Medicine®

OBSERVATIONAL STUDY

OPEN

Risk of Reverse Seroconversion of Hepatitis B Virus Surface Antigen in Rituximab-Treated Non-Hodgkin Lymphoma Patients

A Large Cohort Retrospective Study

Liang-Tsai Hsiao, MD, Tzeon-Jye Chiou, MD, Jyh-Pyng Gau, MD, Ching-Fen Yang, MD, Yuan-Bin Yu, MD, Chun-Yu Liu, MD, PhD, Jin-Hwang Liu, MD, PhD, Po-Min Chen, MD, PhD, Cheng-Hwai Tzeng, MD, Yu-Jiun Chan, MD, PhD, Muh-Hwa Yang, MD, PhD, and Yi-Hsiang Huang, MD, PhD

Abstract: Rituximab causes hepatitis B virus (HBV) reactivation in HBV surface antigen (HBsAg)-seronegative patients with CD20-positive B-cell non-Hodgkin lymphoma ($CD20^+$ NHL), especially for those seropositive to the antibody of core antigen (anti-HBc). Clinical hepatitis usually develops after reverse seroconversion of HBsAg (HBV-RS), indicated by the reappearance of HBsAg in serum. Because of the relatively high prevalence of anti-HBc seropositivity in unvaccinated HBsAg-seronegative adults in an HBV hyperendemic area, we aimed to investigate additional factors influencing the development of rituximab-associated HBV-RS.

Between January 2000 and December 2010, unvaccinated HBsAg-seronegative adults with $CD20^+$ NHL who had received rituximab-containing therapy but not anti-HBV agents were enrolled. Patients with and without HBV-RS were compared in terms of clinical factors and treatments including the number of cycles of rituximab therapy, and transplantation. Competing risk regression was used to identify the factors associated with HBV-RS.

For the 482 patients enrolled, the serological status of anti-HBc was available in 75.9%, with a seropositivity rate of 86.6%. At the last follow-up, a total of 33 (6.85%) patients had HBV-RS, with 95.8% anti-HBc seropositive, 78.9% anti-HBs seropositive, and none anti-HCV seropositive. HBV-RS patients have received more cycles (≥6) and prolonged durations of rituximab therapy, and hematopoietic stem cell transplantation. The overall survival was not different between patients with and those without HBV-RS. At the time of HBV-RS, a total of 25 (78.1%) patients had hepatitis flare, especially when HBV-RS appeared during/after induction therapy (100%, 10 of 10). Three (9.1%) patients had fulminant hepatitis, resulting in death in 1 (3%) patient. A higher rituximab cycle intensity was associated with a higher rate of hepatitis flare at the time of HBV-RS. When death in the absence of HBV-RS was considered as the competing risk, the univariate and multivariate regression analyses showed that several factors were independently associated with the development of HBV-RS, including anti-HCV seronegativity, histological subtype of posttransplant lymphoproliferative disorders, ≥6 cycles of rituximab therapy, and succeeding hematopoietic stem cell transplantation.

The findings of our study identify additional factors influencing the development of rituximab-associated HBV-RS in HBsAg-seronegative adults with $CD20^+$ NHL.

(*Medicine* 94(32):e1321)

Abbreviations: ALT = alanine aminotransferase, anti-HBc = antibody to core antigen, anti-HBs = antibody to HBsAg, $CD20^+$ NHL = CD20-positive B-cell non-Hodgkin lymphoma, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, HBsAg = HBV surface antigen, HBV = hepatitis B virus, HBV-RS = reverse seroconversion of hepatitis B virus surface antigen, HCV = hepatitis C virus, HR = hazard ratio, HSCT = hematopoietic stem cell transplantation, PTLD = posttransplant lymphoproliferative disorders, TB = total bilirubin, ULN = upper limit of normal.

INTRODUCTION

The anti-$CD20^+$ monoclonal antibody rituximab is the backbone of therapy for CD20-positive B-cell non-Hodgkin lymphoma ($CD20^+$ NHL), and causes hepatitis B virus (HBV) reactivation in HBV surface antigen (HBsAg)-seronegative patients.[1–15] The incidence of HBV reactivation varied from 2% to 5% to 23.8% according to the definition based on the appearance of HBsAg (ie, reverse seroconversion of HBsAg, HBV-RS) or HBV DNA.[14] In these events, hepatitis flare usually develop after HBV-RS.[14] Efforts to avoid such events included identification of risk factors, close monitoring, and preemptive and even prophylactic use of anti-HBV agents.[13,14,16]

Editor: Chun-xia Cao.
Received: April 8, 2015; revised: July 3, 2015; accepted: July 17, 2015.
From the Division of Hematology and Oncology, Department of Medicine, Taipei Veterans General Hospital (L-TH, J-PG, Y-BY, C-YL, J-HL, P-MC, C-HT, M-HY); Institute of Clinical Medicine, National Yang-Ming University School of Medicine (L-TH, T-JC, J-PG, C-FY, Y-BY, C-YL, J-HL, P-MC, C-HT, M-HY, Y-HH); Division of Transfusion Medicine, Department of Medicine (T-JC); Department of Pathology and Laboratory Medicine (C-FY); Division of Microbiology, Department of Pathology and Laboratory Medicine (Y-JC); Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital (Y-JC); Institute of Public Health, National Yang-Ming University (Y-JC); and Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital (Y-HH), Taipei, Taiwan.

Correspondence: Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taiwan, No. 201, Sec. 2, Shih-Pai Road, Taipei 11217, Taiwan (e-mail: yhhuang@vghtpe.gov.tw).
This work was supported by grants from the Taiwan Clinical Oncology Research Foundation, Taipei Veterans General Hospital (V99C1–191, V100C-111, and V103E2–002 to L-T), National Science Council (NSC 99-2314-B-075-017-MY2, 101-2325-B-075-008, 102-2325-B-075-005 to L-T), and Ministry of Science and Technology (MOST 103-2325-B-075-004 to L-T).

The authors declare that they have no conflicts of interest.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001321

In terms of risk factors of rituximab-associated HBV reactivation in HBsAg-seronegative CD20$^+$ NHL patients, the patients' HBV serological status before rituximab therapy was especially highlighted. The anti-HBc seropositivity and anti-HBs seronegativity were associated with a significantly increased risk of HBV reactivation.[9,14,16,17] However, these serological factors may not be enough, especially for HBV-hyperendemic areas where there is a high prevalence of anti-HBc seropositivity in CD20$^+$ NHL patients.[9,14–17] Although Taiwan was the first to initiate an HBV mass vaccination in 1984,[18] most of the CD20$^+$ NHL patients were unvaccinated, and >70% of them were seropositive to anti-HBc and anti-HBs at the diagnosis of lymphoma.[10,13,14,17] Second, most of the reports on rituximab-associated HBV-RS usually focus on the risk during/after induction therapy,[9] but did not consider those patients with rituximab therapy extended into so-called maintenance or salvage therapy, or those who further received high-dose therapy and hematopoietic stem cell transplantation (HSCT). Finally, the calculation of HBV-RS risks in previous reports did not consider the so-called competing risk—''death in the absence of HBV reactivation''—and might have overestimated the incidence.[19]

To reduce or resolve the hazard of rituximab-associated HBV-RS in HBV hyperendemic areas, the cost-effective approach may be to identify patients at a higher risk. In the current study, we enrolled all rituximab-treated, unvaccinated HBsAg-seronegative patients with CD20$^+$ NHL from a single institute in Taiwan, one of the HBV-hyperendemic areas since 2000, and used HBV-RS as the indicator of HBV reactivation to identify additional risk factors of rituximab-associated HBV reactivation.

PATIENTS AND METHODS

Patients and Data Collection

Patients with histologically proven CD20$^+$ NHL, who received rituximab alone or in combination with chemotherapy at Taipei Veterans General Hospital between January 2000 and December 2010, were retrospectively reviewed. Patients who fulfilled all of the following inclusion criteria at diagnosis were eligible for inclusion in the study: 18 years of age or older, HBV unvaccinated, seronegative to HBsAg and human immunodeficiency virus, not using prophylactic anti-HBV agents, and with ≥1 cycle(s) of rituximab administered at the last follow-up.

The collected data included demographics, histological subtypes, treatments (chemotherapy, number of cycles of rituximab, and HSCT), blood biochemistry tests including liver function tests, viral serology tests of hepatitis virus (HBsAg, antibody to HBsAg [anti-HBs], anti-HBc, and antibody to HCV [anti-HCV]), virological data (HBV DNA), and outcome. The event of HBV reactivation, determined by the conversion from being HBsAg seronegative to being seropositive, was identified, with additional data collected on liver function, viral serology, and virological tests. This study was approved by the Institutional Regulatory Board of Taipei Veterans General Hospital.

Definition of HBV Reactivation and Hepatitis Flare

Here, HBV-RS was used to represent HBV reactivation and defined as the reappearance of HBsAg in serum. Elevated serum alanine aminotransferase (ALT) level that exceeds the upper limit of normal (ULN, ie, 40 IU/L) was attributed to HBV reactivation if it was preceded or accompanied by HBV-RS and/or an HBV viral load of >2000 IU/mL, if applicable.[13] Hepatitis flare was defined as a >3-fold increase of serum ALT level that exceeds 100 IU/L.[13] Hepatitis was designated as severe when a hepatitis flare with an ALT increase to >10-fold the ULN or bilirubin increase to >1.5-fold ULN was observed,[14] and as fulminant when hepatic encephalopathy and irregular blood coagulation (prothrombin time prolonged for >10 s) were noted.[20]

Biochemistry, Viral Serology, and Virological Tests

Biochemical liver function tests including ALT, aspartate aminotransferase, alkaline phosphate, gamma-glutamyl transferase, and total bilirubin (TB) were checked at baseline, at the start of every new cycle of rituximab-containing therapy, and during the follow-up period after the completion of therapy. Serological tests of HIV, HBsAg, and anti-HCV were routinely done at diagnosis, and were performed if clinically indicated at the discretion of the attending physician. Tests for serum hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe) were usually done when HBsAg was seropositive. Anti-HBs and anti-HBc are usually tested for HSCT recipients; however, they were not universally tested for patients with CD20$^+$ NHL until the late 1990s.

Biochemical liver function tests, viral serology, and virological tests were done as previously described.[13,20] The normal ranges were <40 IU/L for ALT and <1.6 mg/dL for TB. Qualitative analysis of HBsAg and anti-HBc was done by using the microparticle enzyme immunoassay. HBeAg and anti-HBe were tested by using a radioimmunoassay kit (Abbott Laboratories, North Chicago, IL). Anti-HCV was measured by using a second-generation enzyme immunoassay kit (Abbott Laboratories). Detection of HBV DNA in serum was performed with an in-house assay in the early 2000s and with a commercial kit in the late 2000s, by using a Cobas Amplicor HBV monitor (Roche Molecular Systems, Pleasanton, CA) to determine the HBV viral load (detection limit of 12 IU/mL). The data of HBV DNA from the in-house assay were appropriately converted into International Units (IU).

Lymphoma-Related Therapy

The use of rituximab-containing therapy for CD20$^+$ NHL patients was generally based on the guidelines recommended by the National Comprehensive Cancer Network, and, in addition, its coverage by the National Health Insurance of Taiwan. For indolent CD20$^+$ NHL, rituximab was given as salvage therapy since April 2002 and as induction and maintenance therapy of follicular lymphoma (FL) since March 2006 and February 2008, respectively. For diffuse large B-cell lymphoma (DLBCL), the addition of rituximab in induction therapy of the elderly (>60 years) began in January 2004, and then for the remaining patients since March 2006.

Rituximab is used alone or in combination with chemotherapy, with a dose of 375 mg/m^2 every 3 to 4 weeks in induction and salvage therapy, and every 2 to 3 months in maintenance therapy.[21] In combination with chemotherapy, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) or a CHOP-like regimen was usually given as induction therapy for patients with aggressive CD20$^+$ NHL for 6 to 8 cycles if treatment was tolerated. Rituximab was given alone as maintenance after induction therapy for 8 to 12 cycles if treatment was tolerated, or in combination with salvage chemotherapy of relapsed or refractory diseases for 3 to 6 cycles. The

regimens of salvage chemotherapy included ESHAP (etoposide, methylprednisolone, cytarabine, and cisplatin), EPOCH (etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin), and ICE (ifosfamide, carboplatin, and etoposide).

High-dose therapy followed by autologous HSCT was usually given after induction therapy in patients with poor prognostic factors at diagnosis, or salvage therapy for those with relapse or refractory diseases. Allogeneic HSCT was given only to patients with multiple relapses or refractory diseases. The preparative regimens of autologous HSCT were BEAM (BCNU, etoposide, cytarabine, and melphalan) and BEAC (BCNU, etoposide, cytarabine, and cyclophosphamide). The nonmyeloablative regimen containing fludarabine, busulfan, and ATG-Fresenius was used for allogeneic HSCT of $CD20^+$ NHL, especially for indolent $CD20^+$ NHL patients.[22] The regimen for prophylaxis of acute graft-versus-host diseases included a short course of methotrexate and cyclosporine A. The dose of cyclosporine A was decreased by 5% weekly until 6 months after transplantation. Methotrexate was not used in patients receiving a nonmyeloablative regimen.[22] After HSCT, rituximab and an anti-HBV agent was not routinely used to prevent posttransplant lymphoproliferative disorders (PTLD) and HBV reactivation, respectively.

The application of rituximab in maintenance and salvage therapy, and the enforcement of HSCT were determined according to the treatment response at the discretion of the attending physician. Then, modification of chemotherapy dosage and the use of component therapy or granulocyte colony-stimulating factor were performed according to the patients' adverse events.

Statistical Analysis

The first end point was to characterize the incidence, clinical and laboratory features, and rituximab-containing treatments of patients with HBV-RS. First, all patients with and without HBV-RS were compared, and factors with a statistical difference were considered as potential risk factors of rituximab-associated HBV-RS, which will be further evaluated in the regression analysis. Second, patients with HBV-RS were further categorized into 3 groups according to the phase of rituximab therapy administered and whether they have received transplantation, and were compared. Group 1 included HBV-RS that developed during/after induction therapy, and group 2 indicated HBV-RS that did not appear until during/after salvage/maintenance therapy. Group 3 included HBV-RS that developed after transplantation, including 8 patients who had received HSCT before HBV-RS and 2 who received rituximab therapy for PTLD. The number of cycles of rituximab, chemotherapy, and HSCT administered were coded until the development of HBV-RS or the last follow-up if no HBV-RS developed. The overall survival (OS) was calculated from rituximab administration until the last follow-up. Finally, to explore the possible relationship between the dose intensity of rituximab and the severity of hepatitis following HBV-RS, the ''rituximab cycle intensity'' was defined here as the total number of cycles administered according to the duration of rituximab therapy (weeks). The association of rituximab cycle intensity and variable severity of hepatitis at the time of HBV-RS was analyzed. The chi-square test (or Fisher exact test when applicable), Student *t*-test (or 1-way ANOVA when applicable), and Mann-Whitney *U*-test (or Kruskal-Wallis *H*-test when applicable) were applied for categorical, parametrically continuous, and nonparametrically continuous variables between groups, respectively. Log-rank test was used to assess differences in OS between patients with and without HBV-RS.

The second end point was to estimate the risk factors of HBV-RS after rituximab-containing therapy. The Fine and Gray model was used to assess the impact of different variables on the cumulative incidence of HBV-RS, with death in the absence of HBV-RS considered as a competing event.[23] The cumulative incidence of reactivation in the presence of competing risks was calculated. Subhazard ratios with the relative 95% confidence interval were reported for the Fine and Gray analysis, respectively. A P-value of <0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 17.0 (Statistical Package for the Social Sciences; SSPS Inc, Chicago, IL), Stata version 11.0 (Stata Corp, College Station, TX), and XLSTAT (Addinsoft SARL, Paris, France).

RESULTS

Patient Characteristics

As shown in Table 1, 482 unvaccinated HBsAg-seronegative patients with $CD20^+$ NHL were enrolled, with a median age of 69.3 years and 298 (61.8%) being male. The major histological subtypes included DLBCL and FL in 290 (60.2%) and 77 (16%) patients, respectively (also see Table 2). In

TABLE 1. Characteristics of 482 Unvaccinated HBsAg-Seronegative Patients With $CD20^+$ NHL

	Total (%)
No. of patients	482 (100)
Age (y) at diagnosis, median (range)	69.3 (21.5–94.5)
Sex: male/female	298/184 (61.8/38.2)
Histological subtypes	
DLBCL	290 (60.2)
FL	77 (16)
PTLD*	5 (1)
Others†	110 (22.8)
Viral serology	
Anti-HCV, available	470 (97.5)
Anti-HCV, seropositive	33 of 470 (7)
Anti-HBs, available	381 (79)
Anti-HBs, seropositive	297 of 381 (78)
Anti-HBc, available	366 (75.9)
Anti-HBc, seropositive	317 of 366 (86.6)
Rituximab therapy	
Interval since diagnosis (wk), median (range)	4.1 (0–974.9)
Cycle number, mean (SD)	6.55 (3.970)
≥6 cycles	291 (60.4)
Durations (wk), median (range)	20.9 (0–329)
HSCT after rituximab therapy	58 (12.0)
Autologous/allogeneic (% of HSCT in subgroup)	40/18 (69.0/31.0)

Anti-HBc = antibody to hepatitis B virus core antigen, Anti-HBs = antibody to hepatitis B virus surface antigen, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, HBsAg = hepatitis B virus surface antigen, HBV-RS = reverse seroconversion of HBsAg, HCV = hepatitis C virus, HSCT = hematopoietic stem cell transplantation, NHL = non-Hodgkin lymphoma, PTLD = posttransplant lymphoproliferative disorder, SD = standard deviation.

*Including 4 patients with renal transplantation and 1 patient who had severe aplastic anemia before allogeneic HSCT.

†Refer to Table 2.

TABLE 2. Reverse Seroconversion of Hepatitis B According to the Histological Subtypes of $CD20^+$ NHL

	Total, no. of patients (% Of total)	HBV-RS, no. of patients (% Per subtype)
Total	482 (100)	33 (6.8)
Diffuse large B-cell lymphoma	290 (60.2)	17 (5.9)
Follicular lymphoma	77 (16.0)	6 (7.8)
Mature (peripheral) B-cell neoplasm, unclassified	19 (3.9)	3 (15.8)
Mantle cell lymphoma	17 (3.5)	1 (5.9)
Extranodal marginal zone B-cell lymphoma of MALT	13 (2.7)	1 (7.7)
Mediastinal large B-cell lymphoma	13 (2.7)	0 (0)
B-cell chronic lymphocytic leukemia	9 (1.9)	1 (11.1)
Burkitt lymphoma	9 (1.9)	1 (11.1)
Lymphoplasmacytic lymphoma	7 (1.5)	0 (0)
Posttransplant lymphoproliferative disorder	5 (1.0)	2 (40)
Primary DLBCL of CNS	5 (1.0)	0 (0)
Splenic marginal zone B-cell lymphoma	4 (0.8)	0 (0)
B-cell small lymphocytic lymphoma	4 (0.8)	0 (0)
Precursor B-lymphoblastic leukemia	4 (0.8)	0 (0)
Primary effusion lymphoma	2 (0.4)	0 (0)
Precursor B-lymphoblastic lymphoma	1 (0.2)	0 (0)
Nodal marginal zone B-cell lymphoma	1 (0.2)	0 (0)
Intravascular large B-cell lymphoma	1 (0.2)	1 (100)
Lymphomatoid granulomatosis	1 (0.2)	0 (0)

CNS = central nervous system, DLBCL = diffuse large B-cell lymphoma, HBV-RS = reverse seroconversion of hepatitis B virus surface antigen, MALT = mucosa-associated lymphoid tissues, NHL = non-Hodgkin lymphoma.

addition, PTLD was noted in 5 patients, including 4 and 1 who received renal transplantation and HSCT, respectively. The serological status of anti-HCV, anti-HBs, and anti-HBc at diagnosis was available in 97.5%, 79%, and 75.9% of patients, respectively, with seropositivity rates of 7%, 78%, and 86.6%, respectively. Rituximab therapy started at 4.1 weeks after the diagnosis, and consisted of a mean of 6.55 cycles during a period of 20.9 weeks. About 60% of patients had received $\geq$6 cycles of rituximab therapy. At the time of HBV-RS or at the last follow-up, a total of 58 (12%) patients had also received HSCT.

Comparison of Patients With and Without HBV-RS

A total of 33 (6.85%) patients had HBV-RS, with 95.8% of them being anti-HBc seropositive, 78.9% anti-HBs seropositive, and none seropositive to anti-HCV (Table 3). Compared with those without HBV-RS, patients with HBV-RS had a higher proportion of the histological subtype PTLD (also see Table 2), received more cycles and longer durations of rituximab therapy, and more frequently had also received HSCT. There was no significant difference in OS between patients with and those without HBV-RS.

Clinical and Laboratory Features of Patients With HBV-RS

As described above, 33 patients with HBV-RS were categorized into 3 groups: group 1—HBV-RS occurred during/after induction therapy (n = 10) (Table 4), group 2—HBV-RS occurred during/after salvage (n = 4)/maintenance (n = 9) therapy (Table 5), and group 3—transplantation was done before HBV-RS (n = 10) (Table 6). When the 3 groups were compared (Table 7), the patients of group 1 had a higher proportion of the histological subtype DLBCL and a higher ''rituximab cycle intensity.'' Group 2 had a lower ''rituximab cycle intensity'' than group 1, although a higher number of rituximab cycles was administered. Group 3 consisted of 7 patients who had received rituximab therapy before HSCT, 2 with PTLD after allogeneic HSCT and renal transplantation (patients P01 and P02, Table 6), and the 1 with FL who received rituximab therapy for relapsed diseases after allogeneic HSCT (patient H08, Table 6). Four of these 8 non-PTLD patients (50% of 8) had only received <6 cycles of rituximab therapy before HBV-RS (Table 6). At the time of HBV-RS, 25 (78.1%) patients had hepatitis flare, especially in group 1 (100%, 10 of 10); 3 (9.1%) patients had fulminant hepatitis, resulting in death in 1 (3%) patient (Table 7).

The association of the rituximab cycle intensity and the severity of hepatitis at the time of HBV-RS was assessed. As shown in Table 8, a higher rituximab cycle intensity was associated with a higher frequency of ''elevated ALT level'' or ''hepatitis flare'' at the time of HBV-RS in all patients with HBV-RS, or those without transplantation (ie, group 1 and group 2).

Factors Associated With HBV-RS

When death in the absence of HBV-RS was considered as the competing risk, the cumulative incidence of HBV-RS estimated was 0.9%, 2%, and 4.8% at 6, 12, and 24 months after the initiation of rituximab therapy, respectively (Figure 1). The univariate and multivariate regression analyses showed that several factors independently influenced the development of HBV-RS (Table 9), including anti-HCV seropositivity (Figure 2A), the PTLD histological subtype (Figure 2B),

TABLE 3. Comparison of 482 Unvaccinated HBsAg-Seronegative Patients With $CD20^+$ NHL According to the Presence of Rituximab-Associated HBV-RS

	HBV-RS		
	Yes n (%)	**No n (%)**	***P***
Total no. of patients	33 (100)	449 (100)	
Age (y) at diagnosis, median (range)	64.3 (34.9–82.3)	69.9 (21.5–94.5)	0.08
Sex: male/female	17/16 (51.5/48.5)	281/168 (62.6/37.4)	0.27*
Histological subtypes			
Diffuse large B-cell lymphoma	17 (51.5)	273 (60.8)	0.36*
Follicular lymphoma	6 (18.2)	71 (15.8)	0.63*
PTLD	2 (6.1)	3 (0.7)	0.04*
Others†	8 (24.2)	102 (22.7)	0.83*
Viral serology			
Anti-HCV, seropositive	0 (0)	33 of 437 (7.6)	0.15*
Anti-HBs, available	26 (78.9)	355 (79.1)	>0.99*
Anti-HBs, seropositive	19 of 26 (73.1)	278 of 355 (78.3)	0.62*
Anti-HBc, available	24 (72.7)	342 (76.2)	0.67*
Anti-HBc, seropositive	23 of 24 (95.8)	294 of 342 (86.0)	0.23*
Rituximab therapy			
Cycle numbers, mean (SD)	8.61 (3.427)	6.4 (3.969)	0.001
≥6 cycles	29 (87.9)	262 (58.4)	0.001*
Durations (wk), median (range)	39.9 (15.7–1679)	20.3 (0–329)	<0.001‡
HSCT after rituximab therapy§	8 (24.2)	50 (11.1)	0.04*
Autologous/allogeneic (% of HSCT)	2/6 (25/75)	39/11 (78/22)	0.006*
Overall survival (y) (95% CI)	8.11 (6.39–9.83)	6.19 (5.75–6.63)	0.36

Anti-HBc = antibody to hepatitis B virus core antigen, Anti-HBs = antibody to hepatitis B virus surface antigen, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, HBsAg = hepatitis B virus surface antigen, HBV-RS = reverse seroconversion of HBsAg, HCV = hepatitis C virus, HSCT = hematopoietic stem cell transplantation, NHL = non-Hodgkin lymphoma, SD = standard deviation.

* Fisher's exact test.

† Refer to Table 2.

‡ Mann-Whitney test.

§ Included 1 patient with FL who received rituximab therapy for relapsed diseases after allogeneic HSCT (patient H08, Table 6).

administration of ≥6 cycles of rituximab therapy (Figure 2C), and HSCT after rituximab therapy (Figure 2D).

DISCUSSION

The study identified additional factors influencing the development and severity of rituximab-associated HBV reactivation, which would provide clues for a cost-effective approach in reducing or preventing the resulting morbidity and mortality, especially for unvaccinated patients with $CD20^+$ NHL. The impact of these factors can be elucidated according to the clinical course of rituximab-treated $CD20^+$ NHL patients. At diagnosis, a preexisting HCV infection in $CD20^+$ NHL patients would prevent the development of rituximab-associated HBV-RS (Tables 3 and 9, Figure 2A). In a Japanese study of HCV-infected DLBCL patients treated with rituximab-containing chemotherapy, Ennishi et al[24] showed that HCV infection was associated with severe hepatic toxicity; however, at the time of hepatic dysfunction, none of HBV-DNA and HBsAg was seropositive, and in all patients, severe hepatic toxicity improved with no anti-HBV treatment. Similarly, in our $CD20^+$ NHL patients with HCV infection, hepatitis flare was common during rituximab-containing therapy; however, additional serological tests of HBV infection did not show HBV-RS. It was reported that HBV replication was suppressed in patients with HBV and HCV coinfections.[25,26] Kao et al[27] suggested that occult HBV infection does not have clinical significance in chronic hepatitis C patients residing in areas where HBV infection is endemic. On the contrary, patients with $CD20^+$ PTLD might have the highest risk of rituximab-associated HBV-RS (Tables 2, 3 and 9, and Figure 2B), as seen in our 2 (40% of 5) patients (patients P01 and P02, Table 6). Similarly, Mikulska et al[28] recently reported that rituximab therapy of PTLD after allogeneic HSCT caused HBV reactivation in anti-HBc seropositive patients. It is possible that preexisting immunosuppression before rituximab therapy might further increase the risk. Similarly, there was 1 patient with FL who received rituximab therapy for relapsed diseases after allogeneic HSCT, and finally developed HBV-RS (patient H08, Table 6). Because of a relatively small number of PTLD patients in our study, this finding deserves more patients to validate in the future.

Second, during rituximab-containing therapy, the effect of the number of cycles on the development of rituximab-associated HBV-RS was highlighted, especially whenever rituximab was administered at ≥6 cycles, and was continuously given as maintenance and/or salvage therapy. In a recent report on DLBCL patients,[15] a higher number (>8) of rituximab cycles was shown to be associated with an increased risk of HBV reactivation, as defined according to HBV-RS and/or HBV DNA elevation. The number of cycles of rituximab therapy required to induce HBV-RS may vary according to the difference in histologic subtypes, presence and intensity of concomitant chemotherapy, and statistical method used. Except for

TABLE 4. Characteristics of HBV-RS During/After Rituximab-Containing Induction Therapy of 10 Patients With CD20$^+$ NHL (Group 1)

	Baseline					Induction therapy*					HBV-RS						
						Chemotherapy		Rituximab therapy									
Patient	Age, y	Sex	Histological subtypes	Anti-HBc	Anti-HBs	Anthracycline	Fludarabine	Interval since diagnosis, wk	Cycle number	Durations, wk	Latent period since last cycle of rituximab therapy, wk	HBeAg	HBV DNA, Log_{10} IU/mL	ALT, IU/l	Total bilirubin, mg/dL	Antiviral treatment	Outcome
I01	68	F	DLBCL	NA	Neg	Yes	No	7.3	6	19.7	38.4	Neg	NA	389	0.5	Lamivudine	Alive
I02	48	F	BL	NA	NA	Yes	No	0.1	6	20.0	24.4	Pos	NA	953	0.6	Lamivudine	Alive
I03	52	F	DLBCL	Pos	Pos	Yes	No	0.7	6	19.1	7.0	Neg	11.3	1040	28.7	Entecavir	Died of HBV reactivation
I04	80	F	FL	NA	Neg	No	No	0.3	6	24.3	46.7	NA	NA	127	0.3	None	Alive
I05	79	M	Unclassified	NA	NA	Yes	No	0.4	6	17.6	213.6	Pos	6.6	305	0.4	Lamivudine	Alive
I06	55	M	DLBCL	Pos	Pos	No†	No	4.6	7	15.7	4.0	NA	NA	152	0.3	None	Died of lymphoma
I07	82	M	DLBCL	Pos	Pos	Yes	No	0.9	7	18.9	2.7	Pos	7.3	1387	12.8	Lamivudine	Alive
I08	81	M	DLBCL	Pos	Neg	Yes	No	4.6	7	23.1	14.4	Neg	7.9	604	0.7	Entecavir	Alive
I09	70	F	DLBCL	Pos	Pos	Yes	No	0.7	8	34.1	24.3	Neg	NA	211	NA	None	Alive
I10	64	M	DLBCL	Pos	NA	Yes	No	1.0	8	23.4	10.9	Neg	7.2	188	0.6	Entecavir	Died of lymphoma

ALT = alanine aminotransferase, Anti-HBc = antibody to hepatitis B virus core antigen, anti-HBs = antibody to hepatitis B virus surface antigen, BL = Burkitt lymphoma, CLL = B-cell chronic lymphocytic leukemia, DLBCL = diffuse large B-cell lymphoma, F = female, FL = follicular lymphoma, HBeAg = hepatitis B virus e antigen, HBsAg = hepatitis B virus surface antigen, HBV-RS = reverse seroconversion of HBsAg, HCV = hepatitis C virus, HSCT = hematopoietic stem cell transplantation, Intravascular = intravascular large B-cell lymphoma, M = male, MATLoma = extranodal marginal zone B-cell lymphoma of MALT, MCL = mantle cell lymphoma, NA = not available, Neg = seronegative, NHL = non-Hodgkin lymphoma, Pos = seropositive, PTLD = posttransplant lymphoproliferative disorder, Unclassified = mature B-cell neoplasms, unclassified.

* Chemotherapy and rituximab therapy performed before the onset of HBV-RS.

† Anthracycline not given because of the patient's impaired heart function.

TABLE 5. Characteristics of HBV-RS During/After Rituximab-Containing Salvage or Maintenance Therapy of 13 Patients With CD20$^+$ NHL (Group 2)

	Baseline					Chemotherapy*		Rituximab therapy*					HBV Reverser Seroconversion						
Patient	Age, y	Sex	Histological subtypes	Anti-HBc	Anti-HBs	Anthracycline	Fludarabine	Interval since diagnosis, wk	Indication of first cycle	Indication of last cycle	Cycle number	Durations, wk	Latent period since last cycle of rituximab therapy, wk	HBeAg	HBV DNA, $\log_{10}$ IU/mL	ALT, IU/L	Total bilirubin, mg/dL	Antiviral treatment	Outcome
SM01	69	M	DLBCL	NA	NA	Yes	No	3.3	Induction	Maintenance	7	61.4	0.00	Pos	6.3	779	1.00	Lamivudine	Alive
SM02	82	M	DLBCL	Pos	Pos	Yes	No	6.1	Induction	Maintenance	8	167.9	3.4	NA	NA	24	Na	None	Died of lymphoma
SM03	67	F	DLBCL	Pos	Pos	Yes	No	14.9	Induction	Salvage	8	111.4	157.7	Pos	5.7	105	1.2	Lamivudine	Died of lymphoma
SM04	73	F	Unclassified	Pos	Pos	Yes	No	0.3	Induction	Maintenance	9	73.6	7.0	Pos	6.3	19	0.6	Lamivudine	Alive
SM05	71	M	DLBCL	Pos	Pos	Yes	No	0.1	Induction	Maintenance	9	33.6	43.7	Pos	NA	426	0.7	Entecavir	Died of lymphoma
SM06	36	F	DLBCL	NA	NA	Yes	No	1.7	Induction	Salvage	10	39.9	17.3	Neg	5.4	2182	4.3	Lamivudine	Alive
SM07	54	M	MATLoma	Pos	NA	Yes	No	2.0	Induction	Maintenance	10	62.7	28.0	Neg	NA	716	0.8	Entecavir	Alive
SM08	78	M	DLBCL	NA	Neg	Yes	No	6.6	Induction	Salvage	10	82.0	60.4	Neg	10.7	389	1.1	Entecavir	Alive
SM09	73	F	Unclassified	NA	Neg	No	No	0.1	Induction	Maintenance	12	159.4	10.9	Neg	5.8	1283	18.9	Lamivudine	Died of lymphoma
SM10	48	M	DLBCL	Pos	Neg	Yes	No	3.9	Induction	Salvage	12	57.9	0.1	Pos	NA	91	NA	None	Died of lymphoma
SM11	75	M	DLBCL†	NA	NA	Yes	Yes	2.3	Induction	Maintenance	15	61.7	17.0	Neg	9.7	114	1.4	Entecavir	Alive
SM12	71	F	FL	Pos	Pos	Yes	No	0.4	Induction	Maintenance	17	101.1	2.7	Pos	NA	119	0.8	Entecavir	Died of lymphoma
SM13	68	F	DLBCL	Pos	Pos	Yes	No	6.9	Induction	Maintenance	17	114.0	0.0	Pos	7.3	22	0.4	Entecavir	Alive

ALT = alanine aminotransferase, Anti-HBc = antibody to hepatitis B virus core antigen, anti-HBs = antibody to hepatitis B virus surface antigen, BL = Burkitt lymphoma, CLL = B-cell chronic lymphocytic leukemia, DLBCL = diffuse large B-cell lymphoma, F = female, FL = follicular lymphoma, HBeAg = hepatitis B virus e antigen, HBsAg = hepatitis B virus surface antigen, HBV-RS = reverse seroconversion of HBsAg, HCV = hepatitis C virus, HSCT = hematopoietic stem cell transplantation, M = male, MATLoma = extranodal marginal zone B-cell lymphoma of MALT, NA = not available, Neg = seronegative, NHL = non-Hodgkin lymphoma, Pos = seropositive, Unclassified = mature B-cell neoplasms, unclassified.

* Chemotherapy and rituximab therapy performed before the onset of HBV-RS.

† Mixed DLBCL and FL.

TABLE 6. Characteristics of HBV-RS After Rituximab-Containing Therapy and Transplantation of 10 Patients With CD20$^+$ NHL (Group 3)

	Baseline					Chemotherapy*		Rituximab therapy*				Transplantation*				HBV-RS						
Patient	Age, y	Sex	Histological subtypes	Anti-HBc	Anti-HBs	Anthracycline	Fludarabine	Interval since diagnosis, wk	Indication to start	Cycle number	Durations, wk	Chronological vs rituximab therapy	Interval between transplantation and last cycle of rituximab therapy, wk	Type	Source of graft	Latent period since last cycle of rituximab therapy, wk	Latent period since transplantation, wk	HBeAg	HBV DNA, $\log_{10}$ IU/mL	ALT, IU/L	Total bilirubin, mg/dL	Antiviral treatment	Outcome
H01	48	M	FL	Pos	Neg	Yes	Yes	460.1	Salvage	13	90.0	After	1.1	HSCT	Allogeneic, siblings	19.3	18.1	Pos	6.9	198	1.7	Lamivudine	Died of lymphoma
H02	35	F	FL	Pos	Pos	Yes	Yes	178.7	Salvage	4	69.4	After	89.1	HSCT	Allogeneic, siblings	149.3	60.1	Pos	7.4	17	0.5	Lamivudine	Alive
H03	48	F	FL	Pos	Pos	Yes	Yes	277.4	Salvage	11	131.3	After	28.0	HSCT	Allogeneic, unrelated	33.6	5.6	Pos	6.5	17	0.7	Lamivudine	Alive
H04	49	F	Intravascular	Pos	Pos	Yes	Yes	0.1	Induction	8	31.1	After	6.4	HSCT	Allogeneic, siblings	187.6	181.1	NA	NA	16	NA	Entecavir	Alive
H05	472	M	CLL	Pos.	Pos	Yes	Yes	92.1	Salvage	11	61.0	After	18.0	HSCT	Allogeneic, siblings	71.1	53.1	Neg	6.4	292	1.3	Entecavir	Alive
H06	48	M	DLBCL	Pos	Pos	Yes	No	0.1	Induction	3	15.9	After	2.0	HSCT	Autologous	6.9	4.9	Neg	NA	NA	NA	None	Died of lymphoma
H07	54	M	MCL	Pos	Pos	Yes	No	9.4	Induction	5	17.3	After	16.4	HSCT	Autologous	63.3	46.9	Neg	NA	395	NA	Entecavir	Alive
H08	38	M	FL	Pos	Pos	Yes	Yes	297.9	Salvage	5	108.9	Before	90.0	HSCT	Allogeneic, siblings	132.3	222.3	Pos	8.3	1228	4.3	Entecavir	Alive
P01	47	F	PTLD†	Pos	Pos	Yes	No	0.7	Induction	6	21.9	Before	61.0	HSCT	Allogeneic, unrelated	0.3	61.3	Pos	NA	22	0.7	Entecavir	Alive
P02	55	F	PTLD	Neg	Pos	Yes	No	5.9	Induction	7	19.7	Before	75.7	Renal	-	29.6	105.3	Pos	NA	1050	0.8	Lamivudine	Died of lymphoma

ALT = alanine aminotransferase, Anti-HBc = antibody to hepatitis B virus core antigen, anti-HBs = antibody to hepatitis B virus surface antigen, BL = Burkitt lymphoma, CLL = B-cell chronic lymphocytic leukemia, DLBCL = diffuse large B-cell lymphoma, F = female, FL = follicular lymphoma, HBeAg = hepatitis B virus e antigen, HBsAg = hepatitis B virus surface antigen, HBV-RS = reverse seroconversion of HBsAg, HCV = hepatitis C virus, HSCT = hematopoietic stem cell transplantation, HSCT = hematopoietic stem cell transplantation, Intravascular = intravascular large B-cell lymphoma, M = male, MATLoma = extranodal marginal zone B-cell lymphoma of MALT, MATLoma = extranodal marginal zone B-cell lymphoma of MALT, MCL = mantle cell lymphoma, MCL = mantle cell lymphoma, NA = not available, Neg = seronegative, Neg. = negative, NHL = non-Hodgkin lymphoma, Pos = seropositive, Pos. = positive, PTLD = posttransplant lymphoproliferative disorder, PTLD = post-transplant lymphoproliferative disorder, Unclassified = mature B-cell neoplasms, unclassified.

* Chemotherapy, rituximab therapy, and transplantation performed before the onset of HBV-RS.

† The patient with severe aplastic anemia received unrelated HSCT and developed PTLD.

TABLE 7. HBV-RS in 33 Unvaccinated HBsAg-Seronegative Patients With $CD20^+$ NHL According to the Phase of Rituximab Therapy

	Group 1 (%) (Induction)	Group 2 (%) (Salvage/maintenance)	Group 3 (%) (Transplantation)	*P*
No. of patients	10 (100)	13 (100)	10 (100)	
Age (y) at diagnosis, median (range)	69.1 (48.5–81.7)	70.8 (36.2–82.3)	47.8 (34.9–54.6)	<0.001
Sex: male/female	5/5 (50/50)	7/6 (53.8/46.2)	5/5 (50/50)	0.98
Histological subtypes*				
DLBCL	7 (70)	9 (69.2)	1 (10)	0.007
FL	1 (10)	1 (7.7)	4 (40)	0.10
PTLD	0	0	2 (20)	0.09
Others*	2 (20)	3 (23.1)	3 (30)	0.87
Viral serology				
Anti-HBc, seropositive	6 of 6 (100)	8 of 8 (100)	9 of 10 (90)	0.48
Anti-HBs, seropositive	4 of 7 (57.1)	6 of 9 (66.7)	9 of 10 (90)	0.28
Rituximab therapy				
Cycle number, mean (SD)	6.7 (.823)	11.1 (3.353)	7.3 (3.368)	0.001
Durations (wk), median (range)	19.9 (15.7–34.1)	73.6 (33.8–167.9)	46.1 (15.9–131.3)	0.001
Rituximab cycle intensity (cycle/wk), mean (SD)	0.32 (.061)	0.15 (.071)	0.19 (.106)	0.001
HBV-RS				
Latent period since last cycle of rituximab (wk), median (range)	19.4 (2.7–213.8)	10.9 (0–157.7)	48.4 (0.3–187.6)	0.21
Normal ALT level (≤40 IU/L)	0 (0)	3 (23.1)	4 of 9 (44.4)	0.09
Hepatitis flare	10 (100)	9 (69.2)	6 of 9 (55.6)	0.09
HBV DNA (log_{10}) (IU/mL)	7.3 (6.6–11.3)	6.3 (5.4–10.7)	6.9 (6.4–8.3)	0.60
HBeAg seropositivity	3 of 8 (37.5)	7 of 12 (58.3)	7 (70)	0.38
Fulminant hepatitis	2 (20)	1 (7.7)	0 (0)	0.29
Antiviral treatment: none/lamivudine/entecavir	4/4/2 (40/40/20)	2/5/6 (15.4/38.5/46.2)	1/4/5 (10/40/50)	0.43
Death due to HBV-RS	1 (10)	0 (0)	0 (0)	0.31

ALT = alanine aminotransferase, Anti-HBc = antibody to hepatitis B virus core antigen, Anti-HBs = antibody to hepatitis B virus surface antigen, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, HBeAg = hepatitis B virus e antigen, HBsAg = hepatitis B virus surface antigen, HBV-RS = reverse seroconversion of HBsAg, NHL = non-Hodgkin lymphoma, PTLD = posttransplant lymphoproliferative disorder.

* For the other subtypes, refer to Table 2.

TABLE 8. Correlation of Rituximab Cycle Intensity and Hepatitis Flare in 33 CD20$^+$ NHL With Reverse Seroconversion of Hepatitis B Virus Surface Antigen

Subgroups	Severity of Hepatitis		No. of Patients	Rituximab Cycle Intensity (cycle/wk), Mean (SD)	*P*
All (groups 1–3)	Elevated ALT level (ALT >40 IU/L)				0.04
		No	7	0.14 (0.092)	
		Yes	26	0.23 (0.103)	
	Hepatitis flare (ALT >100 IU/L)				0.05*
		No	8	0.15 (0.088)	
		Yes	25	0.24 (0.105)	
Nontransplant (groups 1 and 2)	Elevated ALT level (ALT >40 IU/L)				0.03
		No	3	0.11 (0.053)	
		Yes	20	0.24 (0.101)	
	Hepatitis flare (ALT >100 IU/L)				0.05†
		No	4	0.13 (0.066)	
		Yes	19	0.25 (0.104)	

ALT = alanine aminotransferase; NHL = non-Hodgkin lymphoma.
* $P = 0.047$.
† $P = 0.049$.

the case reported by Koo et al,[29] the risk of HBV-RS during the period of rituximab maintenance therapy was not understood, and an additional 9 cases are shown here (Table 5). In contrast, the severity of hepatitis flare at the time of HBV-RS was possibly related to the ''rituximab cycle density'' rather than the number of cycles of rituximab therapy (Tables 7 and 8). Especially notably, HBV-RS during/after rituximab-containing induction therapy (group 1) tended to have a higher severity of hepatitis flare (Table 7). Although an increased cycle number of rituximab would theoretically lead to a more prolonged B-cell depletion, the concept of ''rituximab cycle intensity'' might provide a more reasonable answer. A higher ''rituximab cycle intensity'' in induction therapy may cause a more rapid and profound B-cell depletion and resulting reduction of anti-HBs, thus rapidly depleting its protective effect on HBV-RS. In contrast, for patients who did not develop HBV-RS during/after rituximab-containing induction therapy, they would have a relatively slow and lower degree of B-cell depletion whenever rituximab is given as maintenance, or would have a period of remission or stable disease to allow at least partial recovery of B cells before rituximab-containing salvage therapy is given for the relapse.

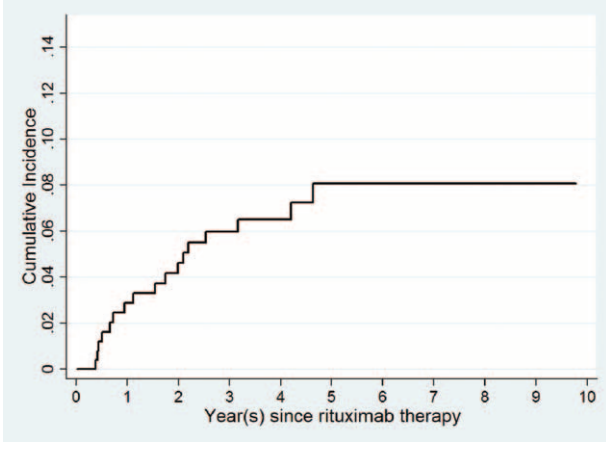

FIGURE 1. Cumulative incidence of rituximab-associated reverse seroconversion of HBsAg in 482 unvaccinated patients with CD20$^+$ NHL, with death in the absence of reverse seroconversion of HBsAg as the competing risk.

Third, similar to the impact of PTLD, a succeeding HSCT would further precipitate the risk of HBV-RS in rituximab-treated CD20$^+$ NHL patients, although <6 cycles of rituximab therapy were administered. Because HBsAg-seronegative/anti-HBc-seropositive patients with other hematological malignancies (other than CD20$^+$ NHL) were shown to have a high risk of HBV-RS after allogeneic HSCT,[30] the development of HBV-RS after allogeneic HSCT in our patients with CD20$^+$ NHL might be simply caused by the immunosuppression after allogeneic HSCT itself, rather than by rituximab therapy. However, the findings—2 patients with autologous HSCT (patients H06 and H07, Table 6) and 4 patients with rituximab therapy of <6 cycles—might provide physicians with additional cautionary examples of the occurrence of HBV-RS after HSCT.

This study has several limitations. First, because of a relatively high prevalence of anti-HBc seropositivity and a low incidence of rituximab-associated HBV-RS, we enrolled all rituximab-treated CD20$^+$ NHL patients who were unvaccinated, including those with unknown serological status of anti-HBc, to have a larger number of patients for analysis. This approach might limit our examination of the impact of anti-HBc seropositivity on rituximab-associated HBV-RS; however, 23 of our 24 HBV-RS patients with known anti-HBc status were seropositive. The only anti-HBc-seronegative patient had PTLD after renal transplantation and developed HBV-RS after rituximab therapy. Several patients with seronegative anti-HBc developed HBV-RS after a different anticancer therapy,[31,32] and the possible causes included a lower titer of anti-HBc and core gene mutation in occult HBV. Second, similar to the findings of our previous reports,[13,15] we did not demonstrate an increased risk of anti-HBs seronegativity in this study, although several recent studies showed such an increase.[9,14,16,17] Third, other factors including the International

TABLE 9. Competing Risk Regression for Factors Influencing Rituximab-Associated HBV-RS, With the Outcome ''Death in the Absence of HBV-RS'' as the Competing Risk

Factor	Univariate		Multivariate	
	SHR	*P* (95% CI)	SHR	*P* (95% CI)
Sex: male	1.486	0.25 (0.752–2.934)	—	—
Histological subtypes				
DLBCL	0.727	0.36 (0.369–1.431)	—	—
FL	1.049	0.91 (0.441–2.497)	—	—
PTLD	12.745	0.002 (2.480–65.486)	32.119	<0.001 (9.176–112.425)
Viral serology				
Anti-HCV seropositivity	7.97e-20	<0.001 (4.75e-20–1.34e-19)	8.50e-08	<0.001 (4.72e-08–1.53e-07)
Anti-HBc seropositivity	3.257	0.25 (0.436–24.323)	—	—
Anti-HBs seropositivity	0.738	0.49 (0.309–1.763)	—	—
Rituximab therapy ≥6 cycles	3.975	0.009 (1.404–11.256)	5.300	0.005 (1.653–16.989)
HSCT after rituximab therapy	2.058	0.07 (0.951–4.452)	2.359	0.03 (1.075–5.177)

– = not applicable, Anti-HBc = antibody to hepatitis B virus core antigen, Anti-HBs = antibody to hepatitis B virus surface antigen, CI = confidence interval, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, HBV-RS = reverse seroconversion of hepatitis B virus surface antigen, HCV = hepatitis C virus, HSCT = hematopoietic stem cell transplantation, PTLD = posttransplant lymphoproliferative disorder, SHR = subdistribution hazard ratio.

Prognostic Index and chemotherapeutic agents used were not analyzed in the present study because of heterogeneities of histological subtypes and treatment modalities in our patients. At present, there is no evidence to support the relation of these factors with rituximab-associated HBV-RS. Finally, the retrospective design of this study might underestimate the incidence of HBV-RS.

In conclusion, we analyzed a large number of unvaccinated HBsAg-seronegative adults with CD20$^+$ NHL in Taiwan—an HBV hyperendemic area—and highlighted several additional factors influencing the development of rituximab-associated HBV-RS, including preexisting HCV infection, the histological type PTLD, the number of rituximab cycles, and succeeding HSCT. In addition, HBV-RS after rituximab-containing induction therapy tended to have a higher severity of hepatitis, and the cycle number-dependent risk should be considered with caution when rituximab is continuously given as maintenance and/or salvage therapy.

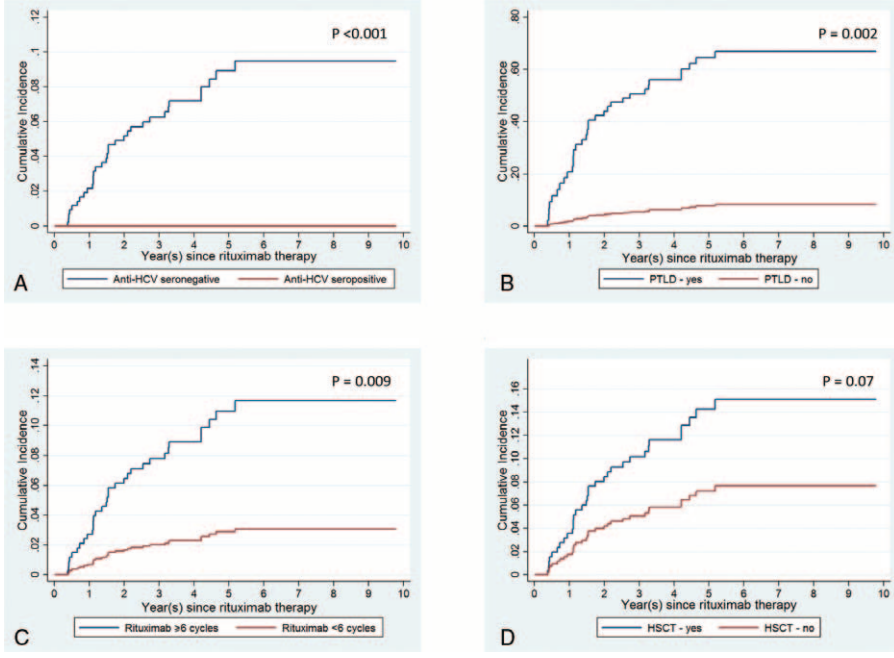

FIGURE 2. Cumulative incidence of rituximab-associated reverse seroconversion of HBsAg in 482 unvaccinated patients with CD20$^+$ NHL, with death in the absence of reverse seroconversion of HBsAg as the competing risk, according to (A) anti-HCV seropositivity, (B) PTLD histological subtype, (C) rituximab therapy ≥6 cycles, and (D) additional hematopoietic stem cell transplantation (HSCT) after rituximab therapy.

ACKNOWLEDGMENTS

The authors thank research assistants Hsiao-Ling Liu and Wen-Chun Chang for their help with experimental analysis.

REFERENCES

1. Dervite I, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatitis B surface antigen who was receiving rituximab. *N Engl J Med.* 2001;344:68–69.
2. Ng HJ, Lim LC. Fulminant hepatitis B virus reactivation with concomitant listeriosis after fludarabine and rituximab therapy: case report. *Ann Hematol.* 2001;80:549–552.
3. Hamaki T, Kami M, Kusumi E, et al. Prophylaxis of hepatitis B reactivation using lamivudine in a patient receiving rituximab. *Am J Hematol.* 2001;68:292–294.
4. Skrabs C, Muller C, Agis H, et al. Treatment of HBV-carrying lymphoma patients with Rituximab and CHOP: a diagnostic and therapeutic challenge. *Leukemia.* 2002;16:1884–1886.
5. Westhoff TH, Jochimsen F, Schmittel A, et al. Fatal hepatitis B virus reactivation by an escape mutant following rituximab therapy. *Blood.* 2003;102:1930.
6. Sarrecchia C, Cappelli A, Aiello P. HBV reactivation with fatal fulminating hepatitis during rituximab treatment in a subject negative for HBsAg and positive for HBsAb and HBcAb. *J Infect Chemother.* 2005;11:189–191.
7. Hui CK, Cheung WW, Zhang HY, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology.* 2006;131:59–68.
8. Khaled Y, Hanbali A. Hepatitis B virus reactivation in a case of Waldenstrom's macroglobulinemia treated with chemotherapy and rituximab despite adefovir prophylaxis. *Am J Hematol.* 2007;82:688.
9. Yeo W, Chan TC, Leung NW, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol.* 2009;27:605–611.
10. Pei SN, Chen CH, Lee CM, et al. Reactivation of hepatitis B virus following rituximab-based regimens: a serious complication in both HBsAg-positive and HBsAg-negative patients. *Ann Hematol.* 2010;89:255–262.
11. Evens AM, Jovanovic BD, Su YC, et al. Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: meta-analysis and examination of FDA safety reports. *Ann Oncol.* 2011;22:1170–1180.
12. Leung C, Tsoi E, Burns G, et al. An argument for the universal prophylaxis of hepatitis B infection in patients receiving rituximab: a 7-year institutional experience of hepatitis screening. *Oncologist.* 2011;16:579–584.
13. Huang YH, Hsiao LT, Hong YC, et al. Randomized controlled trial of entecavir prophylaxis for rituximab-associated hepatitis B virus reactivation in patients with lymphoma and resolved hepatitis B. *J Clin Oncol.* 2013;31:2765–2772.
14. Hsu C, Tsou HH, Lin SJ, et al. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. *Hepatology.* 2014;59:2092–2100.
15. Wu CY, Hsiao LT, Chiou TJ, et al. Lymphocyte/monocyte ratio and cycles of rituximab-containing therapy are risk factors for hepatitis B virus reactivation in patients with diffuse large B-cell lymphoma and resolved hepatitis B. *Leuk Lymphoma.* 2015:1–8.
16. Seto WK, Chan TS, Hwang YY, et al. Hepatitis B reactivation in patients with previous hepatitis B virus exposure undergoing rituximab-containing chemotherapy for lymphoma: a prospective study. *J Clin Oncol.* 2014;32:3736–3743.
17. Pei SN, Ma MC, Wang MC, et al. Analysis of hepatitis B surface antibody titers in B cell lymphoma patients after rituximab therapy. *Ann Hematol.* 2012;91:1007–1012.
18. Chen DS, Hsu NH, Sung JL, et al. A mass vaccination program in Taiwan against hepatitis B virus infection in infants of hepatitis B surface antigen-carrier mothers. *JAMA.* 1987;257:2597–2603.
19. Kim HT. Cumulative incidence in competing risks data and competing risks regression analysis. *Clin Cancer Res.* 2007;13 (2 Pt 1):559–565.
20. Hsiao LT, Chiou TJ, Liu JH, et al. Extended lamivudine therapy against hepatitis B virus infection in hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant.* 2006;12:84–94.
21. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med.* 2002;346:235–242.
22. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood.* 1998;91:756–763.
23. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc.* 1999;94:496–509.
24. Ennishi D, Maeda Y, Niitsu N, et al. Hepatic toxicity and prognosis in hepatitis C virus-infected patients with diffuse large B-cell lymphoma treated with rituximab-containing chemotherapy regimens: a Japanese multicenter analysis. *Blood.* 2010;116:5119–5125.
25. Fong TL, Di Bisceglie AM, Waggoner JG, et al. The significance of antibody to hepatitis C virus in patients with chronic hepatitis B. *Hepatology.* 1991;14:64–67.
26. Shih CM, Lo SJ, Miyamura T, et al. Suppression of hepatitis B virus expression and replication by hepatitis C virus core protein in HuH-7 cells. *J Virol.* 1993;67:5823–5832.
27. Kao JH, Chen PJ, Lai MY, et al. Sequence analysis of pre-S/surface and pre-core/core promoter genes of hepatitis B virus in chronic hepatitis C patients with occult HBV infection. *J Med Virol.* 2002;68:216–220.
28. Mikulska M, Nicolini L, Signori A, et al. Hepatitis B reactivation in HBsAg-negative/HBcAb-positive allogeneic haematopoietic stem cell transplant recipients: risk factors and outcome. *Clin Microbiol Infect.* 2014;20:O694–O701.
29. Koo YX, Tan DS, Tan IB, et al. Hepatitis B virus reactivation in a patient with resolved hepatitis B virus infection receiving maintenance rituximab for malignant B-cell lymphoma. *Ann Intern Med.* 2009;150:655–656.
30. Dhedin N, Douvin C, Kuentz M, et al. Reverse seroconversion of hepatitis B after allogeneic bone marrow transplantation: a retrospective study of 37 patients with pretransplant anti-HBs and anti-HBc. *Transplantation.* 1998;66:616–619.
31. Awerkiew S, Daumer M, Reiser M, et al. Reactivation of an occult hepatitis B virus escape mutant in an anti-HBs positive, anti-HBc negative lymphoma patient. *J Clin Virol.* 2007;38:83–86.
32. Feeney SA, McCaughey C, Watt AP, et al. Reactivation of occult hepatitis B virus infection following cytotoxic lymphoma therapy in an anti-HBc negative patient. *J Med Virol.* 2013;85:597–601.