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COVID-19 antibody production by vaccination in chemotherapy with CD20 antibody for B-cell lymphoma

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Abstract. Most hematologic diseases are immunosuppressed, either by the disease itself or by treatment. As such, the implementation of vaccination is largely at the discretion of the attending physician. In this context, an objective measure is needed, therefore the index of vaccination against coronavirus disease 2019 (COVID-19) in B-cell lymphomas treated with antibody therapy against CD20 (including after the completion of therapy) was examined. A total of 40 patients with B-cell lymphoma during or after antibody therapy against CD20 were vaccinated twice with the BNT162b2 messenger RNA (mRNA) COVID-19 vaccine (Pfizer, Inc. and BioNTech SE.) at 3-week intervals and then again six months later with the same vaccine or mRNA-1273 (Moderna, Inc.). Antibody testing was conducted ~1 month after the third vaccination. Analysis was performed using the antibody titers to the anti-spike immunoglobulin assay, with a titer of 0.8 U/ml or higher (considered positive) and a titer of 264 U/ml or higher (considered the value at which the efficacy of the vaccine can be fully expected). Significant factors of antibody acquisition were identified when i) antibody titers were 0.8 U/ml or higher (CD4 ≥400/μl), ii) no anti-CD20 antibody maintenance therapy was undertaken (CD19 ≥100/μl), iii) patients were not on treatment (CD4 ≥400/μl), or 4) at least six months had passed since treatment ended (CD19 ≥100/μl). When antibody titers were 264 U/ml or higher, the treatment method, the stage of the primary disease and other factors related to the condition treatment method of the patient were relevant. When these were analyzed by multivariate analysis, the significant factor when antibody titers were set to 0.8 U/ml was CD19 ≥100/μl. In contrast, when setting them to 264 U/ml or higher, CD4 ≥400/μl was not significant, but there was a tendency for it to be related. The findings of the present study on vaccine-induced antibody acquisition in patients with B-cell lymphoma indicated that it is desirable to have a CD19 titer of at least 100/μl and a CD4 titer of at least 400/μl (both conditions should be met), and that no maintenance therapy with anti-CD20 antibody should be administered for at least six months after the last treatment or completion of the treatment. Interestingly, when the criteria for antibody titers were compared between 0.8 U/ml, where antibody titer is detected, and 264 U/ml, where vaccine efficacy is expected, several key factors were different. It is possible that these key factors may change depending on the antibody titer used as a criterion.

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Introduction

Most hematologic diseases tend to be immunosuppressed, either by the disease itself or by treatment, but comprehensive reports have demonstrated the positive effect of vaccination in hematologic diseases. While the decision to vaccinate is often left to the discretion of the attending physician, there is a lack of scientific evidence to support the decision making. One troubling study has revealed that the severity of blood diseases caused by recent coronavirus disease 2019 (COVID-19) infections is 58.8% and that the mortality rate is 27% (1). Furthermore, in a study analyzing 3,377 cases of hematologic diseases, the mortality rate was 34% in adults and 4% in children, which is a key aspect to bear in mind when considering the prevention of COVID-19 infection and avoidance of severe disease by vaccination (2). It should also be noted that these results are significantly higher than the 11.8% mortality rate from COVID-19 infection in adults reported in a study on 38,517 cases in USA (3) and cannot be overlooked.

On the other hand, the acquisition rate of antibodies against COVID-19 in healthy individuals or in patients with solid tumors is estimated to be >90% after two doses of the COVID-19 vaccine, whereas the antibody acquisition rate for patients with hematological diseases varies from 66-88% (4). Furthermore, the antibody acquisition rate is reported to decrease to 20-50% when rituximab is used, based on the results of 569 patients immunized with a recombinant zoster vaccine (4). It was hypothesized that B-cell lymphoma treated with anti-CD20 antibody would have a lower rate of antibody acquisition after vaccination and that, if so, it would be appropriate as a model of immunodeficiency.

In the present study, the treatment and immune status related to antibody acquisition after the vaccination of patients with B-cell lymphoma treated with anti-CD20 antibody was investigated.

Materials and methods

Patients. The acquisition of antibodies after COVID-19 vaccination in patients with B-cell lymphoma who were attending the Department of Hematology, Hakodate Municipal Hospital (Hakodate, Japan) and were being or had been treated with CD20 antibodies was prospectively evaluated. Enrollment of patients began on June 2021. The target number of patients for enrollment was set to 40, and patients who did not meet the exclusion criteria were enrolled sequentially until the target number of patients was reached.

The primary endpoint is the antibody-positive rate after three doses of COVID-19 vaccine, and the secondary endpoints are: i) The association with liquid and cellular immunity such as CD4, CD 19, IgG, IgA and IgM in COVID-19 vaccine-positive and -negative cases; ii) the association between COVID-19 vaccine-positive cases and the stage and treatment of B-cell lymphoma; iii) a comparison of various factors between antibody titers at the lower limit of detection and those at which the vaccine is expected to be effective; and (iv) adverse events due to vaccination [Common Terminology Criteria for Adverse Events (CTCAE) version 4.0].

The present study included patients with pathologically diagnosed B-cell lymphoma treated with cyclophosphamide, doxorubicin, Oncovin (vincristine) and prednisone (CHOP), a CHOP-like regimen, or bendamustine, plus rituximab or obinutuzumab. Patients on maintenance therapy with rituximab or obinutuzumab once every two to three months or after the completion of therapy were also eligible for enrollment. Excluded from enrollment were patients with a history of anaphylaxis of any kind, thrombosis of any kind, pregnant women and patients who did not wish to receive the COVID-19 vaccine. A total of 26 patients with diffuse large B-cell lymphoma (DLBCL) and 14 patients with follicular lymphoma (FL) were enrolled. Excluded from the analysis was one case who declined COVID-19 vaccination after declaring participation in the trial, one case of recurrence requiring treatment during the course of the trial and one case of COVID-19 infection that did not proceed to the third and subsequent vaccination. Two additional cases were excluded from the analysis due to a lack of laboratory data on immunity (Fig. 1). The protocol was approved (approval no. 2021-61) by the local Ethics Committee of Hakodate Municipal Hospital Institutional Review Board (Hakodate, Japan) on 21 June 2021, and patient enrollment began the following day. Written informed consent based on The Declaration of Helsinki was obtained from all patients.

Vaccination. Vaccinations were administered according to the regulations of the Japanese vaccine program. Patients were vaccinated twice with the BNT162b2 messenger RNA (mRNA) COVID-19 vaccine (Pfizer, Inc. and BioNTech SE) at 3-week intervals and then again six months later with the same vaccine or the mRNA-1273 (Moderna, Inc.) initial vaccination.

Antibody testing. Antibody testing was performed ~30 days after the third vaccination using the antibody titers to the anti-spike (anti-S) immunoglobulin assay (Roche Diagnostics). In accordance with the manufacturer's recommendations, the cut-off index value for seropositivity was 0.8 U/ml and antibody titers higher than 264 U/ml were considered above the limit for a valid antibody (5-7).

Adverse event assessment. Adverse events due to vaccination were self-evaluated by the patients by filling out a form describing the issues and their severity in accordance with the CTCAE v.4.0. Their responses were then tabulated.

Statistical analysis. Subgroups were compared using the Mann-Whitney U test for continuous variables that do not follow normal distribution and Fisher's exact tests for categorical variables. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed with EZR (version 1.61; Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (version 4.20; The R Foundation). After collection, these data were analyzed by logistic regression analysis. Conditional logistic regression analysis for frequency-matched datasets was utilized to estimate the odds ratio and its respective 95% confidence interval.

Results

Patients. After excluding five patients (as aforementioned), analysis was performed on 35 cases, as listed in Table I. The median age was 69 years (range: 46-83 years) and the number of men and women was almost equal. In total, 23 cases were DLBCL, 12 were FL, 23 were stage III-IV and 21 were undergoing treatment. No progression of lymphomas was observed during treatment, and at the end of the present study, 33 cases had obtained complete response and two cases featured recurrence (one DLBCL and one FL). There were 21 cases under treatment at the time of vaccination and 14 cases after completion of treatment. There were 20 cases with a CD4 count of <400/μl at the time of the first vaccination, which was slightly more common, but 18 cases (~50%) had a CD19 count of <100/μl. Regarding immunoglobulin, IgM was detected to be below normal values in numerous cases, but IgG and IgA were above normal values in the majority of the cases.

Analysis of antibody titers and related actors. Antibodies were detected in 19 cases (54.3%) when the lower limit of detection was 0.8 U/ml of the antibodies acquired with the COVID-19 vaccine. Factors associated with antibody positivity were younger age (P=0.02), rituximab use (P=0.035), no maintenance therapy by anti-CD20 antibody (P=0.03), not on lymphoma therapy (P<0.001), after treatment duration of more than six months (P=0.022), CD4 ≥400/μl (P=0.016), CD19 ≥100/μl (P<0.001), both CD4 ≥400/μl and CD19 ≥100/μl (P<0.001), IgA ≥110 mg/dl (P=0.044) and IgM ≥40 mg/dl (P=0.03) (Table II).

Interestingly, at the antibody titer at which the COVID-19 vaccine was expected to be effective, namely, 264 U/ml, effective antibody titers were only obtained in nine cases (25.7%). The following criteria were utilized for antibody

Table I. Characteristics of patients at the time of vaccination.

Clinicopathological characteristics	Value (%)
Total, n	35
Age, years [median, (range)]	69.00 (46-83)
Sex	
Female	17 (48.6)
Male	18 (51.4)
Disease	
DLBCL	23 (65.7)
FL	12 (34.3)
Clinical stage	
I-II	12 (34.3)
III-IV	23 (65.7)
sIL2R [median (U/ml)]	466.00 (229.00-1000.00)
Anti-CD20 antibody	
Obinutuzumab	4 (11.4)
Rituximab	31 (88.6)
Maintenance therapy	
Without	23 (65.7)
With	12 (34.3)
Bendamustine	
Without	21 (60.0)
With	14 (40.0)
Under treatment	
After	14 (40.0)
Ongoing	21 (60.0)
Duration after treatment (months)	
<6	26 (74.3)
>6	9 (25.7)
Third vaccine	
mRNA1273	23 (65.7)
BNT162b2	12 (34.3)
CD4 count (/μl)	
<400	20 (57.1)
>400	15 (42.9)
CD19 count (/μl)	
<100	18 (51.4)
>10	17 (48.6)
IgG (mg/dl)	
<600	5 (14.3)
>600	30 (85.7)
IgA (mg/dl)	
<110	15 (42.9)
>110	20 (57.1)
IgM (mg/dl)	
<40	23 (65.7)
>40	12 (34.3)
Adverse effect due to vaccine	
Grade 0-1	24 (68.6)
>Grade 2	11 (31.4)

Table I. Continued.

Clinicopathological characteristics	Value (%)
Outcome	
CR	33 (94.3)
Relapse	2 (5.7)

DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; sIL2R, soluble interleukin-2 receptor; mo, months; CR, complete response.

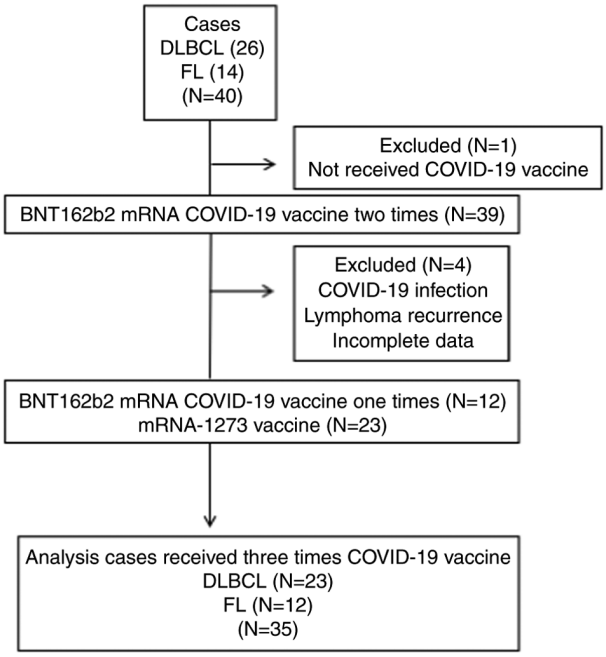

Figure 1. A total of 26 cases of DLBCL and 14 cases of FL were enrolled. Exclusions included one patient who declined COVID-19 vaccination after declaring participation, one patient with recurrent disease, one patient with COVID-19 infection and two patients with insufficient laboratory data. DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; COVID-19, coronavirus disease 2019.

acquisition: DLBCL (P=0.015), clinical stage I-II (P=0.003), no maintenance therapy by anti-CD20 antibody (P=0.015), no bendamustine (P=0.005), not on lymphoma therapy (P<0.001), after treatment duration of more than six months (P<0.001), CD4 ≥400/μl (P=0.002), CD19 ≥100/μl (P<0.001), and both CD4 ≥400/μl and CD19 ≥100/μl (P<0.001). These were identified as the main factors for obtaining effective antibody titers. However, age, type of anti-CD20 antibody, IgG, IgA and IgM were not significantly different, while no bendamustine treatment, DLBCL, and clinical stage I-II were new factors that revealed significant differences (Table III).

Multivariate analysis of the antibody acquisition factors. Multivariate analysis of these factors was performed using logistic regression analysis. After removing confounding factors, three analysis factors for antibody acquisition by vaccine were extracted: i) CD19 ≥100/μl, ii) CD4 ≥400/μl and iii) no maintenance therapy. When antibody titers were set to

Table II. Characteristics of patients (based on antibody titer of 0.8 U/ml).

Clinicopathological characteristics	Antibody titer <0.8 U/ml	Antibody titer >0.8 U/ml	P-value
Total, n	16	19	
Age, years [median (range)]	71.00 (51-83)	63.00 (46-79)	0.02
Sex			0.738
Female, n (%)	7 (20.0)	10 (28.6)	
Male, n (%)	9 (25.7)	9 (25.7)	
Disease			0.09
DLBCL, n (%)	8 (22.9)	15 (42.9)	
FL, n (%)	8 (22.9)	4 (11.4)	
Clinical stage			0.152
I-II, n (%)	3 (8.6)	9 (25.7)	
III-IV, n (%)	13 (37.1)	10 (28.6)	
sIL2R (median: U/ml)	499.50 (229-1000)	466.00 (261-969)	0.83
Anti-CD20 antibody			0.035
Obinutuzumab, n (%)	4 (11.4)	0 (0.0)	
Rituximab, n (%)	12 (34.3)	19 (54.3)	
Maintenance therapy by anti-CD20			0.03
Without, n (%)	7 (20.0)	16 (45.7)	
With, n (%)	9 (25.7)	3 (8.6)	
Bendamustine			0.094
Without, n (%)	7 (20.0)	14 (40.0)	
With, n (%)	9 (25.7)	5 (14.3)	
Treatment of lymphoma			<0.001
After, n (%)	1 (2.9)	13 (37.1)	
Ongoing, n (%)	15 (42.9)	6 (17.1)	
Duration after treatment (mo)			0.022
<6, n (%)	15 (42.9)	11 (31.4)	
>6, n (%)	1 (2.9)	8 (22.9)	
Third vaccine			0.311
mRNA1273, n (%)	9 (25.7)	14 (40.0)	
BNT162b2, n (%)	7 (20.0)	5 (14.3)	
CD4 count (U/μl)			0.016
<400, n (%)	13 (37.1)	7 (20.0)	
>400, n (%)	3 (8.6)	12 (34.3)	
CD19 count (U/μl)			<0.001
<100, n (%)	14 (40.0)	4 (11.4)	
>100, n (%)	2 (5.7)	15 (42.9)	
CD4/CD19 (/μl)			<0.001
<400 or 100, n (%)	16 (45.7)	8 (22.9)	
>400 and 100, n (%)	0 (0.0)	11 (31.4)	
IgG (mg/dl)			0.642
<600, n (%)	3 (8.6)	2 (5.7)	
>600, n (%)	13 (37.1)	17 (48.6)	
IgA (mg/dl)			0.044
<110, n (%)	10 (28.6)	5 (14.3)	
>110, n (%)	6 (17.1)	14 (40.0)	
IgM (mg/dl)			0.03
<40, n (%)	14 (40.0)	9 (25.7)	
>40, n (%)	2 (5.7)	10 (28.6)	

Table II. Continued.

Clinicopathological characteristics	Antibody titer <0.8 U/ml	Antibody titer >0.8 U/ml	P-value
Adverse effect due to vaccine			1
Grade 0-1, n (%)	11 (31.4)	13 (37.1)	
>Grade 2, n (%)	5 (14.3)	6 (17.1)	

DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; sIL2R, soluble interleukin-2 receptor; mo, months.

0.8 U/ml, the significant factor was CD19 ≥100/μl (P=0.008). Although it was not significant at 264 U/ml or higher, there was a tendency for it to be associated with CD4 ≥400/μl (P=0.07) (Table IV).

Adverse events of Grade 2 or higher due to vaccination included chill, fatigue, fever, headache, myalgia, pain at injection point and rash (Table V). Grade 3 rashes were observed in one case of BNT162b2 inoculation while every other case was Grade 2. There was no significant difference in adverse events between BNT162b2 and mRNA1273.

Discussion

As stated in the introduction, the severity rate of COVID-19 infection in hematologic diseases is 58.8% and the fatality rate is ~27%. Risk factors for severe disease include age >70 years, uncontrolled hematologic disease, ECOG 3-4, neutropenia and CRP >20 mg/dl. Analysis of 3,377 patients with hematologic malignancies in a previous study indicated a mortality rate of 34%, especially in patients >60 years, while systemic anticancer therapy did not affect mortality (1,2). These mortality rates are higher than general mortality rates, which suggests that protection against COVID-19 by vaccination is important for hematologic disease cases (3). A previous study on vaccination against hematologic diseases revealed that the antibody acquisition rate of rituximab-treated patients was reduced to 20-50%, compared with 80% for patients who were vaccinated with common hematologic diseases, as a result of the recombinant zoster vaccine. On the other hand, in a small number of cases, COVID-19 vaccination was reported to result in an antibody acquisition rate of 88% in leukemia cases, of 67% in chronic lymphocytic leukemia cases and of 66% in transplant and chimeric antigen receptor T-cell therapy cases (4).

There have been several studies on the acquisition of antibodies by COVID-19 vaccination in B-cell lymphomas, including a study that antibodies were acquired in 89% of patients before rituximab treatment, in 66.7% six months after the end of treatment and in 7.3% during treatment (8). In patients treated with anti-CD20 antibodies, 16 of 17 failed to acquire antibodies. By contrast, 14 of 22 patients acquired antibodies with the COVID-19 vaccine 12 months after the end of treatment (9). It has been reported that during maintenance therapy for B-cell lymphoma with anti-CD20 antibodies, the acquisition rate of antibodies by COVID-19 vaccine was 29.8%, and that it was particularly difficult to acquire antibodies within six months after the last administration of anti-CD20 antibodies (10). According to the findings of the present study, the overall antibody acquisition rate was 54.3% based on an anti-S antibody titer of 0.8 U/ml. Factors including age, no obinutuzumab use, no maintenance therapy for anti-CD20 antibody, no lymphoma treatment, at least six months since completion of treatment, CD4 and CD19 levels and normal levels of IgA and IgM were all important for the acquisition of antibodies. These indices suggested that immunity may be an advantage in acquiring antibodies.

There have been various studies on the factors that determine whether or not antibodies are acquired after COVID-19 vaccination, including CD19 level, CD4 level, time since anti-CD20 antibody treatment, age, CD19 percentage, CD4 percentage, immunoglobulin level and CD4/CD8 ratio, with common factors associated with the success of COVID-19 vaccination including CD19 (regardless of actual number, percentage of lymphocytes), CD4 (regardless of actual number, percentage of lymphocytes) and time since anti-CD20 antibodies treatment (11-15). Notably, a decrease in CD19 is common in all studies and is considered to be a particularly important factor for the success of COVID-19 vaccination.

On the other hand, it has been reported that the antibody titers to the anti-S are important in COVID-19 vaccination and that antibody titers of 264 U/ml or higher are required to control COVID-19 (5-7). The results of the present study were analyzed according to the aforementioned parameters and it was found that there was no difference in antibody acquisition between the BNT162b2 and mRNA1273 vaccines. The acquisition of antibodies by COVID-19 vaccination was also observed to be more likely in patients with CD19 counts of 100/μl or more, CD4 counts of 400/μl or more and six months after treatment with anti-CD20 antibodies, whether based on an anti-S antibody titer of 0.8 or 264 U/ml. Other factors, such as immunoglobulin, IgA and IgM, age and the non-use of obinutuzumab, were significant when the standard value of the anti-S antibody titer was 0.8 U/ml, but not when it was 264 U/ml, which constituted the limit for a valid antibody titer. On the other hand, immunologically unfavorable factors such as the use of bendamustine and the clinical stage resulted in insufficient antibody titers. Multivariate analysis also exhibited that CD19 was a significant factor for the anti-S antibody titer of 0.8 U/ml, but at 264 U/ml, there was no significant factor; however, there is a possibility that it was related to CD4. These findings demonstrated that immune recovery may be important for obtaining antibody titers that enable vaccines to be effective.

Numerous previous studies have utilized 0.8 U/ml as the standard at which the anti-S antibody titer is detected (8,12,16). If an effective antibody titer of 264 U/ml is used, the factors that lead to an effective antibody titer may change (i.e., they

Table III. Characteristics of patients (based on antibody titer of 264 U/ml).

Clinicopathological characteristics	Antibody titer <264 U/ml	Antibody titer >264 U/ml	P-value
N	26	9	
Age, years [median (range)]	71.00 (46-83)	62.00 (51-71)	0.112
Sex			0.264
Female, n (%)	11 (31.4)	6 (17.1)	
Male, n (%)	15 (42.8)	3 (8.5)	
Disease			0.015
DLBCL, n (%)	14 (40.0)	9 (25.7)	
FL, n (%)	12 (34.2)	0 (0.0)	
Clinical stage			0.003
I-II, n (%)	5 (14.2)	7 (20.0)	
III-IV, n (%)	21 (60.0)	2 (5.7)	
sIL2R (median: U/ml)	479.50 (229-1000)	375.00 (261-816)	0.086
Anti-CD20 antibody			0.553
Obinutuzumab, n (n%)	4 (11.4)	0 (0.0)	
Rituximab, n (n%)	22 (62.8)	9 (25.7)	
Maintenance therapy by anti-CD20			0.015
Without, n (%)	14 (0.40)	9 (25.7)	
With, n (%)	12 (34.2)	0 (0.0)	
Bendamustine			0.005
Without, n (%)	12 (34.2)	9 (25.7)	
With, n (%)	14 (40.0)	0 (0.0)	
Treatment of lymphoma			<0.001
After, n (%)	6 (17.1)	8 (22.8)	
Ongoing, n (%)	20 (57.1)	1 (2.8)	
Duration after treatment (mo)			<0.001
<6, n (%)	25 (60.0)	1 (2.8)	
>6, n (%)	1 (2.8)	8 (22.8)	
Third vaccine			0.121
mRNA1273, n (%)	15 (42.8)	8 (22.8)	
BNT162b2, n (%)	11 (31.4)	1 (2.8)	
CD4 count (U/μl)			0.002
<400, n (%)	19 (54.2)	1 (2.8)	
>400, n (%)	7 (20.0)	8 (22.8)	
CD19 count (U/μl)			<0.001
<100, n (%)	18 (51.4)	0 (0.0)	
>100, n (%)	8 (22.8)	9 (25.7)	
CD4/CD19 (U/μl)			<0.001
<400 or 100, n (%)	23 (65.7)	1 (2.8)	
>400 and 100, n (%)	3 (8.6)	8 (22.8)	
IgG (mg/dl)			0.297
<600, n (%)	5 (14.3)	0 (0.0)	
>600, n (%)	21 (60.0)	9 (25.7)	
IgA (mg/dl)			0.244
<110, n (%)	13 (37.1)	2 (5.7)	
>110, n (%)	13 (37.1)	7 (20.0)	
IgM (mg/dl)			0.22
<40, n (%)	19 (54.2)	4 (11.4)	
>40, n (%)	7 (20.0)	5 (14.3)	

Table III. Continued.

Clinicopathological characteristics	Antibody titer <264 U/ml	Antibody titer >264 U/ml	P-value
Adverse effect due to vaccine			1
Grade 0-1, n (%)	18 (51.4)	6 (17.1)	
>Grade 2, n (%)	8 (22.8)	3 (8.5)	

DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; sIL2R, soluble interleukin-2 receptor; mo, months.

Table IV. Logistic regression analysis based on antibody titers of 0.8 U/ml and based on antibody titer of 264 U/ml.

Antibody titer of 0.8 U/ml	Odds ratio	95% confidence interval	P-value
CD 4 (>400 U/μl)	3.8	(0.547, 26.4)	0.177
CD 19 (>100 U/μl)	17.8	(2.07, 152)	0.008
Maintenance therapy	0.874	(0.10, 37.4)	0.901
Antibody titer of 264 U/ml			
CD 4 (>400 U/μl)	2.367	(1.307, 1.811)	0.07
CD 19 (>100 U/μl)	20.191	(5727, 0.004)	0.99
Maintenance therapy	-17.184	(6535, -0.003)	0.99

Table V. Comparison of adverse effects between BNT162b2 and mRNA1273.

	BNT162b2		mRNA1273		
Symptoms	<Grade 1	>Grade 2	<Grade 1	>Grade 2	P-value
Chills	12	0	21	2	0.536
Fatigue	12	0	22	1	1
Fever	12	0	20	3	0.536
Headache	11	1	21	2	1
Myalgia	11	1	20	3	1
Pain (injection point)	10	2	20	3	1
Rash	12	0	18	5	0.141

may be limited or influenced by the treatment method). Further study based on antibody titers may be necessary to interpret the previous studies. In the present study, CD19 was considered a key factor associated with the acquisition of an effective antibody titer, with a highly significant difference between anti-S titers of 0.8 and 264 U/ml. Multivariate analysis also suggested an association between the anti-S titers of 0.8 U/ml and CD19. This has also been shown in studies where CD19 is associated with antibody acquisition at the time of COVID-19 vaccination in CAR-T therapy, in which the actual amount of CD19 is extremely reduced (16,17). The importance of CD19 in COVID-19 vaccination is demonstrated in the present study.

An association between CD4 naïve T cells, especially memory T cells, and COVID-19 vaccination response in immunocompromised and hemodialysis patients has also been reported (18,19). If CD4-positive T cells could be subdivided, the association between antibody titer and CD4-positive T cells may have been further confirmed. However, CD4 >400/μl and CD19 >100/μl were also examined in this analysis, and while 0.8 U/ml was a highly significant factor even when the anti-S antibody titer was detected, the association was more robust when the anti-S titer was 264 U/ml. The multivariate analysis conducted also suggested a relationship between the anti-S titer of 264 U/ml and CD4, but the difference did not reach significance, perhaps due to the small number of cases.

Ishio *et al* (20) examined the acquisition of antibodies after two doses of vaccine for B-cell lymphoma treated with anti-CD20 antibody and observed that antibody production differs depending on the interval between anti-CD20 antibody treatments. They also detected that the antibody titer acquired was lower in cases treated with bendamustine. Liebers *et al* (21) reported that CD19 and CD4 are independent factors that acquire antibodies through vaccines, which appears to agree with the findings of the present study. However,

they also revealed that T cell responses to COVID-19 were observed even in cases that did not seroconvert. A previous study conducted by Nishikubo *et al* (22) revealed that the third vaccine booster can produce a T cell response to COVID-19 regardless of the interval of anti-CD20 antibody treatment, and that regardless of the antibody titer, it was revealed that prevention of COVID-19 can be achieved. In conclusion, for the acquisition of antibodies by vaccination of B-cell lymphomas during or after treatment with anti-CD20 antibodies, a certain amount of CD19 and CD4 is considered necessary (in the present study, CD19 at 100/μl or higher and CD4 at 400/μl or higher, preferably both). In addition, patients with a treatment interval of six months or longer, those who have not received maintenance therapy with anti-CD20 antibodies and those who have completed treatment are more likely to recover from immunosuppression and are expected to acquire antibodies. The antibody titer is also considered an important factor in interpreting the results of vaccination, since the relevant index and its degree differ when the criteria for antibody titer are compared between 0.8 U/ml, the lower limit of detectable antibody titer, and 264 U/ml, the level at which vaccine effect can be expected. However, the present study features only a small number of cases and does not examine the response of T cells to COVID-19, so it is insufficient for a full evaluation of the effectiveness of the vaccine.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to personal information included in the medical record but are available from the corresponding author on reasonable request.

Authors' contributions

YT conceptualized the present study and developed methodology. SI and FH performed software analysis. SI and AM validated data. SI conducted formal analysis and investigation, provided resources and curated data. YT wrote the original draft. TT made substantial contributions to conception and design, acquisition, analysis and interpretation of data wrote, reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. YT and SI confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved (approval no. 2021-61) by the local Ethics Committee of Hakodate Municipal Hospital Institutional Review Board (Hakodate, Japan). Written informed consent was obtained from the patients who participated in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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