

LETTER TO THE EDITOR

Humoral response and safety of the BNT162b2 and mRNA-1273 COVID-19 vaccines in patients with haematological diseases treated with anti-CD20 antibodies: An observational study

In clinical trials, mRNA vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have shown remarkable efficacy and safety in healthy participants.^{1,2} However, patients receiving anti-CD20 antibodies, such as rituximab or obinutuzumab (R/Obi), have been shown to have a poor humoral response to mRNA vaccines, although the response improves gradually if more time has elapsed.^{3,4} R/Obi exerts an immunosuppressive effect through B-cell depletion, which could explain the impaired response to mRNA vaccines. The factors related to vaccine ineffectiveness in patients receiving R/Obi have not been fully investigated. Therefore, this study aimed to assess the effectiveness and safety of COVID-19 mRNA vaccines in patients who

had received R/Obi, and the factors associated with an impaired humoral response.

Patients and healthy volunteers were recruited in June and July 2021. The Institutional Review Board of Kobe City Medical Center General Hospital approved the study. Informed consent was obtained from all participants. Blood samples were collected 14–90 days after the second dose of vaccine. The primary end-point was the proportion of participants who acquired IgG antibodies to the receptor-binding domain in the S1 subunit of the SARS-CoV-2 spike protein (anti-S1 IgG antibodies). We evaluated humoral responses in participants who received R/Obi within less than 6, 6–9, 9–12, or more than 12 months prior to vaccination.⁴ To identify

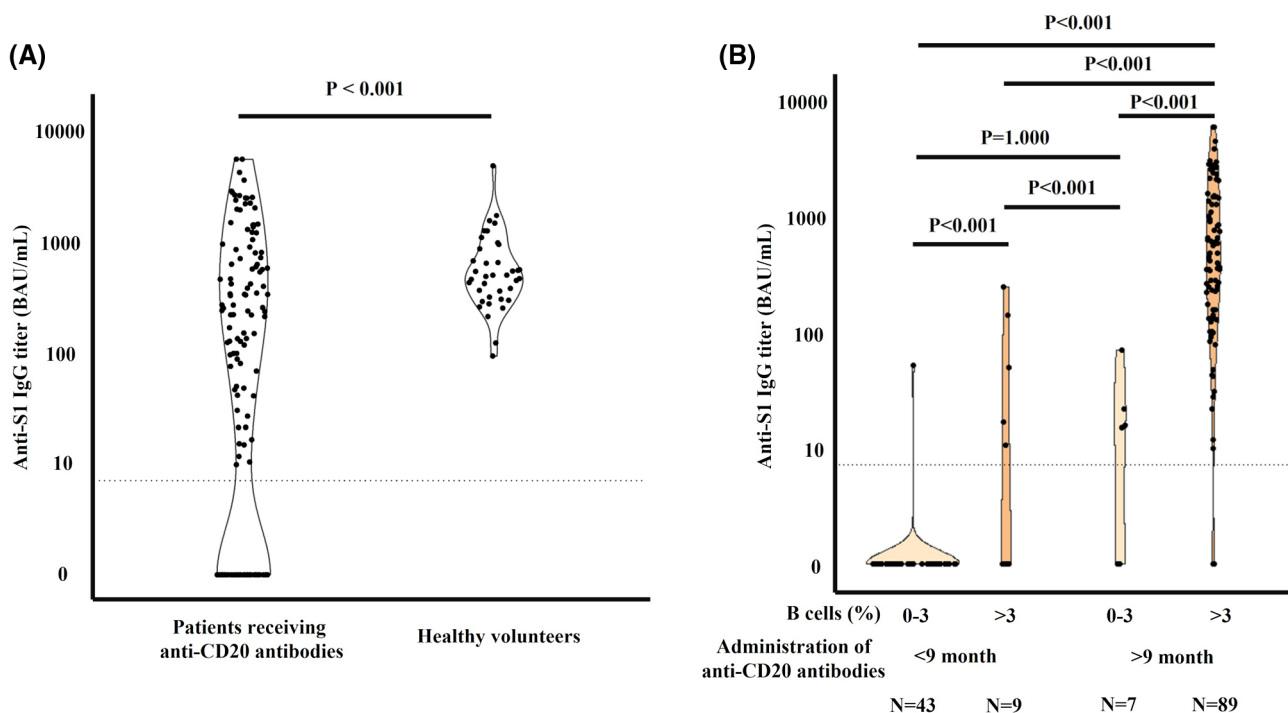


FIGURE 1 (A) Anti-S1 antibody titres in patients who received R/Obi and healthy volunteers. Probability $p < 0.001$ was calculated using either the Mann–Whitney U test or the chi-squared test. (B) Anti-S1 antibody titre in patients ordered in subgroups according to time from last anti-CD20 antibody treatment to vaccination and B-cell fraction. Black dotted lines indicate 7.1 BAU/ml, the cut-off value of serologic reactions according to the manufacturer's instructions. The titre of seronegative patients was 0 AU/ml. The p -values were calculated using the Mann–Whitney U test followed by Bonferroni correction

TABLE 1 Characteristics of study participants

	Patients (N = 148) ^a	Healthy volunteer (N = 38)
Age, median (IQR), years	71 (64, 77)	28 (25, 40)
Sex, n (%)		
Male	71 (48%)	9 (24%)
Female	77 (52%)	29 (76%)
Vaccine ^b , n (%)		
BNT162b2	135 (91%)	38 (100%)
mRNA-1273	13 (8.8%)	0 (0%)
IgG, median (IQR), mg/dl	952 (752, 1184)	1158 (1067, 1386)
>700 mg/dl, n (%)	116 (78%)	38 (100%)
≤700 mg/dl, n (%)	32 (22%)	0 (0%)
IgA, median (IQR), mg/dl	153 (90, 248)	180 (150, 250)
>80 mg/dl, n (%)	117 (79%)	38 (100%)
≤80 mg/dl, n (%)	31 (21%)	0 (0%)
IgM, median (IQR), mg/dl	54 (32, 91)	108 (73, 141)
>40 mg/dl, n (%)	97 (66%)	37 (97%)
≤40 mg/dl, n (%)	51 (34%)	1 (2.6%)
WBC, median (IQR) × 10 ³ /μl	4.7 (3.9, 6.1)	6.156 (5.326, 7.251)
Lymphocytes, median (IQR) × 10 ³ /μl	1.339 (0.936, 1.718)	1.393 (1.164, 1.736)
>1.0 × 10 ³ /μl, n (%)	105 (71%)	34 (89%)
≤1.0 × 10 ³ /μl, n (%)	43 (29%)	4 (11%)
B cells, median (IQR)/μl	138 (0, 266)	151 (113, 212)
B-cell fraction, n (%)		
0%	43 (29%)	0 (0%)
0%–3%	6 (4.0%)	0 (0%)
>3%	99 (67%)	38 (100%)
T cells, median (IQR)/μl	847 (569–1068)	956 (785–1239)
CD4 ⁺ T cells, median (IQR)/μl	378 (224–514)	519 (451–692)
>0.4 × 10 ³ /μl, n (%)	68 (46%)	33 (87%)
≤0.4 × 10 ³ /μl, n (%)	80 (54%)	5 (13%)
NK cells, median (IQR)/μl	210 (122–364)	207 (168–313)
Diseases, n (%)		N/A
DLBCL, NOS	50 (34%)	
FL	49 (33%)	
MALT	11 (7.4%)	
MCL	8 (5.4%)	
LPL/WM	7 (4.7%)	
SMZL	4 (2.7%)	
BCL unclassifiable	4 (2.7%)	

TABLE 1 (Continued)

	Patients (N = 148) ^a	Healthy volunteer (N = 38)
CLL/SLL	3 (2.0%)	
Nodal MZL	2 (1.4%)	
IVLBCL	2 (1.4%)	
BL	1 (0.7%)	
PCNSL	1 (0.7%)	
PMBCL	1 (0.7%)	
TTP	2 (1.4%)	
ITP	1 (0.7%)	
Evans syndrome	1 (0.7%)	
Disease status, n (%)		N/A
Complete remission	107 (72%)	
Not in complete remission	15 (10%)	
On active therapy ^c	26 (18%)	
Time from last anti-CD20 antibody treatment to vaccination, median (IQR), days	658 (122–1488)	N/A
Time from last anti-CD20 antibody treatment to vaccination, n (%)		N/A
<6 months	43 (29%)	
6–9 months	9 (6.1%)	
9–12 months	6 (4.1%)	
>12 months	90 (61%)	
Time from second vaccination to serological assessment, median (IQR), days	44 (29–66)	79 (75–81)

Abbreviations: BCL, B-cell lymphoma; BL, Burkitt lymphoma; CLL, chronic lymphocytic leukaemia; DLBCL, diffuse large B-cell lymphoma, FL, follicular lymphoma; IQR, interquartile range; ITP, idiopathic thrombocytopenic purpura; IVLBCL, intravascular large B-cell lymphoma; LPL, lymphoplasmacytic lymphoma; MALT, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; N/A, not applicable; NK, natural killer; NOS, not otherwise specified; PCNSL, primary central nervous system lymphoma; PMBCL, primary mediastinal large B-cell lymphoma; R/Obi, rituximab or obinutuzumab; SLL, small lymphocytic lymphoma; SMZL, splenic marginal zone lymphoma; TTP, thrombotic thrombocytopenic purpura; WBC; white blood cells; WM, Waldenström's macroglobulinaemia.

^aThree patients were excluded from the final analysis: one did not complete two vaccination doses due to stroke after the first vaccination and two were lost during follow-up due to disease progression.

^bParticipants received two doses of mRNA vaccine as recommended (21 and 28 days apart for BNT162b2 and mRNA-1273, respectively, except two patients who received the second dose of BNT162b2 vaccine after 31 days, and one patient who received the second dose of BNT162b2 vaccine after 37 days.

^cEight patients receiving ongoing R/Obi maintenance therapy were included. None of them exhibited a humoral response or detectable B cells.

factors contributing to higher anti-S1 antibody titres, we investigated lymphocyte subsets including the CD19-positive B-cell fraction. The antibody response in patients whose B-cell fractions of total lymphocytes were 0%, 0%–3% or more than 3% was assessed to estimate the minimal proportion of B cells required to induce a humoral response following B-cell reconstitution.⁵ The inclusion and exclusion criteria, details of the data, kit used for antibody detection, and statistical issues are described in the Supporting Information.

A total of 151 patients with haematological diseases who had received R/Obi, and 38 healthy volunteers were included in the study. Three patients were excluded from the final analysis (Table 1). A positive antibody response to the vaccine was observed in 93/148 patients (64%) and in all 38 healthy controls. The median anti-S1 IgG titre was 98 [interquartile range (IQR) 0–576] and 499 (IQR 311–826) BAU/ml in the patients and controls, respectively. (Figure 1). Among the seropositive patients, 40 (43%) were weak responders (anti-S1 IgG titres < 260 BAU/ml). Based on the results of subgroup analysis (Figures S1 and S2), we set the optimal cut-off at nine months from the last administration of R/Obi and 3% B cells. According to this cut-off, 91.7% of patients receiving R/Obi earlier than in the previous nine months achieved seropositivity, whereas only 11.5% of patients receiving R/Obi within nine months did so. Similarly, 90 patients (89.8%) with more than 3% B cells achieved a seropositive response, while 12% of those with 3% or fewer B cells were seropositive. The combined humoral response to time and B-cell fraction is shown in Figure 1. Of note, patients receiving the last R/Obi within nine months did not exhibit a humoral response even with more than 3% B cells and, conversely, those receiving R/Obi more than nine months earlier but still in a B-cell-depleted state displayed a poor response (Figure 1). Hence, both time from the last R/Obi treatment to vaccination and B-cell reconstitution were important for a humoral response. Details of adverse events and other subgroup analyses are available in the supplementary material (Figures S3 and S4). During the observation period, four cases of symptomatic COVID-19 (one severe and two mild cases in weak and non-responders, and one mild case in a responder) were recorded.

Some guidelines and several prospective studies recommend providing vaccination no earlier than six months after the last administration of B-cell-depleting therapy due to expected impaired humoral response.⁶ The underlying causes of poor humoral response to vaccination in patients with B-cell malignancies are attributed to both disease-related immune dysregulation and therapy-related immunosuppression.⁷ In patients treated with R/Obi, the depletion of B cells, which can last for six months, plays an essential role because B cells drive serologic responses.^{5,7} B-cell reconstitution resumes gradually after approximately 6–9 months, in parallel with the onset of seroconversion.⁵ Our study identified a threshold of 3% B cells as a predictor of the humoral response.

Two findings should be noted. First, patients failed to show a humoral response if the last R/Obi treatment was

within the previous nine months, despite detectable B cells. Second, patients who received their last R/Obi more than nine months earlier, failed to show a humoral response if they did not possess sufficient B cells. Therefore, to accurately estimate vaccine effectiveness in patients receiving R/Obi, a combination of time from the last R/Obi administration and peripheral B-cell fraction (>3%) should be considered.

Administration of a third dose of mRNA vaccine to immunodeficient patients is being promoted in developed countries. However, a single-centre prospective study revealed a low seroconversion rate of only 18.2% in non-responders who had been exposed to rituximab and received a third dose.⁸ In this study, the median interval between the second and third vaccinations was 69 days, which is too short for adequate B-cell recovery.⁸ Therefore, allowing a period of six or nine months before the third dose while implementing strict protective measures, such as wearing masks and social distancing, may benefit the humoral response for non-responders and weak responders who received R/Obi. The use of pre-exposure prophylaxis to the monoclonal tixagevimab–cilgavimab antibody combination was also promising in this group.⁹

This study has several limitations. First, the number of patients was relatively small and regimens used in combination with R/Obi were heterogeneous. Therefore, the potential impact of medications other than R/Obi on the humoral response should be considered. Second, although we excluded participants with reported COVID-19, some participants may have had asymptomatic infections, which might have interfered with the results.¹⁰ Third, we did not assess the cellular immune response.

In summary, we confirmed the safety and the lower effectiveness of mRNA vaccines among patients who received anti-CD20 therapy. A combination of time from the last R/Obi treatment and peripheral B-cell fraction (>3%) can help predict a humoral response to mRNA vaccines.

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CONFLICT OF INTEREST

The authors have no financial or proprietary interest in any material discussed in this article.

AUTHOR CONTRIBUTIONS

Masashi Nishikubo and Yoshimitsu Shimomura initiated the trial, designed the study, and analysed the data. Masashi Nishikubo wrote the manuscript and Yoshimitsu Shimomura supervised the statistical analysis and manuscript writing, editing, and review. Hayato Maruoka, Seiko Nasu, Tomomi Nishioka and Kenji Sakizono performed, managed, and reported the measurements of anti-SARS-CoV-2 antibodies and other related laboratory data. Naoki Okada, Daishi

Nakagawa, Kimimori Kamijo, Hiroharu Imoto, Ryusuke Yamamoto, Yuya Nagai, Nobuhiro Hiramoto, Satoshi Yoshioka, Noboru Yonetani, Akiko Matsushita and Takayuki Ishikawa collected data and provided patient information. Yoshimitsu Shimomura and Takayuki Ishikawa coordinated the project and edited the manuscript. Chisato Miyakoshi provided comments on statistical analysis. Asako Doi provided comments on epidemiology and infection control. All authors have read and approved the final manuscript.

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CLINICAL TRIAL REGISTRATION




This study was registered with the University Hospital Medical Information Network (UMIN) in Japan (UMIN000046043).

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all study participants.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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BNT162b2, COVID-19, haematological disorders, mRNA-1273, obinutuzumab, rituximab

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SUPPORTING INFORMATION

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