The Journal of Rheumatology 2022;49:644–9
doi:10.3899/jrheum.211152
First Release March 15 2022

The Journal of Rheumatology

Time Since Rituximab Treatment Is Essential for Developing a Humoral Response to COVID-19 mRNA Vaccines in Patients With Rheumatic Diseases

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ABSTRACT. ***Objective.*** We aimed to investigate (1) whether patients with rheumatic disease (RD) treated with rituximab (RTX) raise a serological response toward the coronavirus disease 2019 (COVID-19) mRNA vaccines, and (2) to elucidate the influence of time since the last RTX dose before vaccination on this response.

Methods. We identified and included 201 patients with RDs followed at the outpatient clinic at the Department of Rheumatology, Aarhus University Hospital, who had been treated with RTX in the period 2017–2021 and who had completed their 2-dose vaccination series with a COVID-19 mRNA vaccine. Total antibodies against the SARS-CoV-2 spike protein were measured on all patients and 44 blood donors as reference.

Results. We observed a time-dependent increase in antibody response as the interval from the last RTX treatment to vaccination increased. Only 17.3% of patients developed a detectable antibody response after receiving their vaccination ≤ 6 months after their previous RTX treatment. Positive antibody response increased to 66.7% in patients who had RTX 9–12 months before vaccination. All blood donors (100%) had detectable antibodies after vaccination.

Conclusion. Patients with RDs treated with RTX have a severely impaired serological response toward COVID-19 mRNA vaccines. Our data suggest that the current recommendations of a 6-month interval between RTX treatment and vaccination should be reevaluated.

Key Indexing Terms: autoimmune diseases, rheumatic diseases, vaccination, vaccines

Reports of increased risk of a severe outcome and death from coronavirus disease 2019 (COVID-19) in patients treated with B cell–depleting therapy, such as rituximab (RTX),[1,2,3] have raised particular concern for patients with rheumatic diseases (RDs) receiving this treatment. These data favor prioritizing vaccination of patients treated with RTX.

B cell depletion is an established option in a wide range of RDs, such as rheumatoid arthritis (RA), myositis, and vasculitis. However, B cells are crucial for the induction of protective immunity after vaccination and infection.

Since patients receiving immune-inhibiting medication were largely excluded from COVID-19 phase III vaccine trials, there are limited data on the effectiveness of vaccination in patients treated with RTX. The primary concern about RTX treatment is the risk of reduced immunogenicity to COVID-19 vaccination, similar to what has been observed with the influenza vaccine.[4] However, existing knowledge extrapolated from experiences with other vaccines may not translate to the novel mRNA vaccines available for COVID-19.

In our preliminary data for patients with RD receiving the Pfizer/BioNTech mRNA vaccine, we observed that only 4 out of 17 (23.5%) receiving RTX had measurable antibodies against SARS-CoV-2 after vaccination.[5] These findings have recently been confirmed by several other research groups.[6,7,8]

Although the half-life of RTX is approximately 20 days, the recovery of the B cell count typically starts at 6–9 months after

The study was supported by The Danish Rheumatism Association.

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The authors declare no conflicts of interest relevant to this article.

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Accepted for publication February 9, 2022.

the last treatment, and normal levels are obtained after 9–12 months.[9] Thus, RTX treatment may markedly and enduringly debilitate the humoral response to vaccination, and therefore most rheumatology guidelines recommend waiting for 6 months after RTX infusion before vaccinating.[10]

Concerns about RTX treatment and potentially inadequate vaccine response were aired early in the pandemic,[11] and initial data seem to support this concern.[5,12,13,14] So far, most studies regarding patients with RDs and the COVID-19 vaccines have focused on various treatments and included a relatively small number of patients receiving RTX.

The objectives of this study were to investigate if patients with RDs treated with RTX raise a serological response toward the COVID-19 mRNA vaccines, and to elucidate the influence of time since the last dose of RTX before vaccination on this response.

METHODS

Patients. We identified all patients currently followed at the outpatient clinic at the Department of Rheumatology, Aarhus University Hospital (AUH), who had received RTX treatment since 2017. All patients with a systemic autoimmune RD, who had completed a 2-dose mRNA vaccination series, were eligible for inclusion if they received RTX in the specified period. We identified 308 individuals, of whom 257 had received vaccination with 2 doses of an mRNA vaccine with 3 weeks between the first and the second doses (Supplementary Figure 1, available with the online version of this article). Out of the 257 vaccinated individuals, 201 gave their informed consent to participate in the study and had antibodies measured.

Patients received their vaccines between January and June 2021. All patients followed the same vaccination schedule managed by the Danish National Health Authorities and received 2 doses of an mRNA vaccine with 3 weeks between the first and the second doses.

As a reference for a normal serological response, we included a randomly selected group of blood donors (n = 44) from the AUH Blood Bank who had received 2 doses of a COVID-19 mRNA vaccine.

All included patients were asked at inclusion whether they had tested positive for SARS-CoV-2 prior to vaccination (PCR or rapid antigen test).

SARS-CoV-2 antibody testing. We aimed at measuring antibodies 4–6 weeks after the second vaccination, which is the expected time for the highest antibody levels.

Total serum antibodies against recombinant SARS-CoV-2 spike S1 protein were measured in a commercially available assay (VITROS Anti-SARS-CoV-2 Total Test, Ortho Clinical Diagnostics). According to the manufacturer's instructions, the analyses were performed by experienced staff at the AUH Department of Clinical Microbiology.

The assay is a semiquantitative SARS-CoV-2 double-antigen sandwich chemiluminescent immunoassay. It detects total antibodies captured by recombinant SARS-CoV-2 spike S1 protein coated in a microtiter well. A 1-level calibration is lot-specific and links the sample signal (S) to a cut-off value (CO). Signal/cut-off (S/CO) ≥ 1 was considered positive, and S/CO < 1 was considered negative. Results were based on a single test result.

Performance characteristics of the assay have been determined in a Danish validation study.[15] The assay has a sensitivity of 95.3% (95% CI 90.7–97.7) and a specificity of 100% (95% CI 99.4–100.0). No cross-reactivity was observed.

Statistics. Unless otherwise stated, all values reported are medians with IQR. Univariate and multivariate logistic regression analyses were performed with the presence of SARS-CoV-2 antibodies after vaccination as the dependent variable. We included explanatory variables in the univariate model based on previous knowledge of association to vaccination response (age, sex, diagnosis, and treatment) in order to evaluate the effect of RTX treatment (time from last RTX to vaccination, total RTX dose, total number of RTX infusions, total RTX treatment time). Finally, time from vaccination to blood sample was included, as antibody levels vary over time. We did not correct for multiple hypothesis testing, as this was an exploratory design. We included only explanatory variables in the multivariate model that were significant in the univariate models. Differences in antibody concentration were tested with Mann-Whitney *U* test.

Ethics. The study was approved by the Central Denmark Region Committee on Health Research Ethics (1-10-72-238-21) and the regional Danish Data Protection Agency (1-16-02-254-21). All study participants gave informed consent, and the study was conducted according to the Declaration of Helsinki.

RESULTS

Two hundred one patients (78% of eligible patients) were included in the study. Patients were predominantly female (F/M = 135/66) with a mean age of 58.4 years (SD 14.2). The most frequent diagnosis was antineutrophil cytoplasmic antibody–associated vasculitis (n = 65, 32%), RA (n = 63, 31%), and myositis (n = 28, 14%). Seven patients had tested positive for SARS-CoV-2 prior to vaccination. Patient characteristics can be found in Table 1 and in Supplementary Table 1 (available with the online version of this article). The mean age of blood donors was 42 (SD 12.6) years, and they were predominantly female (F/M = 36/8).

We observed a time-dependent increase in antibody response as the interval from the last RTX treatment to vaccination increased (Figure 1A). Eighty-one patients had received RTX within 6 months prior to vaccination, and only 17.3% of these patients (14/81) had a detectable antibody response. Positive antibody response increased to 66.7% in patients who had RTX 9–12 months before vaccination. This was comparable to patients who had received RTX 18–60 months before vaccination (65.9%, 29/44; Figure 1A). These results were independent of age, sex, diagnosis, cumulative RTX dose, and cumulative RTX treatment time, but were influenced by prednisone dose and azathioprine (AZA) treatment in a multivariable logistic regression analysis (Table 2). All blood donors (100%) had a detectable antibody response after vaccination.

The total antibody concentration (median 111 AU/mL) in patients was significantly lower than the concentration in blood donors (median 916 AU/mL; $P < 0.001$; Figure 1B; Supplementary Table 2, available with the online version of this article). This difference was significant even when the last RTX was given 18–60 months prior to vaccination (123 AU/mL; $P < 0.001$). In addition, the antibody response was lower in all RDs compared to blood donors (Supplementary Figure 2). No association between age and serological response was observed in our cohort (data not shown). The median time between the second vaccination and antibody measurement was 42 (IQR 12–64) days for the patients and 33 (IQR 13–52) days for the blood donors.

DISCUSSION

We report the results of the most extensive single-center study, to our knowledge, on patients with RDs treated with RTX during the COVID-19 pandemic who received a 2-dose vaccination

Table 1. Patient demographics (n = 201).

	Values
Female sex	135 (67)
Age, yrs, mean (SD)	58.4 (14.2)
Previous COVID-19 infection	7 (3.5)
Disease duration, yrs, median (IQR)	7.9 (3.0–8.0)
Diagnosis	
Rheumatoid arthritis	63 (31)
Systemic lupus erythematosus	20 (10)
AAV (GPA/EGPA)	61/4 (32)
Polymyositis/dermatomyositis	28 (14)
Systemic sclerosis	10 (5)
Other RDs	14 (7)
mRNA vaccine used	
Pfizer/BioNTech	195 (97)
Moderna	6 (3)
Time from vaccination to blood sample, d, median (IQR)	42 (12–64)
DMARD treatment	
None	52 (25)
Prednisone	84 (42)
Prednisone dose, mg, median (IQR)	5 (5–10)
Methotrexate	51 (25)
Hydroxychloroquine	22 (11)
Azathioprine	21 (10)
Leflunomide	11 (5)
Mycophenolate mofetil	9 (4)
Ig	6 (3)
Cyclophosphamide	2 (1)
Sulfasalazine	1 (0.5)
Biologic treatment	
None	35 (17)
RTX within last 15 months	145 (72)
TNF inhibitors	6 (3)
JAK inhibitors	6 (3)
IL-6 inhibitors	5 (2)
Abatacept	4 (2)
Previous RTX treatment, median (IQR)	
No. of infusions	5 (2–8)
Cumulative total dose, mg	4000 (2000–8000)
Total treatment time[a], d	505 (30–1532)

Values are expressed as n (%) unless otherwise indicated. [a] Time between the first and last RTX treatment before vaccination. AAV: antineutrophil cytoplasmic antibody–associated vasculitis; COVID-19: coronavirus disease 2019; DMARD: disease-modifying antirheumatic drug; EGPA: eosinophilic granulomatosis with polyangiitis; GPA: granulomatosis with polyangiitis; IL: interleukin; JAK: Janus kinase; RD: rheumatic disease; RTX: rituximab; TNF: tumor necrosis factor.

series with an mRNA COVID-19 vaccine. This study confirms a significantly impaired humoral response to mRNA vaccines in patients receiving RTX. Further, ample time from last RTX infusion to time of vaccination is critical in developing a humoral response after vaccination.

Data from other studies on patients with RDs have also observed lower serological response in patients treated with RTX,[6,7,8,13,14] and similar data have been reported in other diseases where B cell–depleting therapy is used.[16,17] When compared to blood donors, the level of antibodies is generally lower in patients. This is likely due to the immunosuppressive treatment[18]; however, it could also be due to the autoimmune disease itself. In our univariate and multivariate model, both prednisone and AZA seemed to have an effect on the vaccine response.

The specific consequences of reduced antibodies are unknown, as we still lack knowledge of how to accurately measure immunity to SARS-CoV-2 infection, including antibody level, antibody type, and T cell memory. In a recent study, Prendecki et al[12] demonstrate that B cell depletion following RTX impairs serological responses, but T cell responses are preserved. Thus, the absence of a serological response does not preclude T cell–mediated immunity. Whether a secluded T cell response is sufficient to protect patients without a serological response remains to be investigated. However, even though a positive antibody response does not necessarily mean protection against COVID-19 disease, undetectable antibodies after vaccination pose an immediate concern since a serological response against SARS-CoV-2 is essential in the early phases of the infection.[19]

Our findings of an increased serological response 9–12 months after RTX treatment are in line with data showing the recovery of the B cell count in most cases starts at 6–9 months after the last treatment, and normal levels are obtained after 9–12 months.[9]

Bonelli et al observed a humoral immune response in patients with RD with measurable peripheral B cells following RTX treatment.[13] This could pose a method of identifying the patients likely to respond to vaccination, although more extensive studies are needed to confirm these findings.

An important finding in this study is that neither cumulative treatment time nor cumulative RTX dose seemed to influence the serological response to the vaccine. Thus, even in patients who have received RTX for a substantial time, expanding the time since the last RTX treatment could prove beneficial for increasing the chance of a serological response.

The limitations of this study include lack of a quantitative assay to measure antibody serum concentrations. In addition, characterization of memory B and T cells were not available, and neither were data on repopulation of B cells at the time of vaccination. Further, there was a lack of serial measurements of antibodies and knowledge of disease activity in patients at the time of vaccination. Last, matching between patients and controls by age was not possible.

However, to our knowledge, this is the first study to examine a large cohort of patients with RD treated with RTX, and our results indicate that the time since last RTX treatment is a crucial element in mounting a serological response. Our data warrant the development of strategies to increase vaccine-induced immunogenicity in patients treated with RTX. Measuring peripheral B cells at the time of vaccination or identifying patients in whom postponing RTX or changing immunomodulatory therapy is clinically feasible could be strategies to increase immunogenicity.

In conclusion, patients with RDs treated with RTX have a severely impaired serological response toward the COVID-19 mRNA vaccine. This is especially true if the interval between RTX treatment and vaccination is < 9 months.

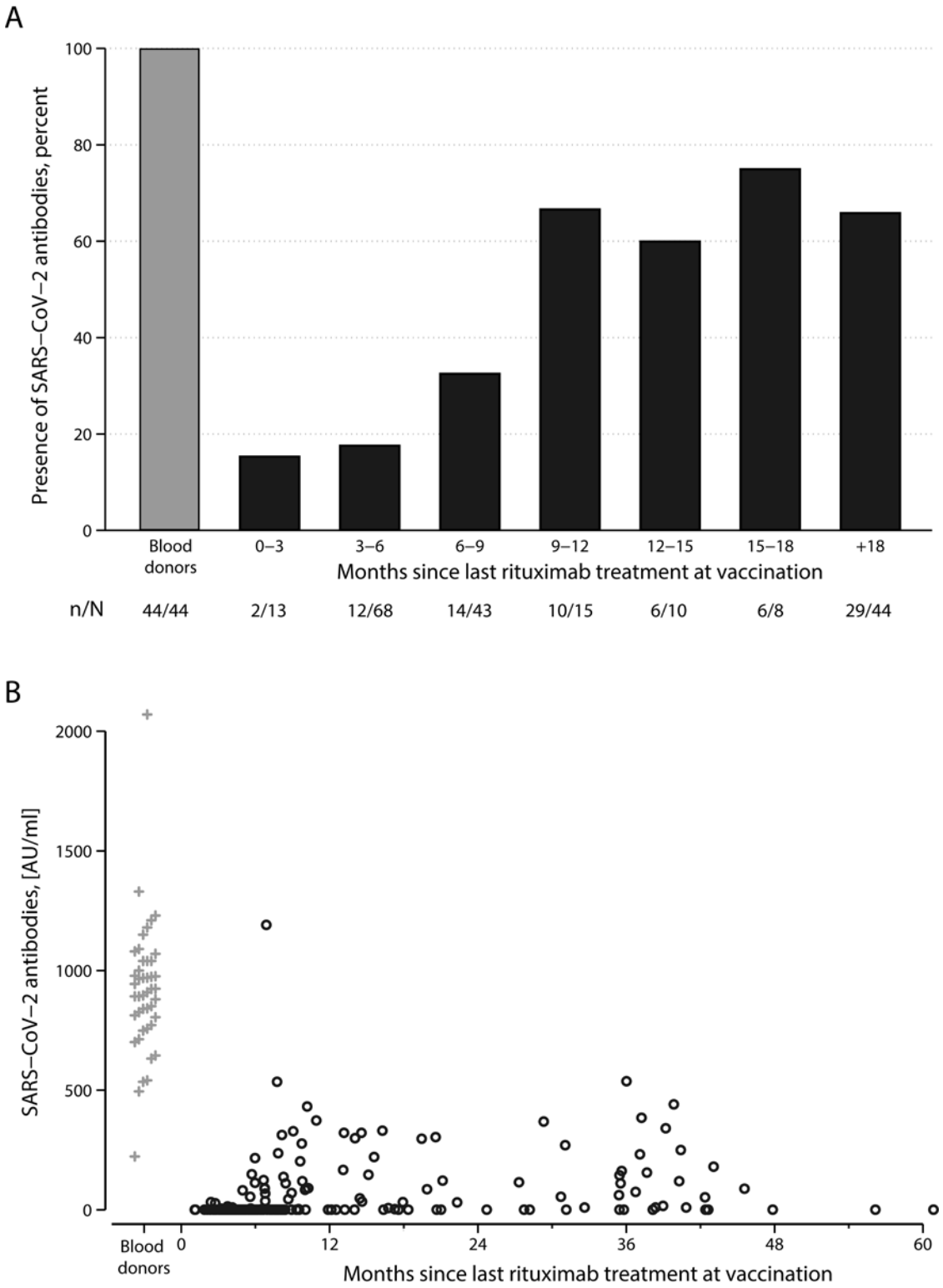

Figure 1. Total SARS-CoV-2 antibody response after receiving 2 vaccinations with COVID-19 mRNA vaccine in patients with rheumatic diseases treated with RTX and controls. (A) Percentage of patients and controls with a measurable antibody response post vaccination. (B) Levels of measured total anti–SARS-CoV-2 antibodies in patients (circles) and controls (crosses). The X-axis shows time since the last treatment with rituximab prior to vaccination. COVID-19: coronavirus disease 2019; n: number of individuals with SARS-CoV-2 antibodies; N: total number of individuals; RTX: rituximab.

Table 2. Univariate logistic regression analysis of antibody response after COVID-19 mRNA vaccination in patients.

	OR	95% CI	*P*
Univariate logistic regression analysis			
Sex, female (ref)	0.91	0.50–1.67	0.77
Age, yrs	0.98	0.96–1.00	0.09
Time from vaccination to blood sample, days	1.00	0.99–1.01	0.61
Diagnosis			
Rheumatoid arthritis	Ref	Ref	Ref
Systemic lupus erythematosus	0.57	0.19–1.69	0.31
AAV (GPA/EGPA)	0.83	0.41–1.69	0.61
Polymyositis/dermatomyositis	0.36	0.13–1.02	0.06
Systemic sclerosis	1.33	0.35–5.07	0.67
Other RDs	2.67	0.82–8.71	0.10
RTX exposure			
RTX treatment within last 15 months	0.21	0.10–0.40	**< 0.001**
Time between last RTX and vaccination, months	1.06	1.03–1.09	**< 0.001**
Total no. of RTX infusions	0.96	0.92–1.01	0.10
Total RTX dose, mg	0.97	0.92–1.02	0.18
Time from first to last RTX treatment, months	1.00	1.00–1.00	0.57
DMARD treatment			
None	0.94	0.50–1.78	0.85
Prednisone, yes/no (no = ref)	0.49	0.27–0.89	**0.02**
Prednisone dose, mg	0.91	0.85–0.99	**0.02**
Methotrexate	1.71	0.90–3.25	0.10
Hydroxychloroquine	0.87	0.35–2.19	0.77
Azathioprine	0.23	0.06–0.80	**0.02**
Multivariate logistic regression analysis			
Time between last RTX and vaccination, months	1.08	1.04–1.11	**< 0.001**
DMARD treatment			
Prednisone dose, mg	0.91	0.84–0.99	**0.03**
Azathioprine	0.10	0.02–0.44	**0.002**

Explanatory variables in the univariate model are based on previous knowledge of association to vaccination response and evaluation of RTX exposure. Specific DMARD treatments were included if ≥ 10% of the patients received it. The multivariate model includes all explanatory variables with statistically significant effects in the univariate analysis. "Time from last RTX treatment to vaccination" and "prednisone treatment" were included as continuous variables. Values in bold are statistically significant. AAV: antineutrophil cytoplasmic antibody–associated vasculitis; COVID-19: coronavirus disease 2019; DMARD: disease-modifying antirheumatic drug; EGPA: eosinophilic granulomatosis with polyangiitis; GPA: granulomatosis with polyangiitis; OR: odds ratio; RD: rheumatic disease; RTX: rituximab.

For the majority of RTX-treated patients, the recommended 6 months since the last RTX treatment is insufficient to develop a humoral response to COVID-19 mRNA vaccines, and our data suggest that the current recommendations of a 6-month interval should be reevaluated.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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