Table 9 Univariate analysis for factors associated with HBV DNA rebound within 24 weeks after discontinuation of NA treatment in the patients in whom ALT levels did not normalize at the end of NA treatment

Factors	ALT relapsed ($n = 7$)	ALT non-relapsed ($n = 4$)	Univariate P value
Gender (M:F)	6:1	4:0	1.000[b]
HBV genotype (B:C:ND)	0:6:1	0:3:1	1.000[b]
Before treatment			
Age (years)[a]	36 (25–56)	50 (30–64)	0.218
Platelet ($\times 10^4/\mu L$)[a]	17.0 (13.1–27.5)	16.1 (15.6–16.5)	0.770
ALT (IU/L)[a]	101 (37–303)	148 (114–270)	0.571
HBsAg (IU/mL)[a]	11,113 (1,180–40,967)	1,384 (406–7,016)	0.197
HBeAg (+: −)	5:2	1:3	0.242[b]
HBcrAg (log U/mL)[a]	5.9 (5.5–8.8)	6.7 (5.0–7.7)	1.000
HBV DNA (log copies/mL)[a]	7.1 (5.0–10.1)	6.7 (5.7–9.7)	0.635
HBV DNA + RNA titers (log copies/mL)	6.9 (5.1–10.0)	6.3 (5.0–9.7)	0.571
DR ratio	−0.1 (−0.2–0.9)	−0.4 (−0.7–0.0)	0.279
After 3 months of treatment			
HBV DNA (log copies/mL)[a]	5.1 (3.8–7.3)	4.2 (2.2–4.4)	0.052
HBV DNA + RNA titers (log copies/mL)	5.7 (3.9–8.2)	4.4 (2.9–6.2)	0.185
DR ratio	0.6 (−0.2–2.7)	0.1 (−0.1–0.6)	0.255
End of treatment			
HBsAg (IU/mL)[a]	4,317 (2,306–11,607)	5,209 (85–5,711)	0.915
HBeAg (+: −)	5:2	1:3	0.242[b]
HBcrAg (log U/mL)[a]	5.4 (3.6–8.2)	5.6 (4.9–5.9)	1.000
HBV DNA (log copies/mL)[a]	4.4 (2.2–9.2)	2.2 (2.2–7.1)	0.178
HBV DNA + RNA titers (log copies/mL)	4.9 (3.1–8.7)	3.0 (2.2–6.5)	0.131
DR ratio	−0.1 (−0.5–2.7)	0.1 (−0.6–1.6)	0.850
Sequential therapy (+: −)	6;1	4:0	1.000[b]
Duration of treatment (weeks)[a]	24 (24–36)	44 (24–110)	0.091

ND not determined, *DR ratio* HBV DNA + RNA titers/HBV DNA

[a] Median (range) univariate analysis was performed with Mann-Whitney *U* test

[b] Chi-square test

ten subjects were assigned to group B. The cumulative ALT rebound rate of group A was significantly lower than that of group B at 24 and 48 weeks after the discontinuation of therapy ($P = 0.008$, $P = 0.024$, respectively, Fig. 5).

Prediction of ALT rebound after discontinuation of therapy using two extracted factors

To predict successful discontinuation of therapy, we analyzed cumulative ALT rebound by using HBV DNA plus RNA levels at 3 months of NA treatment and existence of HBeAg at the end of treatment. Fourteen subjects who achieved both <4.8 log copies/mL of HBV DNA + RNA levels after 3 months of treatment and negative HBeAg at the end of treatment were assigned to group A and the remaining 22 subjects were assigned to group B. The cumulative ALT rebound rate of group A was significantly lower than that of group B among all observation periods ($P = 0.046$, Fig. 6).

Discussion

Since the introduction of NAs, chronic hepatitis B progression has been drastically suppressed. NAs strongly suppress HBV replication in human hepatocytes and rapidly decrease serum HBV DNA titers to undetectable levels [30–33]. However, even if HBV DNA is continuously maintained at undetectable levels, it is difficult to

Table 10 Multiple logistic regression for factors associated with ALT rebound within 24 weeks after discontinuation of NA therapy in HBeAg-positive patients ($n = 16$)

Factors[a]	ALT relapsed ($N = 10$)	ALT non-relapsed ($N = 6$)	Univariate P value[b]	Multiple logistic regression[c]	
				P value	OR (95 % CI)
Gender (M:F)	5:5	3:3	0.696 (chi-square test)		
HBV genotype (B:C)	0:10	0:6	1.000 (chi-square test)		
Before treatment					
Age (years)[d]	35 (25–56)	38 (29–47)	0.957		
Platelets ($\times 10^4/\mu L$)[d]	20.3 (9.6–28.0)	17.3 (14.5–27.5)	0.768		
ALT (IU/L)[d]	148 (37–309)	155 (46–270)	0.958		
HBsAg (IU/mL)[d]	11,113 (462–1,354,400)	6,283 (66–10,109)	0.662		
HBcrAg (log U/mL)[d]	7.1 (5.5–8.8)	7.4 (5.2–7.7)	0.714		
HBV DNA (log copies/mL)[d]	9.1 (6.5–10.1)	8.8 (3.8–9.7)	0.792		
HBV DNA + RNA titers (log copies/mL)	8.3 (6.1–10.0)	8.6 (3.4–9.7)	0.958		
DR ratio	−0.2 (−1.4 to 0.9)	−0.3 (−0.7 to 0.0)	0.776		
After 3 months of treatment					
HBV DNA (log copies/mL)[d]	5.0 (3.5–7.3)	4.1 (2.2–4.4)	0.056	0.897	
HBV DNA + RNA titers (log copies/mL)	5.8 (4.8–8.2)	4.7 (3.7–6.3)	0.011	0.050	8.032 (0.997–64.683)
DR ratio	1.1 (−0.2 to 2.7)	1.1 (−0.6 to 1.9)	0.792		
End of treatment					
HBsAg (IU/mL)[d]	4,736 (823–16,301)	3,523 (48–11,600)	0.529		
HBeAg (+:−)	10:0	4:2	0.125 (chi-square test)		
HBcrAg (log U/mL)[d]	5.6 (4.1–8.2)	5.3 (4.0–6.6)	0.310		
HBV DNA (log copies/mL)[d]	4.4 (2.2–9.2)	3.7 (2.1–6.1)	0.220		
HBV DNA + RNA titers (log copies/mL)	4.9 (3.7–8.7)	3.9 (3.4–5.7)	0.093	0.543	
DR ratio	0.5 (−1.0 to 2.8)	0.2 (−0.8 to 1.6)	0.635		
Sequential therapy (+:−)	7:3	4:2	0.654 (chi-square test)		
Duration of treatment (weeks)[d]	29 (24– 221)	119 (24–175)	0.169		

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Multiple logistic regression analysis was performed using variables that were at least marginally significant ($P < 0.10$) in the univariate analysis

[d] Median (range)

completely eliminate HBV from the liver. The goal of NA therapy is therefore to reduce the HBV DNA titer and to induce an inactive state of hepatitis, but, as a result, it is necessary that NA therapy should be continued for a long period of time. As it is well known that long-term treatment with NAs increases the incidence of HBV drug resistance [14], we propose that patients who maintain an inactive state of hepatitis with NA therapy may be able to discontinue the NA therapy to prevent the appearance of drug-resistant strains. However, as shown in Fig. 1, in our patient cohort, hepatitis was re-activated after discontinuation of the therapy in more than 70 % of the patients who discontinued the NA therapy. Therefore, in this study, we analyzed predictive factors for the safe discontinuation of NA therapy.

After discontinuation of NA therapy, serum HBV DNA titers increased in 91.7 % of our patients within 48 weeks (Fig. 1a). In the multivariate logistic regression, the HBV

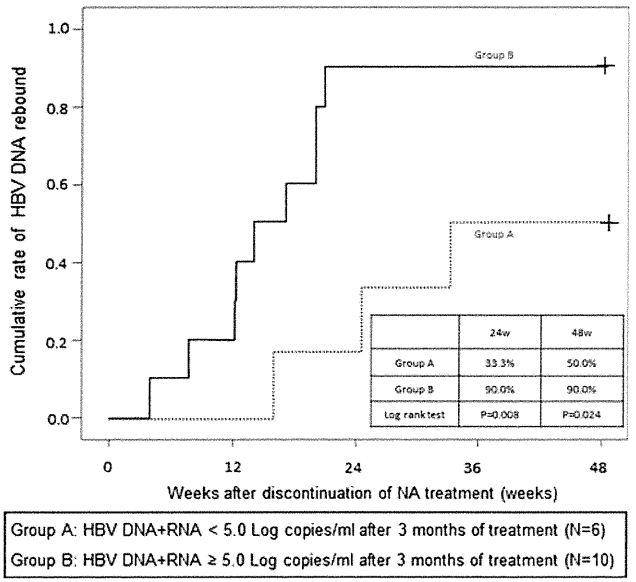

Fig. 5 Cumulative rate of ALT rebound after discontinuation of NA treatment in HBeAg-positive chronic hepatitis B patients. Six patients whose HBV DNA + RNA titers reached <5.0 log copies/mL after 3 months of treatment were assigned to group A; the other ten patients, whose HBV DNA + RNA titers were ≥5.0 log copies/mL after 3 months of treatment, were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

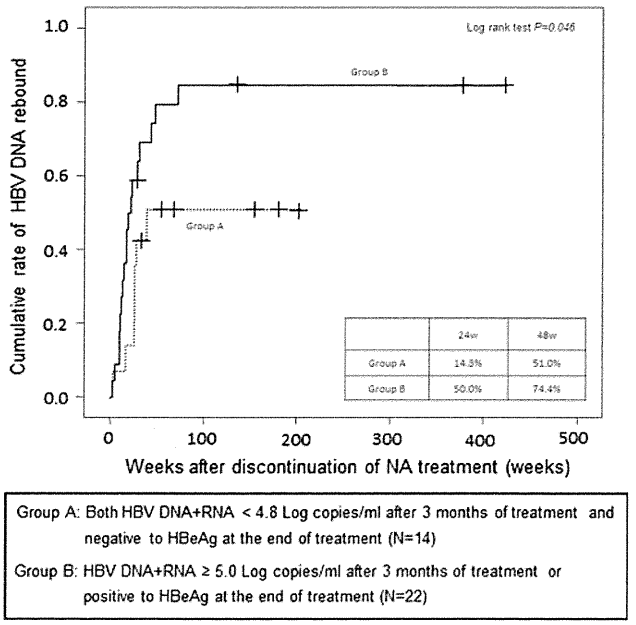

Fig. 6 Cumulative rate of ALT rebound after discontinuation of NA treatment by using combined criteria. The subjects were divided using combined criteria. Fourteen patients whose HBV DNA + RNA titers reached <5.0 log copies/mL after 3 months of treatment and who were HBeAg negative at the end of NA treatment were assigned to group A; the other 22 patients were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

DNA + RNA titer after 3 months of treatment was found to be significantly associated with HBV DNA rebound ($P = 0.043$, OR = 9.474; Table 2). Two other factors, HBV DNA titer after 3 months of treatment and HBsAg titer at the end of treatment, were marginally associated with HBV DNA rebound ($P = 0.074$, $P = 0.070$, respectively). After 3 months of NA treatment, HBV DNA titers decreased in both the HBV DNA relapse and non-relapse groups, but HBV DNA + RNA levels in the relapse group remained high. NA therapy suppressed the production of mature HBV particles in both groups, but in the HBV DNA relapse group, high HBV replication activity was likely maintained during the treatment, and immature HBV particles associated with HBV RNA genomes were continuously produced and accumulated in hepatocytes. After discontinuation of the treatment, these accumulated immature HBV particles may have been maturated and been released from the hepatocytes. Thus, rebound of HBV DNA titers occurred rapidly after the discontinuation of NA therapy.

Although the presence of HBeAg before treatment, HBV DNA and DNA + RNA titers after 3 months of treatment, and the presence of HBeAg, HBsAg titer, and HBV DNA + RNA titer at the end of treatment were all significantly associated with ALT rebound in the univariate analysis, only the presence of HBeAg at the end of treatment was identified as an independent predictive factor for ALT rebound following multivariate analysis (Table 4). HBeAg is commonly strongly associated with the activity of HBV replication, and HBV DNA levels are high in HBeAg-positive HBV carriers. Thus, HBe seroconversion usually indicates suppression of HBV activity, and the absence of HBeAg is thought to indicate the inactivation of HBV replication.

ALT rebound following the discontinuation of NA therapy was not observed in six of the 16 patients (37.5 %) who were HBeAg-positive at the end of treatment. After examining predictive factors for ALT rebound in these HBeAg-positive patients, only the HBV DNA + RNA titer after 3 months of treatment was identified as an independent predictive factor for ALT rebound in HBeAg-positive patients (Table 6). Although the presence of HBeAg indicates high activities of HBV replication and hepatitis, it is expected to be difficult to discontinue NA therapy without ALT rebound in these patients. However, these results indicate that HBV replication activities vary greatly among individuals and suggest that it might be possible to predict future replication activity based on HBV DNA + RNA titers after 3 months of treatment.

A limitation of this study is the small sample size; as such, selection bias might have affected the internal validity of the study. As it is not common to discontinue

NA therapy in Japan, we were only able to examine 36 subjects in our study. Because HBV-related markers such as HBsAg, HBcrAg, and HBV DNA + RNA titers varied widely among individuals, HBeAg an HBV DNA + RNA titers were only marginally associated with HBV DNA or ALT rebound after the discontinuation of NA therapy. In a previous study, Matsumoto et al. [34] analyzed predictive factors for the safe discontinuation of NA therapy in 126 clinical HBeAg-negative subjects from 12 clinical centers. These authors reported that HBsAg and HBcrAg titers at the end of treatment were predictive factors for the safe discontinuation of therapy. In our study, we also found that the absence of HBeAg at the end of treatment was important for the safe discontinuation of NA therapy, but we found no association between safety and HBsAg or HBcrAg titers. However, while HBsAg and HBcrAg are known to be associated with HBV replication activity, our results involving HBeAg and HBV DNA + RNA titers as important factors for safe discontinuation appear to be consistent.

In our study, the duration of NA therapy was quite short (mean duration was 36 weeks). Similar results might be observed if the NA therapy was extended, but it might be difficult to depress the potential of infected HBV replication with long-term NA therapy. HBsAg titers represent HBV replication in human hepatocytes, and it is difficult to decrease HBsAg levels by NA therapy. Thus, HBV DNA + RNA levels might be an important factor for predicting the HBV DNA or ALT rebounds.

As it may be difficult to discontinue therapy in patients with advanced liver fibrosis, our study subjects were selected based on liver spare capacities. As shown in Fig. 1, ALT rebound is likely to occur in most patients following the discontinuation of NA therapy, and severe hepatitis could occur in some patients. Thus, if the liver spare capacity were low, NA therapy would not be discontinued; the patients in this study were selected solely based on clinical aspects, which may have influenced our interpretation of the results.

In conclusion, HBV replication activity was found to be an important predictor of safe discontinuation of NA therapy. These findings suggest that monitoring of serum HBV DNA + RNA levels would be a useful method for predicting the re-activation of chronic hepatitis B following discontinuation of NA therapy.

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Conflict of interest None to declare.

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Research Paper

Efficacy of Lamivudine or Entecavir against Virological Rebound after Achieving HBV DNA Negativity in Chronic Hepatitis B Patients

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Abstract

Nucleos(t)ide analogues (NAs) lead to viral suppression and undetectable hepatitis B virus (HBV) DNA in some individuals infected with HBV, but the rate of virological rebound has been unknown in such patients. We examined the prevalence of virological rebound of HBV DNA among NA-treated patients with undetectable HBV DNA. We retrospectively analyzed 303 consecutive patients [158 entecavir (ETV)- and 145 lamivudine (LAM)-treated] who achieved HBV DNA negativity, defined as HBV DNA < 3.7 log IU/mL for at least 3 months. They were followed up and their features, including their rates of viral breakthrough, were determined. Viral rebound after HBV DNA negativity was not observed in the ETV-group. Viral rebound after HBV DNA negativity occurred in 38.7% of 62 HBe antigen-positive patients in the LAM-group. On multivariate analysis, age was an independent factor for viral breakthrough among these patients (P = 0.035). Viral rebound after HBV DNA negativity occurred in 29.1% of 79 HBe antigen-negative patients in the LAM-group. Differently from LAM, ETV could inhibit HBV replication once HBV DNA negativity was achieved. In contrast, LAM could not inhibit HBV replication even if HBV negativity was achieved in the early phase. Attention should be paid to these features in clinical practice.

Key words: Entecavir, HBeAg, HBV DNA, Lamivudine, Virological rebound.

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major health problem and one of the risk factors for the development of hepatocellular carcinoma (HCC) worldwide [1,2]. Chronic HBV infection has been linked epidemiologically to the development of HCC for more than 30 years [3]. To date, the mechanism of HBV-related hepatocarcinogenesis is not clear. Although effective vaccine exists for preventing HBV

infection [4], acute liver failure due to HBV or acute exacerbation of chronic hepatitis B is also a life-threatening disease [5,6].

Positivity for hepatitis B e antigen (HBeAg), which in serum indicates active viral replication in hepatocytes, is associated with an increased risk of HCC [7]. Chronic HBV carriers with high-titer viremia are also at increased risk for HCC [8]. The risk for cirrhosis and that for HCC increase significantly with increasing HBV DNA levels [9, 10]. Thus, it cannot be overstated that HBV DNA should be directly suppressed to prevent the development of HCC.

There are several nucleos(t)ide analogues (NAs) for the treatment of chronic hepatitis B [11]. Currently, the Japanese national health insurance system approves lamivudine (LAM) and entecavir (ETV) as first-line therapy for treatment-naïve patients with chronic hepatitis B, although some patients are treated with standard interferon-alfa or peginterferon-alfa-2a [6,12]. In general, LAM, the first oral NA available for the treatment of chronic hepatitis B, is associated with high rates of drug-resistance, with ~76% after 8 years of treatment [13,14]. ETV is found to be superior to LAM from the point of view that ETV is stronger than LAM and that resistance to ETV is rare, about 1.2% after 5 years of ETV treatment [14,15].

The aim of this study was to determine the efficacy and the rates of virological rebound after achieving HBV DNA negativity in the use of ETV or LAM in clinical practice. Our study showed that ETV could inhibit HBV replication if HBV DNA negativity had been achieved, but LAM was unable to inhibit HBV replication even if HBV negativity was achieved in the early phase.

MATERIALS AND METHODS

Patients and Study Design

This was a retrospective analysis comparing the rates of virological rebound in patients treated with ETV versus those in patients treated with LAM. A total of 303 patients were examined from Chiba University Hospital, Chiba, Japan, and 4 affiliated hospitals between the period of January 2000 and December 2011. NAs-naïve chronic hepatitis B patients daily receiving 0.5 mg of ETV (ETV group, N=158) or receiving 100 mg of LAM (LAM group, N=145) with undetectable HBV DNA (< 3.7 log IU/mL) for three months were enrolled. Some of the included patients had been previously reported [12, 16]. All patients had serum hepatitis B surface antigen (HBsAg) detectable for at least 6 months, regardless of their HBeAg status. They were negative for hepatitis C virus and human immunodeficiency virus antibodies. This study was approved by the Ethics Committee of Chiba University, Graduate School of Medicine (No. 977).

Definition of Virological Rebound of HBV

We defined virological rebound as $\geq$ 3.7 log IU/mL for at least 3 months after achieving undetectable HBV DNA.

Monitoring of HBV DNA, Serum Liver Function Tests and Hematological Tests

The primary outcome of this study was the virological rebound. Patients were followed up at least every 3 months to examine physical status and to monitor liver biochemistry and virology. All clinical laboratory tests including hematological data, biochemical data, and HBV serologies were performed at the Central Laboratory of Chiba University Hospital. HBsAg, HBeAg and anti-HBe antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [17]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al [18]. HBV DNA was measured by transcription-mediated amplification (TMA) assay, COBAS Amplicor HBV Monitor assay, or COBAS TaqMan (Roche Diagnostics, Branchburg, NJ, USA). The clinical efficacy of NAs was assessed as the proportion of patients achieving HBV DNA negativity, defined as an HBV DNA level of < 3.7 log IU/mL.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD). Differences were evaluated by Student's *t*-test, chi-square test, or Fisher's exact test. $P < 0.05$ was considered statistically significant. Variables with $P < 0.05$ at univariate analysis were retained for multivariate logistic-regression analysis. For all tests, two-sided P-values were calculated and the results were considered statistically significant at $P < 0.05$. Statistical analysis was performed using the Excel-statistics program for Windows, version 7 (SSRI, Tokyo, Japan).

RESULTS

A total 303 patients were recruited into either the ETV group (n = 158) or the LAM group (n = 145), with a follow-up period of 33.7 $\pm$ 11.3 months (28.6 $\pm$ 11.3 months or 39.3 $\pm$ 31.4 months, respectively). Baseline demographic and laboratory data are summarized in Table 1. There were no differences in age, gender, HBV DNA, alanine aminotransferase (ALT) levels, ultrasound findings/presence of cirrhosis, and periods from the initial administration of ETV or LAM to

undetectable HBV DNA, between the ETV and LAM groups, although the proportion of HBeAg-positive patients in the ETV group (55%) tended to be higher than that in the LAM group (44%).

Virological Rebound

The patient flow and outcome are summarized in Figure 1. We excluded 9 patients, whose HBeAg status at baseline was unknown, from this analysis. When comparing the baseline characteristics of patients according to HBeAg status, HBeAg-positive patients were younger, had higher ALT levels and HBV DNA levels, and less cirrhotic findings by ultrasound than HBeAg-negative patients (Table 2). The period from the initial administration of ETV or LAM to the determination of undetectable HBV DNA in the HBeAg-negative group tended to be shorter than that in the HBeAg-positive group (Table 2).

In the ETV group, none of the patients had virological rebound during the follow-up periods. In the LAM group, 24 and 23 patients of 62 HBeAg-positive and 79 HBeAg-negative patients at baseline, respectively, developed evidence of virological rebound. In the 24 HBeAg-positive patients at baseline with virological rebound, 9, 8, 3, 1, 2, and 1 had virological rebound at ≤ 1, $1 \sim \leq 2$, $2 \sim \leq 3$, $3 \sim \leq 4$, $4 \sim \leq 5$, and details unknown, respectively. In the 23 HBeAg-negative patients at baseline with virological rebound, 10, 8, 3, 0, 1, and 1 had virological rebound at ≤ 1, $1 \sim \leq 2$, $2 \sim \leq 3$, $3 \sim \leq 4$, $4 \sim \leq 5$ and details unknown, respectively. Baseline characteristics of patients treated with ETV or LAM according to HBeAg status are shown in Table 3. In the ETV group, the period from the initial administration of ETV to the determination of undetectable HBV DNA in the HBeAg-negative group was the same as that in the HBeAg-positive group (Table 3). In the LAM group, the period from the initial administration of LAM to undetectable HBV DNA in the HBeAg-negative group was shorter than that in the HBeAg-positive group (Table 3). In the HBeAg-positive patients, the period from the initial administration to undetectable HBV DNA in the ETV group was shorter than that in the LAM group (Table 3).

Predictors of Virological Rebound in Patients treated with LAM

To clarify the predictors of virological rebound in patients treated with LAM, we compared the pretreatment factors between patients with and without virological rebound according to HBeAg status (Table 4A & 4B). Univariative analysis showed that age, HBV DNA, ALT levels and the period from the initial administration of LAM to the determination of undetectable HBV DNA in HBeAg-positive patients contributed to the occurrence of virological rebound (Table 4A). Factors significantly associated with virological rebound in HBeAg-positive patients treated with LAM by univariate analysis were also analyzed by multivariate logistic regression analysis. Virological rebound was attained independently of age in HBeAg-positive patients treated with LAM (Table 4C). In HBeAg-negative patients, no significant factors contributing to virological rebound could be found (Table 4B).

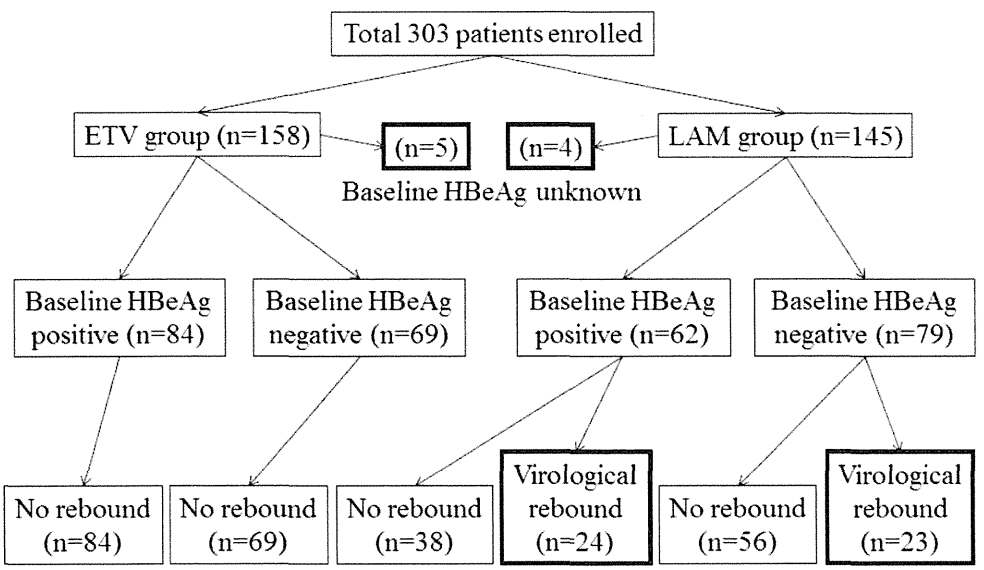

Figure 1. Study design and patient flow for both groups.

Table 1. Baseline characteristics of patients treated with entecavir (ETV) or lamivudine (LAM).

	Total	ETV group	LAM group	*P*-values
Number	303	158	145	
Age (years)	51 ± 12	51 ± 12	50 ± 12	*N.S.*
Gender (male)	205	101	104	*N.S.*
HBeAg (+)	146	84	62	*0.079*
HBV DNA (log IU/mL)	6.5 ± 1.5	6.6 ± 1.7	6.4 ± 1.3	*N.S.*
ALT (IU/L)	203 ± 280	187 ± 290	220 ± 266	*N.S.*
US: Cirrhosis (+)	113	56	57	*N.S.*
Periods to undetectable HBV DNA (months)	10.0 ± 18.2	8.5 ± 11.9	11.8 ± 23.3	*N.S.*

Data are expressed as mean ± SD. ETV group, patients receiving 0.5 mg of ETV daily; LAM group, patients receiving 100 mg of LAM daily; *P*-values, *P*-values between ETV and LAM groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 2. Baseline characteristics of patients according to HBeAg status.

HBeAg	Positive group	Negative group	*P*-values
Number	146	148	
Age (years)	46 ± 12	55 ± 11	*< 0.001*
Gender (male)	101	97	*N.S.*
HBV DNA (log IU/mL)	7.2 ± 1.1	5.8 ± 1.4	*< 0.001*
ALT (IU/L)	257 ± 332	156 ± 211	*0.002*
US: Cirrhosis (+)	41	70	*< 0.001*
Periods to undetectable HBV DNA (months)	11.0 ± 18.1	7.4 ± 14.4	*0.063*

Data are expressed as mean ± SD. *P*-values, *P*-values between HBeAg-positive and HBeAg-negative groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 3. Baseline characteristics of patients treated with entecavir (ETV) or lamivudine (LAM) according to HBeAg status.

	ETV group		LAM group	
HBeAg	Positive	Negative	Positive	Negative
Number	84	69	62	79
Age (years)	48 ± 12	56 ± 11*	44 ± 11 ##	54 ± 11**
Gender (male)	53	45	48	52**
HBV DNA (log IU/mL)	7.5 ± 1.1	5.7 ± 1.5*	6.9 ± 1.1$	5.9 ± 1.3**
ALT (IU/L)	219 ± 325	159 ± 246	309 ± 334	154 ± 174**
US: Cirrhosis (+)	25	29	16	41
Periods to undetectable HBV DNA (months)	8.3 ± 10.5	7.3 ± 11.0	15.0 ± 24.7$$	7.5 ± 16.9#

Data are expressed as mean ± SD. HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *$P < 0.001$, compared to HBeAg-positive of ETV group; **$P < 0.001$ and #$P = 0.034$, compared to HBeAg-positive of LAM group; ##$P = 0.041$, $$P = 0.001$ and $$$P = 0.027$, compared to HBeAg-positive of ETV group.

Table 4A. Predictors of virological rebound in patients treated with lamivudine (LAM). (A) Comparison of HBeAg-positive patients with or without virological rebound by univariate analysis.

Virological rebound	No	Yes	*P-values*
Number	38	23	
Age (years)	42 ± 11	49 ± 11	*0.019*
Gender (male)	30	17	*N.S.*
HBV DNA (log IU/mL)	6.9 ± 1.2	6.8 ± 0.9	*N.S.*
ALT (IU/L)	379 ± 377	196 ± 205	*0.037*
US: Cirrhosis (+)	7	9	*N.S.*
Periods to undetectable HBV DNA (months)	20.6 ± 29.1	4.1 ± 3.1	0.009

Data are expressed as mean ± SD. *P*-values, *P*-values between patients with or without virological rebound groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 4B. (B) Comparison of HBeAg-negative patients with or without virological rebound by univariate analysis.

Virological rebound	No	Yes	*P-values*
Number	56	22	
Age (years)	54 ± 11	54 ± 10	*N.S.*
Gender (male)	40	12	*N.S.*
HBV DNA (log IU/mL)	5.9 ± 1.4	5.9 ± 1.0	*N.S.*
ALT (IU/L)	163 ± 179	137 ± 163	*N.S.*
US: Cirrhosis (+)	30	11	*N.S.*
Periods to undetectable HBV DNA (months)	7.3 ± 14.8	3.1 ± 2.1	*N.S.*

Data are expressed as mean ± SD. *P*-values, *P*-values between patients with or without virological rebound groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 4C. (C) Factor associated with virological rebound among HBeAg-positive patients treated with LAM by multivariate analysis.

Factor	Category	Odds ratio	95% CI	*P-value*
Age ≤ 44.5 (years)	(+/-)	0.222	0.0547-0.9023	0.0354

DISCUSSION

To date, there is not much data regarding virological rebound after achieving HBV DNA negativity in the use of ETV or LAM. A recent report supported the merit of the change from LAM to ETV [14]. This study concluded that prior optimal viral suppression with ETV did not confer any significant advantage for patients who switched to LAM.

The present study revealed that ETV could suppress HBV replication after achieving HBV DNA negativity, although additional longer follow-up studies will be needed. On the other hand, LAM could not suppress HBV replication even after achieving HBV DNA negativity (Figure 1), although most cases with virological rebound were observed within 2 years of the start of LAM medication. We could not check the emergence of YMDD motif mutations [19] in all of the cases because the present study was performed as part of regular clinical practice. Of 2 of the HBeAg-positive patients at baseline with virological rebound, one showed YVDD motif (50%). In 4 of the HBeAg-negative patients at baseline with virological rebound, one YVDD motif (25%) and three YIDD motifs (75%) were seen. Virological rebound may not mean the emergence of NA-resistance mutations [12].

We do not know the reason why virological rebound was attained independently of age in HBeAg-positive patients treated with LAM. HBeAg to anti-HBe antibody seroconversions were found in 20 and 11 patients with and without virological rebound, that is, the HBeAg to anti-HBe antibody seroconversion rates were similar in the two groups (data not shown), although the number of study patients seemed small in the present study. Further studies might be needed. In any event, it might be important to consider the LAM-to-ETV switch in HBeAg-positive patients treated with LAM, although some of our patients in the LAM group remained HBV-negative throughout the observation period.

In the present study, 95.3% (122 of 128), 82.3% (14 of 17) and 89.2% (25 of 28) had an adherence rate >90% [16] in ETV-treated, LAM-treated with virological rebound and LAM-treated patients without virological rebound, respectively. These results supported our previous study that viral breakthrough associated with poor adherence could be a more important issue in the treatment with especially stronger NAs, such as ETV [12,16], although we cannot ensure durable HBV negativity after NAs are discontinued. We and others reported that HBeAg could impair both innate and adaptive immune responses to promote chronic HBV infection [16,20,21]. Of interest, the virological rebound with the use of LAM seemed unrelated to the HBeAg status, suggesting that it was dependent on resistant mutation.

Recently, other effective antiviral therapies such as peginterferon [22,23] and tenofovir [24,25] were reported to be useful for the control of HBV infection. These drugs might also be candidates for treating virological rebound. Fung et al. [14] reported that prior optimal viral suppression with ETV did not confer any significant advantage for patients who switched to LAM. Our results also supported the previous studies that ETV was much more efficient than LAM [26-29]. In conclusion, ETV could inhibit HBV replication if HBV DNA negativity had been achieved. In contrast, LAM could not inhibit HBV replication even if HBV negativity was achieved in the early phase. Attention should be paid to these features in clinical

practice.

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CONFLICT OF INTEREST

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ABBREVIATIONS

ALT: alanine aminotransferase; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; LAM: lamivudine; NA: nucleos(t)ide analogue.

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Reactivation of Hepatitis B Virus in Patients With Undetectable HBsAg Undergoing Chemotherapy for Malignant Lymphoma or Multiple Myeloma

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Despite increasing reports of hepatitis B virus (HBV) reactivation in hematological malignancies, its incidence, and risk factors are still obscure. The aim of this study was to clarify the frequency and risk factors of HBV reactivation in hepatitis B surface antigen (HBsAg) undetectable patients with malignant lymphoma or multiple myeloma, during or after chemotherapy. A total of 109 patients with undetectable HBsAg undergoing chemotherapy for malignant lymphoma or multiple myeloma were enrolled in this study. Anti-hepatitis B surface (anti-HBs) and anti-hepatitis B core (anti-HBc) were checked before treatment, and HBV DNA in sera was quantified monthly during and after chemotherapy. Out of 109 patients, 42 (38.5%) had anti-HBs and 59 (54.1%) had anti-HBc. Among the 59 anti-HBc positive patients, four patients (4/59, 6.8%) showed HBV reactivation during 20.5 median follow-up months. In all four patients with HBV reactivation, peripheral lymphocyte counts before chemotherapy were lower than those without HBV reactivation ($P = 0.033$). HBV reactivation occurred during and after chemotherapy containing rituximab for non-Hodgkin lymphoma. Four patients, who had HBV reactivation, did not develop de novo hepatitis due to HBV reactivation and were able to undergo chemotherapy against malignant lymphoma as scheduled. Monitoring of HBV DNA in sera is useful for the early diagnosis of HBV reactivation, and preemptive therapy is an useful alternative to prevent hepatitis due to HBV reactivation. Patients must be monitored periodically for HBV-DNA levels during and after chemotherapy. ***J. Med. Virol.***

KEY WORDS: reactivation; hepatitis B virus; chemotherapy; lymphocyte

INTRODUCTION

Reactivation of the hepatitis B virus (HBV) is a well-recognized complication following systemic chemotherapy for hematological malignancies [Francisci et al., 2010; Yagci et al., 2010; Sugauchi et al., 2011]. HBV infection has a wide clinical spectrum. Therefore different serologic markers or combinations of markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection, is immune to HBV as a result of prior infection or vaccination, or is susceptible. During acute or chronic hepatitis B infection, hepatitis B surface antigen (HBsAg) can be detected in high levels in serum. The presence of hepatitis B surface antibody (anti-HBs) is generally interpreted as an indication of recovery and immunity from HBV infection. Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B. Total hepatitis B core antibody (anti-HBc) appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with HBV in an undefined time frame. Therefore, in the past, anti-HBc and/or anti-HBs positive patients

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without HBsAg was thought to be eradicated of HBV in the host after resolution of HBV infection. However, recently it has become known that a low level of virus replication continues in hepatocytes even after the end of the acute HBV infection [Yotsuyanagi et al., 1998]. The covalently closed circular DNA (cccDNA) persist for many years in the liver of patients, HBsAg and HBV DNA in the blood has fallen to an undetectable level. Immunosuppressive drugs interfere with the cellular and humoral arm of the immune system controlling hepatocellular HBV infection. In healthy patients, host immunity is able to control HBV in most of the cases. This changes in an immune impaired patient, whether it is due to immunosuppressive drugs or monoclonal antibodies like rituximab. The humoral arm is profoundly affected by rituximab which depletes the body's antibody producing B cells. Other immunosuppressive drugs also impair the innate immune response. Therefore, reactivation of HBV following systemic chemotherapy can develop not only in HBsAg positive patients with a sustained HBV infection, but also in HBsAg undetectable patients with a past history of HBV infection [Wu et al., 2009; Cheung et al., 2010; Matsue et al., 2010; Wursthorn et al., 2010]. Rituximab and corticosteroids are especially well known to cause reactivation of HBV in patients without HBsAg in sera [Kusumoto et al., 2011]. Rituximab is a human monoclonal antibody derived from chimeric mice that inhibits an immune response by attacking CD20 positive B cells [Hiddemann et al., 2005]. Corticosteroids block cytokine synthesis and act as immune-suppressing drugs [Auphan et al., 1995] and, in addition, stimulate HBV DNA, mRNA, and protein production in a stable expression system [Tur-Kaspa and Laub, 1990]. These agents are used for the treatment of malignant lymphoma or other hematological malignancies. Multiple myeloma is recognized as a disease with a risk of HBV reactivation, due to its treatment regime with a high dose of corticosteroids [Yoshida et al., 2010]. To date, there has been increasing reports of HBV reactivation in patients treated with chemo/immunosuppressive therapy including the agents noted above [Hui et al., 2006; Matsubara et al., 2009; Shinkai et al., 2010]. However, the incidence or risk factors of HBV reactivation remain unclear because only a few prospective cohorts have presented for this new clinical entity. This study aimed to assess the incidence and risk factors of HBV reactivation, and analyzing the clinical course of HBV reactivation that occurred in the patients with malignant lymphoma or multiple myeloma during and after treatment.

PATIENTS AND METHODS

Study Patients

Consecutive patients with undetectable HBsAg who received chemotherapy for malignant lymphoma or multiple myeloma from January 2007 to October 2010 were included in this study. After admission, all patients underwent a physical examination and blood chemistry analysis. The study patients consisted of 109 patients (60 male, 55%; 49 female, 45%). The median age was 68-years-old, with a range of 22–91 years. Ninety-six patients (88.1%) had malignant lymphoma and 13 (11.9%) had multiple myeloma. Diagnosis of subtypes in malignant lymphoma included Diffuse large B-cell lymphoma (n = 54, 56.3%), Follicular lymphoma (n = 22, 22.9%), Marginal zone B-cell lymphoma (n = 7, 7.3%), Mantle cell lymphoma (n = 2, 2.1%), Burkitt lymphoma (n = 2, 2.1%), Intravascular large B-cell lymphoma (n = 1, 1.0%), Lymphoplasmacytoid lymphoma (n = 1, 1.0%), Peripheral T-Cell lymphoma (n = 1, 1.0%), Angioimmunoblastic T-Cell Lymphoma (n = 1,1.0%), and Hodgkin lymphoma (n = 5, 5.2%).

Determination of HBV Serological Markers and HBV DNA Quantification

On admission, all patients were screened for HBsAg in sera using a commercially available kit (Architect, Abbott Japan, Tokyo, Japan). Patients with undetectable HBsAg were enrolled in this study. Before treatment of hematological malignancies, patients were tested for anti-HBc, anti-HBs and blood parameters, and were then followed up by monthly monitoring of HBV DNA loads in sera or plasma and blood parameters. All serial sera were stored at −40° C. HBV DNA levels were quantified using Amplicor (range from below 2.6 to 7.6 log copies/ml; Roche Diagnostics, Tokyo, Japan) up to December 2007 and real-time TaqMan PCR (range from below 1.8 to 8.8 log copies/ml; Roche Diagnostics) since then. HBsAg with a highly sensitive chemiluminescent enzyme immunoassay (CLEIA) [Shinkai et al., 2010] was checked in stored sera sampled from patients with HBV reactivation retrospectively.

Definition of HBV Related Hepatitis and HBV Reactivation

Hepatitis was defined as a serum level of alanine aminotransferase (ALT) threefold higher than the normal upper limit of two consecutive determinations, 5 days apart, in the absence of the clinical and laboratory features of acute hepatitis A, hepatitis C, hepatitis E, or other systematic infections [Matsue et al., 2010]. The definition of HBV reactivation was the detection of HBV DNA in sera, including when the DNA load was not quantifiable but a PCR signal was detectable.

Statistical Analysis

In order to assess the risk factors of HBV reactivation, Fisher's exact test was applied for categorical variables, and Mann–Whitney's *U*-test was used for numerical variables. Receiver operating characteristic (ROC) curve was constructed to evaluate the

TABLE I. Clinical Characteristics of HBsAg Undetectable Patients Undergoing Chemotherapy for ML or Multiple Myeloma

Sex (M/F)	60/49
Age of years, median (range)	67.9 (22–91)
Follow-up period, median (range)	20.5 months (1.0–58.5)
Anti-HBc positive	59
Anti-HBs positive	42
Diagnosis	
Multiple myeloma	13
Diffuse large B cell lymphoma	54
Follicular lymphoma	22
Marginal zone B cell lymphoma	7
Burkitt lymphoma	2
T-cell lymphoma	2
Hodgkin lymphoma	5
Others	4
No. of rituximab administration	81
No. of glucocorticoids administration	108

diagnostic ability of HBV reactivation using a measured variable. A *P*-value less than 0.05 was considered significant. The best cutoff was defined as the point on the ROC curve closest to the upper left corner. All statistical analyses were performed using SPSS18 (IBM).

RESULTS

Patient Characteristics

The background characteristics of patients are shown in Table I. Out of 109 patients, 59 (54.1%) had anti-HBc, 42 (38.5%) had anti-HBs, and 47 (43.1%) had neither. Thirty-nine (35.7%) had both anti-HBc and anti-HBs. The number of patients with multiple myeloma were 13, and 96 patients had malignant lymphoma. Of all patients with malignant lymphoma, Hodgkin lymphoma was diagnosed in 5 patients, and non-Hodgkin lymphoma was confirmed in 91 patients. Diffuse large B-cell lymphoma was the dominant subtype of lymphoma. Rituximab was administered in 81 patients and glucocorticoids were used in 108 patients. None were treated by autologous peripheral blood stem cell transplantation or allogenic stem cell transplantation.

Consequences of HBV Serology

Among the 109 patients with undetectable HBsAg at the follow-up period of 20.5 median months (1.0–58.5), 4 (3.7%) showed the emergence of HBV DNA in sera, and were therefore diagnosed as HBV reactivation. They had never received a blood transfusion. The background characteristics and clinical features in patients with HBV reactivation are shown in Table II. None of the 50 patients without anti-HBc revealed HBV reactivation. In contrast, out of the 59 anti-HBc positive patients, 4 (6.8%) became positive for HBV DNA in sera. Among 20 anti-HBc positive and anti-HBs negative patients, 3 (15.0%) patients developed HBV reactivation, and only 1 of the 39 (2.6%) positive for both had an emergence of HBV DNA in sera. Sufficient anti-HBs antibodies in sera among HBV-resolved patients might reduce the incidence of HBV reactivation.

All four patients who developed HBV reactivation had lymphoma and were treated with rituxmab and glucocorticoids containing chemotherapy (Table II).

TABLE II. Clinical Characteristics of HBV Reactivation Patients

	Case 1	Case 2	Case 3	Case 4
Age/sex	75/F	70/M	66/M	83/F
Diagnosis	DLBCL	DLBCL	FL	BL
Stage[a]	IIIB	IIIB	IIA	IVB
Anti-HBc/HBs	+/−	+/−	+/+	+/−
Treatment	R-CHOP like	R-CHOP like	R-CHOP	R-MTX/CPM/VCR/ ADM/DEXA/ETP
Period from initiation of treatment (days)	42	46	398	148
Period from last rituximab (days)	3	26	159	23
Frequency of rituximab administration	5	1	12	8
During or after treatment	During treatment	During treatment	After treatment	During treatment
HBV DNA on the reactivation point (log copy/ml)	3.6	<1.8+[b]	3.6	<1.8+[b]
Peak HBV DNA (log copy/ml)	7.6	2.7	3.6	<1.8+[b]
HBV genotype	C	Ba	Not detected	Not detected
HBV pre-core	Wild	Wild	Not detected	Not detected
HBV core promoter	Wild	Mutant	Not detected	Not detected
Antiviral treatment	+	+	−	−
Outcome	Alive	Alive	Alive	Alive

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; BL, Burkitt lymphoma; R-CHOP, rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone; R-CHOP, rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone; R-MTX/CPM/VCR/ADM/DEXA/ETP, rituximab, methotrexate, cyclophosphamide, vincristine, adriamycin, dexamethasone, and etoposide; ALT, alanine aminotransferase.

[a]Ann Arbor staging.

[b]The DNA load was not quantifiable, but a PCR signal was detectable.

Reactivation of HBV occurred during the course of chemotherapy in three cases, and after maintenance therapy with rituximab in only one case (Fig. 1). Although there is no apparent correlation between HBV reactivation and complete blood count data prior to chemotherapy (data not shown), baseline counts of peripheral lymphocytes were associated with an incidence of HBV reactivation ($P = 0.033$). Nadir levels in peripheral lymphocytes for all subjects during treatment and baseline levels of immunoglobulin G were also assessed. However, none of the parameters were confirmed to be associated with the incidence of HBV reactivation, except for peripheral lymphocytes (Table III). The ROC analysis for the prediction of reactivation using lymphoid counts before treatment showed the area under the curve (AUC) to be 0.814, with the best cut-off to be 860/μl. In four cases with HBV reactivation, based on high sensitive HBsAg assay, HBsAg was examined from stored sera at the time of HBV reactivation, but none was detectable. The detailed clinical course are; (1) Case 1, initially negative for both HBsAg and anti-HBs, became positive for HBV DNA 42 days after the initiation of treatment. She had received chemotherapy using multiple agents such as rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone (R-CHOP like regimen). After an elevation in quantified HBV DNA in sera, the patient was treated with entecavir, at 1mg per day. HBV DNA was immediately undetectable without hepatitis. (2) Case 2 had detectable HBV-DNA 46 days after the initiation of an R-CHOP like regimen. One month after the transient emergence of HBV DNA in serum (signal positive, but not quantified), the HBV DNA became naturally undetectable. However, 6 weeks later, HBV DNA became detectable again, and after the confirmation of a sustained increase in the HBV DNA load, entecavir was continuously given. Thereafter, he showed a decrease in the HBV DNA load below the lower limit for detection without hepatitis, and ALT level became within normal range. (3) Case 3 had maintenance therapy with rituximab after the CHOP regimen. He showed an increase in the HBV DNA load at over 3 log copies/ml just once, 159 days after maintenance therapy with rituximab, but HBV DNA became undetectable again naturally. Although he had an anti-HBs titer of 601.2 mIU/ml before chemotherapy, the titer decreased to 500.8 mIU/ml at the showing of HBV reactivation. (4) Case 4 was not positive for quantified HBV DNA, but had a transient replication signal of HBV DNA in serum at day 148 by real time PCR. These two cases did not present with continuous viremia of HBV, and, as such, antiviral drugs were not administered. Although four cases showed HBV reactivation, they did not develop de novo hepatitis due to HBV reactivation and were able to undergo chemotherapy against malignant lymphoma as scheduled.

DISCUSSION

HBV reactivation is known as a significant complication of chemotherapy for hemodyscrasia [Francisci et al., 2010; Yagci et al., 2010; Sugauchi et al., 2011].

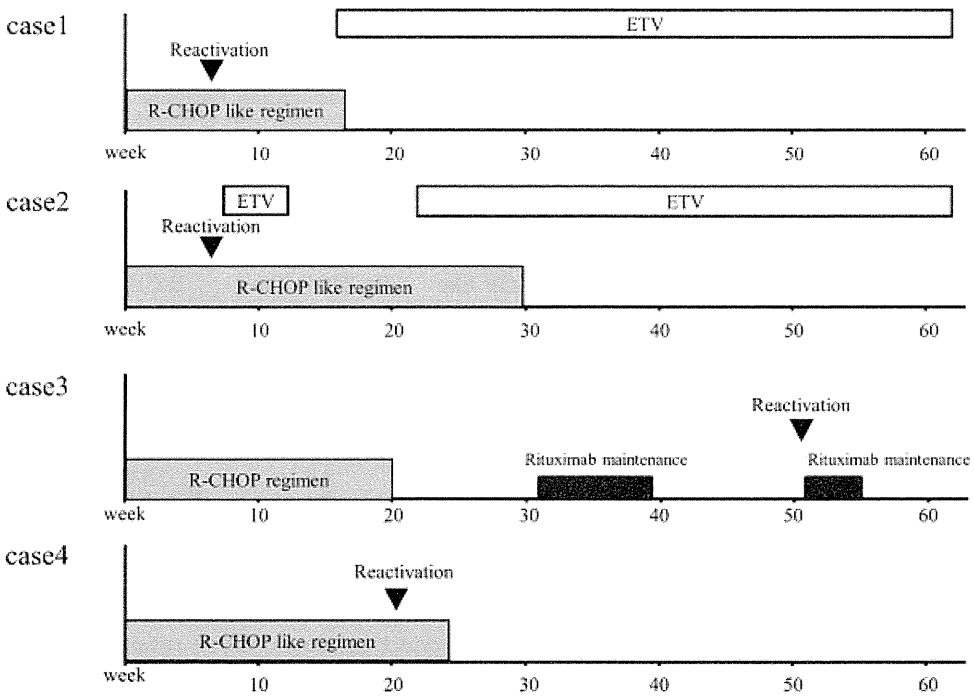

Fig. 1. Reactivation of HBV occurred during the course of chemotherapy in three cases, and after maintenance therapy with rituximab in only one case.

TABLE III. Comparison of Background Between Patients With or Without HBV Reactivation

			Reactivation proportion		
	Reactivation	Non-reactivation	Estimates(%)	95%CI(%)	*P*-value[a]
Number	4	105	3.7	(1.0–9.1)	
Sex(M/F)	2/2	58/47	3.3/4.1	(0.4–11.5)/(0.5–14.0)	1.000
Age[b], mean	73.5	67.7			0.420
Anti-HBc positive	4	55	6.8	(1.9–16.5)	0.123
Anti-HBs positive	1	41	2.4	(0.0–12.6)	1.000
Diagnosis					
Multiple myeloma	0	13	0.0	(0.0–24.7)	1.000
Diffuse large B cell lymphoma	2	52	3.8	(0.5–12.8)	1.000
Follicular lymphoma	1	21	4.8	(0.1–22.8)	1.000
Marginal zone B cell lymphoma	0	7	0.0	(0.0–41.0)	1.000
Burkitt lymphoma	1	1	50.0	(1.3–98.7)	0.072
T-cell lymphoma	0	2	0.0	(0.0–84.2)	1.000
Hodgkin lymphoma	0	5	0.0	(0.0–52.2)	1.000
Others	0	4	0.0	(0.0–60.2)	1.000
No. of rituximab administration	4	84	4.5	(1.3–11.2)	1.000
No. of glucocorticoid administration	4	104	3.7	(1.0–9.2)	1.000
Lymphocyte before chemotherapy median[b] (/μl) (range)	776 (460–1368)	1363 (274–10156)			0.033
Nadir lymphocyte during and after chemotherapy median[b] (/μl) (range)	133 (8–217)	247 (0–1729)			0.130
Immunoglobulin G before chemotherapy median[b] (mg/dl) (range)	1237 (1103–1479)	1421 (82–9085)			0.733

CI, confidence interval.
[a]Fisher's exact test.
[b]Medians and ranges are presented, compared by Mann–Whitney test.

Recently, the risk for development of HBV reactivation after chemotherapy in HBsAg undetectable patients has been reported [Wu et al., 2009; Cheung et al., 2010; Matsue et al., 2010]. Hui et al. [2006] described that 6 of 49 patients with undetectable HBsAg with malignant lymphoma receiving rituxmab plus corticosteroid chemotherapy developed new onset hepatitis B, and the risk factor was rituximab plus corticosteroid administration. Yeo et al. [2009] noted that 5 of 21 HBsAg undetectable, anti-HBc positive patients with diffuse large B-cell lymphoma who were treated with rituximab combination chemotherapy had reactivated HBV, and the risk factors were male, anti-HBs negative, and rituximab combination chemotherapy. It was recently reported that HBV reactivation had occurred in HBsAg undetectable multiple myeloma patients who underwent chemotherapy; 1 of 61 HBsAg undetectable multiple myeloma patients had reactivated HBV following chemotherapy [Yoshida et al., 2010]. However, additional prospective study would be required to know the precise frequency and risk factors for HBV reactivation among patients treated for malignant lymphoma or multiple myeloma. In addition, HBV reactivation was reported to be associated with the presence of anti-HBc and anti-HBs in baseline sera [Hui et al., 2006; Yeo et al., 2009], but the other factors associated with HBV reactivation have not yet been described.

In this study, all four cases with HBV reactivation were positive for anti-HBc before chemotherapy, and three of them were negative for anti-HBs (Table III). However, because of the limitation of size of samples in this study, this study could not evaluate the significance of anti-HBc for HBV reactivation ($P = 0.06$). This might be one of the key results of this study, but it could also be a chance finding. Therefore, serological markers including the titrations of anti-HBc and anti-HBs should be analyzed for the purpose of determining their relationship with HBV reactivation in larger scaled studies.

Out of four patients with HBV reactivation, two patients were treated with entecavir because they showed a persistent increase in the HBV DNA load. In contrast, in the remaining two patients, one patient showed a temporary replication signal of HBV DNA by real-time PCR, and another patient revealed a slight increase in the DNA load during a close follow-up. As HBV DNA of the latter two cases immediately became undetectable by real-time PCR, they were not given antiviral drugs. None of the four patients with HBV reactivation presented de novo hepatitis due to HBV reactivation. All cases were able to receive chemotherapy for underlying diseases as scheduled initially. Although there has been no proposal for the optimal time point for initiation of an anti-HBV treatment for this disease setting, preemptive therapy should be started immediately in patients with sustained viral replication quantified by real-time PCR.

In the present study, periodical quantitation of HBV DNA was useful for monitoring active

replication of HBV in patients receiving chemotherapy. HBsAg was also measured in serial sera in all cases of HBV reactivation, using novel CLEIA which was reported to be highly sensitive. However, HBsAg was not detected in any serum obtained from patients with HBV reactivation, indicating insufficient sensitivity of the assay for detecting HBV reactivation. As shown in the Japanese guidelines [Hirohito Tsubouchi et al., 2009], at present, routine measurement of HBV DNA levels would be preferable to an assay for HBsAg for the purpose of the early diagnosis of HBV reactivation.

Additionally, baseline lymphocyte counts in patients who had HBV reactivation were significantly less than those in patients who did not, although there was no difference in the nadir level of peripheral lymphocytes between patients who developed HBV reactivation and those who did not during or after chemotherapy. Based on these results, it is possible that lower levels of baseline peripheral lymphocytes might have a correlation with the occurrence of reactivated HBV in patients with malignant lymphoma or multiple myeloma. Although there is no similar data, further clinical studies are needed to evaluate the association between HBV reactivation and differential count of lymphocytes. In hepatocytes of chronic hepatitis B patients, cellular and humoral immunity could be associated with viral clearance, especially cytotoxic T lymphocytes (CTL) and natural killer (NK) cells which have roles to suppress proliferation of HBV. Gu et al. [2009] showed that serum HBV DNA levels in chronic hepatitis B patients were correlated to the frequency of HBV-specific CTL. Li et al. [2011] also reported that patients with acute hepatitis B possess a higher frequency of HBV-specific CTL than chronic hepatitis B patients. These reports may indicate that the HBV-specific CTL would be associated with suppression of HBV proliferation. This study could not evaluate differential counts of lymphocytes, and functional analyses of CTL. Further studies with CTL would provide a better understanding of the mechanism of this condition.

In conclusion, among the 59 anti-HBc positive patients with malignant lymphoma or multiple myeloma, four patients (6.8%) showed HBV reactivation during and after chemotherapy. HBV reactivation did not occur among patients without anti-HBc in this study. Monitoring of HBV DNA in sera is useful for the early diagnosis of HBV reactivation, and preemptive therapy is a useful alternative to prevent hepatitis due to HBV reactivation. Patients must be monitored periodically for HBV-DNA levels during and after chemotherapy.

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LETTER TO THE EDITOR

Serum HBV RNA as a possible marker of HBV replication in the liver during nucleot(s)ide analogue therapy

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We read with interest the article by Tsuge et al. [1] published in the recent issue of the Journal of Gastroenterology. Treatment with nucleot(s)ide analogue (NUC) strongly suppresses the replication of hepatitis B virus (HBV) leading to a high rate of serum HBV DNA negativity. However, the incidence of relapse after the cessation of NUCs is high. Criterion for safe discontinuation of NUC therapy after long term therapy is not established to date. In HBe antigen positive patients, seroconversion, HBV DNA negativity and consolidation therapy of >6 months may be a consensus criteria but 30–50 % of patients fulfilling this criteria experience a relapse. In HBe antigen negative patients, NUC therapy is generally recommended until HBs antigen becomes undetected. Tsuge et al. [1] measured serum HBV RNA plus DNA by real time PCR and showed that the serum HBV DNA + RNA titer following 3 months of NUC treatment was a significant predictor of early (within 24 weeks) HBV DNA rebound after discontinuation of NUC. The serum HBV DNA + RNA titer was also associated with ALT rebound in HBe antigen positive patients. The results of the study by Tsuge et al. indicate that serum HBV DNA + RNA titer may serve as predictor of relapse after discontinuation of NUC.

The high rate of relapse after discontinuation of NUC is due to the persistence of HBV replication in the liver even during the NUC therapy. The replicative intermediate form of HBV, covalently closed circular DNA (cccDNA), may not be eliminated by NUC therapy and serves as a template for viral pre-genomic messenger RNA [2]. This concept was proved by a study showing that quantification of intrahepatic HBV cccDNA had a high accuracy of predicting sustained virological response after NUC discontinuation [3]. Still, we need a non-invasive and clinically usable marker for the assessment of HBV replication in the liver during NUC therapy. The measurement of HBV core related antigen may be an alternative [4]. The rationale of measuring HBV RNA in serum was that immature HBV particles including HBV RNA are released from hepatocytes during NUC treatment under the circumstances that pre-genomic HBV RNA are transcribed from HBV cccDNA, packaged into HBV core particles, but not reverse transcribed into plus-strand HBV DNA due to strong interference by NUC, and the excessive amounts of these immature particles are accumulated in hepatocytes [5, 6]. Tsuge et al. showed that serum HBV DNA + RNA titer following 3 months of NUC treatment was significantly lower in patients with no rebound of HBV DNA. By using a cut-off value of 4.8 log copies/mL, the cumulative incidence of HBV DNA rebound was significantly lower in patients with serum HBV DNA + RNA titer < 4.8 log at 3 months of NUC treatment. The same groups previously showed that HBV RNA levels at 3 months of lamivudine treatment were predictor of early emergence of resistant mutations [7]. Taken together, serum HBV DNA + RNA titer may be linked to the level of HBV replication in the liver during NUC therapy. Monitoring of serum HBV DNA + RNA response may be utilized in various decision makings in treatment of HBV patients with NUC therapy.

Based on these important findings, several questions may remain for future elucidation. Commercially available transcription-mediated amplification and hybridization assay

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(TMA) detects both HBV DNA and RNA. We do recognize that detection sensitivity of this assay is not sensitive but could this assay be used in alternative to real time PCR? In the present study, duration of therapy was 36 weeks in average. The question is whether serum HBV DNA + RNA decrease further by a longer duration of therapy and whether monitoring of serum HBV DNA + RNA (at the end of treatment) serve as a predictor of safe discontinuation after long term NUC therapy. Various protocols of sequential interferon therapy starting with NUC are reported in an attempt to enhance the antiviral activity or to achieve drug-free status [8]. However, their outcome varies considerably and negative HBe antigen at the start of interferon is the only predictor of response [9]. Since 26 out of 36 patients in the study by Tsuge et al. received sequential interferon therapy, serum HBV DNA + RNA titer may be an alternative predictor of favorable response to sequential interferon therapy. Further investigation may be necessary to solve these issues but readers of the journal may be interested if comments can be made by the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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