Gut and Liver
https://doi.org/10.5009/gnl210551
pISSN 1976-2283 eISSN 2005-1212

Original Article

Risk of Hepatitis B Virus (HBV) Reactivation in HBsAg-Negative, Anti-HBc-Negative Patients Receiving Rituximab for Autoimmune Diseases in HBV Endemic Areas

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Article Info

Received November 28, 2021
Revised January 25, 2022
Accepted March 15, 2022
Published online October 21, 2022

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Background/Aims: Rituximab is known to be associated with high hepatitis B virus (HBV) reactivation rate in patients with resolved HBV infection and hematologic malignancy. However, data regarding HBV reactivation (HBVr) in rheumatic patients receiving rituximab is limited. To assess the HBVr rate in hepatitis B surface antigen (HBsAg)-negative patients receiving rituximab for autoimmune diseases in a large real-world cohort.

Methods: From March 2006 to December 2019, 900 patients with negative HBsAg receiving at least one cycle of rituximab for autoimmune diseases in a tertiary medical center in Taiwan were retrospectively reviewed. Clinical outcome and factors associated with HBVr were analyzed.

Results: After a median follow-up period of 3.3 years, 21 patients developed HBVr, among whom 17 patients were positive for hepatitis B core antibody (anti-HBc) and four were negative. Thirteen patients had clinical hepatitis flare, while eight patients had HBsAg seroreversion without hepatitis. Old age, anti-HBc positivity, undetectable serum hepatitis B surface antibody level at rituximab initiation and a higher average rituximab dose were associated with a higher HBVr rate. There was no significant difference in the HBVr risk between rheumatoid arthritis and other autoimmune diseases. Among anti-HBc-negative patients, subjects without HBV vaccination at birth had an increased risk of HBVr (4/368, 1.1%) compared with those who received vaccination (0/126, 0%).

Conclusions: In HBV endemic areas where occult HBV is prevalent, anti-HBc-negative patients, may still be at risk for HBVr after rituximab exposure. HBVr may still be considered in HBsAg-negative patients developing abnormal liver function after rituximab exposure, even in patients with negative anti-HBc. **(Gut Liver 2023;17:288-298)**

Key Words: Hepatitis B; Autoimmune diseases; Rituximab

INTRODUCTION

Hepatitis B virus (HBV) infection is a major health issue in endemic areas such as Asian countries, and HBV reactivation (HBVr) is a life-threatening complication.[1] The risk of reactivation not only exists for patients with positive hepatitis B surface antigen (HBsAg); patients with resolved HBV were also reported to have HBVr after chemotherapy or immunosuppressants, especially rituximab. A pooled analysis of studies revealed a 16.9% reactivation rate in patients with resolved HBV receiving rituximab-containing chemotherapy.[2]

Rituximab is one of the most important immunomodulators in rheumatic diseases for its ability to deplete autoreactive B cells and subsequently decrease autoantibody production. It has been approved to be used in rheumatoid

arthritis (RA) and anti-neutrophil cytoplasmic antibody-associated vasculitis by Food and Drug Administration and European Regulatory Agency. It is also widely used and investigated in severe autoimmune diseases, for example, systemic lupus erythematosus (SLE), inflammatory myositis, Sjogren syndrome, systemic sclerosis, and antiphospholipid syndrome.[3-6] Patients with autoimmune diseases may require long-term rituximab administration to maintain remission and prevent relapses, although the optimal dosing timing and interval remain controversial due to the complex nature of autoimmune diseases.[7,8] For HBsAg-positive rheumatic patients receiving rituximab, the risk was as high as 30% to 60%, necessitating antiviral prophylaxis.[9,10] However, for patients with resolved HBV, evidence was available only in RA patients and the reported reactivation rates were inconsistent, ranging from 0% to 10%.[11-16] In patients receiving rituximab for other autoimmune diseases, the risk of HBVr remains unclear.

Therefore, this study aims to assess clinical outcomes and risk factors for HBVr in HBsAg-negative patients receiving rituximab for various autoimmune diseases in a large real-world cohort.

MATERIALS AND METHODS

1. Study population

From March 2006 to December 2019, HBsAg-negative patients receiving at least one cycle of rituximab for autoimmune diseases at National Taiwan University Hospital were retrospectively analyzed (Fig. 1). All patients underwent hepatic function surveillance every 1 to 3 months from rituximab initiation, and HBsAg and hepatitis B surface antibody (anti-HBs) were measured when clinically indicated.

The details of rheumatic diagnosis, age, comorbidity, liver biochemical parameters, viral hepatitis B markers (HBsAg, anti-HBs, and hepatitis B core antibody [anti-HBc]), and immunoglobulin G (IgG) were recorded. The autoimmune diseases of individual patients were defined based on the updated classification criteria. The study was approved by the Ethics Committee of National Taiwan University Hospital (reference number: 202010014RINB) in harmony with the Declaration of Helsinki. The informed consent was waived because this design is a retrospective study.

The universal HBV vaccination program for all newborns started in Taiwan in 1986.[17] All individuals born after 1986 had received a 3-dose course of HBV vaccination at birth. Stratified by their birth date, patients born before 1986 were classified as "unvaccinated cohort," while patients born after 1986 were classified as "vaccinated cohort" for analysis.

2. Rituximab treatment

All patients received the first cycle of rituximab, with 375 mg/m^2 body surface area weekly for 4 consecutive weeks for anti-neutrophil cytoplasmic antibody vasculitis, or 500 to 1,000 mg rituximab twice within 14 days for other autoimmune diseases. Further cycles for treatment consisting of the same regimen were repeated with a 6- to 12-month interval for maintenance as clinically indicated.

3. Definition of HBVr and HBV hepatitis flare

An HBVr was defined as detectable HBV DNA or reappearance of HBsAg in the serum (HBsAg seroreversion). An HBV hepatitis flare was defined as an alanine aminotransferase increase for more than three times of baseline

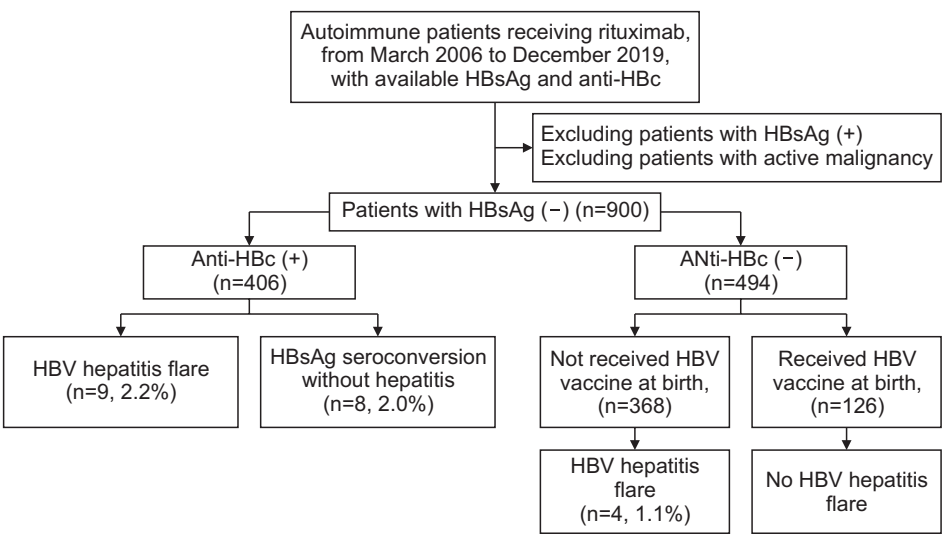

Fig. 1. Patients with negative hepatitis B surface antigen (HBsAg) receiving at least one cycle of rituximab for various rheumatic diseases were enrolled. In addition to hepatitis B core antibody (anti-HBc)-positive patients, four out of 368 (1.1%) anti-HBc-negative patients who did not receive hepatitis B virus (HBV) vaccination at birth had HBV hepatitis flare.

level and >100 U/L and concurrent HBVr,[10] excluding other possible causes.

4. Viral hepatitis B markers testing

The cutoff value for HBsAg positivity was 0.05 IU/mL or 1.0 signal-to-cutoff ratio. The cutoff value for positive anti-HBs and anti-HBc were 10 mIU/mL and 1.0 signal-to-cutoff ratio, respectively. HBV viral load quantification was based on Cobas TaqMan HBV DNA assay (detection limit at 20 IU/mL; Roche, Basel, Switzerland).

5. Statistical analysis

The results are presented as mean with standard deviation or median with interquartile range for continuous

Table 1. Demographics, Baseline Characteristics and Medication in HBsAg-Negative Patients with and without HBV Reactivation

Characteristics	Total (n=900)	HBV reactivation		p-value
		Positive (n=21)	Negative (n=879)	
Demographics, HBV serology and liver function at rituximab initiation				
Age, yr	46.7±14.5	55.5±10.9	46.5±14.5	0.005*
Female sex	733 (81)	18 (86)	715 (81)	0.82
Anti-HBc positivity†	406 (45)	17 (81)	389 (44)	<0.001*
Baseline anti-HBs positivity	247 (75)	3 (14)	244 (27)	0.008*
Baseline anti-HBs titers, mIU/mL	40.2 (10.1–297.7)	6.5 (3.2–26.7)	48.6 (12.1–260.5)	0.06
HBV vaccination at birth	137 (15)	0	137 (15)	0.06
Serum IgG, mg/dL	1,341.9±492.5	1,241.1±413.3	1,344.5±494.3	0.087
ALT, U/L	17.0 (12.0–23.0)	23.0 (19.0–32.0)	17.0 (12.0–23.0)	0.044*
AST, U/L	21.0 (17.0–27.0)	21.0 (15.5–29.0)	21.0 (17.0–26.0)	0.23
Fibrosis-4 score	1.01 (0.65–1.58)	1.22 (0.71–1.71)	1.01 (0.66–1.59)	0.12
Underlying autoimmune diseases				0.83
Rheumatoid arthritis	129 (14)	2 (10)	127 (14)	-
Vasculitis‡	50 (6)	1 (5)	49 (6)	-
Systemic lupus erythematosus	233 (26)	6 (29)	227 (26)	-
Systemic sclerosis	28 (3)	1 (5)	27 (3)	-
Inflammatory myositis	38 (4)	3 (14)	35 (4)	-
Mixed connective tissue disease	15 (2)	0	15 (2)	-
Sjogren syndrome	130 (14)	3 (14)	127 (14)	-
IgG4-related disease	20 (2)	0	20 (2)	-
Antiphospholipid syndrome	115 (13)	2 (10)	113 (13)	-
Unclassified connective tissue disease	69 (8)	1 (5)	68 (8)	-
Other autoimmune disease§	73 (8)	2 (10)	71 (8)	-
Other immunosuppressant exposure during rituximab treatment				
Glucocorticoid	697 (77)	20 (95)	677 (77)	0.06
Hydroxychloroquine	704 (78)	15 (71)	689 (78)	0.40
Azathioprine	297 (33)	9 (43)	288 (33)	0.33
Sulfasalazine	137 (15)	2 (9.5)	135 (15)	0.82
Methotrexate	197 (22)	8 (38)	189 (22)	0.13
Mycophenolate mofetil	21 (2)	0	21 (3)	0.94
Cyclophosphamide	93 (10)	4 (19)	89 (10)	0.32
Cyclosporine	23 (3)	0	23 (3)	0.92
Leflunomide	122 (14)	3 (14)	119 (14)	0.70
Average RTX dose, mg/day‖	4.5 (2.4–5.8)	4.5 (2.4–5.8)	5.0 (4.3–8.1)	0.01*

Data are presented as mean±SD, number (%), or median (interquartile range).
HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody; IgG, immunoglobulin G; ALT, alanine aminotransferase; AST, aspartate aminotransferase; RTX, rituximab.
*The p-value for between-group comparisons was calculated with the chi-square test or Fisher exact test for categorical variables and Mann-Whitney U test or Student t-test for continuous variables. Statistically significant, p<0.05; †All patients had anti-HBc data, with 53% (n=473) obtained at baseline; ‡Includes anti-neutrophil cytoplasmic antibody vasculitis, cryoglobulinemic vasculitis and unclassified vasculitis; §Includes autoimmune hemolytic anemia, immune thrombocytopenia, autoimmune encephalitis, autoimmune peripheral neuropathy, myasthenia gravis, multiple sclerosis and neuromyelitis optica spectrum disorders, autoimmune optic neuropathy, autoimmune pancreatitis, autoimmune hepatitis, primary biliary cirrhosis, interstitial pneumonitis with autoimmune features, autoimmune thyroid disease, and rapid progressive glomerulonephritis; ‖Calculated as the accumulated rituximab dose divided by the total rituximab exposure duration.

variables. The chi-square test or Fisher exact test for categorical variables was used for between-group comparisons. For continuous variables, the Mann-Whitney U test or Student t-test was used. The cumulative incidence stratified by different variables was calculated by the Kaplan-Meier analysis and the significance was determined by the log-rank test. Variables with p-value <0.2 in the univariable Cox regression analysis are selected for multivariable regression analysis. A p-value less than 0.05 was considered statistically significant. All data were analyzed by R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

1. Study population

Nine hundred patients with a negative HBsAg were enrolled. Four hundred and six patients had positive anti-HBc and 494 patients had negative anti-HBc. The mean age at rituximab initiation was 46.7 years, and 81% were female. The diagnoses of autoimmune diseases included RA (n=129) and other autoimmune inflammatory rheumatic diseases (n=771) (Table 1).

2. Incidence of HBVr

After a median follow-up period of 3.3 years (range, 0.5 to 13.9 years) and median cycles of rituximab of 5 (range, 1 to 32), 21 patients developed HBVr. Among them, 17 patients were positive for anti-HBc and four were negative. Thirteen patients encountered clinical hepatitis flare, while eight patients had HBsAg seroreversion without hepatitis (Fig. 1). The incidence of HBV hepatitis flare was 3.4 per 1,000 person-years (total follow-up 3,797 person-years).

3. Factors associated with HBVr in HBsAg-negative patients.

Patients with HBVr had an older age (55.5 years vs 46.5 years, p=0.005), a higher proportion of anti-HBc positivity (81% vs 44%, p<0.001), a lower proportion of anti-HBs positivity (14% vs 27%, p=0.008) at rituximab initiation and a higher average rituximab dose exposure (5.0 mg/day vs 4.5 mg/day, p=0.01). There were no significant differences in baseline IgG level, concomitant use of glucocorticoids or other immunosuppressants during rituximab therapy (Table 1).

The multivariable Cox regression analysis demonstrated that old age (adjusted hazard ratio [HR], 1.05; p=0.005), presence of anti-HBc antibody (adjusted HR, 3.34; p=0.035) and a higher average rituximab dose exposure (adjusted HR, 1.22; p=0.007) were associated with higher risk of HBVr, while anti-HBs positivity was associated with lower risk (adjusted HR, 0.16; p=0.011) (Table 2).

Compared to RA patients, the diagnosis of other autoimmune inflammatory rheumatic diseases was not associated with a higher risk of HBVr (p=0.29) (Fig. 2A).

Table 2. Univariable and Multivariable Cox Regression Analysis for Risk Factors for HBV Reactivation in HBsAg-Negative Patients Receiving RTX for Autoimmune Diseases

Factor	Univariable analysis		Multivariable analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	1.06 (1.03–1.10)	<0.001*	1.05 (1.02–1.09)	0.005*
Sex (male vs female)	0.79 (0.23–2.69)	0.73	-	-
Anti-HBc positivity	5.54 (1.86–16.5)	0.002*	3.34 (1.09–10.3)	0.035*
Baseline anti-HBs positivity	0.17 (0.04–0.68)	0.012*	0.16 (0.04–0.65)	0.011*
Baseline serum IgG level	1.00 (1.00–1.00)	0.34	-	-
Baseline ALT level	1.00 (0.09–1.01)	0.60	-	-
Other autoimmune diseases versus RA	2.16 (0.50–9.31)	0.34	-	-
Average RTX dose	1.28 (1.11–1.47)	<0.001*	1.22 (1.06–1.41)	0.007*
Concomitant glucocorticoid use during RTX	5.18 (0.69–38.7)	0.11	6.23 (0.83–46.5)	0.075
Concomitant immunosuppressant during RTX				
Hydroxychloroquine	0.49 (0.19–1.28)	0.14	-	-
Azathioprine	1.16 (0.49–2.78)	0.72	-	-
Sulfasalazine	0.42 (0.10–1.81)	0.23	-	-
Methotrexate	1.66 (0.68–4.04)	0.32	-	-
Cyclophosphamide	1.69 (0.55–5.14)	0.42	-	-
Leflunomide	0.82 (0.24–2.81)	0.83	-	-

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; RTX, rituximab; HR, hazard ratio; CI, confidence interval; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody; IgG, immunoglobulin G; ALT, alanine aminotransferase; RA, rheumatoid arthritis.
*Statistically significant, p<0.05.

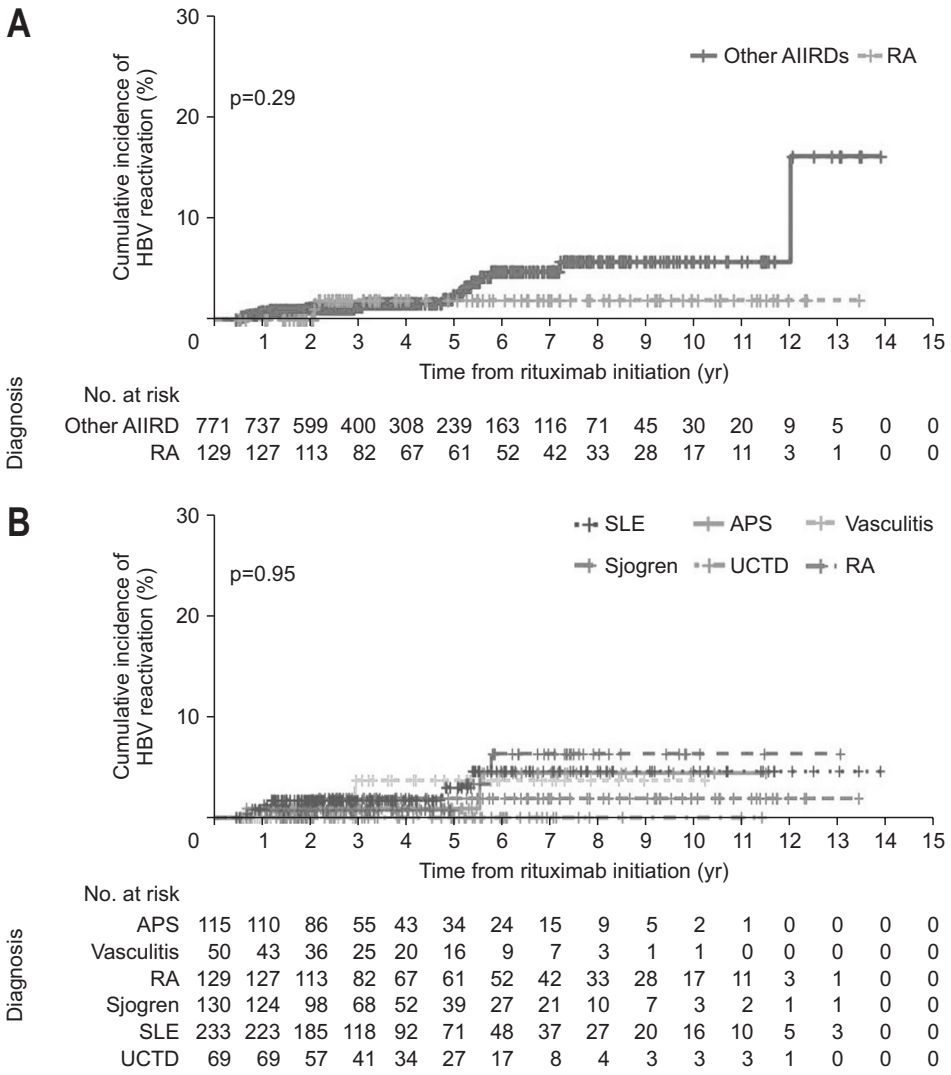

Fig. 2. Cumulative incidence of hepatitis B virus (HBV) reactivation stratified according to different autoimmune diseases. (A) Comparison between rheumatoid arthritis (RA) and other autoimmune inflammatory rheumatic disease (AIIRD). (B) Comparison between six AIIRDs (n>50 for each individual diagnoses), including RA, antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE), Sjogren syndrome and unclassified connective tissue disease (UCTD).

Additionally, major autoimmune inflammatory rheumatic diseases (with over 50 patients per disease in the study, including systemic lupus erythematosus, Sjogren syndrome, antiphospholipid syndrome, vasculitis, and unclassified connective tissue disease) were not associated with higher HBVr risks when compared with RA (Fig. 2B, Supplementary Table 1).

In patients with positive anti-HBc, a positive anti-HBs antibody was associated with a lower risk of HBVr (HR, 0.13; 95% confidence interval, 0.03 to 0.56). No significant associations were observed regarding age, baseline IgG level, different autoimmune diseases, and concomitant steroid use in the Cox proportional hazard model (Supplementary Table 2).

4. Clinical outcome of patients with HBVr

Tables 3 and 4 summarized the patient characteristics of 21 individuals with HBVr. The time to HBVr from the first rituximab ranged from 7 to 144 months (median, 58.5 months), and the median number of accumulated rituximab cycles was 5 (Table 3).

Ten out of 13 patients with hepatitis received nucleoside/nucleotide analogues, either entecavir or tenofovir alafenamide. Among these patients, eight had resolved hepatitis within 3 to 9 months, one had persistent hepatitis, and one died from hepatic failure. The other three patients with hepatitis did not receive nucleoside/nucleotide analogues, but their hepatitis resolved spontaneously within 2 to 3 months after discontinuation of rituximab. In patients who developed HBsAg seroreversion without hepatitis, six did not receive nucleoside/nucleotide analogues and did not develop hepatitis after a median follow-up of 9.2 months from seroreversion (range, 4.3 to 19.2 months) (Table 4).

5. HBVr in patients with negative anti-HBc antibody

Four patients developed hepatitis flare among 494 anti-HBc-negative individuals (patient no. 1-4 in Tables 3 and

Table 3. Demographics, Baseline Characteristics and RTX Exposure of 21 Patients with HBV Reactivation

No	Age, yr* /sex	Diagnosis	Time from autoimmune disease diagnosis to first RTX, yr	Baseline IgG, mg/dL	Baseline ALT, U/L	Anti-HBc (S/CO)†	Steroid daily dose, mg‡	Concomitant immunosuppressant	Time from first RTX to HBV reactivation, mo	Time from last RTX to HBV reactivation, mo	RTX	
											Cycle	Accumulated dose, mg
1	57/F	AON	2.2	823	27	0.50	10	HCQ, CYC	23	3.9	5	5,000
2	46/F	IIM	5.4	1,030	22	0.74	2.5	HCQ	60	1.8	9	9,000
3	69/F	PSS	8.0	1,040	19	0.11	10	CYC, AZA, HCQ	66	4.6	11	11,000
4	59/F	IIM	9.1	972	53	0.19	2.5	MTX	87	5.9	8	8,000
5	35/M	APS	1.6	1,570	44	11.70	26	CYC, HCQ	8	1.5	2	2,000
6	37/F	SLE	9.2	1,590	11	6.10	10	HCQ	76	14.0	5	5,000
7	52/F	SLE	9.1	1,120	11	9.20	2.5	HCQ	57	6.0	8	8,000
8	71/F	SLE	1.3	1,110	20	8.40	15	HCQ	12	3.6	3	3,000
9	56/F	SLE	8.9	1,220	18	18.70	10	HCQ	7	6.5	1	1,000
10	65/F	RA	3.3	1,300	28	7.51	2.5	MTX, SSZ	25	7.3	3	3,000
11	58/F	IIM	0.8	846	58	9.20	15	MTX, HCQ	7	6.3	1	1,000
12	52/F	PSS	6.8	844	11	7.00	2.5	HCQ, colchicine	80	4.0	3	3,000
13	49/F	SLE	8.7	1,350	16	17.50	0	HCQ, colchicine	64	3.2	10	10,000
14	62/F	UCTD	14.2	NA	42	9.60	10	ADA, LEF, HCQ	144	5.4	19	19,000
15	66/F	SLE	22.7	1,760	14	1.83	5	AZA, LEF	70	9.0	6	6,000
16	55/F	APS	6.0	997	15	8.30	5	HCQ	67	6.3	9	9,000
17	65/F	AE	6.2	1,110	36	6.66	2.5	HCQ, CYC	62	3.4	8	8,500
18	68/F	PSS	11.4	1,300	21	1.15	2.5	LEF, HCQ	62	5.1	10	10,000
19	68/M	SSc	7.8	2,710	57	5.60	10	MTX, HCQ	48	33.9	3	3,000
20	75/M	AAV	3.4	1,140	30	3.78	5	HCQ	35	6.7	6	6,000
21	68/F	RA	20.8	1,090	28	8.31	5	LEF, SSZ, HCQ	25	10.6	4	4,000

RTX, rituximab; HBV, hepatitis B virus; IgG, immunoglobulin G; ALT, alanine aminotransferase; anti-HBc, hepatitis B core antibody; S/CO, signal-to-cutoff ratio; F, female; M, male; AON, autoimmune optic neuropathy; HCQ, hydroxychloroquine; CYC, cyclophosphamide; IIM, idiopathic inflammatory myositis; PSS, primary Sjogren syndrome; AZA, azathioprine; MTX, methotrexate; APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SSZ, sulfasalazine; UCTD, unclassified connective tissue diseases; ADA, adalimumab; LEF, leflunomide; AE, autoimmune encephalopathy; SSc, systemic sclerosis; AAV, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis; NA, not available.

*Age at HBV reactivation; †The value of anti-HBc is considered positive if ≥1 S/CO; ‡The dose was calculated as mean daily dose within 1 year before the time of reactivation.

Table 4. Clinical Condition and Outcome of 21 Patients with HBV Reactivation

No	Clinical condition at HBV reactivation					Treatment and outcome after HBV reactivation			
	Peak ALT, U/L	Peak T-bilirubin, mg/dL	HBV DNA, IU/mL	HBeAg	IgG, mg/dL	Antiviral treatment	Clinical course	Outcome	Follow-up time from reactivation, mo
1	1,400	0.92	10,500,000	+	876	ETV	Hepatitis resolved 9 mo after NUC use	Alive	49.2
2	127	1.01	>170,000,000	+	757	TAF	Improved but persistent hepatitis despite NUC use for a year; HBV viral load decreased to 21,300 IU/mL	Alive	12.3
3	1,613	12.20	13,300,000	+	1,100	ETV	Liver decompensation despite NUC use, resulting in morality 3 mo after seroreversion	Dead	3.4
4	279	0.83	123,000,000	+	NA	ETV	Hepatitis resolved 6 mo after NUC use	Alive	43.0
5	147	0.89	>170,000,000	+	862	No	Hepatitis spontaneously resolved	Alive	33.6
6	255	0.76	74	–	1,060	No	Hepatitis spontaneously resolved after 2 mo	Alive	9.3
7	635	1.20	>170,000,000	+	1,140	ETV	Hepatitis resolved 6 mo after NUC use	Alive	17.1
8	748	0.69	3,590,000	–	835	ETV	Hepatitis resolved 9 mo after NUC use	Alive	52.6
9	371	3.20	56,000	–	1,190	ETV	Liver decompensation and hepatitis improved after NUC use for 2 mo	Alive	50.9
10	104	1.20	31,300,000	+	1,290	ETV	Hepatitis resolved 3 mo after NUC. Recurrent episode of hepatitis after 2 mo, but HBV viral load already decreased significantly; the hepatitis spontaneously resolved again 2 mo later	Alive	22.2
11	175	0.80	428,00,000	+	942	No	Hepatitis spontaneously resolved after 3 mo	Alive	13.2
12	114	0.48	70,800,000	+	963	ETV	Hepatitis spontaneously resolved after 2 mo; another episode of hepatitis recurred after 15 mo with persistent elevated HBV viral load, NUC was thus initiated	Alive	26.4
13	432	4.81	15,200,000	+	1,150	TAF	Hepatitis with hepatic decompensation resolved after 3 mo	Alive	5.0
14	21	0.35	261,000,000	+	657	No	No clinical hepatitis	Alive	7.0
15	20	NA	NA	NA	1,510	No	No clinical hepatitis	Alive	4.3
16	23	NA	NA	NA	893	No	No clinical hepatitis	Alive	5.9
17	13	NA	108,000,000	+	742	No	No clinical hepatitis	Alive	8.4
18	16	NA	71,000,000	+	990	No	No clinical hepatitis	Alive	8.0
19	19	0.45	4,100,000	+	NA	ETV	No clinical hepatitis	Alive	2.2
20	15	NA	NA	NA	597	No	No clinical hepatitis	Alive	4.6
21	20	0.74	38,200,000	+	699	ETV	No clinical hepatitis	Alive	13.2

HBV, hepatitis B virus; ALT, alanine aminotransferase; T-bilirubin, total bilirubin; HBeAg, hepatitis B surface antigen; IgG, immunoglobulin G; ETV, entecavir; NUC, nucleoside/nucleotide analogues; TAF, tenofovir alafenamide; NA, not available.

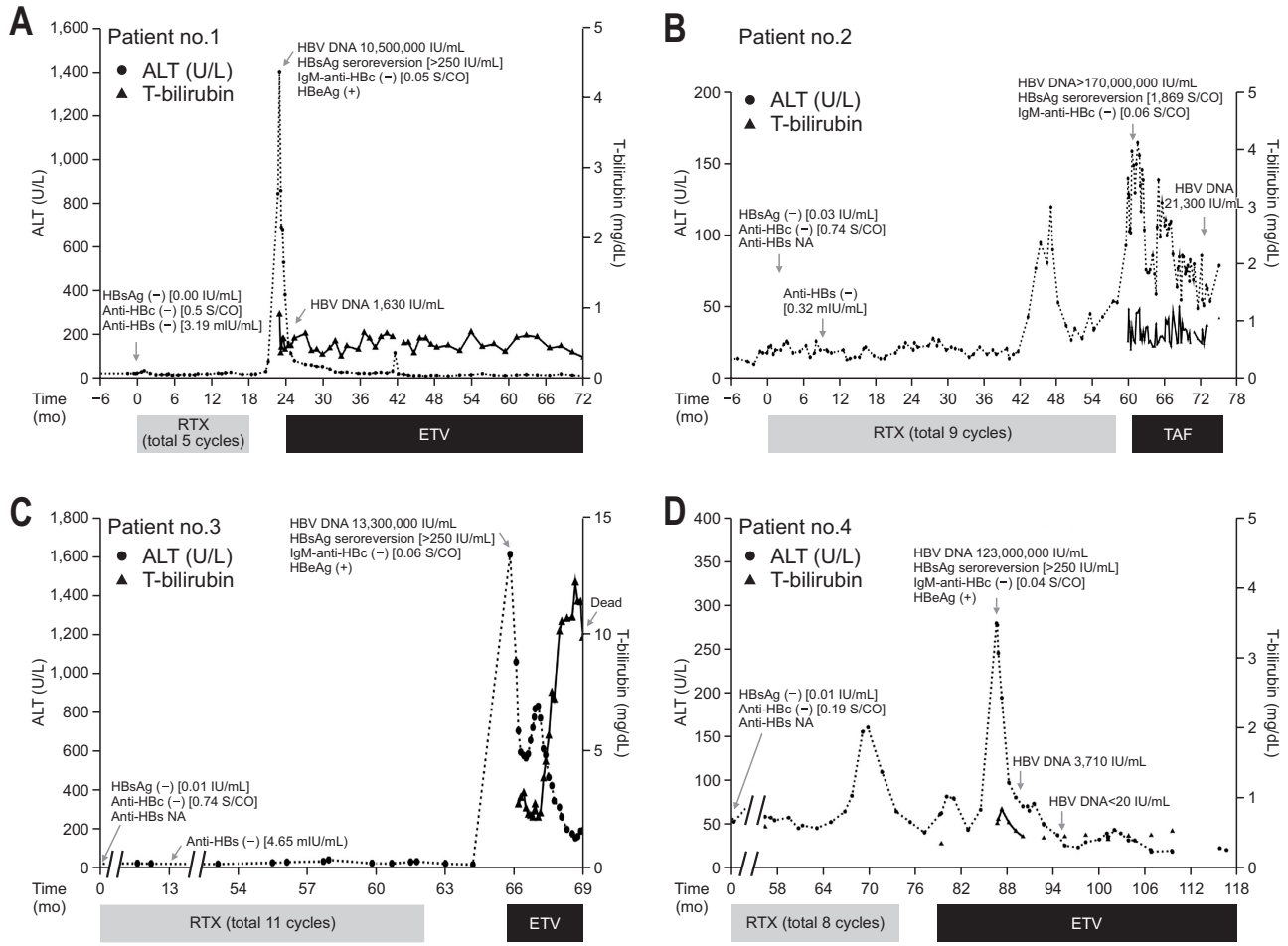

Fig. 3. The clinical course of four hepatitis B core antibody (anti-HBc)-negative patients experiencing hepatitis B virus (HBV) hepatitis flare (patient nos. 1-4 in Table 1). (A) A 57-year-old woman diagnosed with autoimmune optic neuropathy had HBV-associated hepatitis flare after five cycles of rituximab (RTX). (B) A 46-year-old woman with inflammatory myositis; prior to HBV reactivation, there was an episode of alanine aminotransferase (ALT) elevation with spontaneous resolution attributed to her myositis disease flare at that time. (C) A 69-year-old woman with Sjogren syndrome and interstitial lung disease had hepatitis flare and died from hepatic decompensation. (D) A 59-year-old woman with inflammatory myositis also had an episode of ALT elevation attributed to her myositis diseases flare prior to HBV reactivation.
T-bilirubin, total bilirubin; HBsAg, hepatitis B surface antigen; anti-HBs, hepatitis B surface antibody; S/CO, signal-to-cutoff ratio; ETV, entecavir; TAF, tenofovir alafenamide; NA, not available.

4). In addition to a negative IgM-anti-HBc and high viral loads at reactivation, all patients did not have a recent blood transfusion or unprotected sexual behaviors, which suggested that they had HBVr rather than acute HBV infection. The detailed clinical courses of these patients were summarized in Fig. 3.

No predominant clinical features were observed in these four patients, except that they did not receive prior HBV vaccination. Stratified by the vaccination status based on their birth date, anti-HBc-negative patients without HBV vaccination had a higher HBV hepatitis flare rate (4/368, 1.1%) than those with prior vaccination (0/126, 0%).

DISCUSSION

In this study, 21 out of 900 HBsAg-negative patients developed HBVr after rituximab treatment for their autoimmune diseases after a median follow-up of 3.3 years. There were 13 (1.4%) hepatitis flares and eight HBsAg seroreversion without hepatitis. While most of the reactivation developed in patients with positive anti-HBc antibody (n=17), four reactivation events were observed in anti-HBc-negative individuals. Stratified by vaccination status (whether receiving HBV vaccination at birth), anti-HBc-negative patients without vaccination were more likely to have reactivation (4/368) than patients with vaccination (0/126).

Current consensus recommended HBV status assess-

ment before initiation of immunosuppressive therapy to stratify the patients' risk of HBVr. In cases of positive HBsAg or anti-HBc, either initiating antiviral prophylaxis or close monitoring should be provided.[10,18,19] However, our study revealed that patients in HBV endemic areas with negative anti-HBc, who should presumably be naïve to HBV infection, were also at risk of HBVr. This finding is consistent with a prospective study from Japan, which reported three RA patients with negative anti-HBc having HBVr after receiving immunosuppressants.[20] In HBsAg-negative patients, one possible mechanism of HBVr after rituximab treatment is the presence of replication-competent HBV DNA in liver or blood, which is known as the definition of occult HBV infection (OBI). Considering the liver sample is not available in most patients and the fluctuation of serum HBV DNA level, anti-HBc is the commonly used surrogate marker for diagnosing OBI. However, the absence of anti-HBc does not exclude OBI in HBV endemic area.[21] This is supported by epidemiologic studies in Taiwan and Iran, where the OBI is observed in 4.8% to 16% of patients with negative anti-HBc.[22,23] Recent studies also reported that 1% to 20% OBI patients are "seronegative" (negative anti-HBc and negative anti-HBs), which is possibly caused by a progressive loss of HBc and HBs antibodies over time.[21,24,25] In addition, a study in Taiwan showed that universal HBV vaccination at birth decreased OBI prevalence; in anti-HBc-negative patients without vaccination, the OBI prevalence is as high as 4.8%, in contrast to 0% with HBV vaccination.[22] Our data also showed that, the "unvaccinated group" had higher HBVr rate (4/368, 1.1%) compared to that in the vaccinated group (0/126). Therefore, we believe that a certain proportion of anti-HBc-negative patients, especially those without prior vaccination in HBV-endemic area, may have OBI, like the four anti-HBc-negative patients with HBVr in our study. While the definite diagnosis of OBI is sometimes challenging at HBV-endemic area, the risk of HBVr in patients with seronegative OBI should not be overlooked. Regular follow-up of HBV serology should be considered in these patients, when they are going to receive high-risk agents such as rituximab.

Previous studies regrading rituximab-associated HBVr primarily focused on patients with hematologic malignancies and RA, and the data of other autoimmune diseases are scarce.[2,11-13] It was reported that systemic autoimmune diseases, such as SLE, had higher rates of opportunistic infections than RA.[26] A retrospective study also revealed 3/157 (1.9%) anti-HBc-positive patients with SLE experiencing HBsAg seroreversion after receiving immunosuppressants.[27] Our study shows that the HBVr rate is numerically higher in patients with some autoimmune diseases (including SLE, Sjogren syndrome, antiphospholipid syndrome, and vasculitis) compared to RA, but the difference was statistically insignificant (Fig. 2B, Supplementary Table 1).

We found the absence of serum anti-HBs antibody at rituximab initiation was associated with HBVr, consistent with previous reports.[12,13] While hypogammaglobulinemia was reported as a predictor for severe infection in rheumatic patients receiving rituximab, we did not find significant correlations between the baseline IgG and the HBVr.[28] Taking the above findings, it is postulated that the vitality of anti-HBs-secreting plasma cell clones and the serum level of anti-HBV immunoglobulin, as reflected by anti-HBs, might serve as a better indicator of anti-HBV immunity than the overall status of humoral immunity (judged by baseline IgG). Rituximab induces peripheral B cell depletion, which leads to the loss of anti-HBs, might explain the higher HBVr rates in individuals receiving rituximab. However, further translational studies are required to explore this concept.

Although the concurrent steroid use with other biologics increases the risk of HBVr in RA patients with chronic HBV, the impact of steroid in HBsAg-negative patients remains controversial.[29,30] In our study, all patients with HBVr received mean daily steroid dose less than 10 mg, and the concomitant steroid use with rituximab was not associated with HBVr in the Cox regression analysis. Nonetheless, given the variable course and dose change of steroid treatment in rheumatic patients during long follow-up period, the exact effect of concomitant steroid usage was difficult to clarify and was also difficult to analyze with Cox regression model.

There are a few limitations in this study. First, we used the semi-quantitative kit for anti-HBc measurement and the impact of quantitative anti-HBc level on HBVr risk could not be evaluated. In addition, not all of the anti-HBc data were measured at baseline. Nevertheless, there were 196 patients in our study with repeated measurement of their anti-HBc data. Only a small number of patients (9/196, 3.6%) had different anti-HBc status after rituximab treatment, suggesting the qualitative anti-HBc is a stable marker even after rituximab exposure. Secondly, there were some missing data regarding the HBV serology, due to the retrospective study design and the lack of universal monitoring strategy for HBV during study period (from 2006 to 2019). The incidence of HBVr without hepatitis might be underestimated, especially for anti-HBc-negative patients, whose HBV serology was rarely regularly checked. Nonetheless, because the liver function tests were monitored at a 1- to 3-month interval for every patient, events of HBV-associated hepatitis were not likely to be missed. Thirdly,

the HBV viral load at rituximab initiation was not available for most of the patients. Although single HBV DNA measurement is not sufficient to exclude OBI in HBV endemic areas, combining HBV viral load and prior HBV vaccination status may stratify patients' risk better for anti-HBc-negative individuals. Further studies are required to explore this hypothesis. Fourthly, we divided the patient to vaccinated or unvaccinated cohorts by using the national vaccination campaign starting time as the index date. The limitation of this definition is that patient belonging to "unvaccinated cohort" may have HBV vaccination and patients belonging to vaccination cohort might not have received HBV vaccination. Finally, the patients with some rheumatic diagnoses were limited, making detailed analysis and comparison in these patients difficult.

In conclusion, anti-HBc-negative patients, especially those without vaccination at birth, were still at risk of HBVr after receiving rituximab for their autoimmune diseases. Risk stratification based on HBsAg and anti-HBc at rituximab initiation is insufficient to identify all patients at risk in HBV endemic areas, where occult HBV is prevalent. HBVr may still be considered in HBsAg-negative patients developing abnormal liver function during rituximab exposure, even for patients with negative anti-HBc.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Department of Medical Research, National Taiwan University Hospital for the Integrated Medical Database.

AUTHOR CONTRIBUTIONS

Study concept and design: T.Y.L. Data acquisition: T.Y.L., Y.C.L., T.C.T., H.C.Y., S.C.H. Data analysis and interpretation: T.Y.L., Y.C.L., T.C.T., H.C.Y., J.H.K., C.F.C., C.H.L., K.J.L., S.C.H. Drafting of the manuscript: T.Y.L., T.C.T., T.J.L. Critical revision of the manuscript for important intellectual content: T.C.T., H.C.Y., S.C.H. Statistical analysis: T.Y.L. Administrative, technical, or material support; study supervision: S.C.H. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl210551.

REFERNCES

1. Yuen MF, Chen DS, Dusheiko GM, et al. Hepatitis B virus infection. Nat Rev Dis Primers 2018;4:18035.
2. Perrillo RP, Gish R, Falck-Ytter YT. American Gastroenterological Association Institute technical review on prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. Gastroenterology 2015;148:221-244.
3. Fanouriakis A, Kostopoulou M, Alunno A, et al. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. Ann Rheum Dis 2019;78:736-745.
4. Ramos-Casals M, Brito-Zerón P, Bombardieri S, et al. EULAR recommendations for the management of Sjögren's syndrome with topical and systemic therapies. Ann Rheum Dis 2020;79:3-18.
5. Fasano S, Gordon P, Hajji R, Loyo E, Isenberg DA. Rituximab in the treatment of inflammatory myopathies: a review. Rheumatology (Oxford) 2017;56:26-36.
6. Rodríguez-Pintó I, Cervera R, Espinosa G. Rituximab and its therapeutic potential in catastrophic antiphospolipid syndrome. Ther Adv Musculoskelet Dis 2015;7:26-30.
7. Tieu J, Smith R, Basu N, et al. Rituximab for maintenance of remission in ANCA-associated vasculitis: expert consensus guidelines. Rheumatology (Oxford) 2020;59:e24-e32.
8. Smolen JS, Landewé RB, Bijlsma JW, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis 2020;79:685-699.

9. Di Bisceglie AM, Lok AS, Martin P, Terrault N, Perrillo RP, Hoofnagle JH. Recent US Food and Drug Administration warnings on hepatitis B reactivation with immunesuppressing and anticancer drugs: just the tip of the iceberg? Hepatology 2015;61:703-711.
10. Terrault NA, Lok AS, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560-1599.
11. Kuo MH, Tseng CW, Lee CH, Tung CH, Tseng KC, Lai NS. Moderate risk of hepatitis B virus reactivation in HBsAg-/HBcAb+ carriers receiving rituximab for rheumatoid arthritis. Sci Rep 2020;10:2456.
12. Chen YM, Chen HH, Huang WN, et al. Reactivation of hepatitis B virus infection following rituximab treatment in HBsAg-negative, HBcAb-positive rheumatoid arthritis patients: a long-term, real-world observation. Int J Rheum Dis 2019;22:1145-1151.
13. Tien YC, Yen HH, Li CF, et al. Changes in hepatitis B virus surface antibody titer and risk of hepatitis B reactivation in HBsAg-negative/HBcAb-positive patients undergoing biologic therapy for rheumatic diseases: a prospective cohort study. Arthritis Res Ther 2018;20:246.
14. Varisco V, Viganò M, Batticciotto A, et al. Low risk of hepatitis B virus reactivation in HBsAg-negative/anti-HBc-positive carriers receiving rituximab for rheumatoid arthritis: a retrospective multicenter Italian study. J Rheumatol 2016;43:869-874.
15. van Vollenhoven RF, Fleischmann RM, Furst DE, Lacey S, Lehane PB. Longterm safety of rituximab: final report of the Rheumatoid Arthritis Global Clinical Trial Program over 11 years. J Rheumatol 2015;42:1761-1766.
16. Barone M, Notarnicola A, Lopalco G, et al. Safety of long-term biologic therapy in rheumatologic patients with a previously resolved hepatitis B viral infection. Hepatology 2015;62:40-46.
17. Ni YH, Chang MH, Wu JF, Hsu HY, Chen HL, Chen DS. Minimization of hepatitis B infection by a 25-year universal vaccination program. J Hepatol 2012;57:730-735.
18. Loomba R, Liang TJ. Hepatitis B reactivation associated with immune suppressive and biological modifier therapies: current concepts, management strategies, and future directions. Gastroenterology 2017;152:1297-1309.
19. Koutsianas C, Thomas K, Vassilopoulos D. Reactivation of hepatitis B virus infection in rheumatic diseases: risk and management considerations. Ther Adv Musculoskelet Dis 2020;12:1759720X20912646.
20. Fukuda W, Hanyu T, Katayama M, et al. Incidence of hepatitis B virus reactivation in patients with resolved infection on immunosuppressive therapy for rheumatic disease: a multicentre, prospective, observational study in Japan. Ann Rheum Dis 2017;76:1051-1056.
21. Raimondo G, Locarnini S, Pollicino T, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol 2019;71:397-408.
22. Hsu HY, Chang MH, Ni YH, Chiang CL, Wu JF, Chen HL. Universal infant immunization and occult hepatitis B virus infection in children and adolescents: a population-based study. Hepatology 2015;61:1183-1191.
23. Ghaziasadi A, Fakhari Z, Aghcheli B, et al. High prevalence of occult hepatitis B infection (OBI) among healthy children and their parents in Alborz province, Iran; vertical OBI, myth or truth? Liver Int 2020;40:92-100.
24. Hsu HY, Chang MH, Ni YH, et al. Chronologic changes in serum hepatitis B virus DNA, genotypes, surface antigen mutants and reverse transcriptase mutants during 25-year nationwide immunization in Taiwan. J Viral Hepat 2017;24:645-653.
25. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis 2002;2:479-486.
26. Hsu CY, Ko CH, Wang JL, Hsu TC, Lin CY. Comparing the burdens of opportunistic infections among patients with systemic rheumatic diseases: a nationally representative cohort study. Arthritis Res Ther 2019;21:211.
27. Chen HC, Chung CH, Wang CH, et al. Increased risk of sudden sensorineural hearing loss in patients with hepatitis virus infection. PLoS One 2017;12:e0175266.
28. Md Yusof MY, Vital EM, McElvenny DM, et al. Predicting severe infection and effects of hypogammaglobulinemia during therapy with rituximab in rheumatic and musculoskeletal diseases. Arthritis Rheumatol 2019;71:1812-1823.
29. Chen MH, Chen MH, Liu CY, et al. Hepatitis B virus reactivation in rheumatoid arthritis patients undergoing biologics treatment. J Infect Dis 2017;215:566-573.
30. Wong GL, Wong VW, Yuen BW, et al. Risk of hepatitis B surface antigen seroreversion after corticosteroid treatment in patients with previous hepatitis B virus exposure. J Hepatol 2020;72:57-66.