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Off-Therapy Durability of Response to Entecavir Therapy in Hepatitis B e Antigen-Negative Chronic Hepatitis B Patients

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The optimal duration of nucelos(t)ide analog (Nuc) treatment in hepatitis B e antigen (HBeAg)-negative patients with chronic hepatitis B virus (HBV) infection is unknown. The Asian Pacific Association for the Study of the Liver (APASL) guidelines recommend that treatment can be discontinued if undetectable HBV-DNA has been documented on three occasions ≥6 months apart. This study aimed to test this stopping rule in HBeAg-negative chronic hepatitis B (CHB) patients treated with entecavir (ETV). Ninety-five patients (39 cirrhosis) were treated with ETV for a median of 721 (395-1,762) days before stopping therapy and were then monitored with serum HBV DNA and alanine aminotransferase (ALT) at least every 3 months. Within 1 year after stopping ETV therapy, "clinical relapse" (an episode of ALT elevation >2 × upper limit of normal plus HBV-DNA >2,000 IU/mL) occurred in 43 (45.3%) of the 95 patients. Of the 39 cirrhosis patients, 17 (43.6%) relapsed and one (2.6%) developed decompensation. The median duration until relapse was 230 days (74.4% >6 months). Logistic regression analysis showed that baseline HBV-DNA ≤2 × 10^5 IU/mL was the only significant independent factor for sustained response. The 1-year relapse rate was 29% in patients with a baseline HBV DNA ≤2 × 10^5 IU/mL versus 53% in those with HBV DNA >2 × 10^5 IU/mL (P = 0.027). For the latter, consolidation therapy >64 weeks reduced the relapse rate to 33.3% in patients without cirrhosis. *Conclusion*: With an overall 1-year relapse rate of 45% and 29% in those with a baseline serum HBV DNA ≤2 × 10^5 IU/mL, the APASL stopping rule for HBeAg-negative CHB patients with proper off-therapy monitoring is adequate even in patients with cirrhosis. Consolidation therapy >64 weeks seems more appropriate for those with higher baseline HBV DNA. (HEPATOLOGY 2013;58:1888-1896)

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The advent of effective antiviral agents with different mechanisms of action has led to better therapeutic strategies for chronic hepatitis B virus (HBV) infection. Among the currently available oral nucleos(t)ide analogs (Nuc), entecavir (ETV) and tenofovir disoproxil fumarate (TDF) are the preferred first-line agents.[1-3] For hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB), long-term Nuc therapy is usually required but the optimal duration of treatment is still unknown and is under debate. The American Association for the Study of Liver Disease (AASLD) and European Association for the Study of the Liver (EASL) recommend long-term Nuc therapy until the patient has achieved hepatitis B surface antigen (HBsAg) clearance, which is a remote and unrealistic endpoint because it occurs in <1% per year.[1,3] Several earlier studies in Asian patients treated with lamivudine (LAM) showed that the sustained

Abbreviations: AASLD, American Association for the Study of Liver Disease; ADV, adefovir; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; CHB, chronic hepatitis B; EASL, European Association for the Study of the Liver; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LAM, lamivudine; Nuc, nucleos(t)ide analog; TDF, tenofovir

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response rate 6-12 months after cessation of LAM therapy was around 50% if patients had achieved a maintained virological response before stopping LAM therapy.[4-6] Based on these, the Asian Pacific Association for the Study of the Liver (APASL) guidelines suggested that cessation of Nuc therapy can be considered if undetectable HBV-DNA by real-time polymerase chain reaction (PCR) has been documented on three separate occasions at least 6 months apart.[7]

There are only a few studies on the durability of LAM or adefovir (ADV) in HBeAg-negative CHB patients after cessation of therapy by the stopping rule of the APASL.[8,9] Compared to LAM and ADV, ETV is a more potent Nuc with a high genetic barrier to drug resistance. Of the HBeAg-negative patients in the phase 3 trial treated with ETV for 1 year and who stopped treatment after achieving the protocol-defined response (HBV DNA <0.7 MEq/mL and serum alanine aminotransferase [ALT] <1.25 times upper limit of normal [ULN]), only 48% sustained this response for >24 weeks after treatment cessation.[10] It was not known whether the off-therapy durability of response to the more potent ETV using the more stringent stopping rule of APASL was similar to or better than that of the patients treated with LAM or ADV. We therefore conducted this study, which also aimed to validate the APASL stopping rule in our HBeAg-negative patients with CHB treated with ETV.

Materials and Methods

Patients and Study Design. This study used a retrospective-prospective cohort, approved by the Institutional Review Board of the Chang Gung Memorial Hospital, Taiwan. Excluding patients with coexisting HCV or HDV infection, alcoholism, autoimmune hepatitis, and malignancy, all HBeAg-negative, anti-HBe-positive patients with CHB who had been treated with ETV and were followed for a minimum of 12 months (48 weeks) after cessation of ETV therapy by the stopping rule of APASL (undetectable HBV-DNA by PCR had been demonstrated on three occasions at least 6 months apart[7]) were included. After cessation of ETV therapy, serum ALT was monitored every 1-1.5 months in the first 3 months and then at least every 3 months along with serum HBV DNA assay every 3 months during off-therapy follow-up. Alfafetoprotein and ultrasonography were performed every 3-6 months. If serum HBV DNA increases over 2,000 IU/mL or ALT level increases over ULN during off-therapy follow-up, HBV DNA and/or ALT were retested for confirmation and further evaluation. The "consolidation duration" was calculated from the first demonstration of undetectable HBV DNA to the end of treatment. According to the APASL guidelines, "clinical relapse" was defined as an event with an increase of serum HBV-DNA level over 2,000 IU/mL and serum ALT levels >2 × ULN, which is the AASLD and APASL indication of anti-HBV therapy for CHB.[1,2]

Age, gender, presence of cirrhosis, prior treatment, baseline biochemical data and viral features, serum HBV DNA and ALT at the end of 3 and 6 months on therapy, serum HBsAg, HBV-DNA and ALT levels at baseline and at end of therapy, as well as treatment duration and consolidation duration were compared between patients with clinical relapse (relapsers) and those with sustained response (nonrelapsers).

Since there was no APASL stopping rule for HBeAg-negative patients before 2008[7] and most of our patients have been treated with ETV after 2008, only 22 LAM-treated and 30 telbivudine (LdT)-treated HBeAg-negative patients had stopped drug therapy after a consolidation therapy >1 year and were followed for 1 year off-therapy, as the ETV cohort in the present study did. The occurrences of clinical relapse in these 52 patients were searched by chart review retrospectively for comparison.

Biochemistry and Laboratory Methods. The biochemical tests were performed using routine automated techniques at our clinical pathology laboratories. The serum ALT ULN was set by the laboratory at 36 U/L for both male and female. Serum hepatitis markers including HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HDV, and anti-HCV were assayed using the EIA kit (Abbott Diagnostics, North Chicago, IL). HBV genotype was determined using PCR-restriction fragment length polymorphism of the surface gene of HBV.

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Serum HBV DNA was assayed using Roche Cobas Amplicor HBV Monitor test (Roche COBAS TaqMan HBV Test, lower limit of detection: 69 or 1.84 $\log_{10}$ copies/mL; 12 or 1.08 $\log_{10}$ IU/mL, Roche Diagnostics, Pleasanton, CA). Serum HBsAg level was measured using the Roche Elecsys HBsAg II quant assay (detection limit 0.05 IU/mL, Roche Diagnostics, Mannheim, Germany).

Statistical Analyses. Statistical analysis was performed with chi-square test or Fisher exact test and independent Student *t* test for the categorical and continuous variables, respectively, between groups of patients with sustained remission and relapse. The Mann-Whitney *U* test and Wilcoxon test were used for nonparametric analysis. Continuous variables are shown as median (range). Logistic regression analysis was performed to find the predictor of clinical relapse. The Kaplan-Meier method with log-rank test was used to compare cumulative relapse rates. Statistic procedures were performed with SPSS software (v. 17.0, Chicago, IL). $P < 0.05$ was considered significant. Receiver operating characteristic (ROC) curve and the Youden Index were applied for summary measures of optimal discriminative levels of pretreatment/end of treatment HBsAg, baseline HBV-DNA, and HBV DNA at 3 months posttreatment.[11]

Results

Of the HBeAg-negative CHB patients who had been treated with ETV in our unit, 408 have ever stopped ETV therapy. Excluding those whose consolidation therapy was <1 year (120 patients) and those whose off therapy follow-up was <48 weeks (193 patients), 95 patients met the inclusion criteria of the present study. The majority (87.4%) of the patients were males. The median age was 52.1 (28.3-82.2) years. Thirty-nine (41.1%) of the 95 patients showed histologic or clinical evidence of cirrhosis. Fifty-six patients (58.9%) experienced prior treatment with Nuc (five had rtM204I and two had rtM204I/V mixed mutations) or interferons. Of the 92 patients assayed for HBV genotype, 66 (71.7%) and 24 (26.1%) were infected with genotype B and C HBV, respectively, and two were of undetermined type. Of the 69 patients assayed, 78.3% were detected to have pre-core G1896A mutation (93 of the 95 patients received pre-core G1896A mutation assay, but it could not be detected in 24 of them due to lower serum HBV DNA levels), and 27 (36.5%) of 74 had basal core promoter mutations (A1762T/G1764A) (91 of the 95 patients received A1762T/G1764A analysis, but BCP mutation could not be detected in 17 of the 91 patients due to lower HBV DNA levels). rtM204I/V mutation existed in seven patients (7.4%). The median baseline ALT level was 158 (19-2,155) U/L. Compared with noncirrhosis patients, cirrhosis patients were older (54.5 ± 10.84 versus 49.5 ± 9.55 years, $P = 0.02$) and had lower baseline serum HBV DNA level (median 3.14×10^5 or 5.496 $\log_{10}$ IU/mL versus 5.35×10^6 or 6.728 $\log_{10}$ IU/mL, $P = 0.003$; $\leq 2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL in 46.2% versus 23.2%, $P = 0.019$). All other features were comparable between cirrhosis and noncirrhosis patients.

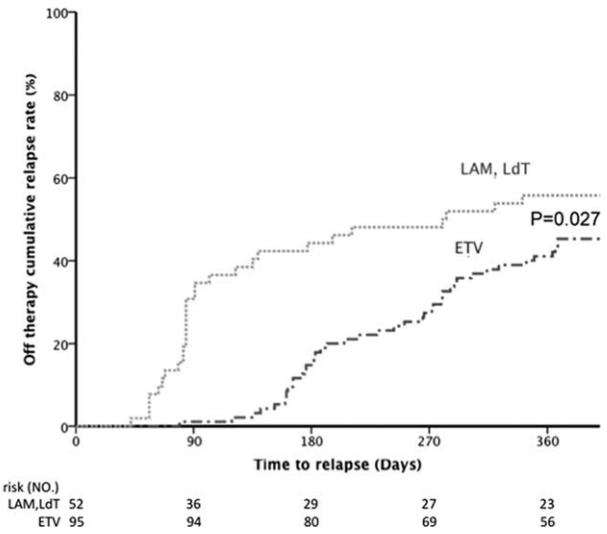

Fig. 1. One-year cumulative relapse rate after cessation of ETV therapy was 45.3%, significantly lower and relapses occurred later than those after cessation of LAM or LdT.

During ETV treatment, serum HBV DNA became undetectable in 45.2% and 84.1%, ALT normalized in 64.2% and 81.7% of the patients by the end of 3 and 6 months, respectively. The mean treatment duration was 721 days (24 months), with 48 patients (50.5%) being treated for more than 2 years. The median duration of consolidation therapy was 448 (345-1,678) days. None of the patients lost HBsAg during treatment and the 1-year off-therapy period.

Clinical relapse occurred in 43 patients and virologic relapse with normal ALT occurred in additional 12 patients (six cirrhosis). The cumulative off-therapy clinical relapse rate was 45.3% in 1 year with a median duration to relapse of 230 days (79-368 days). Most relapses (74.4%) occurred beyond 6 months after stopping ETV therapy (Fig. 1). The baseline and on-treatment features of the patients with or without clinical relapse (relapsers versus nonrelapsers) are compared in Tables 1 and 2. All baseline features were comparable between relapsers and nonrelapsers except

Table 1. Comparisons of Baseline Features Between Relapsers and Nonrelapsers

	Total (N = 95)	Nonrelapser (N = 52, 54.7%)	Relapser (N = 43, 45.3%)	*P* Value
Age	52.1 (28.3-82.2)*	51.2 (28.3-79.2)*	52.2 (33.1-82.2)*	0.358
	51.6 ± 10.3†	50.7 ± 10.9†	52.7 ± 9.6†	
Gender (male, %)	83 (87.4)‡	48 (92.3)‡	35 (81.4)‡	0.131
Cirrhosis (+)	39 (41.1)‡	22 (42.3)‡	17 (39.5)‡	0.785
Prior treatment	56 (58.9)‡	35 (67.3)‡	21 (48.8)‡	0.069
rtM204 I/V mutation I/V mutant	7 (7.4)‡	6 (11.5)‡	1 (2.3)‡	0.123
Genotype (N = 92) B	66/92 (71.7)‡	37/51 (72.5)‡	29/41 (70.7)‡	0.891
C	24/92 (26.1)‡	13/51 (25.5)‡	11/41 (26.8)‡	
G1896A mutation (N = 69)	54/69 (78.3)§	28/35 (80)§	26/34 (76.5)§	0.722
A1762T/G1764A mutations (N = 74)	27/74 (36.5)§	19/42 (45.2)§	8/32 (25)§	0.073
HBV DNA(10^5IU/mL)	12.5(0.00114-5214)*	9.7 (0.00238-5214)*	30.6 (0.00114-1828)*	0.063
>2×10^5 IU/mL	64 (67.4)‡	30 (57.7)‡	34 (79.1)‡	0.027
HBsAg (IU/mL)	1540.2 (0.026-19505.2)*	1194.5 (5.5-15186.6)*	2012.0 (0.03-19505.2)*	0.083
>100	84 (88.4)‡	45 (86.5)‡	39 (90.7)‡	0.749
>200	80 (84.2)‡	41 (78.8)‡	39 (90.7)‡	0.159
>500	68 (71.6)‡	35 (67.3)‡	33 (76.7)‡	0.365
>1000	56 (58.9)‡	29 (55.8)‡	27 (62.8)‡	0.489
>1500	49 (50.5)‡	23 (44.2)‡	26 (60.5)‡	0.080
ALT (U/L)	158.0 (19-2155)*	147 (19-873)*	165.0 (27-2155)*	0.580
	230.3 ± 270†	203.2 ± 184.3†	263 ± 346.2†	
>5X ULN	39 (41.1)‡	19 (36.5)‡	20 (46.5)‡	0.325
Bilirubin (mg/dl)	1.9 (0.4-8.6)*	0.9 (0.5-2.4)*	1.0 (0.4-8.6)*	0.130
Prolonged PT (second)	1.0 (0-5)*	1.0 (0-3.1)*	1.0 (0-5)*	0.329

*Median (range).
†Mean ± SD.
‡No. (%).
§Data as No./No. assayed (%). PT: prothrombin time; N: number.

that relapsers had a marginally higher baseline HBV DNA (30.6×10^5 or 6.485 $\log_{10}$ versus 9.7×10^5 or 5.986 $\log_{10}$ IU/mL, $P = 0.063$) and significantly more relapsers (79.1 versus 57.7%, $P = 0.027$) had a baseline serum HBV DNA $>2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL, a level determined by the Youden Index method showing an area under the ROC curve (AUC) of 0.611 (95% confidence interval [CI]: 0.498-0.725; $P = 0.063$). Of the seven patients who had had rtM204 mutations during prior LAM or LdT therapy,

Table 2. Comparisons of On and Off Treatment Features Between Relapsers and Nonrelapsers

On-Treatment Factors	Total (N = 95)	Nonrelapsers (N = 52)	Relapers (N = 43)	*P* Value
DNA undetectable in 3M	28/62 (45.2)§	17/32 (53.1)§	11/30 (36.7)§	0.193
in 6M	74/88 (84.1)§	41/47 (87.2)§	33/41 (80.5)§	0.56
ALT normalization in 3M	61 (64.2)‡	34 (63.0)‡	29 (67.4)‡	0.550
in 6M	76 (81.7)‡	43 (84.3)‡	33 (78.6)‡	0.476
Treatment duration (days)	721 (395-1762)*	747 (446-1762)*	688 (395-1276)*	0.184
	721 ± 215†	748 ± 230†	689 ± 193†	
>2 years	48 (50.5)‡	29 (55.8)‡	19 (44.2)‡	0.261
Consolidation (days)	448 (345-1678)*	462 (362-1678)*	434 (345-1199)*	0.322
	519.5 ± 205.3†	538.8 ± 220.7†	496.5 ± 185.5†	
>18 months	33 (34.7)‡	20 (38.5)‡	13 (30.2)‡	0.363
EOT HBsAg (IU/mL) (N = 89)	682.7 (0.02-5394.4)*	565.6 (25.7-3520)*	828.7 (0.02-5394.4)*	0.193
>100	81/89 (91.0)§	42/48 (87.5)§	39/41 (95.1)§	0.279
>200	73/89 (82.0)§	37/48 (77.1)§	36/41 (87.8)§	0.189
>500	54/89 (60.7)§	26/48 (54.2)§	28/41 (68.3)§	0.174
>1,000	34/89 (38.2)§	15/48 (31.2)§	19/41 (46.3)§	0.144
Log reduction from baseline	0.2 ± 0.91†	0.16 ± 0.63†	0.25 ± 1.15†	0.818
HBV DNA (IU/mL) 3 months off therapy (N = 62)				
>20	43/62 (69.4)§	22/32 (68.8)§	21/30 (70)§	0.915
>200	26/62 (41.9)§	9/32 (28.1)§	17/30 (56.7)§	0.023
>2,000	13/62 (21.0)§	5/32 (15.6)§	8/30 (26.7)§	0.357

*Median (range).
†Mean ± SD.
‡No. (%).
§Data as No./No. assayed (%). EOT: end of treatment.

Table 3. Logistic Analysis of Factors for Clinical Relapse

Variables	UV			MV		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Gender (M = 1, F = 0)	0.365	0.102-1.307	0.12			
Prior treatment	0.464	0.202-1.066	0.07			
Baseline DNA>2x10^5 IU/mL	2.77	1.106-6.937	0.03	3.934	1.345-11.508	0.012
A1762T/G1764A mutations	0.404	0.148-1.102	0.077	0.362	0.121-1.079	0.068
Treatment duration	0.99	0.99-1.00	0.19			
Duration of consolidation therapy	0.99	0.99-1.00	0.33			
Baseline HBsAg>1,500 IU/mL	2.09	0.92-4.75	0.08			
EOT HBsAg >1,000 IU/mL	1.9	0.80-4.52	0.146			

Abbreviation: EOT, end of treatment.

one with rtM204I/V mixed mutation relapsed. There was no statistically significant difference between patients with or without prior mutation ($P = 0.123$) nor between patients with different rtM204 mutations ($P = 0.286$). There was no significant difference in the duration of consolidation therapy between relapsers and nonrelapsers. The 1-year relapse rate was 39.4% (13 of 33) and 48.4% (30 of 62) in patients with a consolidation therapy >18 months and 12-18 months, respectively ($P = 0.402$). Using logistic regression analysis, baseline HBV DNA $>2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL (odds ratio [OR]: 3.934, 95% CI: 1.345-11.508; $P = 0.012$) was the only significant independent predictor for clinical relapse (Table 3). Of the 31 patients with a baseline serum HBV DNA $\leq 2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL, 29% encountered clinical relapse, as compared to 53.1% of the 64 patients with HBV DNA $>2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL (log-rank test $P = 0.036$; Fig. 2). There was no difference between relapsers and nonrelapsers in the magnitude of the decline in the levels of HBsAg and HBV DNA from baseline to 6 months of ETV therapy ($P = 0.364$ and 0.83, respectively). Of the five patients who achieved HBsAg level reduction >1 $\log_{10}$ during ETV therapy, three relapsed.

Logistic regression multivariate analysis in the 56 noncirrhosis patients revealed that both consolidation duration (OR: 0.99, 95% CI: 0.99-0.99; $P = 0.034$) and baseline HBV-DNA $>2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL (OR: 14.5, 95% CI: 1.945-108.173; $P = 0.009$) were independent predictive factors for relapse. A consolidation duration longer than 64 weeks, which was determined by the Youden Index method with an AUC of 0.689 (95% CI: 0.548-0.831, $P = 0.015$), was associated with a much lower relapse rate (28.6% versus 64.3% in those <64 weeks; $P = 0.007$). No significant predictor of relapse was found in cirrhosis patients. Of the noncirrhosis patients with a baseline HBV DNA $>2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL, the 1-year relapse rate in those with consolidation therapy >64 weeks was only 33.3% (7 of 21 patients), significantly lower than 72.7% of 22 patients with a consolidation therapy <64 weeks ($P = 0.01$) (Fig. 3A).

Among the 43 relapsers, nine patients experienced spontaneous remission after a short hepatitis episode. One cirrhosis patient who had not followed the off-therapy monitoring schedule developed hepatic decompensation (total bilirubin 11.2 mg/dL and prothrombin time prolongation of 9 seconds) and was successfully rescued with ETV retreatment. A total of 34 patients (35.8% of 95 patients) were retreated with ETV. The therapeutic response was similar between ETV retreatment and the first-round ETV therapy. One patient who had had rtM204I/V mixed mutations during prior LAM therapy developed ETV resistance at 9 months on ETV retreatment. No mortality was encountered in this ETV cohort of patients.

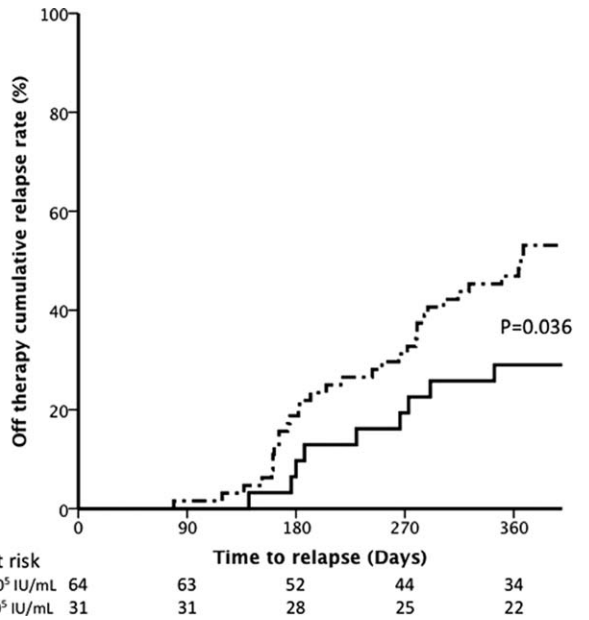

Fig. 2. The cumulative relapse rate in patients with a baseline serum HBV DNA $\leq 2 \times 10^5$ IU/mL (solid line) was significantly lower than those with a level $>2 \times 10^5$ IU/mL (broken line).

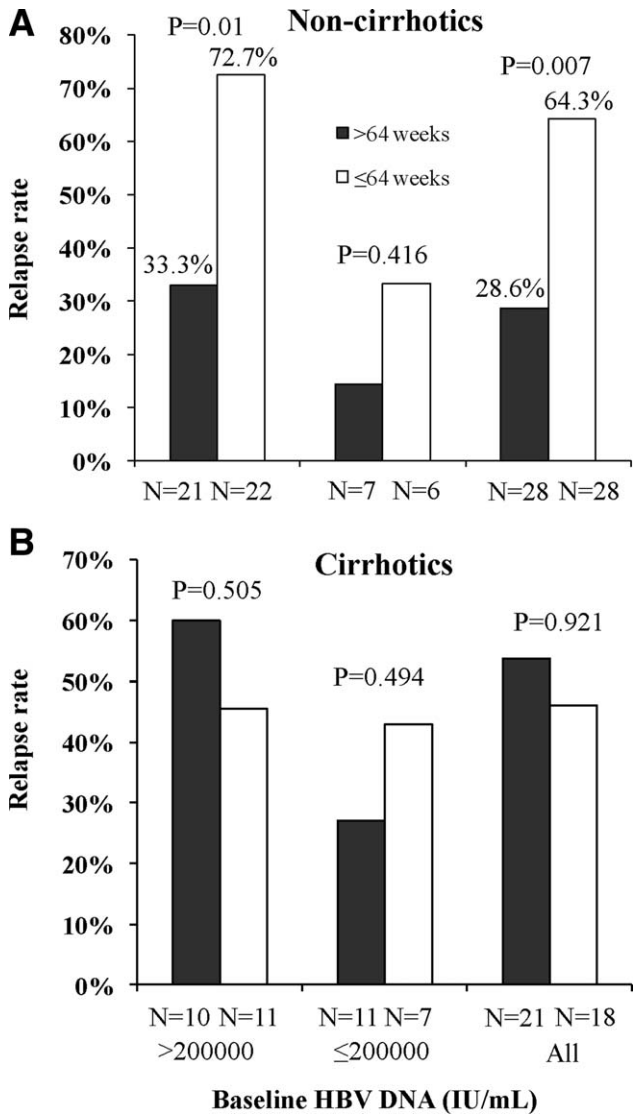

Fig. 3. A consolidation therapy >64 weeks was associated with a much lower relapse rate in patients without cirrhosis with a serum baseline HBV DNA $>2 \times 10^5$ IU/mL (A), but not in patients with cirrhosis (B).

In comparison, clinical relapse occurred in 12 (54.5%) of the 22 LAM-treated patients and 17 (56.7%) of the 30 LdT-treated patients within 1 year after cessation of drug therapy. Of these 29 clinical relapses, 16 (55%) and 23 (79%) occurred within 3 and 6 months, respectively. Because the number of patients was too small and their timing of relapse was similar, they were grouped together to be compared with the ETV cohort in Fig. 1.

Discussion

The results of the present study have shown that the 1-year clinical relapse rate was around 45% in both treatment-naïve and experienced (mostly Nuc) HBeAg-negative patients with CHB who had stopped ETV therapy according to the APASL guidelines.[2] The relapse rate was even less than 30% in our patients with a baseline serum HBV DNA $\leq 2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL (Fig. 2). As such, only one-third of the patients in this ETV cohort required retreatment during this follow-up period and had similar excellent responses. Together with the observation that increasing duration of consolidation therapy longer than 12 months was not a factor for clinical relapse, these findings support the clinical validity of the APASL stopping rule. This stopping rule is very important for patients who had great concern about the cost of long-term Nuc therapy.[12] It is also important for patients who are fully reimbursed for their Nuc therapy but cannot tolerate long-term therapy of indefinite and unpredictable duration. Like other chronic diseases requiring long-term therapy, persistence and adherence to oral anti-HBV therapy are also issues of great concern.[13-15] Given a 1-year Nuc persistence rate (drug refill rate) of 81% and only 74.7% in new patients[13] and a medication possession rate ≧80% in only 53.7% of patients treated with ETV or TDF,[14] it is anticipated that hepatitis flare and, even worse, decompensation may likely develop because the patients who stopped Nuc therapy by themselves are conceivably not monitored properly. This stopping rule may help to convince the patients to persist and adhere to Nuc therapy in a foreseeable finite duration of only 2-3 years. In addition, viral breakthrough occurred in 33% of 191 HBeAg-negative patients who had maintained undetectable HBV DNA (<12 or 1.08 $\log_{10}$ IU/mL in 148 patients, <380 or 2.58 $\log_{10}$ IU/mL in the remaining 43 patients) for 5 years and continued LAM therapy for a median of 15 months.[16] Although Nucs with very low resistance rates such as ETV are now available, there is no guarantee that viral breakthrough or other unknown/unexpected adverse event(s) will not occur during the indefinite long-term Nuc therapy in real-world clinical practice.[15] One of our patients with LAM resistance at baseline of the first-round ETV therapy developed ETV resistance at 9 months of ETV retreatment. Perhaps patients who had had LAM resistance should be treated with TDF instead of ETV from the beginning.[2,3]

There are only a few studies on stopping Nuc therapy in HBeAg-negative CHB using stringent stopping rules. The virologic relapse (defined as reappearance of HBV DNA $>1.4 \times 10^5$ or 5.146 $\log_{10}$ copies/mL by hybridization assay) rate was 50% in an earlier LAM cohort of 50 patients.[5] When virologic relapse was defined as a rise of HBV DNA over 2,000 or 3.3

$\log_{10}$ IU/mL in other studies, the 1-year relapse rate was 43.6% in 61 patients after stopping LAM therapy and 24 (39.3%) of them required retreatment,[8] while the relapse rate was 61.4% after stopping ADV therapy in 145 patients and 88 (60.1%) of them required retreatment.[9] Of the 4- to 5-year ADV treatment cohort, 33 genotype D HBV infected Greek patients who had stopped ADV therapy after achieving long-term undetectable HBV DNA were followed for 69 months (range, 67-72). Fifteen (45%) of them experienced biochemical and virological relapse and were retreated.[17] The two studies from China involving mostly genotype C HBV-infected patients showed that patients younger than 25 years old had a much lower (around 20% in 1 year) virologic relapse rate.[8,9] In contrast, the 1-year virologic relapse rate after stopping LAM therapy was greater than 60% in patients over 40 years of age.[8] The mean age of our ETV cohort, mainly (71.7%) infected with genotype B HBV, was 52.1 years, which is close to that of the Greek patients[17] but much older than 32 years in the LAM cohort[8] and 33 years in the ADV cohort.[9] The 1-year relapse rate in our 83 patients over age 40 was 48.2% (compared to 25% in patients younger than 40 years; $P = 0.214$), clearly lower than a relapse rate of >60% in those over 40 years old who stopped LAM therapy.[8] Of note, the overall relapse rate of 45% in this ETV cohort and the LAM cohort from China[8] is very close to the overall 1-year reactivation (HBeAg reversion plus HBeAg-negative hepatitis) rate of 148 HBeAg-positive patients from Taiwan (mean age at HBeAg seroconversion 35.5 years) who stopped Nuc (93% LAM) therapy according to APASL guidelines, that is, after a post-HBeAg seroconversion consolidation therapy >12 months.[18] All guidelines of the major liver associations agree to stop Nuc therapy after >12 months consolidation therapy in HBeAg-positive CHB patients.[1-3] Given the similar relapse rate observed in HBeAg-positive and -negative patients, there seems no reason that Nuc therapy must continue indefinitely only in HBeAg-negative patients.

Although the duration of consolidation therapy was longer than 18 months in the studies on LAM or ADV therapy, 48% of the virological relapses in the LAM cohort and 65% of the relapses in the ADV cohort occurred within 3 months off therapy.[8,9] Similarly, >50% of the clinical relapses occurred within 3 months in our combined LAM and LdT-treated cohorts meeting the APASL stopping rule (Fig. 1). In contrast, the median time to clinical relapse was 230 days posttreatment and 74.4% of the relapses occurred after 6 months off therapy in our ETV cohort. Different definitions of relapse in different studies may be one of the reasons for this discrepancy. HBV genotype is not a likely factor, as there was no difference in clinical relapse rate between genotype B and C HBV-infected patients (29 of 66 or 43.9% versus 11 of 24 or 45.8%) in our ETV cohort (Table 1). Comparing the reported potency of LAM, ADV, and ETV, these data suggest that relapses occur earlier when less potent Nuc was used. In addition, the detection limit of serum HBV DNA assay was higher (1×10^3 or 3 $\log_{10}$ copies/mL) in the LAM and ADV cohorts[8,9] than 69 or 1.84 $\log_{10}$ copies/mL in the present ETV cohort. Conceivably, patients with an end of treatment serum HBV DNA level higher than 69 copies/mL will relapse earlier than our patients with sustained low-level <69 copies/mL over 1 year.

Both AASLD and EASL guidelines suggest that Nuc therapy should continue indefinitely in patients with cirrhosis and patients with hepatic decompensation.[1,3] Based on their most recent long-ADV treatment/discontinuation study, Hadziyannis et al.[17] suggested a paradigm shift that Nuc therapy can be carefully stopped with close monitoring in HBeAg-negative CHB patients with compensated liver disease but not in patients with cirrhosis or advanced fibrosis. Contrary to these notions, 41% of our ETV cohort were cirrhosis patients and they did not have a higher relapse rate or worse outcome than their noncirrhosis counterparts. Furthermore, the relapses in cirrhosis patients responded similarly well to ETV retreatment, including the cirrhosis patient who had not followed the off-therapy monitoring schedule and consequently developed decompensation. In other words, ETV can be safely stopped using the APASL stopping rule even in compensated cirrhosis patients if they are properly monitored off therapy. Since the HBV DNA level increased over 200 or 2.3 $\log_{10}$ IU/mL within 3 months after stopping ETV therapy was significantly ($P = 0.023$, Table 2) associated with subsequent clinical relapse, more frequent monitoring is required in cirrhosis patients who show an increase of off-therapy serum HBV DNA level over this level.

Although an increasing duration of consolidation therapy longer than 12 months was not a significant factor in our ETV cohort, subgroup analysis showed that a consolidation duration more than 64 weeks was associated with a much lower relapse rate (28.6% versus 64.3%; $P = 0.007$) in the noncirrhosis patients, even in those with higher baseline serum HBV DNA $>2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL (33.3%, Fig. 3A). With these findings, it seems safer to recommend a longer consolidation therapy (>64 weeks, 16 months;

rounded up to 18 months) for patients with a baseline HBV DNA $>2 \times 10^5$ IU/mL.

It has been shown that the serum HBsAg level declines minimally during 1-year Nuc therapy, especially in HBeAg-negative patients.[19] However, a Hong Kong study involving 53 HBeAg-negative patients treated with LAM for a mean of 34 (range, 12-76) months and then stopped LAM therapy for 47 ± 35 months showed that both end-of-treatment HBsAg $\leq$100 or 2 $\log_{10}$ IU/mL and a reduction by >1 log from the baseline were associated with a 1-year sustained HBV DNA $\leq$200 or 2.3 $\log_{10}$ IU/mL in 78% of the patients with an NPV of 96%.[20] These findings were not confirmed by the present study in the ETV cohort.

The current study has some limitations. First, not all patients had stored serum sufficient for retrospective assays of HBV factors (Table 1). Second, the prospective off-therapy follow-up duration was only 12 months. Earlier studies showed that the relapse rate increased to 50% at 2 years and 56% at 5 years off-LAM[8] and to 65.5% at 2 years off-ADV therapy.[9] It is possible that the clinical relapse rate may increase over time during longer off-ETV follow-up. Therefore, continuous monitoring at least every 3 months is needed, especially for cirrhosis patients. Third, the present study examined "clinical relapse" instead of "virological relapse" (HBV DNA >2,000 or 3.3 $\log_{10}$ IU/mL), which was used in the LAM and ADV cohorts.[8,9] A truly valid comparison of relapse rate between this ETV cohort and the reported LAM or ADV cohort is therefore not possible. However, "clinical relapse" is the indication for anti-HBV therapy in both the AASLD and APASL guidelines,[1,2] and thus is of real clinical significance. In addition, studies on HBeAg-negative HBsAg carriers have suggested that 20,000 or 4.3 $\log_{10}$ IU/mL is a more appropriate cutoff level to define inactive chronic HBV infection in the setting of persistently normal ALT.[21] Then, "virological relapse" with an HBV DNA level >2,000 or 3.3 $\log_{10}$ IU/mL without ALT elevation is of much less clinical relevance except in cirrhosis patients for whom antiviral therapy may be indicated according to current guidelines.[1-3] Finally, given that a baseline HBsAg level >1,500 IU/mL has marginal significance in predicting clinical relapse in our ETV cohort, the number of patients (95) may still be too small to verify the value of HBsAg level in this setting.

In summary, the 1-year clinical relapse rate was around 45% in HBeAg-negative CHB patients who had stopped ETV therapy by the APASL stopping rule. This relapse rate is similar to the 1-year reactivation rate of a younger cohort of HBeAg-positive CHB who stopped Nuc therapy by APASL guidelines.[18] Furthermore, the 1-year relapse rate was 29% and 33%, respectively, in patients with a baseline serum HBV DNA $\leq 2 \times 10^5$ or 5.3 $\log_{10}$ IU/mL and noncirrhosis patients with serum HBV DNA $>2 \times 10^5$ IU/mL plus consolidation therapy >64 weeks. A longer consolidation therapy seems more appropriate for patients with higher baseline HBV DNA. With proper off-therapy monitoring, ETV therapy can be safety stopped in HBeAg-negative CHB, including patients with compensated cirrhosis, as their HBeAg-positive counterparts usually do. Proper monitoring is of paramount importance in cirrhosis patient for timely retreatment to prevent decompensation. Of note, recent studies have shown reversal of liver cirrhosis in patients treated with ETV or TDF $\geq$5 years.[22,23] In this regard, it would be beneficial to continue therapy in cirrhosis patients.

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