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Title:

Humoral immunogenicity of COVID-19 vaccines in patients with inflammatory rheumatic diseases under treatment with Rituximab: a case-control study (COVID-19VacRTX)

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Key words:

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Rheumatology key messages:

- 44% of the patients treated with rituximab showed no humoral immune response to the COVID-19 vaccines.
- There is a clear relationship between anti-SARS-CoV-2 (S1) IgG antibody level and interval of rituximab infusion and vaccination.
- Parameters of B-cell depletion help to improve synchronization of vaccination and therapy regimen with rituximab.

Abstract

Objectives

Patients with inflammatory rheumatic diseases (IRD) treated with the monoclonal anti-CD20 antibody rituximab (RTX) have been identified as high-risk for severe COVID-19 outcomes. Additionally, there is increased risk due to reduced humoral immune response, induced by therapeutic B-cell depletion. This study sought to quantify humoral response after vaccination against SARS-CoV-2 in patients with IRD treated with RTX. It also sought to elucidate the influence of timeframe between the last RTX dose and the first vaccination or the status of B-cell depletion on antibody titre.

Methods

In this case-control study patients with IRDs previously treated with RTX were examined for humoral immune response after completing the first series of vaccinations with approved vaccines (BNT162b2 (Biontech/Pfizer), RNA-1273 (Moderna), (AstraZeneca/Oxford), Ad26.COV2.S (Janssen/Johnson & Johnson). Antibody levels were quantified using the Euroimmun Anti-SARS-CoV-2 QuantiVac ELISA [EI-S1-IgG-quant]. Blood samples were taken just before the next infusion with RTX after the vaccination. The interval between the last RTX infusion and the first vaccination against SARS-CoV-2 and other possible influencing factors on the antibody levels were evaluated.

Results

102 patients were included. 65 (64%) showed a negative antibody level (<24IE/ml) after the vaccination. The comparative univariate analysis of the antibody levels achieved a significant result (p=0.0008) for the time between last RTX infusion and first vaccination against SARS-CoV-2. No CD19+ peripheral B-cells could be measured in 73 of the patients (72%).

Conclusion

The study confirms the negative impact of RTX on antibody level after vaccination against SARS-CoV-2. A clear relationship exists between antibody titre and interval of the last infusion to the first

vaccination, number of peripheral B-cells and immunoglobulin quantity. These parameters help improve synchronization of vaccination and RTX therapy regimen.

Introduction

The ongoing SARS-CoV-2 pandemic represents a significant challenge in the treatment of patients with inflammatory rheumatic diseases (IRD). Patients with IRD treated with the monoclonal anti-CD20 antibody rituximab (RTX) are considered to be at particularly high risk for severe COVID-19 outcomes.(1) This resulted in a series of research that provides new information about the clinical management of these patients during the pandemic.(2–5) There is currently no sufficient therapy against COVID-19. Generally, the main solution for this problem would be an effective vaccination against SARS-CoV-2.

However, previous studies about other seasonal vaccinations highlight the need for a special approach considering patients who are being treated with RTX. A reduced humoral immune response to influenza and pneumococcal vaccines under B-cell depleting therapy have been noticed.(6) Initial studies that indicate a reduced immune response for vaccination against SARS-CoV-2 during therapy with RTX have been published.(7) Furthermore, RTX induces a selective cell depletion of the CD20-positive B-cell subpopulations. Since B-cells also react to neoantigens, a reduced humoral immune response to vaccinations under therapy with RTX can be assumed.(8) Regarding other disease-modifying anti-rheumatic drugs (DMARDs) such as TNF inhibitors, IL-17- or IL-6 inhibitors, the available studies did show much better humoral immune responses than under therapy with RTX, which is partly due to the normal number of B-cells under these therapies.(9)

When it comes to a method of detection for immunity against SARS-CoV-2, presently, no clear recommendations can be made. Both cellular and humoral immune responses are an indication of the possible immunity after the vaccination. The gold standard for demonstrating humoral immunity is to determine the neutralizing antibodies that can block the uptake of the virus into the cell, for instance, by using a plaque reduction neutralization test (PRNT).(6) Many serological tests, as those used in our study, consist of the detection of non-specific IgG antibodies against the receptor binding domain (RBD) of the spike protein using enzyme-linked immunosorbent assay (ELISA).(10) But this method does not provide direct information about the sterilizing immunity produced by the antibodies.(11) Although, there are initial indications that high levels of neutralizing antibodies against SARS CoV-2 can lead to an effective protection against a symptomatic COVID-19 infection.(12) In addition, a positive correlation between the measured SARS-CoV-2 IgG titre and the neutralizing antibodies after vaccination could already be seen.(12, 13, 7)

Markewitz et al were able to demonstrate a relevant SARS-CoV-2 igG value after vaccination with mRNA vaccines in 531 vaccinees, recruited from healthcare professionals. By using kits by EUROIMMUNE there was an inverse correlation of the IgG level with age. (14) Geisen et al also used the IgG-Test QuantVac (Euroimmun) and showed positive IgG levels in patients

with chronic inflammatory conditions. No patients treated with rituximab were included in this study. (2)

The primary measure of this study was the quantification of the humoral response (IgG antibody levels) after vaccination against SARS-CoV-2 in patients with IRD treated with RTX. Secondly, we sought to discover whether the time frame between the last RTX dose and the first vaccination had an impact on the antibody levels. Moreover, the influence of co-medication and other parameters such as existing IRD, age and B-cell levels and immunoglobulins have been measured. The main intention of evaluation of the parameters is to achieve a better synchronization of vaccinations against SARS-CoV-2 and the therapy regimen with RTX.

Materials and Methods

This study is a case-control study. At the time of the next infusion with RTX after vaccination against SARS-CoV-2, the patient's humoral immune response and other laboratory values were measured. Retrospectively, possible characteristics of the patient were assessed in relation to the presence of a relevant humoral immune response. 102 patients from the academic teaching hospital of the University of Cologne, "Krankenhaus Porz am Rhein gGmbH", in Germany, were included, in the period from January to December 2021. The inclusion criteria were: age ≥18 years, the diagnosis of a IRD, current therapy with rituximab and the fully completed vaccination against SARS-CoV-2 with approved vaccines (BNT162b2 (Biontech/Pfizer), RNA-1273 (Moderna), (AstraZeneca/Oxford), Ad26.COV2.S (Janssen/Johnson & Johnson)). The vaccination itself and the determination of the time of vaccination were not part of this study.

Antibody quantification was carried out as part of routine clinical practice in the infusion clinic. The participants gave their written informed and verbal consent to the evaluation of their data as part of the study. After the patients' declaration of informed consent, the necessary data regarding the rheumatological disease, co-medication and the dates of vaccination against SARS-CoV-2 were collected. Blood samples were taken immediately before the first RTX infusion after the completed vaccination against SARS-CoV-2. The following parameters have been determined: Anti-SARS-CoV-2 antibody level (IgG), differential blood count, CD19+ peripheral B cells, CD20+ peripheral B cells, kidney parameters, liver and inflammation parameters and the quantitative immunoglobulins (IgG, IgM, IgA).

The quantification of anti-SARS-CoV-2 antibodies (IgG) had been determined by the quantitative ELISA (Euroimmun Anti-SARS-CoV-2 QuantiVac enzyme-linked immunosorbent assay [EI-S1-IgG-quant]).(10) The test is calibrated on the "First WHO International Standard positive ≥24IE/ml for anti-SARS-CoV-2 Immunglobulin" (NIBSC code: 20/136), (1IE/ml = 1BAU/ml=1ABU/ml). The maximum of quantitation is 1000IE/ml.

The quantification of CD19+ peripheral B-cells was performed by fluorescence-activated cell sorting (FACS). Lymphocyte subsets were characterised with Klon SJ25C1 (Becton Dickinson). Results were expressed as proportion of CD19+ B-cells among total lymphocytes. The normal range of peripheral B-cells was 7-23%.

Ethical approval for this study was granted by the Ethics Committee of the Medical University of Gießen, Germany (AZ 52/21).

Statistical analysis: categorical variables were reported by absolute and relative frequency. Continuous variables were reported by median and lower and upper quartile. Univariate models were applied to single explanatory variables by logistic regression. From the results of univariate analysis, selected explanatory variables were analysed by a multivariate model (Supplementary Table S1, available at *Rheumatology* online). The data were analysed using SAS 9.4 software (SAS Institute, Cary NC) Graphics and Kaplan-Meier analysis with Log-Rank Tests (R-package "Survival") were performed by the statistical software R-4.1.1. (20) All tests applied were two-tailed. P-values have not been adjusted for multiplicity.

Results

Demographics

102 patients (mean age 65.37 ± 12.08 years, 56% women) were included in the study. Further characteristics of the patients are shown in Table 1. Two of the examined patients had a proven COVID 19 infection prior to vaccination.

Medical therapy

All patients had received at least one cycle (min. 1 cycle, max. 24 cycles) of B-cell depletion therapy with RTX before the vaccination, the mean duration of therapy being 1347 days (SD 1104.4). The dose of the last RTX cycle administered before vaccination was between 500 and 2000 mg (Supplementary Table S2, available at *Rheumatology* online). 81 patients (79%) had a co-medication with prednisolone ≤ 7.5mg, the others had no therapy with prednisolone at the time the antibodies were determined. 23 patients (23%) had concomitant therapy with methotrexate (Table 1). There were no other immunosuppressive co-medications in the group. The univariate analysis of the anti-SARS-CoV-2 antibody titre in relation to co-medication with methotrexate (p=0.9103) or prednisolone (p=0.7345) did not reach statistical significance (Table 2).

COVID-19 vaccines

Regarding the vaccine, most of the patients (N=85, 82%) received 2 doses of BNT162b2 (Biontech / Pfizer). The median of the interval between vaccinations was 27.00 (Q1=21, Q2=42) days. 4 Patients (4%) were vaccinated with 2 doses of RNA-1273 (Moderna), 7 patients (7%) with 2 doses of AZD1222 (AstraZeneca / Oxford), 1 patient with single dose of Ad26.COV2.S (Janssen / Johnson & Johnson). 2 patients (2%) received the first dose of AZD1222 and the second dose of BNT162b2 and 1 patient (2%) received the first does of AZD1222 and the second dose of RNA-1273. A total of 4 patients (4%) received 3 vaccinations (one patient first and second vaccination of BNT162b2 and third with AZD1222, 3 patients received 3 doses of BNT162b2). The time-period between first and second vaccinations are shown in Supplementary Table S3, available at *Rheumatology* online.

Peripheral CD 19+ B-cells

Only 29 patients (28%) showed measurable peripheral CD 19+ B-cells (Supplementary Figure S1, available at *Rheumatology* online). In the univariate analysis there was a significant effect of the level of peripheral B-cells on the SARS-CoV-2 antibodies titre (>=24IE/ml) (p=0.0005) (Table 2). The anti-SARS-CoV-2 level according to the B-cell level is shown in Supplementary Figure S2, available at *Rheumatology* online.

Humoral immune response to vaccination

In total, 65 patients (64%) showed a negative antibody level (<24IE/ml) after vaccination. The anti-SARS-CoV-2 level according to the interval (last RTX infusion and first vaccination) are shown in Supplementary Figure S3, available at *Rheumatology* online. The proportion of a positive humoral immune response was higher in the group of patients with rheumatoid arthritis (41%) than in the group of ANCA-associated vasculitis (26%). In total, the group of negative anti-SARS-CoV-2 antibodies had a higher age (median 66.00, Q1=59.00, Q3=79.00 vs. 60.00, Q1=57.00, Q3=67.00 years) and lower quantitative IgG levels (median 773.00, Q1=661.00, Q3=954.00 vs. 903.00, Q1=731.50, Q3=1066.00 mg/dl). There was a significant effect in the univariate analysis from the quantitative IgG levels (p=0.0142) on the anti-SARS-CoV-2 antibody level (Table 2). A supplementary multivariate analysis is shown in Supplementary Table S1, available at *Rheumatology* online.

Interval of last Rituximab infusion and vaccination

In the group with a negative antibody determination, there was a shorter interval between the last RTX infusion and the date of the first vaccination against SARS-CoV-2 than in the group with a positive humoral immune response (median 5.07 Q1=3.97, Q3=6.10 vs. 6.83, Q1=5.00, Q3=9.13 in month). With regard to the interval, the patients were also divided into a group with an interval ≤ 180 days and a group with >180 days. 180 days (6 months) correspond to the recommended treatment interval in the long-term therapy of rheumatoid arthritis and ANCA-associated vasculitis.

The group with short interval (<6month) contained 48 patients (76%) with negative antibody levels and 15 patients (24%) with positive antibody levels. The group with a long interval contained 17 patients (44%) with negative antibody levels and 22 patients (56%) with positive antibodies. This dichotomization achieved statistical significance with regard to seroconversion in the univariate analysis (p=0.0012).

The comparative univariate analysis of the antibody levels achieved a significant result (p=0.0008) for the time between last rituximab infusion and first vaccination against SARS-CoV-2 (in months) and for the time between last rituximab infusion and second vaccination (p=0.0012). Ultimately, a relevant influence on humoral immunogenicity can be derived from the extension of the time interval between the last RTX administration and the vaccination (Figure 1). Obviously, no difference between the positive and negative B-cell groups are detectable in this analysis (Log-Rank p = 0.56). A relevant difference of the evaluation with regard to the first or the second vaccination cannot currently be determined for patients with and without detectable B cells.

Discussion

For patients with IRD under therapy with DMARDs other than RTX, there is already evidence from current studies regarding an existing humoral immune response to the vaccination against SARS-CoV-2. In comparison to controls without IRDs, patients under DMARD therapy (depending on the exact medication) show either slightly reduced or no reduction of COVID-19 IgG antibody levels.(2, 15) In small cohorts there has already been noted a significantly reduced humoral immune response to the vaccination in this group of patients.(16, 17) In a larger multicentre study with a subgroup of 87 examined patients with IRD under Anti-CD-20 therapy, seroconversion was shown in 36% of the patients treated with RTX in monotherapy and in 41.3% under co-medication with methotrexate. This matches with our data as well. In our cohort positive antibodies could be measured in 35% of the patients under monotherapy and in 65% of the patients under co-medication with methotrexate. Furthermore, Mrak et al. also showed a correlation between the circulating B-cells and the measured antibody levels. In this cohort patients with circulating B-cells also had detectible antibodies (except for one patient).(13) This is not the case in our cohort, in which 10 patients (35%) with measurable CD19+ B-cells did not show seroconversion (Supplementary Figures S1 and S2, available at *Rheumatology* online). The last-mentioned study also shows a good agreement with our data with regard to antibody levels and its correlation with the time frame between the last infusion with RTX and the date of vaccination. (13) Fuhrer et al. also showed a connection between the distance to the last RTX therapy and a seroconversion of the examined patients.(6) In our study, which is closely related to everyday clinical practice, we were able to confirm the influence of time elapsed between last infusion of RTX on vaccination with the amount of antibody response to it. There is also a significant effect of the measured B-cell levels on the immunogenicity after vaccination against COVID-19. But there are also

patients without measurable CD19+ B-cells, who show positive anti-SARS-CoV-2 antibodies and we also see patients with measurable CD19+ B-cells, who do not show positive anti-SARS-CoV-2 IgG.

Depending on the possible time of vaccination and disease activity, the interval of the RTX infusion can be extended in certain cases. A vaccination that was administered in a short interval after the last RTX therapy has an increased risk of an inadequate humoral immune response. Since the measured antibodies show a good correlation to the neutralizing antibodies(18, 7), the measuring method of anti-SARS-CoV-2 antibodies (IgG) could represent a realistic component in the therapy planning of our patients. These results could also be important regarding the possible benefits of further vaccination against SARS-CoV-2. An important factor for assessing a precise meaning of antibody levels, will be the topic of further research about COVID-19 protection. In addition, it should be noted that a certain proportion of patients who do not show any relevant antibody levels can be assumed to have cellular immunity.(7)

The limitations of this study include the number of included patients and the lack of information regarding the cellular immune response and comorbidities of the patients. Further examinations with a larger examination group as well as long-term examinations are necessary. It remains to be seen which constellation of the humoral and cellular immune response is associated with the best protection against a severe course of a COVID-19 infection. In addition, our study design as a case-control study means that there is no constant interval between the completed vaccination and the antibody measurement, which makes it difficult to compare the data. Information on the different effects of RTX that inhibits mostly circulatory B-cells, and less the ones located in other compartments such as the bone marrow or synovium, would also be of interest. (19)

In summary this study confirms the negative impact of therapy with RTX on antibody level after vaccination against SARS-CoV-2. A clear relationship has been noticed between the antibody titre and the interval of the last infusion to the first vaccination, the number of peripheral B-cells and the quantification of immunoglobulins (IgG). These parameters could be helpful for better synchronization of vaccination and therapy regimen with rituximab.

Disclosure statement: The authors have declared no conflicts of interest.

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Data availability: All the primary data and evaluations on which this study is based are accessible to the investigators at any time and are archived for at least 10 years.

Tables and Figures

Table 1. Patients characteristics

n	**102**
Age (mean) (SD)	**65,37 (12.08)**
Gender: woman, n (%)	**57 (56)**
Diagnosis, n (%)	
Rheumatoid arthritis	**66 (65)**
ANCA-associated vasculitis	**27 (26)**
Jo-1 syndrome	**3 (3)**
Polymyositis	**2 (2)**
Felty's syndrome	**2 (2)**
Sjögren's syndrome	**1 (1)**
IgG4-related disease	**1 (1)**
Co-medication, n (%)	
Prednisolone ≤7.5mg	**81 (79)**
Methotrexate	**23 (23)**
CD19+ peripheral B-cells, n, (%)	
0	**73 (72)**
1-14	**29 (28)**

Co-medication, prednisolone ≤7.5mg: 74% of the included patients had a long-term therapy with 7.5 mg prednisolone p.o. or less, 26% of the patients did not have any prednisolone in therapy; Co-medication, Methotrexate: The dose was between 5 and 20 mg, form of application was not taken into account

Table 2. Univariate analysis of the anti-SARS-CoV-2 (S1) IgG antibody +/- (positive: ≥24IE/ml, negative <24IE/ml)

Effect	Odds Ratio	Lower 95% confidence limit of Odds Ratio	Upper 95% confidence limit of Odds Ratio	p-value
Age	0.961	0.927	0.996	0.0285
Diagnosis: Rheumatoid arthritis vs. ANCA-associated vasculitis	0.506	0.188	1.362	0.1772
Interval: last RTX infusion – first vaccination	1.503	1.184	1.908	0.0008
Interval: last RTX infusion –second vaccination	1.432	1.153	1.779	0.0012
Cumulative lifetime RTX dose	1.000	1.000	1.000	0.7370
Dose of Methotrexate	0.996	0.923	1.074	0.9103
Dose of Prednisolone	1.034	0.853	1.253	0.7345
CD19+ peripheral B-cell level	1.987	1.347	2.932	0.0005
CD19+ peripheral B-cells +/-	5.102	2.017	12.904	0.0006
IgG-level	1.002	1.000	1.004	0.0142

Age (years): was calculated using the date of birth and the date of the next RTX infusion after vaccination; **interval of last RTX infusion and first vaccination (months):** calculation from the date of the last infusion of the last cycle of RTX therapy and the date of the first vaccination against SARS-CoV-2; **Interval of last RTX infusion and second vaccination:** calculation from the date of the last infusion of the last cycle of RTX therapy and the date of the second vaccination against SARS-CoV-2; **cumulative lifetime RTX dose (mg):** calculated from the duration of therapy with RTX and dose for each cycle; **dose of Methotrexate (mg):** was defined based on the medical history of the patients for the date the vaccination series against SARS-CoV-2 started, form of application was not taken into account; **dose of Prednisolone (mg):** was defined based on the medical history of the patients for the date the vaccination series against SARS-CoV-2 started, application form was oral in all patients; **CD19+ peripheral B-cell level (%):** measurement took place immediately before the intravenous administration of the next therapy with RTX after vaccination against SARS-CoV-2; **CD19+ peripheral B-cells +/-:** it was only recorded whether or not B cells were present; **IgG-level (mg/dl):** measurement took place immediately before the intravenous administration of the next therapy with RTX after vaccination against SARS-CoV-2

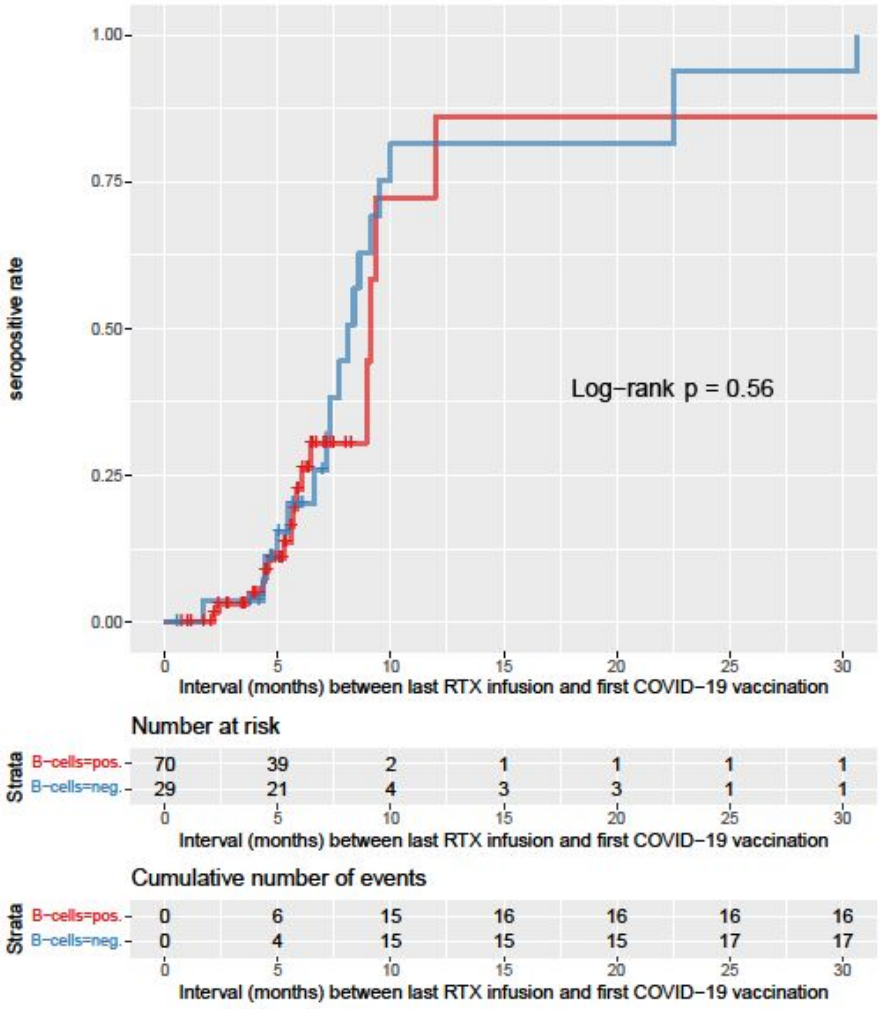

Figure 1: Cumulative seropositive rate according to the interval between the last RTX infusion and the first vaccination

Excerpt from the graphic up to month 30, one patient at month 60. **Red:** seropositive rate of patients with a measurable proportion of B-cells at the time of the first infusion after vaccination; **blue:** seropositive rate of patients with no measurable proportion of B-cells at the time of the first infusion after vaccination; **number at risk:** number of patients under observation at the respective interval; **cumulative number of events:** number of patients in whom positive SARS-COV-2 antibodies were found at the respective interval; **Log-Rank p = 0.56:** no significant difference between B-cell= pos. and B-cell=neg. can be found

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