Seroconversion after a third COVID-19 vaccine is affected by rituximab dose but persistence is not in patients with rheumatoid arthritis

Céleste JT van der Togt [1,2], David F Ten Cate [2], Bart JF van den Bemt [3,4], Janette Rahamat-Langendoen[5], Nathan den Broeder [1,2], and Alfons A den Broeder [2,6].

[1]Radboud Institute for Health Sciences, Department of Rheumatology, Radboud university medical center, Nijmegen, The Netherlands. [2]Department of Rheumatology, Sint Maartenskliniek, Ubbergen, The Netherlands. [3]Department of Pharmacy, Sint Maartenskliniek, Ubbergen, The Netherlands. [4]Department of Clinical Pharmacy, Radboudumc, Nijmegen, The Netherlands. [5]Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands. [6]Department of Rheumatic Diseases, Radboudumc, Nijmegen, The Netherlands.

Correspondence to: Céleste van der Togt, MD, Sint Maartenskliniek, department of Rheumatology, PO Box 9011, 6500 GM Nijmegen, The Netherlands. E-mail: c.vandertogt@maartenskliniek.nl

Abstract

Objectives. In patients with rheumatoid arthritis (RA) treated with (ultra-)low dose rituximab (RTX), we investigated (1) the association of dosing and timing of rituximab (RTX) on seroconversion after third COVID-19 vaccination, and (2) persistence of humoral response after two-dose vaccination.

Methods. In this monocentre observational study, patients from the COVAC-cohort were included in the third vaccine analysis if humoral response was obtained 2-6 weeks after third vaccination in previous non-responders, and in the persistence analysis if a follow-up humoral response was obtained before third vaccination in previous responders. Dichotomization between 'positive' and 'negative' response was based on the assay cut-off. The association between latest RTX dose before first vaccination, timing between latest rituximab and vaccination, and response was analysed with univariable logistic regression.

Results. Of the 196 patients in the cohort, 98 were included in the third vaccine analysis and 23 in the persistence analysis. Third vaccination response was 19/98 (19%) and higher for 200 mg RTX users (5/13, 38%) than 500 and 1000 mg (7/37, 19% and 7/48, 15%). Non-significant trends were seen for higher response with lower dosing (200 versus 1000 mg: OR 3.66, 95% CI 0.93-14.0) and later timing (per month since infusion: OR 1.16, 0.97-1.35). Humoral response persisted in 96% (22/23) and in 89% (8/9) of patients who received RTX between the two measurements.

Conclusion. Repeated vaccination as late as possible after the lowest RTX dose possible seems the best vaccination strategy. A once positive humoral response after COVID-19 vaccination persists irrespective of intercurrent rituximab infusion.

Trial registration. Netherlands Trial Register, https://www.trialregister.nl/, NL9342

Key words: rheumatoid arthritis, rituximab, biologic therapies, COVID-19, vaccination

Key messages:

- Approximately 20% of rheumatoid arthritis patients treated with rituximab seroconverted after third COVID-19 vaccination.
- Seroconversion numbers were higher for patients treated with 200 mg rituximab.
- Persistence of humoral response was high, irrespective of intercurrent rituximab infusion.

Introduction

The coronavirus disease-19 (COVID-19) pandemic has led to large numbers of COVID-19-related hospitalizations and death. Several vaccines against COVID-19 are available, which have shown to induce humoral and cellular response and to reduce the risk and severity of COVID-19. Rheumatoid arthritis (RA) patients treated with rituximab (RTX) have both an increased risk of COVID-19 hospitalization[1] and a reduced humoral response after two-dose vaccination,[2] when compared to other disease modifying antirheumatic drugs (DMARDs). Therefore, to optimise management of COVID-19 risk in these patients, it is important to identify strategies for increasing response in this population.

Previously, we demonstrated that both use of an ultra-low dose of 200 mg RTX and a longer time between latest RTX and vaccination are associated with positive humoral response after two-dose vaccination, with the effect of timing also confirmed by a recent meta-analysis.[3,4] Now that (at least) a third dose vaccination has been advised for these patients, it is of interest whether these factors also positively influence humoral response after follow-up COVID-19 vaccines. Previous studies found a seroconversion in approximately 20% of patients, but mostly included patients treated with registered dose RTX (≥1000mg).[5-7]

Additionally, there is scarce data on humoral response persistence in RA patients treated with rituximab. So far, persistence of humoral response after two-dose vaccination was investigated in one study, which found a persistence rate of 88% after 6 months in a population with a median dose of 1000 mg RTX.[7]

Therefore, we aimed to investigate (1) the association of dosing and timing of RTX on humoral response after three dose vaccination in previous non-responders, and (2) the persistence of an initial positive humoral response after two dosages of the COVID-19 vaccination in RA patients treated with (ultra-)low dose RTX.

Methods

Study design and participants

This is a follow-up study of the RTX-COVAC cohort in which we demonstrated that the humoral response after two-dose COVID-19 vaccination in rheumatoid arthritis patients treated with (ultra-) low dose rituximab is dependent on both dosage and timing.[4] In the current study, the first aim was to investigate the efficacy of a third vaccine and the second to investigate persistence of response after two-dose COVID-19 vaccination. Patients were included in the first analysis if they had a negative humoral response after two doses, had received a third COVID-19 vaccination and had drawn a blood sample 2-6 weeks thereafter ('third dose sample'). Patients were included in the second analysis if they had a previous positive humoral response and have drawn a blood sample ≥ 6 weeks after second SARS-CoV-2 vaccination but before the third vaccination ('persistence sample'). All participants provided written informed consent. This study was registered at the Netherlands trial register (www.trialregister.nl, NL9342). The follow-up study took place from June 2021 to January 2022 in the Sint Maartenskliniek, Nijmegen, the Netherlands and was approved by the local ethics committee (CMO Arnhem-Nijmegen, 2021-7406).

Procedures

All participants received their COVID-19 vaccinations through the Dutch national vaccine programme. For the first two vaccinations, patients in the cohort either received BNT162b2 (Comirnaty; Pfizer-

BioNtech), ChAdOx1 nCoV-19 (Vaxzevria; AstraZeneca), or mRNA-1273 (Spikevax; Moderna). For the third vaccination, only the mRNA vaccines BNT162b2 and mRNA-1273 were approved for RTX patients in the Netherlands. Relevant demographics and RA disease characteristics were obtained at baseline. Also, we recorded relevant treatment characteristics including concomitant conventional synthetic DMARD (csDMARD) use, prednisolone use, current biological/targeted synthetic DMARD (b/tsDMARD), cumulative RTX dose, and dosage and date of the latest RTX administration. Details on a previous COVID-19 infection (including date of positive test, and dichotomized between before or after second vaccination) and COVID-19 vaccination dates were provided by the participant. The 'persistence samples' were evaluated using the Wantai SARS-CoV-2 Ab assay measuring ratio of total immunoglobulin (IgT) with a cut-off of positive (≥ 1.1).[8] Most samples of the 'third dose sample' were evaluated with the prementioned assay, however, not all patients were able to visit our clinic during the established time frame. Therefore, humoral response measured with routinely used and validated assays at a local laboratory was also accepted, and results were categorised as either 'positive' or 'negative' based on assay specific cut-offs. Follow-up of patients ended after the last blood sample was drawn for the study.

Outcomes

The main outcomes of the study were to assess (1) the proportion of patients with a seroconversion after third vaccination and the association with dosing and timing of rituximab, and (2) the proportion of patients with persistence of humoral response after second vaccination.

Statistical analysis

All eligible patients from the first study were included.[4] Three dosage groups (200mg, 500mg, and 1000mg) were defined based on the last received RTX dose before first vaccination similar to our first study. We used a dichotomous outcome to assess seroconversion, based on the cut-off of ≥ 1.1 of the IgT index number for the Wantai assay, and for other assays we used the dichotomous outcome as provided by the local laboratory.[8] Descriptive statistics were appropriately used to assess group characteristics. We used the 'third dose sample' (see fig. 1) for the efficacy after third vaccine analysis. Fisher's Exact Test was used to test the differences between the vaccine types on third vaccine efficacy. To assess the associations between dosing and timing on humoral response in the 'efficacy after third vaccine analysis', we used univariable logistic regression with humoral response 2-6 weeks after third vaccination as dependent variable, and latest RTX dose before baseline and time between latest RTX and first vaccination as central determinant. The 'persistence sample' was used for the secondary analysis, investigating persistence of humoral response. All data were entered in an electronic data capture database (Castor EDC, Amsterdam, Netherlands) and subsequently exported to StataIC (version 13, StataCorp LLC, TX, USA) for statistical analyses.

Results

Patients

Ninety-eight patients provided a 'third dose sample' for third vaccine analysis and twenty-three a 'persistence sample' for persistence analysis (see Figure 1). The baseline characteristics of the patients included in this study are displayed in Supplementary Table S1, available at *Rheumatology* online.

Factors associated with seroconversion after third vaccine dose

Third vaccinations took place between 5 October 2021 and 9 January 2022. RTX dose at baseline did not differ between before and after the respective vaccinations in 89% of patients. The median time

between second and third vaccination was 145 days (IQR 130-160). Samples for third vaccine efficacy were drawn in 98 patients 2-6 weeks after third vaccination and took place between 28 October 2021 and 9 February 2022. Of those 98 patients, 13 (13%) had received 200 mg as latest RTX dose before first vaccination, 37 (38%) 500 mg, and 48 (49%) 1000 mg (Table 1). Nineteen patients (19%) reached a positive response after third vaccination, of which two had a COVID-19 infection between second and third vaccination. Response rates were numerically higher for patients who received AstraZeneca as the first two COVID-19 vaccines (5/19, 26%) versus the Pfizer and Moderna (combined; 10/79, 13%, *p*=0.16). Between 200 mg and 1000 mg as latest rituximab dose for first vaccination, the percentage of humoral response after third vaccination was higher for the 200 mg group (5/13, 38%) versus 1000 (7/48, 15%) although not significantly (OR 3.66, 95% CI 0.93 to 14, p=0.06). Between 500 mg and 1000 mg, response rates were similar: 19% (7/37) versus 15% (7/48) respectively (OR 1.37, 95% CI 0.43 to 4.3, p=0.59). These values were similar when analysing with the latest RTX dose before third vaccination. The median time between third vaccination and the latest RTX prior was 138 days (IQR 111-156) for responders and 119 days (IQR 91-147) for non-responders, resulting in a non-significant association between humoral response and timing (OR 1.16, 95% CI 0.97 to 1.35 per month increased time, p=0.10).

Humoral response persistence

Samples for humoral response persistence were drawn between 30 June and 4 November 2021, with a median time after second vaccination of 83 days (IQR 66-122). Detectable response persisted in 96% (22/23; Table 2). Nine patients with a previous positive response had received a RTX dose between both samples, of which four a dose of 1000 mg (44%), three 500 mg (33%), and two 200 mg (22%). Response persisted in 8/9 patients who retrieved intercurrent rituximab (89%), except for one patient who received 500 mg.

Discussion

Our main results illustrate that humoral response after third vaccination occurs in a relevant proportion of patients who did not respond to earlier vaccination. Also, with a similar odds ratio as in our first study[4] – although not significantly so due to a smaller study population – humoral response was associated with 200 mg RTX and longer time between RTX infusion and vaccination. Additionally, we have shown that persistence of humoral response is very high even in context of intercurrent RTX infusions.

The association between 200 mg RTX and positive response after two-dose and three-dose vaccination could be explained by faster B-cell repletion. Previous studies showed that B-cell repopulation is associated with humoral response[3,9], and that B-cell numbers are non-significantly higher at six months after a dose of 200 mg RTX compared to 1000 mg.[10] Unfortunately, B-cell counts were not performed in our current study. We also found a non-significant higher response rate after third vaccination for patients receiving AstraZeneca for the first two vaccinations in comparison to Pfizer or Moderna. This may be explained by the beneficial effect of a heterologous booster,[11] as only mRNA vaccines were approved for third vaccination.

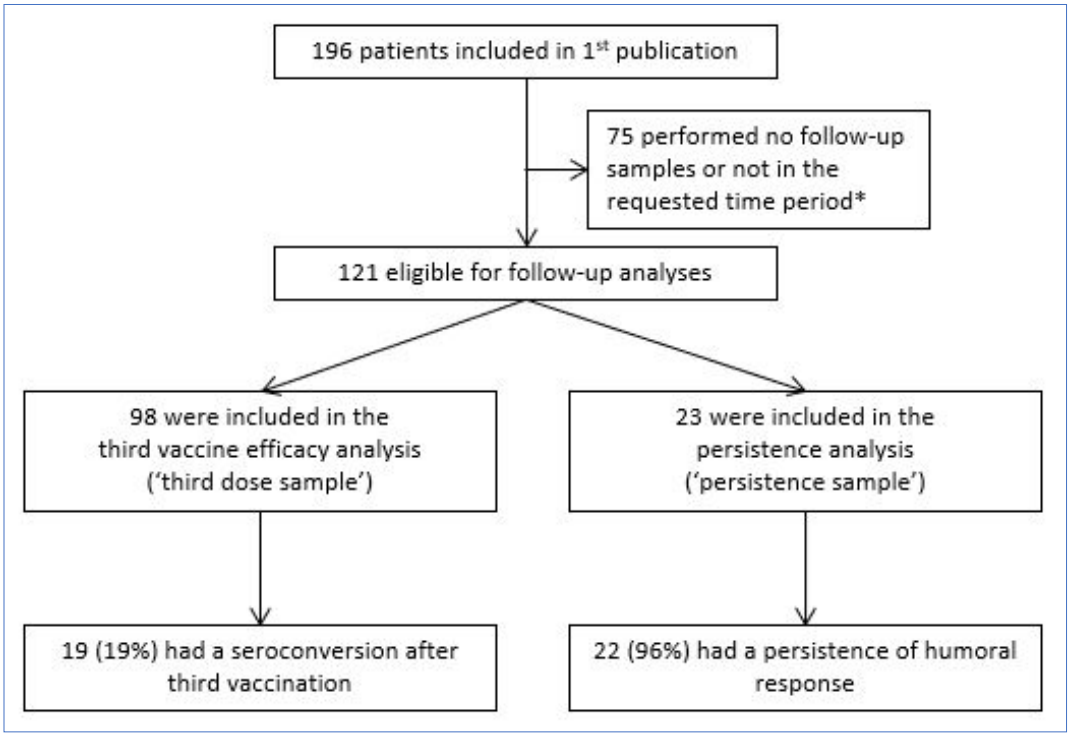
A limitation of this study is the smaller sample size compared to the first study, possibly leading to reduced power. Also, T-cell measurements were not performed which may lead to an underrepresentation of responders in our study, as T-cell responses are present in the majority of RTX patients after two-dose vaccination.[12] To extend this, the optimal outcome would of course be the COVID-19 occurrence, but this would require a longer follow-up, more patients, and is dependent on COVID-19 incidence in the population. Of note, this study did not include patients with other

diseases in which RTX is used, therefore extrapolation of our recommendations to treatment with RTX in general may be difficult.

Based on the results of our study, repeated vaccination as late as possible after the lowest RTX dose possible seems the best vaccination strategy. Once seroconversion is achieved, humoral response persists despite rituximab continuation.

Figures and tables

Figure 1 Study flow chart



* for third vaccine analysis: non-response after two vaccines and blood sample taken 2-6 weeks after third vaccination. for persistence analysis: previous response after two vaccines and follow-up blood sample taken after second COVID-19 vaccination but before third vaccination.

Table 1 Third vaccine efficacy

RTX dose*	Positive response	Negative response	Total
200 mg	5 (38%)	8 (62%)	13
500 mg	7 (19%)	30 (81%)	37
1000 mg	7 (15%)	41 (85%)	48
Total	19	79	98

Displayed as number (percentage). *Latest RTX dose before first COVID-19 vaccination

Table 2 Humoral response persistence

RTX dose*	Positive response	Negative response	Total
200 mg	5 (100%)	0 (0%)	5
500 mg	5 (83%)	1 (17%)	6
1000 mg	12 (100%)	0 (0%)	12
Total	22	1	23

Displayed as number (percentage). *Latest RTX dose before first COVID-19 vaccination

Statements

Acknowledgements

We thank Kasper Jolink and the staff of the rheumatology outpatient clinic of the Sint Maartenskliniek for performing additional blood sampling for this study, and Paul Daemen for performing the assays.

Contributors

CJTvdT, DFTC, BJFvdB, NdB and AAdB designed the study. CJTvdT, DFTC and AAdB informed and included patients. JR supervised and interpreted the antibody measurements. CJTvdT had access to all the data and performed the statistical analyses. CJTvdT, DFTC, and AAdB drafted the manuscript, and all other authors critically revised the final version of the manuscript.

Funding statement: No funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

Conflicts of interest: AAdB reports grants from Abbvie, Pfizer, Lilly, Novartis, and Sanofi to the institution, outside of the submitted work. The other authors declare no competing interests.

Ethics: The study was approved by the Ethics Committee of the Radboudumc 'CMO Arnhem-Nijmegen' (protocol number 2021-7406) and the National Ethics Committee of the Netherlands 'CCMO' (protocol number NL76709.091.21). The study was conducted in accordance with the Declaration of Helsinki and International Council for Harmonisation Good Clinical Practice guidelines.

Data availability statement

Data are available upon request from Alfons den Broeder, PhD, clinical research lead: a.denbroeder@maartenskliniek.nl . Researchers that are interested in doing additional analyses using these data can contact the corresponding author. Data can only be used for scientific research without conflict of interests.

References

1. Avouac J, Drumez E, Hachulla E, et al. COVID-19 outcomes in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. *Lancet Rheumatol*. 2021;3(6):e419-e26.
2. Furer V, Eviatar T, Zisman D, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis*. 2021;80(10):1330-8.
3. Schietzel S, Anderegg M, Limacher A, et al. Humoral and cellular immune responses on SARS-CoV-2 vaccines in patients with anti-CD20 therapies: a systematic review and meta-analysis of 1342 patients. *RMD Open*. 2022;8(1).
4. van der Togt CJT, Ten Cate DF, den Broeder N, et al. Humoral response to Coronavirus Disease-19 vaccines is dependent on dosage and timing of rituximab in patients with rheumatoid arthritis. *Rheumatology (Oxford)*. 2022.
5. Bonelli M, Mrak D, Tobudic S, et al. Additional heterologous versus homologous booster vaccination in immunosuppressed patients without SARS-CoV-2 antibody seroconversion after primary mRNA vaccination: a randomised controlled trial. *Ann Rheum Dis*. 2022.
6. Jyssum I, Kared H, Tran TT, et al. Humoral and cellular immune responses to two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis: a prospective, cohort study. *Lancet Rheumatol*. 2021.
7. Sidler D, Born A, Schietzel S, et al. Trajectories of humoral and cellular immunity and responses to a third dose of mRNA vaccines against SARS-CoV-2 in patients with a history of anti-CD20 therapy. *RMD Open*. 2022;8(1).
8. Beijing Wantai Biological Pharmacy Enterprise Co. L. WANTAI SARS-CoV-2 Ab ELISA https://www.fda.gov/media/140929/download Access date: 07-10-2021.

9. Avouac J, Miceli-Richard C, Combier A, et al. Risk factors of impaired humoral response to COVID-19 vaccination in rituximab treated patients. *Rheumatology (Oxford)*. 2021.
10. Wientjes MHM, Gijzen TMG, den Broeder N, et al. Drug levels, anti-drug antibodies and B-cell counts were not predictive of response in rheumatoid arthritis patients on low dose rituximab. *Rheumatology (Oxford)*. 2022.
11. Liu X, Munro APS, Feng S, et al. Persistence of immunogenicity after seven COVID-19 vaccines given as third dose boosters following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK: Three month analyses of the COV-BOOST trial. *J Infect*. 2022;84(6):795-813.
12. Prendecki M, Clarke C, Edwards H, et al. Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. *Ann Rheum Dis*. 2021;80(10):1322-9.