MAJOR ARTICLE

Monitoring of Hepatitis B Virus (HBV) DNA and Risk of HBV Reactivation in B-Cell Lymphoma: A Prospective Observational Study

Shigeru Kusumoto,[1,a] Yasuhito Tanaka,[2,a] Ritsuro Suzuki,[3] Takashi Watanabe,[4] Masanobu Nakata,[5] Hirotaka Takasaki,[6] Noriyasu Fukushima,[7] Takuya Fukushima,[8] Yukiyoshi Moriuchi,[9] Kuniaki Itoh,[10] Kisato Nosaka,[11] Ilseung Choi,[12] Masashi Sawa,[13] Rumiko Okamoto,[14] Hideki Tsujimura,[15] Toshiki Uchida,[16] Sachiko Suzuki,[17] Masataka Okamoto,[18] Tsutomu Takahashi,[19] Isamu Sugiura,[20] Yasushi Onishi,[21] Mika Kohri,[22] Shinichiro Yoshida,[23] Rika Sakai,[24] Minoru Kojima,[25] Hiroyuki Takahashi,[26] Akihiro Tomita,[27] Dai Maruyama,[4] Yoshiko Atsuta,[3] Eiji Tanaka,[28] Takayo Suzuki,[29] Tomohiro Kinoshita,[30] Michinori Ogura,[16] Masashi Mizokami,[31] and Ryuzo Ueda[32]

[1]Department of Hematology and Oncology, and [2]Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, [3]Department of Hematopoietic Stem Cell Transplantation Data Management and Biostatistics, Nagoya University Graduate School of Medicine, [4]Department of Hematology, National Cancer Center Hospital, Tokyo, [5]Department of Internal Medicine, Sapporo Hokuyu Hospital, [6]Department of Medical Oncology, Kanagawa Cancer Center, Yokohama, [7]Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Saga University, [8]Department of Hematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University, [9]Department of Hematology, Sasebo City General Hospital, [10]Divisions of Oncology and Hematology, National Cancer Center Hospital East, Kashiwa, [11]Department of Hematology and Infectious Diseases, Kumamoto University School of Medicine, [12]Division of Hematology, National Hospital Organization Kyushu Cancer Center, Fukuoka, [13]Department of Hematology and Oncology, Anjo Kosei Hospital, [14]Department of Chemotherapy, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, [15]Division of Hematology-Oncology, Chiba Cancer Center, [16]Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, [17]Department of Hematology, National Hospital Organization Hokkaido Cancer Center, Sapporo, [18]Department of Hematology and Medical Oncology, Fujita Health University School of Medicine, Toyoake, [19]Department of Oncology/Hematology, Shimane University Hospital, Izumo, [20]Division of Hematology and Oncology, Toyohashi Municipal Hospital, [21]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai, [22]Department of Hematology, International Medical Center, Saitama Medical University, Hidaka, [23]Department of Hematology, National Hospital Organization Nagasaki Medical Center, Ohmura, [24]Department of Hematology, Yokohama City University Medical Center, [25]Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, [26]Department of Internal Medicine and Clinical Immunology, Yokohama City University Graduate School of Medicine, [27]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, [28]Department of Medicine, Shinshu University School of Medicine, Matsumoto, [29]Department of Hematology and Oncology, Shiga Medical Center for Adults, Moriyama, [30]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, [31]The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, and [32]Department of Tumor Immunology, Aichi Medical University School of Medicine, Japan

Background. There is no standard management of reactivation of hepatitis B virus (HBV) infection in HBV-resolved patients without hepatitis B surface antigen (HBsAg), but with antibodies against hepatitis B core antigen and/or antibodies against HBsAg (anti-HBs).

Methods. We conducted a prospective observational study to evaluate the occurrence of HBV reactivation by serial monthly monitoring of HBV DNA and to establish preemptive therapy guided by this monitoring in B-cell non-Hodgkin lymphoma (B-NHL) treated with rituximab plus corticosteroid-containing chemotherapy (R-steroid-chemo). The primary endpoint was the incidence of HBV reactivation defined as quantifiable HBV DNA levels of ≥11 IU/mL.

Results. With a median HBV DNA follow-up of 562 days, HBV reactivation was observed in 21 of the 269 analyzed patients. The incidence of HBV reactivation at 1.5 years was 8.3% (95% confidence interval, 5.5–12.4). No hepatitis due to HBV reactivation was observed in patients who received antiviral treatment when HBV DNA levels

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[a]S. K. and Y. T. contributed equally to this work.

Correspondence: Masashi Mizokami, MD, PhD, The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1 Kounodai, Ichikawa 272-8516, Japan (mmizokami@hospk.ncgm.go.jp).

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were between 11 and 432 IU/mL. An anti-HBs titer of <10 mIU/mL and detectable HBV DNA remaining below the level of quantification at baseline were independent risk factors for HBV reactivation (hazard ratio, 20.6 and 56.2, respectively; $P < .001$). Even in 6 patients with a rapid increase of HBV due to mutations, the monthly HBV DNA monitoring was effective at preventing HBV-related hepatitis.

Conclusions. Monthly monitoring of HBV DNA is useful for preventing HBV reactivation–related hepatitis among B-NHL patients with resolved HBV infection following R-steroid-chemo (UMIN000001299).

Keywords. HBV DNA monitoring; HBV reactivation; resolved HBV infection; rituximab; preemptive antiviral therapy.

Reactivation of hepatitis B virus (HBV) infection has been reported to occur not only in hepatitis B surface antigen (HBsAg)–positive patients undergoing systemic chemotherapy [1–3] but also in a proportion of patients with resolved HBV infection who are negative for HBsAg but seropositive for antibodies against hepatitis B core antigen (anti-HBc) and/or seropositive for antibodies against hepatitis B surface antigen (anti-HBs) [1, 4–9]. HBV reactivation has also been reported as a potentially fatal complication in B-cell non-Hodgkin lymphoma (B-NHL) patients with resolved HBV infection who received anti-CD20 antibody, rituximab plus corticosteroid-containing chemotherapy (R-steroid-chemo) [5–9].

Because antiviral treatments after hepatitis onset are often insufficient to control HBV reactivation, prophylaxis with antiviral drugs is essential for preventing liver failure in HBsAg-positive patients [2, 3, 10, 11]. However, for patients with resolved HBV infection, no standard strategy has yet been established to prevent HBV reactivation [12–15]. The following 2 options have been considered: prophylaxis with antiviral drugs and preemptive therapy guided by serial HBV DNA monitoring, whereby the antiviral drug is given immediately when HBV DNA becomes detectable. Preemptive antiviral therapy may be a reasonable strategy; however, there is little evidence regarding the optimal interval and period of monitoring [7, 16–18].

Therefore, we conducted a multicenter prospective observational study to evaluate the risk of HBV reactivation by serial HBV DNA monitoring among B-NHL patients with resolved HBV infection during and just after R-steroid-chemo. We also wanted to obtain information to aid in establishing the preemptive antiviral therapy.

METHODS

Study Population and Design

The major eligibility criteria were previously untreated CD20-positive B-NHL in patients seronegative for HBsAg and seropositive for anti-HBc and/or anti-HBs including seropositivity only for anti-HBs. None of the patients had a history of vaccination for HBV because there is no universal vaccination program in Japan. All patients were scheduled to receive treatment with 6 to 8 cycles of R-steroid-chemo defined by the protocol (see Supplementary materials). The doses and schedules of R-steroid-chemo are listed (see Supplementary Table 1). The baseline HBV status was established upon enrollment based on the serological results for HBsAg, anti-HBc, and anti-HBs using various methods at each institute. The details of the inclusion and exclusion criteria are listed (see Supplementary Table 2). The review board of each participating institution approved the protocol. All patients gave written informed consent. For an ancillary study, additional written informed consent was also obtained before enrollment.

Plasma HBV DNA levels were determined using real-time polymerase chain reaction (PCR) assay after enrollment. If HBV DNA levels of <11 IU/mL were confirmed at baseline, the serial monitoring of HBV DNA (without prophylaxis of antiviral drugs) was performed, with assessments every 4 weeks after enrollment for 1.5 years. The measurement of HBV DNA was allowed to be delayed up to a maximum of 2 weeks, specifically every 6 weeks. A 1-year follow-up (from 1.5 years to 2.5 years after enrollment) was conducted.

HBV reactivation was predefined as HBV DNA levels of ≥11 IU/mL. Prompt antiviral treatment with an anti-HBV nucleoside analogue (entecavir, 0.5 mg/day) was highly recommended in patients with confirmed HBV reactivation. If a patient developed HBV reactivation or received salvage lymphoma treatment within 1.5 years of enrollment, the monitoring was extended for an additional year, for a total of 2.5 years.

Endpoints

The primary endpoint was the incidence of HBV reactivation by means of the serial HBV DNA monitoring. HBV DNA levels in plasma were measured independently by a central laboratory (SRL, Inc., Tokyo, Japan) that used an automated real-time TaqMan PCR assay. This assay was performed using the COBAS AmpliPrep/COBAS TaqMan HBV test (v1.0) [19] from August 2008 to September 2009; the revised version (v2.0) [20] was used starting in October 2009. The quantitative range of the assay was 11 to 1.1E + 08 IU/mL for v1.0 and 11 to 2.3E + 08 IU/mL for v2.0. Each investigator and the Center for Supporting Hematology-Oncology Trials (C-SHOT) Data Center were immediately informed of the results. The secondary endpoint was the incidence of hepatitis due to reactivation, which was predefined as exacerbation of hepatitis clinically with increased HBV DNA levels. Overall survival was defined

as the time from enrollment until death from any cause or until the date of the last follow-up. Adverse events were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0.

Ancillary Study

An ancillary study was conducted to identify risk factors for HBV reactivation. Additional 5-mL specimens were collected on the days of HBV DNA monitoring and stored at −70°C at Nagoya City University. The preserved specimens were centrally reanalyzed using the HBsAg, immunoglobulin (IgM)-anti-HBc, anti-HBc (IgM and IgG classes), and anti-HBs assays (ARCHITECT; Abbott Laboratories, North Chicago, Illinois). For the analysis of HBV sequences, nucleic acids were extracted from the preserved serum specimens (200 μL) and subjected to PCR to amplify HBV genomes within the short S region (nucleotides [nt] 427 to 607) and the basal core promoter (BCP)/precore (PC) regions (nt 1628 to 2047) followed by direct sequencing using the ABI Prism Big Dye v3.1 kit in an ABI 3100 DNA automated sequencer. HBV genotypes were determined by molecular evolutionary analysis [21].

Statistical Analysis

The probability of HBV reactivation was estimated using the Kaplan–Meier method, defined as the time from enrollment until HBV reactivation or until the date of the last HBV DNA measurement. A total of 292 eligible patients were required to estimate the probability of HBV reactivation within ± 2.5% of the point estimation in consideration of point estimation of 5% with exact binomial confidence limits. The accrual goal was set to 321 patients in expectation that 10% of patients would be deemed ineligible for the enrollment period of 2 years at the designing of this study protocol. Subsequent to the interim analysis, the steering committee members confirmed and certified the utility and safety of preemptive antiviral therapy guided by the serial HBV DNA monitoring and decided to extend the enrollment period by 1 year. Finally, 275 patients were enrolled for 3 years.

Multivariate analysis was performed using the Cox proportional hazard model to identify subsets of independent factors related to the risk of HBV reactivation. The factors with $P < .2$ by univariate analysis were included in the multivariate analysis. The serial change in anti-HBs titers at baseline was compared with those at the end of follow-up among the patients who were seropositive for anti-HBs at baseline. Among the patients with HBV reactivation, these anti-HBs titers were measured at baseline and at confirmed HBV reactivation. The 2-sided Fisher exact test was used for comparing categorical data, and the Mann–Whitney *U* test was used for comparing continuous variables. All statistical analyses were performed on a personal computer with SPSS (version 22.0) and Stata (version 12.0, Stata Corp) statistical software for Windows.

All case report forms were collected and managed at the C-SHOT Data Center. This trial was registered at the University Hospital Medical Information Network-Clinical Trial Registry System (http://www.umin.ac.jp/ctr/ as 000001299; 10 August 2011, date last accessed). This final analysis was performed using data fixed on 31 October 2013.

RESULTS

Patients

A total of 275 patients from 50 institutes were enrolled from September 2008 through July 2011. Six patients were excluded from the final analysis for the reasons given in Figure 1, resulting in 269 patients analyzed in detail. The baseline characteristics of these 269 patients are listed in Table 1. Baseline HBV status judged at each institute was categorized into 3 groups: anti-HBc positive and anti-HBs positive (n = 194), anti-HBc positive and anti-HBs negative (n = 48), and anti-HBc negative and anti-HBs positive (n = 26). One patient was anti-HBc positive but lacked anti-HBs measurement. Baseline HBV DNA below the level of quantification was detected in 6 patients.

Of the 269 patients analyzed, 236 (87.7%) received the planned 6 to 8 cycles of R-steroid-chemo. However, 29 patients (10.8%) discontinued lymphoma treatment due to adverse events in 11, disease progression in 8, and other reasons in 10. The remaining 4 patients (1.5%) did not receive any lymphoma treatment. Thirty-six patients received salvage chemotherapy for relapse or refractory to protocol treatment.

Assessment of HBV DNA Monitoring

A total of 223 patients (82.9%) completed the entire serial HBV DNA monitoring for 1.5 years, with median interval of 30 days (interquartile range [IQR], 28–35). Four patients (1.5%) continued the monitoring for more than 1.5 years. The remaining 42 patients (15.6%) discontinued the HBV DNA monitoring due to lymphoma progression in 15, for patient's convenience in 8, transfer to another hospital in 7, adverse events in 5, and other reasons in 7. The median HBV DNA follow-up time from the last cycle of R-steroid-chemo to the last measurement of HBV DNA levels was 436 days (IQR, 391–463) among the 269 analyzed patients.

No patients received antiviral prophylaxis, except for one who was given entecavir for 4 days because HBV DNA below the level of quantification was detected during the monitoring.

Endpoints and Characteristics of HBV Reactivation

Among the cohort of 269 patients (the median HBV DNA follow-up of 562 days; IQR, 552–580), HBV reactivation was observed in 21 patients per protocol. The incidence of HBV reactivation at 1.5 years was 8.3% (95% confidence interval [CI], 5.5–12.4; Figure 2). HBV reactivation was diagnosed at a median time of 97 days (IQR, 67–204) after enrollment. HBV reactivation developed

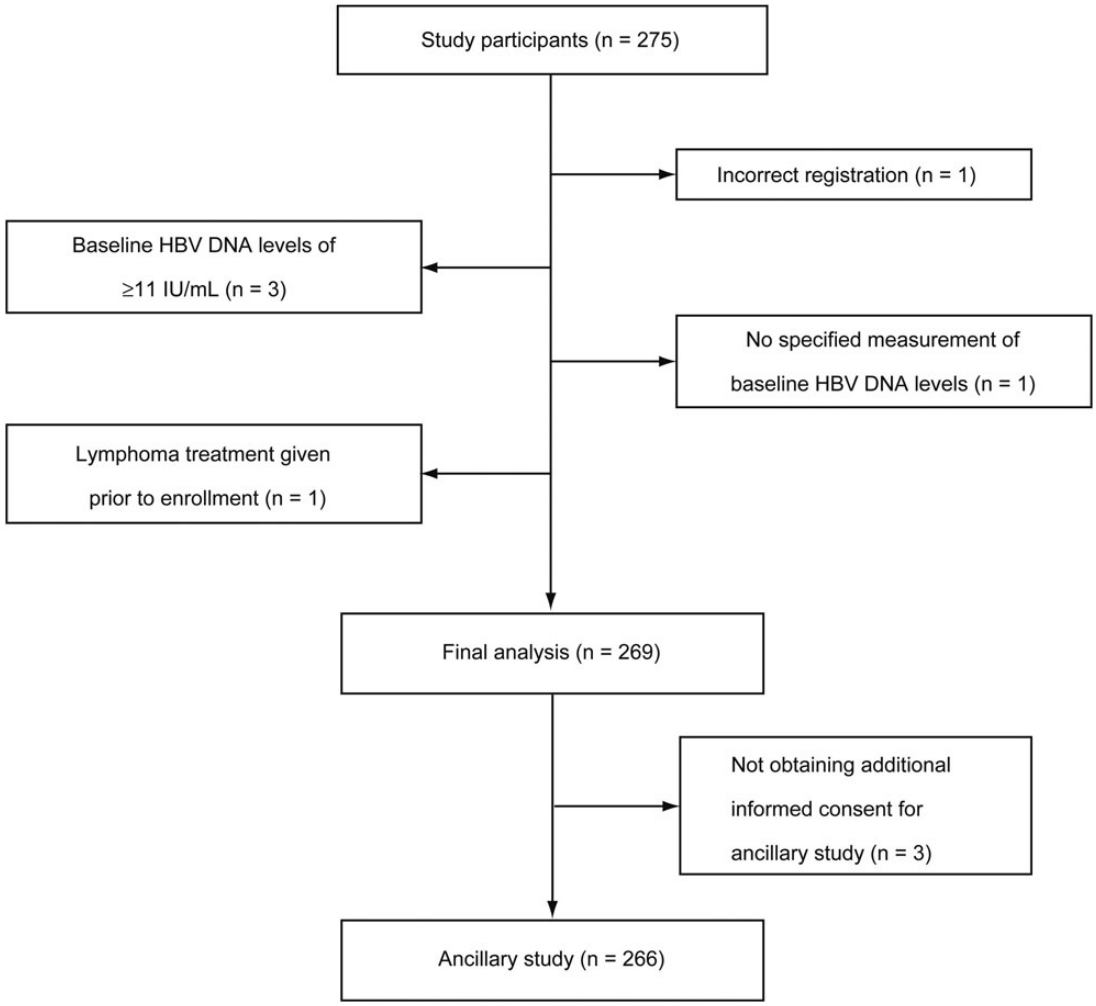

Figure 1. Study flow diagram from enrollment to the final analysis and the ancillary study. Of the 275 participants in this study, 6 were excluded from the final analysis for the following reasons: incorrect registration (n = 1), baseline hepatitis B virus (HBV) DNA levels ≥11 IU/mL (n = 3), no specified measurement of baseline HBV DNA levels (n = 1), and lymphoma treatment given prior to enrollment (n = 1). Additional informed consent for the ancillary study was obtained from 266 of the 269 patients in the final analysis.

during the first year in 19 patients and more than 1 year after enrollment in 2 patients. In addition, 1 patient showed HBV reactivation 2 years after enrollment by HBV DNA measurement not per protocol, although HBV DNA below the level of quantification had been detected at the last HBV DNA measurement per protocol (patient 22; Table 2).

In all 22 patients with HBV reactivation (17 male and 5 female; median age of 69.0 years; IQR, 65–75), the median HBV DNA level at reactivation was 27 IU/mL (IQR, 21–86). At baseline, detectable HBV DNA below the level of quantification was present in 5 patients, whereas it was undetectable in the remaining 17 patients (Table 2). Among the 13 patients with HBV reactivation during R-steroid-chemo, 12 completed chemotherapy and 1 discontinued lymphoma treatment early because of septic shock.

No hepatitis due to HBV reactivation occurred when antiviral treatment was initiated for patients with HBV DNA levels between 11 and 432 IU/mL (Table 2). HBV DNA levels fell below the detection limit at the first or second measurement after initiation of antiviral treatment, and no further reactivation was observed over the treatment period (the median antiviral treatment period was 647 days; IQR, 242–841, with a median HBV DNA follow-up of 928 days; IQR, 842–936). Fifteen patients were continuing with antiviral treatment at the last follow-up. Among the remaining 7 patients who discontinued antiviral treatment, the median HBV DNA follow-up time from the last antiviral treatment to the last measurement of HBV DNA was 323 days (IQR, 28–497). Two patients received blood transfusions during R-steroid-chemo prior to HBV reactivation; HBV DNA was undetectable by real-time PCR in all the transfused blood.

Table 1. Baseline Characteristics of the 269 Patients in the Final Analysis

Characteristic	No. (n = 269)	%
Sex		
Male/ Female	142/127	52.8/47.2
Age, y		
Median (interquartile range)	65 (59–70)	
Diagnosis (based on World Health Organization classification)		
Diffuse large B-cell lymphoma	165	61.3
Follicular lymphoma	87	32.3
MALT lymphoma	11	4.1
Mantle cell lymphoma	2	0.7
Intravascular large B-cell lymphoma	2	0.7
Nodal marginal zone B-cell lymphoma	1	0.4
Lymphoplasmacytic lymphoma	1	0.4
HBV status[a]		
Anti-HBc positive and anti-HBs positive	194	72.1
Anti-HBc positive and anti-HBs negative	48	17.8
Anti-HBc negative and anti-HBs positive	26	9.7
Anti-HBc positive and anti-HBs not available	1	0.4
HBV DNA levels[b]		
Undetectable	263	97.8
Detectable but not quantifiable	6	2.2
Performance status (Eastern Cooperative Oncology Group)		
0	184	68.4
1	71	26.4
2	14	5.2
International prognostic index		
Low risk	96	35.7
Low–intermediate risk	85	31.6
High–intermediate risk	58	21.6
High risk	30	11.2
Planned lymphoma treatment		
R-CHOP		
6–8 cycles	237	87.8
R-THP-COP		
6–8 cycles	23	8.5
R-CVP		
6–8 cycles	7	2.6
R-C-MOPP		
6–8 cycles	2	0.7

Abbreviations: anti-HBc, antibody against hepatitis B core antigen; anti-HBs, antibody against hepatitis B surface antigen; HBV, hepatitis B virus; MALT lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; R-C-MOPP, rituximab, cyclophosphamide, procarbazine, vincristine, and prednisolone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; R-CVP, rituximab, cyclophosphamide, vincristine, and prednisolone; R-THP-COP, rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone.

[a] Baseline HBV status was measured based on serological results of hepatitis B surface antigen (HBsAg), anti-HBc, and anti-HBs using various methods at each institute upon enrollment. Except for 1 patient, the serological results for HBsAg, anti-HBc, and anti-HBs were available. Excluding 6 patients from the total 275 participants, all the 269 patients in the final analysis were HBsAg negative and either anti-HBc positive or anti-HBs positive, although 1 patient was anti-HBc positive without anti-HBs measurement.

[b] HBV DNA levels in plasma were measured independently by a central laboratory (SRL, Inc.; Tokyo, Japan) that used an automated real-time TaqMan polymerase chain reaction assay after enrollment. If baseline HBV DNA levels <11 IU/mL were confirmed, the serial monitoring of HBV DNA (without prophylaxis of antiviral drugs) was performed.

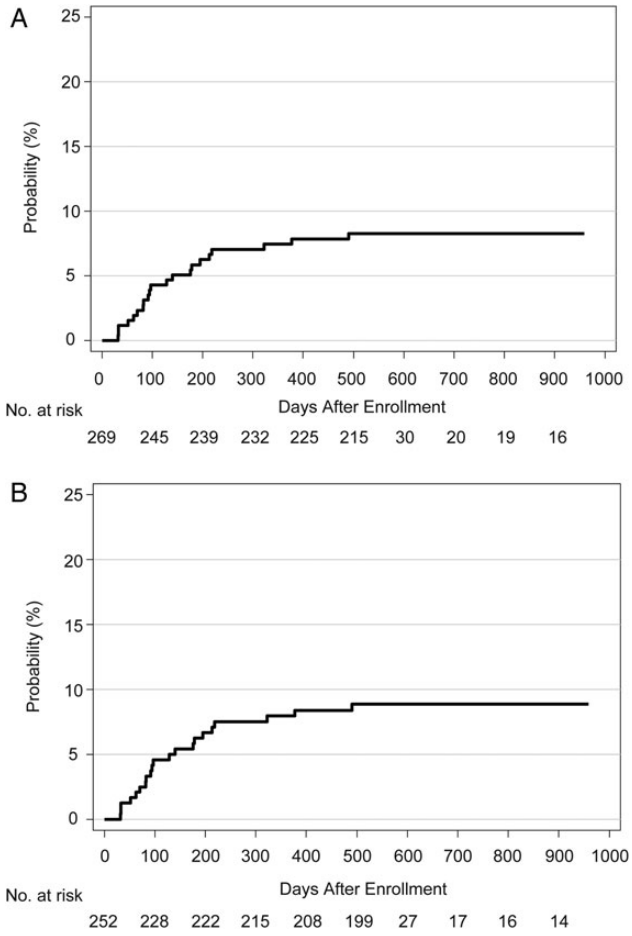

Figure 2. Kaplan–Meier analysis of hepatitis B virus (HBV) reactivation. *A*, The probability of HBV reactivation among 269 patients. *B*, The probability of HBV reactivation among 252 patients in the ancillary study.

Adverse Events and Overall Survival

The following severe adverse events were observed: 1 patient died from R-steroid-chemo–related infectious complication, 14 patients had grade 3 elevations of aspartate aminotransferase/alanine aminotransferase and 1 had grade 4 elevation (all transaminase elevations resolved without parallel increases in HBV DNA levels during follow-up), 1 patient had septic shock, and 1 had pulmonary embolism.

Twenty-two patients died from lymphoma progression and 7 died from other diseases or accident. With a median follow-up of 1163 days (IQR, 17–1831), the probability of overall survival was 94.9% (95% CI, 91.4–97.0) at 1.5 years and 91.2% (95% CI, 86.9–94.1) at 3 years after enrollment (see Supplementary Figure 1).

Risk Factors of HBV Reactivation and Mutations in HBV DNA

The ancillary study was performed for 266 patients. No HBV reactivation was seen in the 14 patients (5.3%) who were both anti-HBc negative and anti-HBs negative at baseline (see

Table 2. Baseline Characteristics and Courses of the 22 Patients With Hepatitis B Virus Reactivation

	At Baseline							After HBV Reactivation				
Patient	Age, y	Sex	Diagnosis	Anti-HBc[a] (s/co)	Anti-HBs [a] (mIU/mL)	HBV DNA[b] (IU/mL)	Lymphoma Treatment (cycles)[c]	Timing at Reactivation (from Enrollment)[d]	HBV DNA at Reactivation (peak) (IU/mL)	Hepatitis due to Reactivation[e]	Last HBV DNA Measurement (day)[f]	Outcome of Lymphoma[g]
1	68	F	DLBCL	pos	pos	ND	R-CHOP (8)	During cycle 5 (day 97)	27 (216)	No	937	Alive (CR1)
2	73	M	DLBCL	pos	pos	NQ	R-CHOP (8)	During cycle 2 (day 52)	86 (86)	No	931	Alive (CR1)
3	70	M	DLBCL	pos	neg	ND	R-THP-COP (8)	During cycle 4 (day 94)	108 (108)	No	548	Death from lymphoma
4	60	M	DLBCL	pos	pos	NQ	R-CHOP (6)	During cycle 3 (day 82)	34 (216)	No	266	Death from lymphoma
5	62	M	DLBCL	pos	neg	ND	R-CHOP (6)	3 mos after Cx (day 213)	108 (108)	No	920	Alive (CR1)
6	68	M	DLBCL	pos	neg	ND	R-CHOP (4)	4 mos after Cx (day 195)	14 (14)	No	930	Alive (CR1)
7	75	M	DLBCL	neg	pos	ND	R-THP-COP (6)	During cycle 3 (day 92)	27 (27)	No	792	Death from lymphoma
8	69	M	DLBCL	pos	pos	NQ	R-CHOP (8)	During cycle 6 (day 128)	27 (136)	No	929	Alive (CR1)
9	79	M	DLBCL	pos	neg	ND	R-CHOP (8)	0.5 mo after Cx (day 176)	22 (22)	No	927	Death from lymphoma
10	78	M	DLBCL	pos	neg	ND	R-THP-COP (6)	During cycle 5 (day 140)	22 (22)	No	920	Alive (CR1)
11	67	F	DLBCL	pos	neg	ND	R-CHOP (8)	During cycle 7 (day 178)	11 (11)	No	933	Alive (CR1)
12	55	F	FL	pos	pos	ND	R-CHOP (6)	7 mos after Cx (day 322)	17 (17)	No	945	Alive (CR2)
13	67	M	MALT	pos	pos	NQ	R-CHOP (4)	During cycle 2 (day 33)	68 (68)	No	109	Alive (CR1)
14	77	M	DLBCL	pos	pos	ND	R-CHOP (6)	13 mos after Cx (day 490)	14 (14)	No	945	Alive (CR1)
15	69	M	DLBCL	pos	pos	ND	R-CHOP (8)	2 mos after Cx (day 218)	27 (34)	No	953	Alive (CR1)
16	61	M	DLBCL	pos	pos	ND	R-CHOP (3) followed by salvage Cx	11 mos after Cx (day 377)	22 (22)	No	936	Alive (CR2)
17	63	M	DLBCL	pos	neg	NQ	R-CHOP (8)	During cycle 2 (day 32)	68 (272)	No	928	Alive (CR1)
18	76	F	DLBCL	pos	neg	ND	R-THP-COP (6)	During cycle 3 (day 63)	34 (34)	No	912	Alive (CR1)
19	70	F	FL	pos	pos	ND	R-CHOP (6)	During cycle 4 (day 83)	86 (86)	No	938	Alive (CR1)
20	66	M	DLBCL	pos	neg	ND	R-CHOP (8)	During cycle 2 (day 33)	343 (343)	No	845	Alive (CR1)
21	78	M	MCL	pos	neg	ND	R-THP-COP (2) followed by salvage Cx	1 mo after Cx (day 70)	11 (86)	No	833	Alive (CR2)
22[h]	70	M	DLBCL	pos	pos	ND	R-CHOP (6)	21 mos after Cx (day 754)	432 (432)	No	871	Alive (CR1)

Abbreviations: anti-HBc, antibody against hepatitis B core antigen; anti-HBs, antibody against hepatitis B surface antigen; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HBV, hepatitis B virus; MALT, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma); MCL, mantle cell lymphoma; neg, negative; pos, positive; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; R-THP-COP, rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisolone; CR1, first complete response; CR2, second complete response after salvage therapy.

[a] Serological results for anti-HBc and anti-HBs were reanalyzed using preserved specimens at baseline in the ancillary study.

[b] ND indicates that HBV DNA was not detected by real-time polymerase chain reaction assay for HBV DNA at baseline. NQ indicates that detectable but not quantifiable HBV DNA at baseline.

[c] Salvage Cx indicates rituximab-combined salvage chemotherapy for refractory lymphoma. HBV reactivation was observed in 2 patients (16, 21) after salvage chemotherapy; 1 patient (16) had received allogeneic hematopoietic stem cell transplantation prior to HBV reactivation.

[d] Cx indicates systemic chemotherapy defined by protocol.

[e] Hepatitis due to reactivation was predefined as exacerbation of hepatitis clinically, with increased HBV DNA levels as a specified secondary endpoint in the study protocol.

[f] Last HBV DNA measurement (day) was defined as the duration from enrollment to the last measurement of HBV DNA levels.

[g] Outcome of lymphoma was confirmed on 31 October 2013, and parentheses indicate the last assessment of lymphoma response to treatment at the same time.

[h] In patient 22, HBV reactivation was confirmed 21 months after 6 cycles of R-CHOP regimen (2 years after enrollment) by HBV DNA measurement, not per-protocol.

Supplementary Table 3). In the remaining 252 patients who were anti-HBc positive and/or anti-HBs positive, the incidence of HBV reactivation at 1.5 years was 8.9% (95% CI, 5.9–13.3; Figure 2).

HBV reactivation was significantly associated with being older ($P = .008$), male sex ($P = .02$), diffuse large B-cell lymphoma ($P = .04$), lower baseline titers of anti-HBs ($P < .001$), and HBV DNA detected below the level of quantification at baseline ($P < .001$; see Supplementary Table 4). Multivariate analysis showed that anti-HBs baseline titers of <10 mIU/mL or of 10–100 mIU/mL were independent risk factors for HBV reactivation, relative to a titer of ≥100 mIU/mL (adjusted hazard ratio [HR], 20.6; 95% CI, 3.9–105.8 and adjusted HR, 5.2; 95% CI, 1.0–25.7, respectively). HBV DNA detected below the level of quantification at baseline was also an independent risk factor (adjusted HR, 56.2; 95% CI, 15.3–207.0; Table 3).

Of the 22 patients with HBV reactivation, 21 were anti-HBc positive and 12 were anti-HBs positive at baseline. One patient

Table 3. Risk Factors for Hepatitis B Virus Reactivation

Variable	Crude HR[a]	95% CI	*P* Value	Adjusted HR[b]	95% CI	*P* Value
Age, per 10-y increase	2.2	1.2–4.0	.01	1.4	.7–2.9	.404
Sex						
Male	3.2	1.2–8.7	.022	1.8	.6–5.2	.289
Female	1.0 (ref.)			1.0 (ref.)		
Type of lymphoma						
DLBCL	3.5	1.2–10.3	.025	2.0	.6–6.5	.23
Non-DLBCL[c]	1.0 (ref.)			1.0 (ref.)		
Performance status (Eastern Cooperative Oncology Group)						
2	0.9	.1–6.9	.943	. . .	. . .	. . .
0 or 1	1.0 (ref.)					
International prognostic index						
High–intermediate risk or high risk	1.3	.5–3.0	.607	. . .	. . .	. . .
Low risk or low–intermediate risk	1.0 (ref.)					
Type of regimen						
R-CHOP	0.4	.2–1.2	.093	0.5	.1–1.6	.221
Other regimens[d]	1.0 (ref.)			1.0 (ref.)		
Anti-HBc[d]						
≥1.0 s/co	1.8	.2–13.3	.572	. . .	. . .	. . .
<1.0 s/co	1.0 (ref.)					
Anti-HBs[e]						
<10 mIU/mL	19.5	4.3–88.9	<.001	20.6	3.9–105.8	<.001
≥10 and <100 mIU/mL	6.9	1.5–31.4	.013	5.2	1.0–25.7	.044
≥100 mIU/mL	1.0 (ref.)			1.0 (ref.)		
Hepatitis B virus DNA below the level of quantification[f]						
Detected	34.5	12.2–97.3	<.001	56.2	15.3–207.0	<.001
Not detected	1.0 (ref.)			1.0 (ref.)		

Abbreviations: anti-HBc, antibody against hepatitis B core antigen; anti-HBs, antibody against hepatitis B surface antigen; CI, confidence interval; DLBCL, diffuse large B-cell lymphoma; HR, hazard ratio; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; ref., reference.

[a] Univariate analysis was used to identify candidate risk factors for hepatitis B virus (HBV) reactivation.

[b] Multivariate Cox proportional hazard analysis was used to determine the relative contribution of various factors to the risk of HBV reactivation. Risk factors were limited to those that were closely associated with HBV reactivation in the univariate analysis ($P < .2$).

[c] Non-DLBCL included the following types of lymphoma: follicular lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue, mantle cell lymphoma, lymphoplasmacytic lymphoma, nodal marginal zone B-cell lymphoma, and intravascular large B-cell lymphoma.

[d] Other regimens included the following types of systemic chemotherapy: rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisolone; rituximab, cyclophosphamide, vincristine, and prednisolone; and rituximab, cyclophosphamide, procarbazine, vincristine, and prednisolone.

[e] Serological results for anti-HBc and anti-HBs were analyzed using preserved specimens at baseline for the 249 patients. However, the specimens within 6 weeks from the baseline were used for the remaining 3 patients because baseline specimens were not obtained. Anti-HBc was defined as a dichotomous variable in which 1.0 s/co was used as the cutoff value. Anti-HBs was defined as a categorical variable and divided into 3 groups, in which 10 mIU/mL and 100 mIU/mL were used as the cutoff values.

[f] HBV DNA levels in plasma were measured independently by a central laboratory (SRL, Inc.; Tokyo, Japan) that used an automated real-time TaqMan polymerase chain reaction assay after enrollment. If baseline HBV DNA levels <11 IU/mL were confirmed, the serial monitoring of HBV DNA (without prophylaxis of antiviral drugs) was performed.

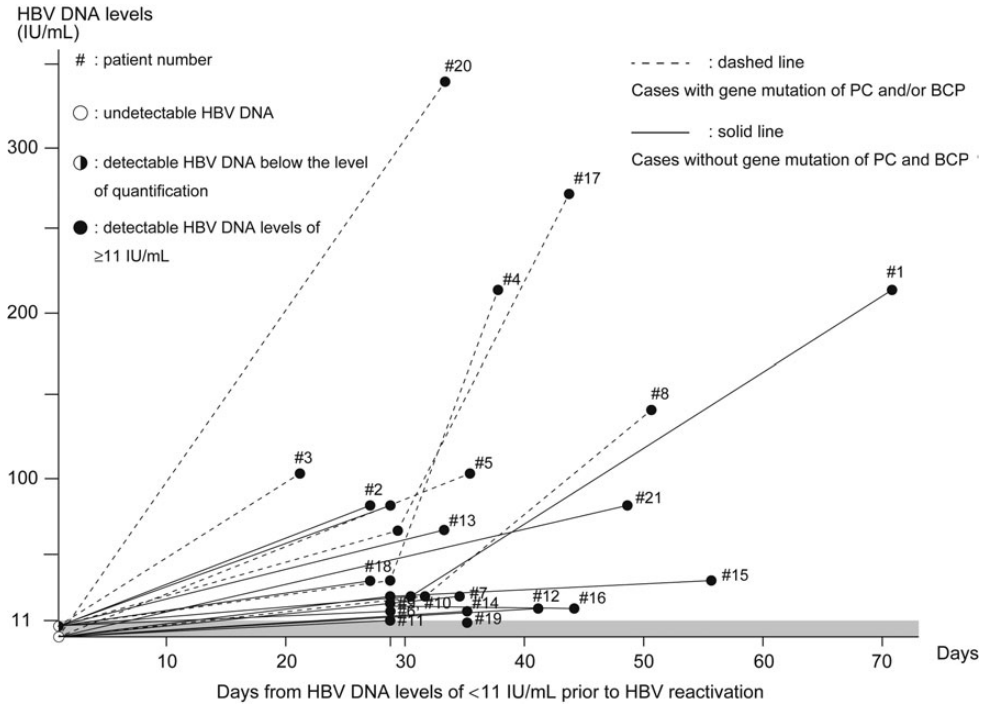

Figure 3. Kinetics of hepatitis B virus (HBV) DNA level from <11 IU/mL just prior to reactivation. The vertical axis shows the plasma HBV DNA levels determined by real-time polymerase chain reaction assay, and the horizontal axis shows the number of days from the time HBV DNA levels were <11 IU/mL just prior to reactivation to the time they reached ≥11 IU/mL ("HBV reactivation"). Open circles, undetectable HBV DNA during the serial monitoring; half-filled circles, detectable HBV DNA below the level of quantification; and filled circles, detectable HBV DNA levels ≥11 IU/mL. The pound symbol (#) indicates the same patient number as shown in Table 2; however, patient 22 was excluded because HBV reactivation was confirmed by HBV DNA measurement not per-protocol. Dashed lines between the circles, patients with detectable precore (PC) and/or basal core promoter (BCP) mutations; solid lines, patients without detectable PC or BCP mutations. A gray zone indicates no HBV reactivation; that is, HBV DNA levels were <11 IU/mL defined by protocol.

was seropositive only for anti-HBs at baseline (patient 7; see Supplementary Table 5). At the time of HBV reactivation, 3 patients were HBsAg positive and all were IgM-anti-HBc negative. Titers of anti-HBs at reactivation decreased from baseline in the 12 patients who were anti-HBs positive, except for 1 (patient 2). There was no difference in the frequency of decrease of anti-HBs titers in patients with or without HBV reactivation (11 of 12 [91.7%] and 175 of 196 [89.3%], respectively; $P = 1.00$). In addition, there was no difference in the absolute change and the percentage change of anti-HBs titers in patients with or without HBV reactivation.

Sequencing analysis showed that 3 patients had typical escape mutations in the viral S region at reactivation; 2 with a point mutation of G145R and 1 with a point mutation of G145A. One of the 2 patients with G145R mutation maintained an anti-HBs titer of >100 mIU/mL at reactivation (patient 7).

HBV genotypes were determined in these 22 patients, identifying 18 with C2, 3 with B1, and 1 with B2 infections. Viral mutations of PC (G1896A) or BCP (A1762T/G1764A) were identified in 6 patients, 3 of whom had both mutations. The kinetics of HBV DNA and the detection of these mutations in patients with reactivation are shown in Figure 3. There is a difference in the rates of viral replication during HBV reactivation depending on the different mutations. Thus, a rapid increase in HBV DNA from <11 IU/mL just prior to HBV reactivation might be seen in the 6 patients with PC and/or BCP mutations.

DISCUSSION

This study showed that the incidence of HBV reactivation at 1.5 years was 8.3% in B-NHL patients with resolved HBV infection who received R-steroid-chemo. Preemptive antiviral therapy, timed depending on monthly HBV DNA monitoring using a cutoff value of 11 IU/mL, was effective at preventing hepatitis in all patients with HBV reactivation. A Taiwanese prospective study showed that monthly monitoring of HBV DNA, using a cutoff value of 3.0 log copies/mL and with reactivation defined as a 1.0-log increase of HBV DNA relative to nadir levels, could be used to manage HBV reactivation [18]. However, HBV-related severe hepatitis occurred in 7 (4.6%) of the 150 patients with resolved HBV infection who received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone in

that study. Patients with HBV reactivation might have a poorer prognosis than those without reactivation, indicating that our strategy of monthly monitoring might be more appropriate for preventing HBV-related hepatitis.

The ancillary study presented here demonstrated that the results of anti-HBc and anti-HBs serology performed centrally using the same assay kit on specimens banked at baseline provides more accurate data than when assessed at each institute individually; this is due, in part, to variation among different commercial kits [22]. We also conducted sequence analyses of reactivated HBV strains, because viral mutations of PC and BCP have been reported to be associated with enhanced viral replication in patients with fulminant hepatitis B [23, 24]. We identified 6 patients with these mutations that may have been associated with enhancement of replication during HBV reactivation. Importantly, even in those patients with such enhanced replication, the monthly HBV DNA monitoring was effective at preventing HBV-related hepatitis.

Regarding risk factors for HBV reactivation, multivariate analysis showed that patients with anti-HBs titers of <10 mIU/mL had a 20-fold higher risk of HBV reactivation than those with titers of ≥100 mIU/mL at baseline (Table 3). This corroborates previous findings that the absence of anti-HBs is an important risk factor in patients with B-NHL treated with rituximab-containing chemotherapy and those with hematological malignancies receiving allogeneic hematopoietic stem cell transplantation [6, 25]. In healthy immunocompetent individuals vaccinated against HBV, an anti-HBs titer of ≥10 mIU/mL is generally considered to be protective [26], and a titer of ≥100 mIU/mL is a useful surrogate marker of a high response [26, 27]. The present study showed that quantitative measurement of the baseline anti-HBs titer is important for predicting the risk of HBV reactivation. Patients with detectable but not quantifiable HBV DNA at baseline had a greater risk of HBV reactivation; 5 of 6 suffered reactivation in this study, suggesting that they may be considered as having occult HBV infection. Antiviral prophylaxis can be recommended for such patients to prevent HBV reactivation.

This study had several limitations. The eligibility criteria allowed the inclusion of participants who were seropositive only for anti-HBs without a history of HBV vaccination. In general, individuals who are seropositive only for anti-HBs (HBsAg negative and anti-HBc negative) are considered as having a history of HBV vaccination and are at no risk for HBV reactivation. However, HBV reactivation has been reported to occur in patients who were seropositive for anti-HBs alone [5, 28, 29]. In fact, HBV reactivation was observed in patients who were seropositive only for anti-HBs in the ancillary study (see Supplementary Table 5). In Japan where universal HBV vaccination has not been introduced, approximately 10% of patients are seropositive only for anti-HBs among those with resolved HBV infection. Even in the United States where universal HBV vaccination has been adopted, the proportion of elderly patients vaccinated with HBV was reported to be only 17%, 10%–15%, and 5%–7% of persons aged 60–64, 65–69, and ≥70 years, respectively [30]. The coverage rate of HBV vaccination among elderly patients is therefore not high in the United States. Because lymphoma patients are generally older, many may have no history of HBV vaccination, suggesting that those who are seropositive only for anti-HBs without a history of HBV vaccination should be considered at risk for HBV reactivation following R-steroid-chemo.

Antiviral prophylaxis is an alternative approach that may be easier and more convenient than the regular HBV DNA monitoring-guided preemptive antiviral therapy in preventing HBV reactivation in patients with resolved HBV infection [31]. However, there are some concerns such as emergence of drug resistance and cost effectiveness [32]. Recently, a Taiwanese randomized controlled trial showed that antiviral prophylaxis could prevent HBV reactivation during and just after R-steroid-chemo, but late onset HBV reactivation was observed after withdrawal of the antivirals [31]. This suggests that regular monitoring of HBV DNA is also necessary for such patients to prevent HBV reactivation.

In conclusion, this study demonstrated that preemptive antiviral therapy guided by serial monthly HBV DNA monitoring using highly sensitive assays is a useful approach for preventing HBV reactivation–related hepatitis in B-NHL patients with resolved HBV infection following R-steroid-chemo.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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