration (weeks)	155.1 (1.9-613.9)	150.3 (1.9-574.0)	268.6 (49.9-613.9)	<0.0001
VR	547 (79.16%)	527 (81.2%)	20 (47.6%)	<0.0001
CR	419 (60.64%)	408 (62.9%)	11 (26.2%)	<0.0001
Retreatment	281 (40.67%)	279 (43%)	2 (4.8%)	<0.0001
CR+ reTx+	269 (38.93%)	267 (41.14%)	2 (4.76%)	<0.0001
CR+ reTx–	150 (21.71%)	141 (21.76%)	9 (20.93%)	
CR– reTx+	12 (1.74%)	12 (1.85%)	0 (0%)	
CR– reTx–	260 (37.63%)	229 (35.34%)	31 (72.09%)	

Abbreviation: CR, clinical relapse; reTx, retreatment; Tx, treatment; ud, DNA undetectable; VR, virologic relapse.

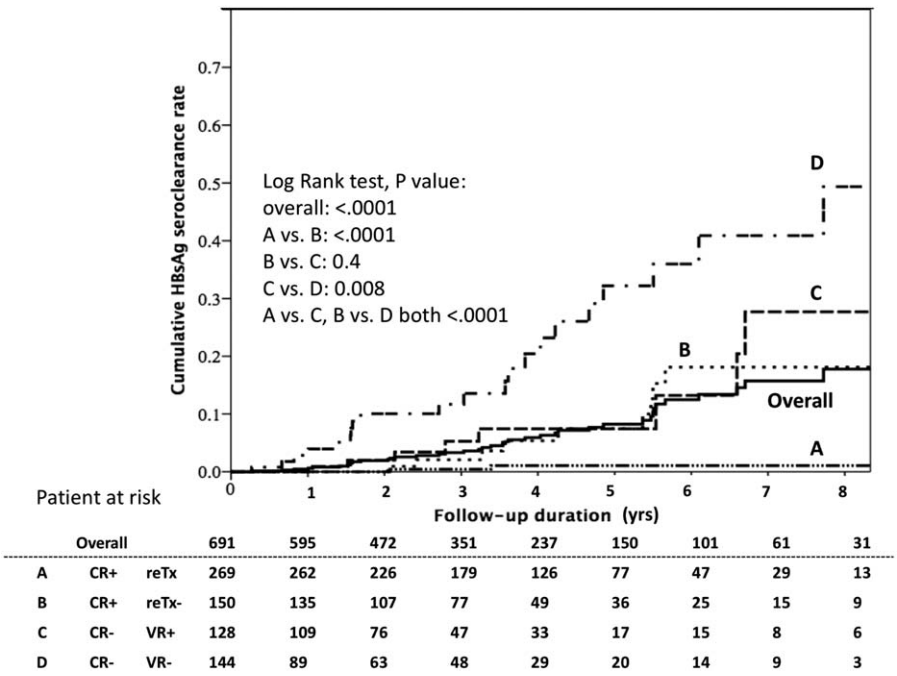

FIG. 3. Cumulative HBsAg seroclearance rate in overall patients (solid line): A, patients with clinical relapse and retreatment; B, patients with clinical relapse but no retreatment; C, patients with virologic relapse but no clinical relapse; D, patients with no virologic relapse (HBV DNA <2,000 IU/mL). Abbreviations: CR, clinical relapse; reTx, retreatment; VR, virologic relapse.

relapse were statistically significant factors. In multivariate analysis, time to HBV DNA undetectable <12 weeks, EOT qHBsAg <100 IU/mL, and qHBsAg reduction >1 log_{10}IU/mL were independent factors for HBsAg seroclearance. Analysis using variables at EOT and post-EOT showed that EOT qHBsAg

TABLE 3. Cox Regression for Predictors of HBsAg Seroclearance Using Variables Available at EOT but Excluding Pretherapy (Baseline) Variables

Variables	All	SC	IR per 10^4 py	Crude HR (95%CI)	*P*	Adjusted HR (95% CI)	*P*
Time to undetectable DNA							
≥12 weeks	603	29	139.661	Referent		Referent	
<12 weeks	88	13	468.087	3.36 (1.73-6.5)	0.0003	3.04 (1.53-6.03)	0.0015
Treatment duration							
<3 years	274	27	227.599	Referent			
≥3 years	417	15	128.437	0.71 (0.37-1.36)	0.3025		
HBsAg reduction (log)							
<1	549	24	122.828	Referent		Referent	
≥1	142	18	449.746	3.95 (2.14-7.31)	<0.0001	2.34 (1.2-4.57)	0.0126
EOT HBsAg (IU/mL)							
≥500	303	7	60.105	Referent		Referent	
100-499	274	12	123.999	2.02 (0.71-5.78)	0.1878	1.89 (0.72-4.93)	0.1961
<100	114	24	793.545	15.11 (6.46-35.34)	<0.0001	9.85 (3.98-24.39)	<0.0001

Abbreviation: CI, confidence interval; IR, incidence rate; py, person-year; SC, seroclearance; VR, virologic relapse.

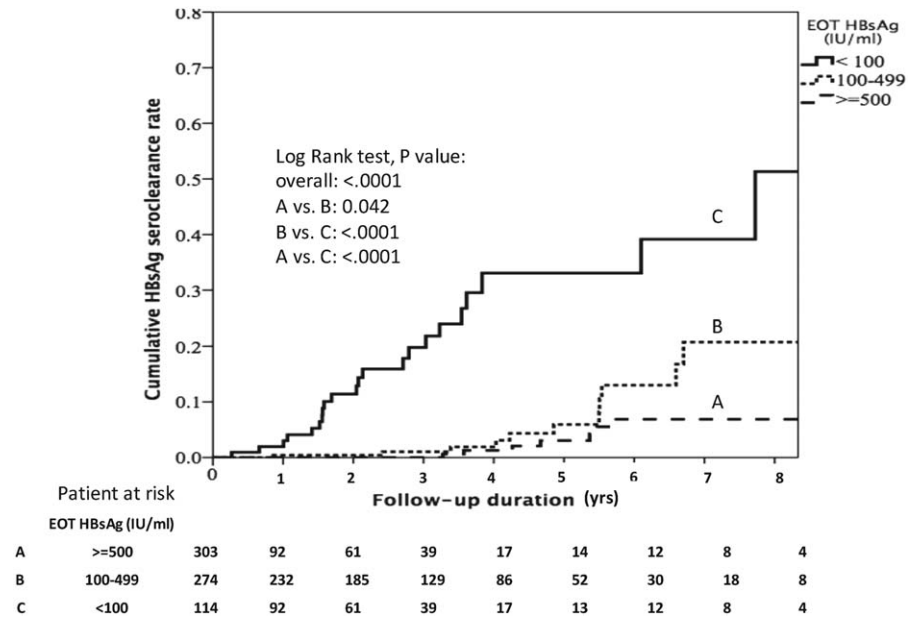

FIG. 4. Cumulative HBsAg seroclearance rate in patients with EOT HBsAg <100, 100-499, and ≥500 IU/mL (log-rank test, $P < 0.0001$).

<100 IU/mL, clinical relapses not retreated, virologic relapse alone, and no virologic relapse were significant factors. When pretherapy baseline variables were also included in the analysis, baseline qHBsAg was an additional factor of borderline significance ($P = 0.056$) (Supporting Table S2). A stepwise analysis is shown in Supporting Table S3. The cumulative incidence rates of HBsAg seroclearance in patients with EOT qHBsAg <100 IU/mL and other levels are compared in Fig. 4. Of these factors, sustained response (no virologic relapse and no clinical relapse) had the highest adjusted hazard ratio (HR).

FACTORS PREDICTIVE OF OFF-THERAPY HBsAg SEROCLEARANCE IN PATIENTS WITH AND WITHOUT CIRRHOSIS

Of the 308 patients with liver cirrhosis, 19 showed HBsAg seroclearance during off-therapy follow-up, with a median time to HBsAg loss of 162.6 (39.3-519) weeks. The 6-year cumulative incidence was 9%, and the estimated annual incidence was 1.69%. No relapse with no retreatment was the only factor predictive of off-therapy HBsAg seroclearance. Of the 383

CHB patients without evidence of cirrhosis, 23 showed HBsAg seroclearance, with a median time to HBsAg loss of 170 (13-322) weeks. The 6-year cumulative incidence was 16%, and the estimated annual incidence was 1.87%. In CHB patients without cirrhosis, multivariate analysis showed genotype C (HR versus genotype B, 5.42; $P = 0.0083$), time to HBV DNA undetectable during treatment (≥12 versus <12 weeks; HR, 0.2; $P = 0.0063$), EOT qHBsAg <100 IU/mL (HR, 4.9; $P = 0.0345$), and no virologic relapse (HR, 12.5; $P = 0.0196$) were independent factors predicting off-therapy HBsAg seroclearance.

LONG-TERM CLINICAL EVENTS AND SAFETY

During follow-up after cessation of Nuc therapy, 7 of the 308 patients with cirrhosis suffered from hepatic decompensation at a median of 41 (7-183) weeks after EOT. They were retreated promptly, but one TDF-treated patient died 3 months after EOT. Two additional patients with cirrhosis treated with ETV developed hepatic decompensation and died 15 and 32 months after EOT, respectively, despite being retreated with ETV. The annual incidence of decompensation after cessation of Nuc was 0.28%, with a 5-year cumulative incidence of 1% (0% in patients without cirrhosis and 2.95% in patients with cirrhosis). No HBsAg loss was observed in patients who encountered hepatic decompensation.

Twenty-one patients developed HCC at a median of 2.04 (0.18-6.73) years after EOT. When the 3 patients who developed HCC within 1 year after EOT were considered as preexisting HCC developed during Nuc therapy and excluded, the annual, 3-year, and 6-year cumulative incidence rates were 0.69%, 2%, and 4% (0.15%, 1%, and 1% for patients without cirrhosis; 1.3%, 4%, and 9% for patients with cirrhosis), respectively. The annual incidence and 3-year cumulative HCC incidence rates were 0.7% and 2%, respectively, during Nuc therapy (0.083% and 0.3% for patients without cirrhosis; 1.52% and 3.4% for patients with cirrhosis). No HBsAg loss was observed in patients who developed HCC after cessation of Nuc therapy.

Discussion

The results of the present large prospective study in 691 HBeAg-negative CHB patients (44.6% with cirrhosis) have shown a higher incidence of HBsAg seroclearance after cessation of Nuc therapy at an estimated annual incidence of 1.78% during a median follow-up period of 155 (2-614) weeks. It is unexpected that the incidence is so much higher than the on-treatment annual incidence of 0.15% observed in the present study and 0.33% in a Korean cohort of 5,409 (49.6% with cirrhosis) patients during 6-year ETV therapy.[6] A recent small randomized controlled trial in TDF-treated patients also showed a significantly higher HBsAg seroclearance rate (19% versus 0) in patients who discontinued therapy for 144 weeks than in those who continued TDF therapy.[13]

The incidence of HBsAg seroclearance after cessation of Nuc therapy has been reported in only a few studies with a substantial number of HBeAg-negative patients. It appears that the current study is the largest one to address the incidence and factors of HBsAg seroclearance after cessation of Nuc therapy. The incidence of reported HBsAg seroclearance varied among the studies with more than 33 patients (the number of patients in the pivotal study of Hadziyannis et al.[12]) with a follow-up period >3 years after EOT (Table 4). The cumulative incidence varied from 13% at year 6 in the present study of 691 patients to 23% at year 5 in a Hong Kong study of 53 patients[18] to 39% at year 3 in

TABLE 4. Summary of Studies on Off-Therapy HBsAg Seroclearance

Source	Year	No.	Cirrhosis	No Cirrhosis	Male (%)	Age (Years)	Tx Duration	Follow-up Duration	HBsAg Loss	Incidence
Chan et al.[18]	2011	53	18	35	81.1	56	27 months	47 months	11/53*	5-year 23%
Hadizyannis et al.[12]	2012	33	0	33	81.8	52	4-5 years	5.5 years	13/33	3-year 39%
Chen et al.[19]	2014	105	32	73	78.3	48.8	92.8 weeks	49 months		6-year 30.3%
Patwardhan et al.[23]	2014	33	0	33	72.7	42	5.28 years	3 years	0/33	
Hung et al.[20]	2017	73	73	0	78.1	51.7	30.3 months	66.8 months	20/73	6-year 46.3%
Yao et al.[21]†	2017	119	28	91	79	52	151.5 weeks	6 years	44/119	6-year 54.9%
Current study	2017	691	308	383	86	52.3	156 weeks	155 weeks	42/691	6-year 13%

*Two of the 11 patients achieved HBsAg loss at the EOT.
†All patients with EOT qHBsAg <200 IU/mL.
Abbreviation: HBsAg, seroclearance; Tx, treatment.

a Greek study of 33 patients.[12] Another group of investigators reported a 6-year cumulative incidence of 30.3% in a cohort of 105 patients including 32 patients with cirrhosis.[19] The same group of investigators reported a 6-year cumulative HBsAg seroclearance incidence of 46.7% during a median follow-up of 5.6 years after cessation of Nuc therapy in 73 HBeAg-negative patients with cirrhosis.[20] However, they also reported a 6-year cumulative incidence of 54.9% in 119 patients (28 had cirrhosis) who were HBeAg-negative, with EOT qHBsAg $\leq$200 IU/mL.[21] A study involving 59 HBeAg-negative CHB patients (22% had cirrhosis) showed HBsAg seroclearance of 14% in 3 years.[22] Another study showed that 0 of 33 CHB patients (no cirrhosis) lost HBsAg during 3-year follow-up.[23] The reasons for these widely variable results are not clear. It seems unlikely to be explained by the Nuc used for treatment or the status of liver cirrhosis. Of note is that the highest HBsAg seroclearance rate was observed in the smallest study[12] and in the study with the longest off-therapy follow-up duration.[20] Perhaps the number of study patients and the duration of follow-up can partly explain the difference. The HBsAg seroclearance rate in our patients may increase further upon follow-up for a longer duration.

It is not unexpected that the incidence of HBsAg clearance was highest in patients with sustained response (no virologic relapse), followed by those with virologic relapse but no clinical relapse, compared to clinical relapsers. The sustained responders had the highest HBsAg seroclearance rate, which may reflect that such patients may have already achieved better immune control before EOT. Perhaps the more important finding is that the HBsAg seroclearance rate is significantly higher in clinical relapsers who remained untreated than those who received Nuc retreatment. These findings support the concept that patients with transient ALT elevation and subsequent decrease in viral load and HBsAg level had better immune control.[12,14] It seems appropriate to suggest that clinical relapse could be beneficial in terms of driving immune-mediated elimination of infected hepatocytes to achieve immune control. Therefore, it may be recommended to retreat patients with clinical relapse only if the relapse is severe or expected to be severe or if ALT elevation persists or is recurrent.[24] It is important to find the optimal timing for retreatment that is able to prevent premature treatment, which may hamper the chance of effective immune control and may prevent severe flares.[14,24,25]

HCC development may be a concern after Nuc cessation, especially in patients with cirrhosis. Our results showed that the HCC incidence was not higher than that during treatment of patients with or without cirrhosis. This finding is consistent with the observation in ETV-treated patients with cirrhosis from three independent medical centers that cessation of ETV therapy in 205 patients did not increase the incidence of HCC compared with that in 381 patients who continued ETV therapy.[17] Another Taiwanese study on Nuc cessation in 73 patients with cirrhosis also concluded that the HCC risk did not increase compared with patients matched for age, gender, and viral factors who continued Nuc therapy.[20] Further, the 5-year cumulative HCC incidence of 4% in the current study is at least not higher than the 5.7%-8.4% reported in 1,815 Caucasian patients during 5-year ETV/TDF therapy.[26]

As for the safety issue of Nuc cessation, hepatic decompensation was observed exclusively in patients with cirrhosis. Of note is that hepatic decompensation in 7 (2.3%) of 308 patients with cirrhosis after cessation of Nuc therapy in the present study was not higher than the 7 (3.4%) of 205 patients with cirrhosis during long-term ETV treatment[17] and the 5 (8.2%) of 61 patients with cirrhosis continuing ETV therapy.[27] In addition, more stringent off-therapy monitoring may minimize the risk of decompensation and rescue such patients in time.

In conclusion, the HBsAg seroclearance rate is much higher during follow-up after cessation of Nuc therapy. Those with sustained viral and clinical remission had the highest off-therapy HBsAg seroclearance rate. Of the patients with clinical relapse, a wait-and-watch strategy may increase the chance of HBsAg seroclearance in those not requiring immediate retreatment. Because the patients in the current study were infected with genotype B or C HBV, further studies in patients from other countries or in patients infected with HBV of another genotype(s) are needed to confirm our results. Studies are also important to find the optimal timing for retreatment that is not too early and not too late.

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Supporting Information

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