Virological Breakthrough and Resistance in Patients with Chronic Hepatitis B Receiving Nucleos(t)ide Analogues in Clinical Practice

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Virological breakthrough (VBT) is the first manifestation of antiviral drug resistance during nucleos(t)ide analogue (NUC) treatment of chronic hepatitis B (CHB), but not all VBTs are due to drug resistance. This study sought to determine the incidence of VBT and genotypic resistance (GR) in patients with CHB who were receiving NUCs in clinical practice. Records of patients with CHB who were receiving NUCs were reviewed. All patients with VBT were tested for drug resistance mutations. Of 148 patients included, 73% were men and mean age was 44.9 years. During a mean follow-up of 37.5 ± 20.1 months, 39 (26%) patients had at least 1 VBT. Of these 39 patients, 15 (38%) were not confirmed to have VBT on retesting, and 10 of these 15 had no evidence of GR. The cumulative probability of VBT, confirmed VBT, and GR at 5 years was 46.1%, 29.7%, and 33.9%, respectively. In multivariate analysis, failure to achieve undetectable hepatitis B virus (HBV) DNA was the only factor significantly associated with VBT. Among the 10 patients who had VBT but no confirmed VBT or GR and who were maintained on the same medications, serum HBV DNA decreased in all 10, and nine had undetectable HBV DNA at a mean of 6.8 months after the VBT. Four patients had persistently undetectable HBV DNA, six had transient increase in HBV DNA during follow-up, and none had GR. *Conclusion:* VBT was common in patients with CHB receiving NUCs in clinical practice, but nearly 40% of the VBTs were not related to antiviral drug resistance. Counseling of patients with CHB on medication adherence and confirmation of VBT and/or GR can avoid unnecessary changes in antiviral medications. (HEPATOLOGY 2011;53:1854-1863)

Five nucleos(t)ide analogues (NUCs)—lamivudine, adefovir, entecavir, telbivudine, and tenofovir—have been approved for the treatment of chronic hepatitis B (CHB). NUCs are administered orally and have very few side effects; however, these medications suppress but do not eradicate hepatitis B virus (HBV). Therefore, most patients with CHB will require long-term treatment to derive clinical benefit. However, long-term NUC treatment is associated with increasing risk of drug resistance, particularly when NUCs with low genetic barrier to resistance are used as monotherapies.

Virological breakthrough (VBT) is the first clinical manifestation of antiviral drug resistance and may precede biochemical breakthrough (BBT).[1-3] Phase 3 clinical trials of NUCs in NUC-naive patients revealed that 0%-87.5% of patients with VBT had confirmed genotypic resistance (GR).[4-9] In the phase 3 trial of telbivudine versus lamivudine, 32 of 680 (4.7%) and 99 of 687 (14.4%) patients who received telbivudine and lamivudine, respectively, experienced VBT after 1 year of treatment, but only 28 (87.5%) and 75 (75.8%) patients with VBT were confirmed to have GR.[9] In the phase 3 trial of entecavir versus lamivudine, 11 of 679 (1.6%) and 88 of 668 (13.2%)

Abbreviations: ALT, alanine aminotransferase; Anti-HBe, antibody to hepatitis B e antigen; BBT, biochemical breakthrough; CHB, chronic hepatitis B; CI, confidence interval; GR, genotypic resistance; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HR, hazard ratio; NUC, nucleos(t)ide analogue; ULN, upper limit of normal; VBT, virological breakthrough.

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patients who received entecavir and lamivudine, respectively, experienced VBT after 1 year of treatment, but 0 (0%) of the entecavir-treated and 65 (73.9%) of lamivudine-treated patients with VBT were confirmed to have GR.[7,8] In the phase 3 trial of tenofovir, 10 of 426 (2.3%) patients who received tenofovir experienced VBT after 1 year of treatment, but none of these patients were confirmed to have GR.[4] These data indicate that not all VBTs are related to antiviral drug resistance.

Possible explanations for the discrepancy between the rates of VBT and GR include poor adherence to medications, failure to detect drug-resistance mutations due to insensitive assays, and failure to recognize new mutations associated with antiviral drug resistance. VBT during the first year of treatment was attributed to medication nonadherence in patients who received entecavir or tenofovir in the phase 3 trials.[4,7] Medication adherence is likely to be lower in clinical practice than in phase 3 clinical trials, where highly motivated patients are recruited and closely monitored. Differentiating between VBT due to medication nonadherence and VBT due to drug resistance is important, because virological response can be restored by reinforcement of adherence in the former case whereas rescue therapy is needed in the latter situation.

The aims of this study were (1) to determine the incidence of VBT and GR in patients with CHB who were treated with NUCs in clinical practice, (2) to determine the factors associated with VBT in patients with CHB who were receiving NUCs, and (3) to determine the outcomes of patients with VBTs that were not confirmed to be associated with antiviral drug resistance mutations.

Patients and Methods

Consecutive adult patients with CHB seen at the liver clinic of the University of Michigan Health System between January 2000 and July 2010, who had received NUC treatment for at least 1 year and who had serum HBV DNA <10,000 IU/mL after 1 year of treatment were included. Patients who were receiving combination therapy of NUCs and interferon; patients receiving NUCs to prevent reactivation of hepatitis B during immunosuppressive or cancer treatment or to prevent recurrent hepatitis B after liver transplantation; patients with human immunodeficiency virus (HIV), hepatitis C virus, or hepatitis D virus coinfection; and patients with impaired renal function requiring dose adjustment of NUCs were excluded. The study was approved by the Institutional Review Board of the University of Michigan.

Medical records were reviewed, and information on patient demographics (age, sex, race), body weight, HBV markers (hepatitis B e antigen [HBeAg], hepatitis B e antibody [anti-HBe], HBV DNA), hepatic panel (albumin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, and alkaline phosphatase), complete blood count, prothrombin time/international normalized ratio, and liver histology were recorded. HBV treatment history was reviewed. Index treatment was defined as the first course of NUC therapy initiated at our liver clinic. Start and stop dates of index and prior HBV treatment, medications used during each course of treatment, and serial results of HBeAg, anti-HBe, HBV DNA, AST, and ALT measurements during treatment were recorded.

During treatment, serum HBV DNA and the hepatic panel were tested every 3 months and HBeAg/anti-HBe every 6-12 months. Serum HBV DNA was quantified by commercial polymerase chain reaction assays: Amplicor HBV monitor test (Roche Molecular Diagnostics, Indianapolis, IN) with a lower limit of detection of 200-1000 copies/mL (40-200 IU/mL) between 2000 and 2005, and real-time polymerase chain reaction assays, COBAS TaqMan HBV (Roche Molecular Diagnostics) with a lower limit of detection of 29 IU/mL from July 2005 onward.

Tests for Antiviral Drug Resistance Mutations. HBV DNA from serum samples of patients with VBT was amplified and sequenced as described.[10] The DNA sequences were aligned with Seqman II and EditSeq software (DNASTAR Inc., Madison, WI) and compared to consensus sequences of the respective HBV genotype. All samples were also tested for antiviral drug resistance mutations by a line probe assay, INNO-Lipa HBV DR version 2 and version 3 (Innogenetics NV, Gent, Belgium) according to the manufacturer's instructions.[10,11]

Definition of Virological Breakthrough, Genotypic Resistance, and Biochemical Breakthrough. VBT was defined as any increase in serum HBV DNA by >1 $\log_{10}$ from nadir or redetection of serum HBV DNA at levels ≥10-fold the lower limit of detection of the HBV DNA assay after having an undetectable result. Thus, a patient who previously had undetectable serum HBV DNA by an assay with a lower limit of detection of 29 IU/mL would be considered to have a VBT if serum HBV DNA is subsequently detected at levels ≥290 IU/mL. A cutoff ≥10-fold the lower limit of detection was chosen because the consensus definition of VBT required a 10-fold increase in HBV

DNA.[2,12] In addition, HBV DNA levels slightly above the limit of detection may not be reproducible on retesting of the same serum sample. Confirmed VBT was defined as persistence of VBT on repeat test (fulfilling the same criteria) 1-3 months later (with or without further increase in serum HBV DNA). GR was defined as detection of signature resistance mutations by direct sequencing. Signature resistance mutations included substitution of alanine to threonine or valine at codon 181 (rtA181T/V), threonine to alanine, cysteine, glycine, isoleucine, leucine, methionine, or serine at codon 184 (rtT184A/C/G/I/L/M/S), alanine to threonine at codon 194 (rtA194T), serine to cysteine, glycine, or isoleucine at codon 202 (rtS202C/G/I), methionine to valine or isoleucine at codon 204 (rtM204V/I), asparagine to threonine at codon 236 (rtN236T), and methionine to isoleucine or valine at codon 250 (rtM250I/V). Compensatory mutations such as substitution of leucine to methionine at codon 180 (rtL180M) were not included.[13]

BBT was defined as ALT above the upper limit of normal (ULN) (35 IU/L) in patients who had normalized ALT and ALT >2 times nadir in those who never had normal ALT. ALT flare was defined as ALT >5 times ULN in patients who had normalized ALT and ALT >5 times nadir in those who never had normal ALT.

Statistical Analyses. Continuous variables were expressed as mean ± standard deviation, or median and range. Serum HBV DNA was expressed as $\log_{10}$ IU/mL. Categorical variables were expressed as number and percent. Continuous variables were compared with two-tailed student *t* test or Mann-Whitney test depending on the distribution, and categorical variables were compared with chi-square test or Fisher's exact test. The Kaplan-Meier method was used to estimate the cumulative probability of VBT, confirmed VBT, and GR. For these analyses, patients were censored when index treatment was changed. The log-rank method was used to compare the cumulative probability of VBT, confirmed VBT, and GR of different treatment groups. Cox regression analysis was used to identify factors associated with VBT. The following variables were included in the analysis: HBV markers (HBeAg, HBV DNA) at the start of treatment, HBV DNA levels after 1 year of treatment, nadir virological response, history of HBV treatment (NUC-naive or NUC-experienced), and medication used in the index regimen (lamivudine versus others for NUC-naive patients and combination therapy or tenofovir versus others for NUC-experienced patients). Variables with a $P < 0.1$ on univariate analysis were further analyzed by multivariate Cox regression to determine the independent factors associated with VBT. Data analyses were performed using SPSS software, version 15 (SPSS Inc., Chicago, IL).

Results

Characteristics of Patients at the Start of the Index Regimen. A total of 148 patients with CHB were included. Five patients who met other criteria but whose serum HBV DNA exceeded 10,000 IU/mL after 1 year of treatment were excluded, all five patients had >1 $\log_{10}$ decrease in HBV DNA after 6 months of treatment. Characteristics of the patients at the start of the index regimen are shown in Table 1. Most 73% of the patients were men, and the mean age was 44.9 ± 12.3 years. Approximately half (48.7%) of the patients were Asian, 41.2% were Caucasian, and the remainder were of other races. Roughly half (52.7%) of the patients were positive for HBeAg, and the mean HBV DNA was 6.2 ± 1.8 $\log_{10}$ IU/mL. A majority (129, or 87.2%) of patients had an elevated serum ALT based on our hospital laboratory reference range and 42 (28.4%) had ALT level >5 times ULN.

Index Regimen and Initial Response. Table 2 lists the medications used in the index regimen. Among the 81 NUC-naive patients, entecavir alone (n = 43) and lamivudine alone (n = 26) were the most common medications. Of the 67 NUC-experienced patients, 19 received combination therapy, 16 received adefovir, 15 received tenofovir, 13 received entecavir, and four received lamivudine. The mean duration of follow-up was 37.5 ± 20.1 months (median 31.5 [range 12-102] months). Forty-two (28.4%) patients had been receiving the index treatment regimen for more than 48 months.

After 1 year on the index regimen, 70.9% of the patients had undetectable HBV DNA (Table 2). With continued treatment, 86.5% (87.7% NUC-naive and 85.1% NUC-experienced) of patients achieved undetectable HBV DNA. The mean interval from the start of the index treatment to nadir virological response was 9 ± 6.3 months. Of the 136 patients who had baseline elevated ALT, 105 (77.2%) achieved ALT normalization after a mean of 8.4 ± 8.3 months.

Virological Breakthrough During Treatment. Thirty-nine (26.4%) patients experienced at least one VBT after the first year of treatment, 24 (62%) of these patients had confirmed VBT, 24 (62%) had GR by direct sequencing, 13 (33%) had BBT, and only one had ALT flare (Fig. 1). Of the 36 patients who had repeat HBV DNA after VBT, the mean interval from

Table 1. Baseline Characteristics of Patients

	Virological Breakthrough	No Virological Breakthrough	All Patients	*P* Value
Number of patients	39	109	148	
Age (year)	48.1 ± 12.4	43.7 ± 12.1	44.9 ± 12.3	0.06
Sex (male)	31 (79.5)	77 (70.6)	108 (73)	0.40
Race				
Caucasian	15 (38.5)	46 (42.2)	61 (41.2)	0.36
Asian	22 (56.4)	50 (45.9)	72 (48.7)	
Other	2 (5.1)	13 (11.9)	15 (10.1)	
Weight (pounds)	159 ± 40	169 ± 46	167 ± 45	0.35
Previous HBV treatment				
None	21 (53.8)	61 (56)	82 (55.4)	0.95
1 Regimen	11 (28.2)	28 (25.7)	39 (26.4)	
>1 Regimen	7 (18)	20 (18.3)	27 (18.2)	
Liver Histology	24	64	88	
Cirrhosis	12 (30.8)	29 (26.6)	41 (27.7)	0.68
HBV DNA (log IU/mL)	6.6 ± 1.7	6.0 ± 1.9	6.2 ± 1.8	0.11
<5 log	7 (17.9)	34 (31.2)	41 (27.7)	0.28
5-7 log	15 (38.5)	35 (32.1)	50 (33.8)	
>7 log	17 (43.6)	40 (36.7)	57 (38.5)	
HBeAg-positive	21 (55.8)	57 (52.3)	78 (52.7)	0.99
Laboratory values				
Total bilirubin (mg/dL)	1.7 ± 4.4	1.0 ± 1.3	1.2 ± 2.5	0.11
AST (IU/L)	76 (23-920)	62 (15-2854)	66 (15-2854)	0.24
ALT (IU/L)	97 (22-1720)	97(13-4324)	97 (13-4324)	0.19
Albumin (g/dL)	3.9 ± 0.6	4.2 ± 0.5	4.1 ± 0.5	***0.03***
Alkaline phosphatase (IU/L)	102 ± 53	91 ± 46	94 ± 48	0.16
Platelets (10^3/mm^3)	177 ± 84	194 ± 75	189 ± 78	0.08

Results are expressed as number (%), mean ± SD, or median (range).

VBT to confirmed VBT was 2.4 ± 1.6 months. Line probe assay revealed two additional patients had GR. Both were receiving adefovir and found to have the rtN236T mutation (Fig. 1). The first patient had confirmed VBT and was switched to combination of tenofovir and emtricitabine with undetectable HBV DNA 6 months after start of rescue therapy (Supporting Table 1, patient T). The second patient (patient 8) was not confirmed to have VBT on retesting and continued to receive adefovir monotherapy.

The baseline characteristics of the patients who did or did not experience VBT were similar, except for lower serum albumin among the patients who subsequently experienced VBT (Table 1). The cumulative probability of VBT at 3 and 5 years was 21.5% and 46.1%, confirmed VBT was 13.7% and 29.7%, and GR was 10.7% and 33.9%, respectively (Fig. 2). If the traditional definition of VBT (increase in HBV DNA by >1 log from nadir or redetection of any level of HBV DNA after becoming undetectable) was used, 45 (30%) patients would be considered to have experienced at least one VBT, and 26 (58%) of these patients would have confirmed VBT.

Among the NUC-naive patients, the cumulative probability of VBT, confirmed VBT, and GR at 3 and 5 years was 18% and 54%, 12% and 31%, and 13% and 39%, respectively. NUC-naive patients who experienced VBT were more likely to be receiving lamivudine than other NUCs (Table 2). Fourteen of 26 (53.8%) patients receiving lamivudine monotherapy experienced at least one VBT compared to three of 43 (7%) patients receiving entecavir monotherapy. Antiviral drug resistance mutations were detected in 12 of the 14 patients receiving lamivudine and one of the three patients receiving entecavir who experienced VBT. The cumulative probability of VBT, confirmed VBT, and GR at 3 years was 37%, 30%, and 33%, respectively, among the patients receiving lamivudine monotherapy (Fig. 3A), and 13%, 3.1%, and 3.1%, respectively, among the patients receiving entecavir monotherapy (Fig. 3B) ($P = 0.001$, 0.001, and <0.001, respectively).

Among the NUC-experienced patients, the cumulative probability of VBT, confirmed VBT, and GR at 3 and 5 years was 22% and 33%, 14% and 28%, and 8% and 29%, respectively. Sixteen of 33 (48.5%) patients who were receiving lamivudine, adefovir, or entecavir monotherapy but only two of 34 (5.9%) patients receiving combination therapy or tenofovir monotherapy experienced VBT. The cumulative probability of VBT, confirmed VBT, and GR at 3 years was 32.3%, 23.6%, and 13.8%, respectively, among the

Table 2. Index Treatment Regimen and Response to Treatment

	Virological Breakthrough (N = 39)	No Virological Breakthrough (N = 109)	All Patients (N = 148)	*P* Value
Regimen				
NUC-naive (N = 81)				
Lamivudine	14 (35.9)	12 (11)	26 (17.6)	***<0.001***
Adefovir	3 (7.7)	1 (0.9)	4 (2.7)	
Entecavir	3 (7.7)	40 (36.7)	43 (29.1)	
Tenofovir	0	6 (5.5)	6 (4.1)	
Telbivudine	1 (2.6)	1 (0.9)	2 (1.3)	
NUC-experienced (N = 67)				
Lamivudine	2 (5.1)	2 (1.8)	4 (2.7)	***0.002***
Adefovir	9 (23)	7 (6.4)	16 (10.8)	
Entecavir	5 (12.8)	8 (7.3)	13 (8.8)	
Tenofovir	1 (2.6)	14 (12.8)	15 (10.1)	
Combination	1 (2.6)	18 (16.5)	19 (12.8)	
Duration of index treatment (month), mean ± SD	45.5 ± 18.4	34.6 ± 20	37.5 ± 20.1	***0.001***
≤ 24	3 (7.7)	47 (43.1)	50 (33.8)	***<0.001***
25-48	20 (51.3)	37 (33)	56 (37.8)	
≥49	16 (41)	26 (23.9)	42 (28.4)	
Response at 1 year				
HBV DNA undetectable				
All patients	21/39 (53.8)	84/109 (77.1)	105 (70.9)	***0.008***
NUC-naive	12/21 (57.1)	50/60 (83.3)	62/81 (76.5)	***0.03***
NUC-experienced	9/18 (50)	34/49 (69.4)	43/67 (64.2)	0.16
HBV DNA* (log IU/mL)	2.4 ± 0.7	2.4 ± 0.6	2.4 ± 0.7	0.91
ALT < ULN†	25/37 (67.6)	54/99 (54.5)	79/136 (58.1)	0.24
Nadir Response				
HBV DNA undetectable				
All patients	26/39 (66.7)	102/109 (93.6)	128 (86.5)	***<0.001***
NUC-naive	15/21 (71.4)	56/60 (93.3)	71 (87.7)	***0.02***
NUC-experienced	11/18 (61.1)	46/49 (93.9)	57 (85.1)	***0.003***
HBV DNA* (log IU/mL)	2.3 ± 0.7	1.7 ± 0.7	2.1 ± 0.8	0.09
ALT < ULN†	31/37 (83.8)	74/99 (74.7)	105/136 (77.2)	0.36
Interval from nadir to first VBT (months), Mean ± SD	20.5 ± 17	0	20.5 ± 17	
Duration of on-treatment follow-up after nadir (months), Mean ± SD	35.6 ± 19.5	25.8 ± 20.7	28.4 ± 23	***0.003***

Results are expressed as number (%), mean ± SD.

*Among patients who had detectable HBV DNA.

†For patients who had elevated baseline ALT.

patients receiving lamivudine, adefovir, or entecavir monotherapy, and 8.7%, 3.8%, and 0%, respectively, among the patients receiving combination therapy or tenofovir monotherapy ($P = 0.005$, 0.01, and 0.04, respectively).

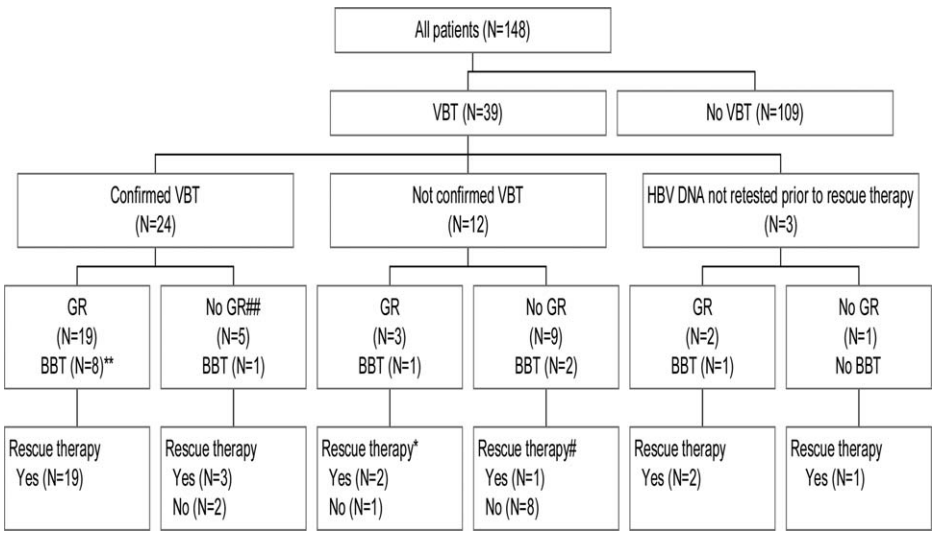

Fig. 1. Outcome of patients with VBT. *This patient was lost to follow-up. **One patient had ALT flare. #One patient was found to have N236T mutation by line probe assay but did not receive rescue therapy (patient 8). ##One patient was found to have N236T mutation by line probe assay and received rescue therapy. BBT, biochemical breakthrough.

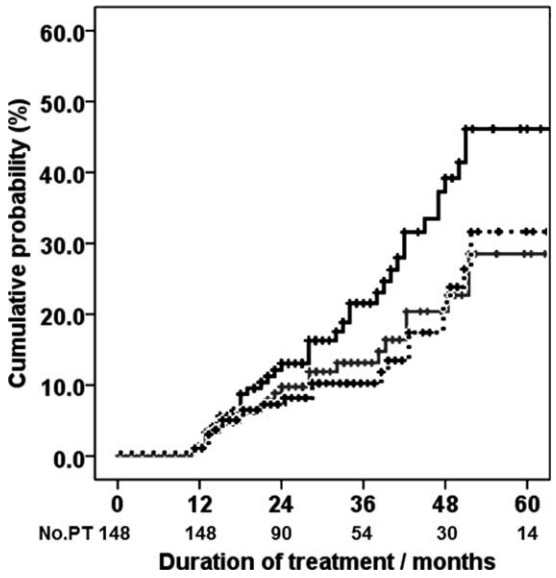

Fig. 2. Cumulative probability of VBT (black line), confirmed VBT (gray line), and GR (dotted line) (n = 148). No. PT, number of patients.

Univariate analyses found that failure to achieve undetectable HBV DNA after 1 year of treatment (hazard ratio [HR] = 2.6, 95% confidence interval [CI] = 1.39-5.0, $P = 0.003$) and at nadir response (HR = 6.92, 95% CI = 3.4-14.1, $P < 0.001$) significantly increased the risk of VBT. Multivariate analyses showed that failure to achieve undetectable HBV DNA at nadir response was the only factor significantly associated with VBT in the overall population (HR = 5.5, 95% CI = 2.49-12.28, $P < 0.001$). For subgroup analyses, the predictors of VBT among NUC-naive patients were failure to achieve undetectable HBV DNA at 1 year (HR = 2.79, 95% CI = 1.11-7.01, $P = 0.03$) and at nadir response (HR = 3.95, 95% CI = 1.46-10.71, $P = 0.007$), and receipt of lamivudine monotherapy (HR = 3.19, 95% CI = 1.22-8.33, $P = 0.02$). The predictors of VBT among the NUC-experienced patients were failure to achieve undetectable HBV DNA at nadir response (HR = 9.59, 95% CI = 1.64-56.07, $P = 0.01$), and monotherapy with drugs other than tenofovir (HR = 8.15, 95% CI = 1.61-41.29, $P = 0.01$) (Table 3).

Outcome of Patients Who Had Virological Breakthrough. Of the 39 patients who had at least 1 episode of VBT, 24 (62%) had confirmed VBT, 12 were not confirmed to have VBT on retesting, and three were not available for retesting because they received rescue therapy when VBT was first detected (Fig. 1). Two of the latter three patients had GR. BBT was observed in nine (37.5%) of 24 patients with confirmed VBT, three (25%) of 12 not confirmed to have VBT, and one of the three patients who received rescue therapy when VBT was first detected (Fig. 1). Only one patient had an ALT flare. This patient had confirmed VBT, nadir ALT was 30 IU/L, and ALT at the time of VBT was 34 IU/L. This patient was noncompliant and did not have repeat HBV DNA testing until 8 months after the initial VBT. At the time of confirmed VBT, ALT was 258 IU/L.

Among the 24 patients who had confirmed VBT, 19 patients had GR, and all 19 received rescue therapy. The index treatment, mutations detected at the time of VBT, rescue therapy administered, and

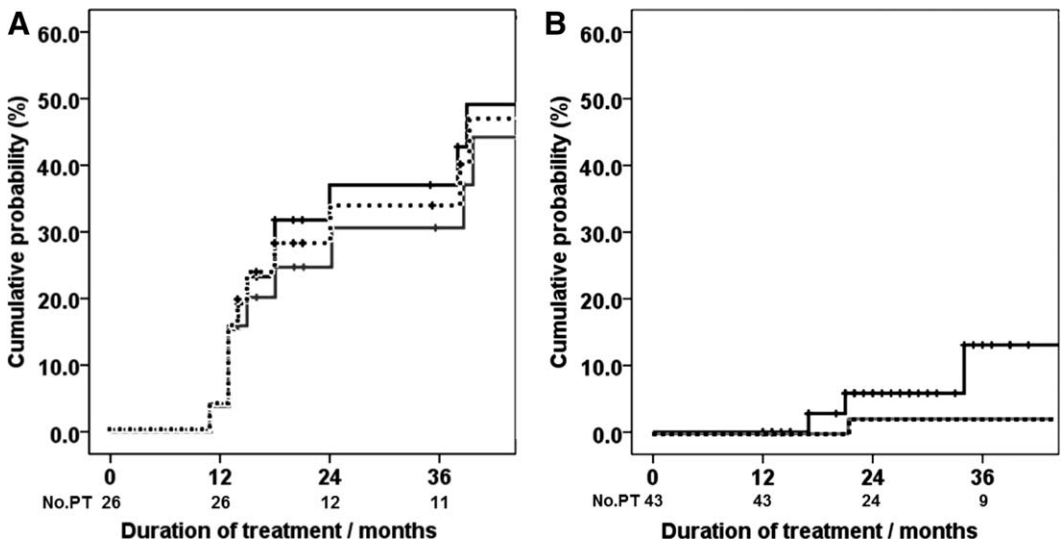

Fig. 3. Cumulative probability of VBT (black line), confirmed VBT (gray line), and GR (dotted line) in NUC-naive patients receiving (A) lamivudine monotherapy (n = 26) and (B) entecavir monotherapy (n = 43). No. PT, number of patients.

Table 3. Multivariate Analyses of Factors Associated With Virological Breakthrough

	All Patients		NUC-Naive Patients		NUC-Experienced Patients	
Variables	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Undetectable HBV DNA after 1 year	1.57 (0.75-3.26)	0.23	2.79 (1.11-7.01)	***0.03***	1.92 (0.36-10.34)	0.45
Undetectable HBV DNA at nadir response	5.53 (2.49-12.28)	***<0.001***	3.95 (1.46-10.71)	***0.007***	9.59 (1.64-56.07)	***0.01***
Index regimen						
Lamivudine monotherapy versus others	–	–	3.19 (1.22-8.33)	***0.02***	–	–
Other monotherapy versus tenofovir monotherapy or combination therapy	–	–	–	–	8.15 (1.61-41.29)	***0.01***

response to rescue therapy in these 19 patients are shown in Supporting Table 1 (patients A-S). Seventeen of these 19 patients had undetectable HBV DNA within 6 months of initiating rescue therapy.

Of the five patients who did not have GR by direct sequencing, two (patients 1 and 2) continued on the same treatment (Fig. 4) and the other three received rescue therapy (Supporting Table 1, patients T-V). Patient 1 had a further decrease in HBV DNA level during continued treatment with the same medication, but HBV DNA remained detectable. Serum HBV DNA at the last visit was 2.2 $\log_{10}$ IU/mL 12 months after the VBT. Patient 2 had a slow decline in serum HBV DNA level, retesting for GR 10 months later did not reveal any signature antiviral resistance mutations and HBV DNA became undetectable 19 months after the VBT. This patient subsequently developed two more episodes of VBT, but testing for GR did not reveal any signature antiviral resistance mutations and HBV DNA became undetectable again after each episode of VBT. No BBT was observed during subsequent episodes of VBT. The three patients who received rescue therapy had undetectable HBV DNA within 6 months.

Among the 12 patients who had VBT that was not confirmed on retesting, three were found to have signature antiviral resistance mutations. All three patients were receiving lamivudine monotherapy and were found to have the Met204Ile mutation. Two patients (Supporting Table 1, patients W and X) had undetectable HBV DNA within 6 months after rescue therapy, whereas the third was lost to follow-up. The remaining nine patients who did not have confirmed VBT had no evidence of GR, two had BBT with ALT of 56 IU/L and 65 IU/L. Eight of these nine patients (patients 3-10) continued on the same treatment, whereas one patient received rescue therapy. HBV DNA levels in four patients (patients 3-6) who continued on the same treatment became consistently undetectable during follow-up. Three (patients 7-9) patients' HBV DNA levels became undetectable during continued treatment with the same medication, but HBV DNA subsequently became detectable at levels below our VBT criteria. Retesting did not reveal any signature antiviral resistance mutations, and HBV DNA levels of

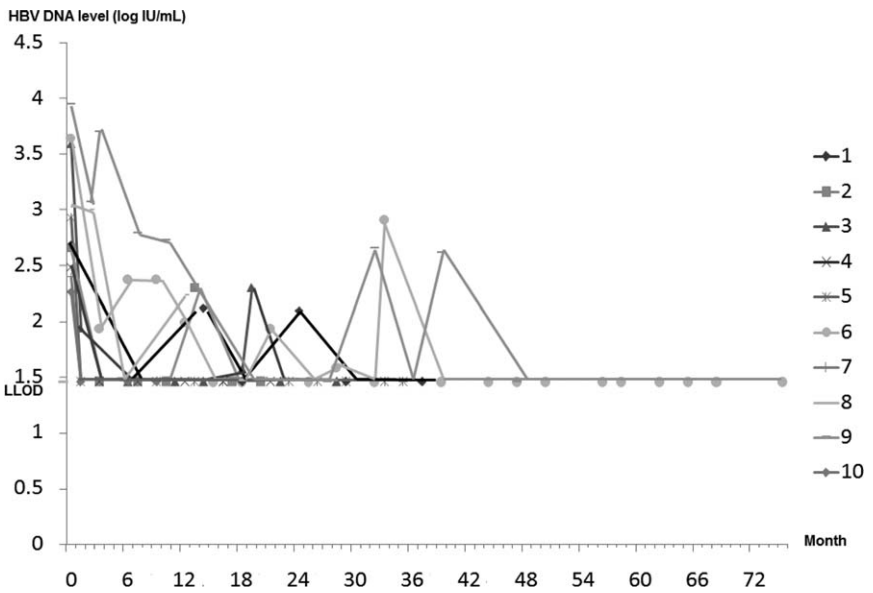

Fig. 4. Serum HBV levels in 10 patients who had VBT but no confirmed VBT or GR during continued treatment with the same medication. LLOD, lower limit of detection.

these three patients became undetectable again with continued treatment on the same medication. The last patient (patient 10) had an initial decline in HBV DNA during continued treatment with the same medication but developed a second VBT 33 months after the first episode of VBT. This second episode of VBT was not confirmed on retesting and no signature antiviral resistance mutation was detected. HBV DNA level subsequently became undetectable (Fig. 5).

In total, 10 patients who experienced VBT continued on the same treatment and had been followed for a mean of 29.3 ± 20.5 months (median 24.5 (range 9-75) months) after the initial episode of VBT. All 10 patients had further decrease in serum HBV DNA and nine had undetectable HBV DNA a mean of 6.8 ± 6.3 months after the VBT. Six patients had one or more episodes of transient increase in serum HBV DNA; of these, two met our criteria for VBT but none had GR. Two of these six patients had a mild increase in ALT with peak values of 56 and 65 IU/L during subsequent episodes of HBV DNA increase.

Of the remaining 29 patients with VBT, 28 received rescue therapy and all but three had undetectable HBV DNA within 6 months of initiating rescue therapy whereas one was lost to follow-up.

Discussion

This study examined the rates of VBT, confirmed VBT and GR in 148 CHB patients treated with NUCs in clinical practice. We found a high rate of VBT, 39 (26%) patients experienced at least 1 episode of VBT with a cumulative probability of 46% at 5 years. Twenty-four (16%) patients had confirmed VBT; of these, 19 (79%) had GR by direct sequencing. An additional five patients had GR but were not confirmed to have VBT on retesting or received rescue therapy without retesting. Thus, in total 24 (16%) patients had GR. The finding that 38% of patients who experienced VBT were not confirmed to have VBT on retesting and 38% did not have antiviral resistance mutations on direct sequencing suggests that medication nonadherence may be the cause of the VBT in these patients.

We acknowledge that direct sequencing is insensitive and will not detect viral variants that comprise <20% of the viral population. In this study, all patients were also tested by a line probe assay which is more sensitive and can detect viral variants that comprise >5% of the viral population. Two additional patients were found to have N236T mutation by the line probe assay. Thus, signature antiviral resistance mutations were not detected in 13 (33%) patients with VBT by both direct sequencing and line probe assay. None of these 13 patients was noted to have other substitutions in the reverse transcriptase region of the HBV polymerase gene. Only one had BBT. In phase 3 clinical trials of NUCs for CHB, 65.7% to 87.5% of patients receiving lamivudine or telbivudine and 0% of patients receiving entecavir or tenofovir, who experienced VBT during the first year had GR.[4,7-9]

Although antiviral resistance mutations may be detected if we had used more sensitive methods such as single genome sequencing or pyrosequencing, follow-up data suggest that most of the patients who experienced VBT but did not have confirmed VBT or GR were not adherent to their antiviral medication(s). Because this was a retrospective study, data on medication adherence was not available; however, follow-up data suggest that non adherence was an important cause of these unconfirmed VBTs. All 10 patients who continued treatment with the same medication(s) had further decrease in serum HBV DNA levels and all but one had undetectable HBV DNA after the VBT. Six of these 10 patients experienced ≥1 episode of transient increase in serum HBV DNA level during follow-up despite counseling on the importance of medication adherence. None of the five retested for GR was found to have antiviral resistance mutations and all had subsequent decline in serum HBV DNA during continued treatment with the same medication(s). We acknowledge that our attribution that nonadherence was an important cause of VBT is speculative. In a separate prospective study of 105 patients in whom adherence was evaluated using a self-administered questionnaire, 26% admitted to missing their medication at least once in the past 30 days and patients who had <100% adherence on serial assessments had a trend toward a higher rate of VBT.[14] Medication adherence has been shown to be important in maintaining response in patients receiving treatment for other conditions. Published studies showed that patients who were adherent to antihypertensive medications were more likely to have adequately controlled blood pressure.[15-17] Several studies of antiretroviral medications in patients with HIV infection also revealed that failure to adhere to HIV treatment regimens and repeated drug holidays (defined as stopping treatment entirely for ≥48 hours) were associated with a higher rate of virological failure.[18,19] In one study of HIV treatment that included a protease inhibitor, 80% of patients with <80% adherence had virological failure, compared to 22% of those with ≥95% adherence.[20]

There are very little data on adherence to NUC treatment for hepatitis B. In a previous study of a pharmacy claims database that included 11,100 patients receiving NUCs for CHB, we found that mean adherence 1 year after enrollment, defined as percent of days in which the patient had medications during that year, was 87.8%.[21] In that study, we were not able to correlate medication adherence with virological response or occurrence of VBT. In the current study, we found that nadir response and type of medication used were significantly associated with the occurrence of VBT. Thus, patients who had rapid response with undetectable serum HBV DNA within 1 year of treatment and those who had undetectable serum HBV DNA at some point during the course of treatment were less likely to experience VBT. These data are in accord with previous studies showing that undetectable serum HBV DNA after 24 weeks of treatment is associated with significantly lower rates of antiviral resistance.[22,23] As expected, among NUC-naive patients, those receiving entecavir monotherapy were less likely to experience VBT than those receiving lamivudine monotherapy. Among NUC-experienced patients, those receiving combination therapy (lamivudine + adefovir or emtricitabine + tenofovir) or tenofovir monotherapy were less likely to experience VBT than those receiving lamivudine, adefovir, or entecavir monotherapy. This is not surprising, because these patients had previous nonresponse or resistance to lamivudine or adefovir, and it is now known that switching from lamivudine to adefovir or entecavir monotherapy in patients with prior lamivudine resistance is associated with a high rate of subsequent resistance to adefovir or entecavir.[24-26] In this study, all the patients who received entecavir or tenofovir alone or in combination with another NUC had undetectable HBV DNA within 6 months of rescue therapy, whereas three patients with lamivudine resistance still had detectable HBV DNA 6 months after rescue therapy with adefovir alone or in combination with lamivudine.

In conclusion, this study revealed that VBT was common in clinical practice. However, VBT was not always related to antiviral drug resistance. Patients with CHB receiving NUC therapy who experienced VBT should be counseled on medication adherence, and for patients who are immunocompetent and have compensated liver disease, confirmation of VBT and/or determination of GR is prudent before the initiation of rescue therapy to avoid unnecessary changes in antiviral medications. We acknowledge that this study is limited by the small number of patients, the heterogeneity in treatment regimens, and the lack of data on medication adherence. However, the results of this study highlight the importance of HBV DNA monitoring and counseling on medication adherence throughout the course of NUC treatment and the fine balance between prompt initiation of rescue therapy versus avoidance of unnecessary changes to the treatment regimen.

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