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Original Article

Antibody response to SARS-CoV-2 mRNA vaccines in patients with rheumatic diseases in Japan: Interim analysis of a multicentre cohort study

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ABSTRACT

Objectives: To evaluate the impact of medication on antibody response to severe acute respiratory syndrome coronavirus-2 mRNA vaccines in Japanese patients with rheumatic diseases.

Methods: This prospective multicentre cohort study evaluated the humoral response in 12 different medication groups. Antibody levels before the first vaccination and 3–6 weeks after the second vaccination were measured using the Elecsys Anti-SARS-CoV-2 S assay. Statistical analysis included comparing antibody titres among the different medication groups using the Kruskal–Wallis test followed by the Bonferroni–Dunn test and multiple linear regression analysis.

Results: 295 patients were analysed. The seroconversion rate was 92.2% and the median antibody titre was 255 U/ml (interquartile range, 34.1–685) after the second mRNA vaccination. Antibody levels were significantly lower in the groups treated with Tumour necrosis factor inhibitor with methotrexate, abatacept, mycophenolate mofetil (MMF), MMF or mizoribine combined with calcineurin inhibitor, and rituximab or cyclophosphamide compared with those treated with sulfasalazine and/or bucillamine or calcineurin inhibitor ($p < 0.01$). The correlation between antibody titre and treatment was significant after adjusting for age, gender, and glucocorticoid dose ($p < 0.01$).

Conclusions: Additional early vaccination is required in patients treated with Tumour necrosis factor inhibitor and methotrexate, abatacept, MMF, MMF or mizoribine combined with calcineurin inhibitor and rituximab or cyclophosphamide.

KEYWORDS: Rheumatic diseases; COVID-19 vaccines; antirheumatic agents; immunosuppressive agents

Inroduction

Vaccination is one of the most important preventive strategies against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). In December 2021, the vaccination rate was more than 60% in many countries [1], and many patients with rheumatic disease treated with immunosuppressive or antirheumatic agents had already received a vaccination. Most of these are mRNA vaccines and are created using relatively new technology. They have proven efficacy in healthy individuals. However, little information is available about the immunogenicity of mRNA vaccines in patients with rheumatic disease, especially in Asians [2].

The novelty of mRNA vaccines provides a unique opportunity to observe the immune responses of many patients to novel antigens in a real clinical setting. Many types of immunosuppressive agents and anti-rheumatic drugs are used to treat rheumatic diseases. The mechanisms of action are different, and their effects on adaptive immunity are also different in theory. However, it was difficult to interpret the actual effects of drugs on patients‘ adaptive immunity because large numbers of patients were not simultaneously exposed to new antigens. Although influenza and pneumococcal vaccines are administered to patients with rheumatic diseases, they should have been exposed to these antigens throughout their lives. SARS-CoV-2 is a novel human infection, and its protein

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has never elicited an immune response in unaffected individuals. By monitoring the immune response before and after the SARS-CoV-2 vaccination, we observed the actual effect of the drugs on adaptive immunity.

Most previous reports have insufficiently interpreted the effects of drugs because the participants were registered regardless of medication intake and the number of patients receiving specific medications was small [3]. Furer et al. reported the seroconversion rate in 686 patients with rheumatic disease in Israel, but their report included only a small number of cases treated with interleukin-6 inhibitor (IL6i) monotherapy and abatacept (ABT) monotherapy [4]. Moreover, it did not contain a number of conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), such as bucillamin (BUC), iguratimod (IGUR), mizoribine (MZR), and calcineurin inhibitors (CNIs), which are commonly used for rheumatic diseases in Japan and the Asia-Pacific region [4, 5]. In order to resolve these problems, we defined 12 different medication groups in Japanese patients with rheumatic diseases and planned to measure antibody levels before vaccination and 3–6 weeks and 6 months after the second SARS-CoV-2 mRNA vaccination to study its humoral immunogenicity. Here, we report the results of the interim analysis up to 3–6 weeks after the second vaccination.

Materials and methods

Patients

All patients were Japanese outpatients from Kyushu University Beppu Hospital, Kyushu University Hospital, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, and Osaka Minami Medical Center. We defined 12 medication groups according to the inclusion criteria as follows: 1. Sulfasalazine (SSZ) and/or BUC (SSZ/BUC); 2. Methotrexate (MTX); 3. IGUR; 4. Tumour necrosis factor inhibitors (TNFi) with MTX; 5. TNFi without MTX; 6. IL6i without MTX; 7. ABT without MTX; 8. Janus kinase inhibitors (JAKis) without MTX; 9. CNI; 10. Mycophenolate mofetil (MMF); 11. MMF or MZR (MMF/MZR) combined with CNI; and 12. Rituximab (RTX) or cyclophosphamide (CPA) was administered within the previous year (RTX/CPA). The exclusion criteria were glucocorticoids > 10 mg/day (prednisone equivalent), biologics not mentioned in the inclusion criteria, severe anaemia, history of COVID-19, age < 20 years, and an absence of paired serum. Concomitant use of hydroxychloroquine, colchicine, apremilast, SSZ, BUC, and glucocorticoids with a prednisone-equivalent dose of 10 mg/day or less was not excluded in all groups. Concomitant use of immunosuppressive agents other than MTX was not excluded in groups treated with biologic therapy, JAKis, MMF/MZR combined with CNI, or RTX/CPA. Up to 50 patients receiving each treatment and mRNA vaccination were eligible from four institutions.

Immunogenicity of the mRNA vaccine

Serum samples were collected before vaccination and at 3–6 weeks after the second vaccination. Serum samples were stored at −80°C, and antibody titres were measured using Elecsys Anti-SARS-CoV-2 S assays (Roche Diagnostics, Basel, Switzerland) at Kyushu University Hospital. Elecsys anti-SARS-CoV-2 S assays are chemiluminescent immunoassays measuring antibodies against a recombinant protein comprising the receptor-binding domain (RBD) of the S antigen. The quantitative results are interpreted as follows: <0.8 U/mL: negative or ≥0.8 U/mL: positive. The major outcomes of this study were seroconversion rate and antibody titres after the second mRNA vaccination in all patients. This was an exploratory analysis of 12 groups.

This multicentre prospective cohort study was approved by the ethics committee of Kyushu University [#2021-128] and written informed consent was obtained from all patients before inclusion.

Statistical analysis

The results were analysed using Stata Statistical Software Release 14 (StataCorp, College Station, TX, USA). Continuous variables are reported as median (interquartile range [IQR]). Categorical and continuous variables were compared using Fisher's exact test and the Mann-Whitney U test. Antibody titres among the medication groups were compared using the Kruskal–Wallis test followed by the Bonferroni–Dunn test for multiple comparisons. Multiple linear regression analysis was performed to evaluate the effects of treatment on antibody titres after adjustment for sex, age, and glucocorticoid dose, following log (x + 1) data transformation of antibody titres. A quantile-quantile (QQ) plot was used to check the normality of the residuals. Statistical significance was set at $p < 0.05$.

Results

Between May and November 2021, 372 patients were enrolled and 300 paired titres of antibodies were measured before vaccination and 3–6 weeks after the second mRNA vaccination (273 patients were vaccinated with BNT162-2b (Pfizer/Biontech) twice, 6 with mRNA-1273 (Moderna) twice, and 21 with either of the two mRNA vaccines, twice). Patients seropositive before vaccination ($n = 1$), receiving other immunosuppressive therapy of specified treatment ($n = 3$), and affected by COVID-19 ($n = 1$) were excluded. Therefore, 295 patients were included in the analysis.

Patients and major outcomes

Patient characteristics are shown in Table 1. All patients were Japanese, and the median age was 57 (IQR, 48–67). The seroconversion rate of anti-SARS-CoV-2 RBD antibody 3–6 weeks after the second mRNA vaccination was 92.2% (272/295), and the median antibody titre was 255 (IQR, 34.1–685) U/ml in total patients. Medication group, immunologic diagnosis, and glucocorticoid dose were statistically associated with the seroconversion rate ($p < 0.01$; Fisher's exact test). The seroconversion rates were lower in the MMF, MMF/MZR combined with CNI, and RTX/CPA groups (64.3%, 57.9%, and 66.7%, respectively) than in the SASP/BUC group (100%). However, the seroconversion rates were > 90% in the other groups.

Medication group and antibody titres

Details of drugs and demographic characteristics among the medication groups are shown in Supplementary Table 1 and Supplementary Table 2. TNFi with MTX, ABT without MTX, MMF, MMF/MZR combined with CNI, and RTX/CPA group

Table 1. Demographic characteristics of patients and anti-SAR-CoV2 RBD antibody after the second mRNA vaccination.

Characteristics	Total number of patients	Seroconversion		p value	Anti-SARS-CoV2 RBD antibody titer
		Number	Rate (%)		
Gender					
Male	78	74	94.9	0.46	250.5 (64.5–641)
Female	217	198	91.2		255 (24.9–718)
Age					
20–39	36	34	94.4	0.95	572 (67.3–1657)
40–64	169	155	91.7		317 (51.5–800)
≥ 65	90	83	92.2		134.5 (23.8–325)
Race					
Asian	295	–	–		–
Immunologic diagnosis					
Rheumatoid arthritis	176	172	97.7	<0.01	250.5 (51.7–641.5)
Systemic lupus erythematosus	43	33	76.7		122 (2.09–674)
Spondyloarthritis	15	15	100		536 (246–763)
Polymyositis/Dermatomyositis	14	12	85.7		80.7 (13–1954)
Scleroderma	7	6	85.7		31.9 (2.7–481)
Vasculitis	7	4	57.1		19.5 (0–194)
Behçet's disease	7	7	100		288 (111–475)
Mixed connective tissue disease	6	5	83.3		206 (49.8–2159)
Castleman disease	5	4	80		126 (78.2–1124)
Other	15	14	93.3		824 (338–1683)
Medication group					
SSZ and/or BUC	20	20	100	<0.01	831.5 (451–1451.5)
MTX	40	40	100		228.5 (59.5–742.5)
IGUR	11	11	100		457 (245–1170)
TNFi with MTX	42	41	97.6		104 (33.2–260)*
TNFi without MTX	24	24	100		317.5 (169.5–594.5)
IL6i without MTX	43	41	95.4		348 (131–857)
ABT without MTX	21	19	90.5		48.2 (17.9–182)*
JAKi without MTX	14	13	92.9		310.5 (71.2–626)
CNI	38	37	97.4		833 (164–1882)
MMF	14	9	64.3		3.24 (0–34)*
MMF or MZR combined with a CNI	19	11	57.9		5.5 (0–21)*
RTX or CPA in the past year	9	6	66.7		19.5 (0–142)*
Glucocorticoid dose (prednisone equivalent)					
0 mg/day	147	146	99.3	<0.01	317 (104–820)
>0 ≤ 5 mg/day	111	98	88.3		193 (22.4–674)
>5 ≤ 10 mg/day	37	28	75.7		15.5 (2.1–338)

p-values were calculated using Fisher's exact test. The asterisked treatment groups had significantly lower antibody titres than the SSZ/BUC and CNI groups ($p < 0.01$, Kruskal–Wallis test followed by Bonferroni–Dunn test).

patients showed significantly lower antibody titres than those in the SSZ/BUC group and the CNI group ($p < 0.01$) (Figure 1, Supplementary Table 3). Multiple linear regression analysis included the medication groups with significantly higher or lower antibody titre in Bonferroni–Dunn test as variables. Consequently, SSZ/BUC and CNI were correlated with high anti-SARS-CoV-2 RBD antibody, and TNFi with MTX, ABT without MTX, MMF, MMF/MZR combined with CNI, and RTX/CPA were correlated with low anti-SARS-CoV-2 RBD antibody titres after adjusting for age, sex, and glucocorticoid dose ($p < 0.01$) (Table 2). Immunologic diagnoses were excluded from the analysis because they were correlated with the medication groups (Supplementary Table 2). The QQ plot of the residuals was almost linear (Supplementary Figure 1).

Discussion

This is the first large-scale demonstration of mRNA vaccine responsiveness in Asian patients with rheumatic diseases. For an accurate evaluation of the effect of treatments on antibody titre, the background of each group was made as uniform as possible. The absence of a history of COVID-19 was assessed

Table 2. Multiple linear regression analysis for log transformed SARS-CoV2 RBD antibody titers.

Variable	Beta coefficient (95% CI)	*p* value
Gender: female	−0.04 (−0.24 to 0.16)	0.71
Age: per 1-year increase	−0.01 (−0.02 to −0.01)	<0.01
Glucocorticoid dose: per 1 mg/body increase (prednisone equivalent)	−0.08 (−0.12 to −0.04)	<0.01
Medication group		
Other than below	0 (Ref.)	–
SSZ or BUC	0.54 (0.18 to 0.90)	<0.01
CNI	0.43 (0.14 to 0.73)	<0.01
TNFi with MTX	−0.50 (−0.77 to −0.23)	<0.01
ABT without MTX	−0.65 (−1.00 to −0.29)	<0.01
RTX or CPA	−0.80 (−1.35 to −0.25)	<0.01
MMF or MZR combined with a CNI	−1.21 (−1.61 to −0.80)	<0.01
MMF	−1.26 (−1.70 to −0.82)	<0.01

Adjusted R2 = 0.34. All variables are adjusted by each other.

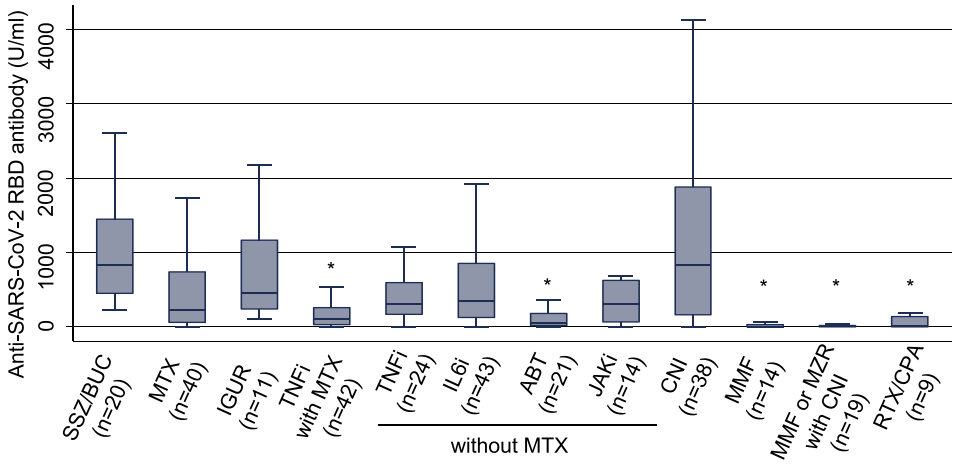

Figure 1. Box plot distribution of anti-SARS-CoV2 RBD antibody titres among medication groups.

The asterisked treatment groups had significantly lower antibody titers than the SSZ/BUC and CNI groups ($p < 0.01$, Kruskal–Wallis test followed by Bonferroni–Dunn test). Data are represented as median and interquartile range.

not only by patient interviews but also by measuring antibody titres before vaccination. Patients treated with high-dose glucocorticoids and belimumab were excluded. Low racial diversity is suitable for assessing purely drug-induced effects. Our study findings demonstrate that drugs elicit different adaptive immune responses in patients with rheumatic diseases, with some keeping the antibody response while others markedly diminishing it. This is an interim study up to 3–6 weeks after the second vaccination, with a third sample scheduled for 6 months after vaccination to be reported in a future study.

CNI is one of the most used drugs for rheumatoid arthritis and other rheumatic diseases in the Asia-Pacific region [4–6]. To the best of our knowledge, this is the first report to examine the effect of CNI on humoral response to the mRNA vaccine in patients with rheumatic diseases. Rozen-Zvi et al. reported lower CNI blood level (less than $\leq$7 ng/ml) was associated with antibody response to SARS-CoV-2 mRNA vaccine among kidney transplant recipients [7]. In our report, the CNI group showed good humoral response; the median dose of tacrolimus was 3 mg/day (IQR, 2–3), and that of cyclosporine was 150 mg/day (IQR, 150–200). These results suggest that low doses of CNI used for rheumatic diseases do not reduce humoral response.

In most studies on seroconversion rates of mRNA vaccination in patients with rheumatic disease, MMF and RTX have been reported to reduce seroconversion rates after the second vaccination [3, 8, 9], which is consistent with the results of our study. ABT has also been reported to reduce the seroconversion rate in two large-scale studies [3, 8], whereas the seroconversion rate remained at 90.5% in our patients. Furer et al. reported a seroconversion rate of 40% (2/5) for ABT with MTX and 71% (5/7) for ABT monotherapy [3]. Inconsistencies in our findings from those in previous reports may be largely owing to the exclusion of patients with concomitant MTX use in our study.

The combination of MZR and CNI was enrolled as part of the same group as the combination of MMF and CNI in the present study, because it is a commonly used multitargeted therapy for lupus nephritis in Japan [10, 11]. Seroconversion rate and antibody titres were 75% and 473.5 U/ml (IQR, 233–1524.5) in patients with MZR and CNI ($n = 4$), and 53.3% and 2.67 U/ml (IQR, 0–16.8) in those with MMF and CNI ($n = 15$), respectively. Although patients with MZR and CNI tended to have lower antibody titres than those with MMF and CNI ($p = 0.054$), the small numbers of patients make evaluation difficult and further studies are needed.

Whether MTX reduces the seroconversion rate remains controversial. Although Haberman et al. reported a decrease in adequate vaccine response to MTX, the adequate response in the study was assessed by the presence of antibody production above a certain level, not by true non-response [12]. Bugatti et al. also reported that MTX was associated with a lower seroconversion rate after the first mRNA vaccination, but this was not evaluated after the second vaccination [13]. On the other hand, in large-scale studies referring to seroconversion rate after the second mRNA vaccination, univariate analysis of seroconversion rates showed significant differences, and these differences disappeared in multivariate analysis [3, 8]. In our study, we also observed a high seroconversion rate in MTX monotherapy after the second vaccination. In the present study, MTX alone failed to reduce the seroconversion rate because of a lower dose of MTX used in Japan than that in other countries. For example, the median MTX dose was 10 mg/week (IQR, 8–12). We also revealed that the antibody titre significantly decreased in patients treated with TNFi in combination with MTX, which was not observed with TNFi alone. MTX in combination with other drugs may further reduce the antibody titre and seroconversion rate after the second mRNA vaccination.

It is not known at what dose corticosteroids actually affect vaccine responsiveness. In many studies included in a systematic review of immunosuppressive agents on the immunogenicity of pneumococcal vaccination, the study design allowed for concomitant corticosteroid use; however, doses were <10 mg/day of prednisolone equivalent in 99% of reported cases [14]. In the present study, high doses of steroids were excluded by exclusion criteria and small doses of steroids were also treated as variables in the multivariate analysis. The results show that even steroids below 10 mg showed a negative correlation in the multivariate analysis, indicat-

ing that a cut-off of 10 mg was not sufficient to exclude the influence on antibody response.

Anti-SARS-CoV-2 RBD antibody titre measured by the Elecsys assay correlates with the effectiveness in preventing viral infections in ex vivo experiments [15], although it remains unclear what level of anti-SARS-CoV-2 RBD antibody titre would be effective in preventing severe disease or disease onset in the general population and in immunosuppressed patients. In a large observational study that included patients receiving immunosuppressive treatment, a third vaccination administered five months after the second vaccination was also reported to reduce the risk of severe illness and death [16]. Even in post-renal transplant patients without antibody response to the second mRNA vaccination, seroconversion was observed in 27–38% after a third mRNA vaccination was received 1 month after the second vaccination [17, 18]. Our results indicate that an early third vaccination is desirable in patients under treatment that significantly reduce antibody titres.

In this observational study, it was not fixed and was decided by the physician and patient whether the drug was temporary ceased or not. Although the American College of Rheumatology recommends withdrawal of immunosuppressive agents for 1–2 weeks after vaccination [19], it is not clear whether this is truly beneficial. Aroujo et al. reported that stopping MTX for 2 weeks after COVID-19 inactivated vaccination increased antibody titres, seroconversion rates, and RA flares [20]. It is theoretically uncontroversial that the temporary cessation of drugs that reduce antibody titres can improve antibody titres to some extent. An important question is what extent these changes improve the clinical prophylactic effect of COVID-19 and outweigh the risk of disease flare. Studies involving a large number of patients are needed to examine this problem.

There are limitations to this study. Foremost, this is an observational study. There may be bias relating to the type of mRNA vaccine used, as more than 90% of vaccinations in this study used BNT162b2, healthy controls were not included, and confounding factors other than sex, age, and glucocorticoid dose have not been considered. Although only cases with negative antibody titres beforehand are included, it is possible that some cases with affected but negative antibody titres due to immunosuppressive therapy may be included. Other factors that may affect the antibody titres include the wide range in timing of blood sampling (3–6 weeks) and the inclusion of heterogenous rheumatic diseases. Additionally, although the Bonferroni–Dunn test is a robust method of analysis, it is difficult to achieve statistical significance. No significant differences were observed in treatments other than those in the five groups outlined previously; however, other study designs may have observed differences in other treatments, especially MTX.

In conclusion, patients with rheumatic disease treated with TNFi and MTX, ABT, MMF, MMF or MZR combined with CNI, and RTX or CPA had significantly lower anti-SARS CoV-2 RBD antibody levels after the second mRNA vaccination. Strict precautions should be taken to prevent infection, even after vaccination, and an early third vaccination is recommended for patients receiving these treatments. Meanwhile, patients treated with CNI or SASP and/or BUC alone may be safer among patients with rheumatic diseases.

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Supplementary data

Supplementary data is available at *Modern Rheumatology* online.

Conflict of interest

None declared.

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