Revaccination of patients with systemic lupus erythematosus or rheumatoid arthritis without an initial COVID-19 vaccine response elicits seroconversion in half of the patients

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Abstract

Objective

To investigate the effect of COVID-19 mRNA revaccination (two doses) on the antibody response in patients with rheumatic diseases (RD) who were initial vaccine non-responders. Further, to examine if B-cell levels or T-cell responses before revaccination predicted seroconversion.

Methods

From a RD cohort vaccinated with the standard two-dose COVID-19 vaccinations, we enrolled cases without detectable antibody responses (n=17) and controls with detectable antibody response (n=29). Blood donors (n=32) were included as additional controls. Samples were collected before and six weeks after completed revaccination. Total antibodies and specific IgG, IgA, and IgM against SARS-CoV-2 spike protein, SARS-CoV-2 neutralising antibodies, and SARS-CoV-2 reacting CD4+ and CD8+ T-cells were measured before and after revaccination. B-cells (CD19+CD45+) were quantified before revaccination.

Results

Forty-seven percent of cases had detectable neutralising antibodies after revaccination. However, antibody levels were significantly lower than in controls and blood donors. Revaccination induced an antibody class switch in cases with a decrease in IgM and increase in IgG. No significant difference was observed in T-cell responses before and after revaccination between the three groups. Only 29% of cases had measurable B-cells compared to 100% of controls and blood donors. Fifty percent of revaccinated cases who seroconverted had measurable B-cells before revaccination.

Conclusion

Forty-seven percent of initial non-responders seroconverted after two-dose revaccination but still had lower levels of SARS-CoV-2 antibodies compared with controls and blood donors. RD patients without a detectable serological response after the initial COVID-19 mRNA vaccine had a T-cell response similar to immunocompetent controls and blood donors.

Key words

COVID-19, vaccine, rituximab, B-cell depleting therapy, rheumatic diseases, autoimmune disease

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Introduction

Immunosuppressive treatment in patients with rheumatic diseases (RD) has presented a challenge during the COVID-19 pandemic. Soon after SARS-CoV-2 vaccines came into use, it became evident that patients receiving B-cell depleting therapy, like rituximab (RTX), had markedly reduced and often undetectable antibody responses after vaccination (1-5).

Extensive population-based studies document that RD patients have an increased risk of hospitalisation and death after SARS-CoV-2 infection compared to the background population (6-8) and that RTX treatment is a risk factor for death (6). The rate of COVID-19 breakthrough infections in patients with RD is relatively low (1–5%). However, such infections are associated with a significant increase in mortality and post-COVID morbidity (9-11).

Despite the emergence of SARS-CoV-2 variants, 'Delta' and 'Omicron", where evidence points to decreased neutralisation by antibodies generated during previous infection or vaccination, the original vaccines still effectively prevent severe disease course and death 12,13. Additionally, booster vaccination also conveys an additional lower risk of symptomatic disease due to Delta and Omicron variants (14).

RTX-treated RD patients may demonstrate an impaired antibody response but an intact T-cell response (15, 16). It is unclear if solitary T-cell immunity alone provides sufficient protection. However, breakthrough COVID-19 infections are observed in vaccinated RTX-treated patients (11), and breakthrough infections in RD patients are associated with seronegativity after vaccination (17).

The American College of Rheumatology (ACR) recommends that RD patients on immunosuppressants receive an extra third mRNA vaccine dose minimum 28 days after their second dose, followed by an additional fourth dose at least five months later (18). However, these recommendations are not evidence-based but rely on findings in healthy individuals not receiving immunosuppressants. Such data may not be translatable to RD patients.

Therefore, our primary aim was to investigate whether revaccination with an mRNA SARS-CoV-2 vaccine (two doses three weeks apart) in RD patients who had not seroconverted after a standard vaccination regimen (two doses of SARS-CoV-2 mRNA vaccine three weeks apart), could improve vaccine-induced immunity. Our secondary aims were to examine if the pre-vaccination level of peripheral blood B-cells predicted seroconversion, the neutralising antibody capacity, and the changes in T-cell responses against SARS-CoV-2 spike protein.

Methods

Participants

We identified cases and controls from the COPANARD cohort (19-22) who previously participated in COVID-19 vaccine studies. All COPANARD participants had received two vaccine doses as part of the national vaccination schedule managed by the Danish National Health Authorities. The group defined as cases had not developed detectable antibodies after their initial two-dose mRNA vaccines, (VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total test, see below). Controls were defined as participants from the COPANARD cohort who had detectable antibodies after the same vaccine regimen. All eligible cases and controls were followed at the outpatient clinic at the Department of Rheumatology, Aarhus University Hospital (AUH), Denmark, with a diagnosis of systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA).

The controls received a single booster vaccination (3rd dose), whereas cases received full revaccination (3rd and 4th vaccine doses three weeks apart). BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) COVID-19 mRNA vaccines were used. In addition, blood donors from the hospital blood bank who had received a two-dose mRNA vaccine were used as a reference for a normal immune response (referred to as blood donors).

COPANARD cases and controls received their booster or revaccination between beginning of October and end of December 2021. At inclusion, par-

ticipants were asked whether they had ever tested positive for SARS-CoV-2 in any test.

Total antibodies against SARS-CoV-2
Total antibodies against recombinant SARS-CoV-2 spike S1 protein were measured in serum with a commercial assay (VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total test, Ortho Clinical Diagnostics, USA). Assay performance characteristics were reported previously (23). The sensitivity was 95.3% (95% confidence interval (CI): 90.7–97.7), and the specificity was 100% (95% CI: 99.4–100).

Quantification of IgG against SARS-CoV-2 spike protein
Specific IgG against recombinant trimeric SARS-CoV-2 spike protein in serum was analysed using a commercial assay (LIAISON® SARS-CoV-2 TrimericS IgG, DiaSorin SpA, Italy) on the LIAISON®XL platform. Samples results ≥33.8 BAU/mL were considered positive, and <33.8 BAU/mL was negative. The assay range is 4.81–2,080 BAU/mL. Results were based on a single test result. Assay performance characteristics reported by Bonelli *et al*. (24): Clinical sensitivity 98.7% (≥15 days post positive PCR) and specificity 99.5% (95% CI: 99.0%–99.7%).

Quantification of IgG, IgM, and IgA antibodies against SARS-CoV-2 RBD
An in-house ELISA-based assay was used to quantify the IgG, IgM, and IgA antibody levels against the receptor-binding domain (RBD) of the Spike (S) protein as previously described (25).

ACE-2/RBD antibody inhibition measurement
An in-house ELISA-based assay was used to determine the capacity of the antibodies measured to inhibit the binding of RBD to the ACE-2 receptor, as described previously (26). A normal human plasma pool from convalescent individuals at a starting dilution of 1:10 in PBS-T was used as a positive control. A normal human serum pool from uninfected/unvaccinated individuals was used as a negative control. Assay positivity threshold was set at 57 AU/ml. Interpolation of IgG, IgM, and IgA antibody levels and neutralisation levels was executed using the non-linear regression four-parameter curve fitting (GraphPad v. 9.3.1 [GraphPad Software, La Jolla, CA]). IgG, IgM, and IgA results were given in AU/ml, where the highest concentration of the calibrator was given a value of 200 AU/ml. Neutralisation results were given in AU/ml, where the highest concentration of the calibrator was given a value of 100 AU/ml.

B-lymphocyte measurements
B-cells were quantified in EDTA-stabilised peripheral blood by flow cytometry at the Department of Clinical Immunology, AUH, Denmark, as previously described (27).

T-cell response
The frequency of antigen-specific cells was determined by the AIM (Activation Induced Marker) assay at the department of infectious medicine, AUH, Denmark (28). Cryopreserved PBMCs were thawed, washed, and rested at 37°C for 3 hours. Cells were then plated into wells of a 96-well plate, at a total of 10^6 cells per well. For each assay, three conditions were used: vehicle control as a negative control, one SARS-CoV-2 spike antigen stimulation, and staphylococcal enterotoxin B (SEB, 1 µg/ml) as a positive control. The antigen stimulation was an overlapping peptide pool corresponding to SARS-CoV-2 spike (JPT, PepMix™ product code PM-WCPV-S-1) used at a final concentration of 2 µg/mL of total peptide. Following 20 hours of incubation at 37°C, and cells were washed with PBS and stained for viability with Near IR Live/Dead for 20 minutes. Cells were then incubated with Human TruStain FcX (BioLegend) in PBS 2% FBS for 10 minutes and stained for 30 minutes with antibodies against surface markers: CD3 (PerCP/Cy5.5 anti-human CD3, SK7, BioLegend), CD4 (BV650 anti-human CD4, RPA-T4, BioLegend), CD8 (BV605 anti-human CD8a, RPA-T8, BioLegend), 4-1BB (PE anti-human CD137, BioLegend) CD69 (APC anti-human CD69, FN50, BioLegend) and OX40 (BV421, anti-human OX40, ACT35, BioLegend). Cells were washed twice and analysed on a MACSQuant Analyzer 16 flow cytometer. Data were analysed in FlowJo 10.1. CD4+ and CD8+ T cells were gated as follows: Live cells, singlets, CD3+ T cells, and CD4+ or CD8+ T cells. Then the frequency of antigen-specific CD4 and CD8 T cells (AIM+ cells) was defined as the frequency of cells that were either CD69+OX40+4-1BB+, CD69+OX40+, CD69+4-1BB+ or OX40+4-1BB+. Total SARS-CoV-2 spike-specific AIM+ cells were calculated as a summation of the four populations for antigen stimulation. The frequency of AIM+ cells in the non-stimulation condition was subtracted. A cutoff value was calculated based on data from 247 individuals previously not infected with SARS-CoV-2. A positive T-cell response was defined as median+1*SD (CD4=0.107, CD8=0.078).

Statistics
All values reported are medians with interquartile range (IQR) unless otherwise stated. Because the outcomes were not normally distributed and because of the small sample size, nonparametric tests were used to test for differences. Wilcoxon rank-sum test and Kruskal-Wallis test was used in unpaired observations, and Wilcoxon matched-pairs signed-rank test was used in matched pairs of observations. Fisher's exact test and McNemar test were used on nominal observations. A *p*-value below 0.05 was considered significant.
Univariate logistic regression analyses were performed with detectable total SARS-CoV-2 antibodies after revaccination as the dependent variable. The presence of B-lymphocytes, CD4+ and CD8+ specific reactive T-cells, and active RTX treatment before revaccination were included as explanatory variables in the model.

Ethics
Participants were offered participation in the study after informed written consent. The regional Danish Data Protection Agency (1-16-02-254-21) and the Central Denmark Region Committee on Health Research Ethics (1-10-72-

Table I. Demographics.

	Cases Revaccination, n=17		Controls Boost, n=29	
Female sex, no (%)	14	82%	21	72%
Age, median (IQR)	65	49 - 70	67	62 - 72
COVID-19 infection previous, no (%)	1	6%	0	
Disease duration, years	15	10 - 18	22	9 - 31
Diagnosis, no				
Rheumatoid arthritis	13	76%	10	34%
Systemic lupus erythematosus	4	24%	19	66%
mRNA vaccine used initially, no (%)				
Pfizer/BionTec	16	94%	26	90%
Moderna	1	6%	1	3%
Astra Zeneca/Oxford			2	7%
Time from initial vaccination to 1. blood sample, weeks	26	22 - 28	27	24 - 28
DMARD treatment				
None	5	29%	8	28%
Prednisone	4	24%	1	3%
Methotrexate	7	41%	12	41%
Hydroxychloroquine	2	12%	4	14%
Leflunomide	2	12%	3	10%
Azathioprine	1	6%	3	10%
Mycophenolatmofetile	0		1	3%
Biologic treatment				
None	4	24%	9	31%
Rituximab	12	71%	0	
TNF-inhibitors	1	6%	7	24%
JAK-inhibitors	0		6	21%
IL-6-inhibitors	0		4	14%
Abatacept	0		2	7%
Benlysta	0		1	3%
Previous rituximab treatment				
Any rituximab treatment	16	94%	1	3%
RTX within the last 15 months, no	14	88%	0	
Number of infusions	14	6 - 25	2	
Cumulative total dose, mg	13	4-24	2	
Total treatment time *, months	70	15-84	1	
Time from RTX to revaccination, months	9	5-12	49	

IQR: interquartile range; DMARD: disease-modifying anti-rheumatic drug; GPA: granulomatosis with polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis.
*Time between the first and last rituximab treatment before revaccination.

238-21) approved the study. The study followed the Helsinki declaration.

Results

Participants

We included 17 cases and 29 controls from the COPANARD cohort and 32 blood donors in the study (Table I). Thirteen (76%) cases had RA compared to 10 (34%) controls. Cases had a median age of 65 years, were predominantly female (82%), and had an average disease duration of 15 years. Sixteen out of 17 (96%) cases had received RTX, compared to 1 out of 29 (3%) controls. Twelve (71%) cases were in ongoing RTX treatment, while none of the controls were. The cases had an increased interval between the re-vaccination and the preceding RTX treatment of 280 days (146–367), compared to 122 days (107–189) between the primary vaccination and the preceding RTX treatment.

The blood donors had a median age of 49 (45–52), and 17 (53%) were women. The median time from vaccination to blood sample for the blood donors was 16 weeks (IQR 15–18).

Antibody response

SARS-CoV-2-spike antibodies were measured with five different assays at baseline and follow-up. The results are presented in Figure 1 and Supplementary Table S1.

Total SARS-CoV-2-antibodies

None of the cases had detectable antibodies in the total SARS-CoV-2 antibody assay at baseline, compared to 8 (47%) at follow-up (Fig. 1A). All controls and blood donors were seropositive at baseline. Both cases and controls had increased levels of total SARS-CoV-2-abs between baseline and follow-up (all $p<0.008$), but concentrations were significantly lower in cases compared to controls both at baseline and follow-up (all $p<0.001$). Controls had lower levels of total SARS-CoV-2-abs at baseline ($p<0.001$) but similar at follow-up ($p=0.50$) compared to blood donors at baseline.

SARS-CoV-2 IgG antibodies

SARS-CoV-2 IgG antibodies (abs) were measured using a commercially available assay (Fig. 1B) and an in-house ELISA-based assay (Fig. 1F). We found a correlation of 0.93 between the two assays (Suppl. Fig. S1).

In the commercially available assay, all blood donors (100%) had measurable antibodies at baseline compared to 72% (18 of 25) of controls and 12% (2 of 17) of cases. The frequency of seropositivity increased significantly in both cases and controls. Seroprevalence among cases and controls increased significantly to 100% for controls and 53% (9 of 17) for cases. Both cases and controls had a significant increase in SARS-CoV-2 IgG abs between baseline and follow-up (all $p<0.004$), but concentrations were significantly lower in cases compared to controls both at baseline and follow-up (all $p<0.001$). Controls had lower levels of SARS-CoV-2 IgG abs at baseline ($p<0.001$) but similar at follow-up ($p=0.25$) compared to blood donors at baseline.

SARS-CoV-2 neutralising antibodies

At baseline, all blood donors and 79% (23/29) of controls had sufficient antibodies to inhibit the binding of RBD to the ACE-2 receptor (Fig. 1C). Two of 17 (12%) cases had measurable neutralising abs at baseline, which increased to 8 of 17 (47%) at follow-up ($p=0.002$). All three groups had significantly different levels of SARS-CoV-2 neutralising abs at baseline. Cases had the lowest levels of SARS-CoV-2 neutralising-abs, followed by controls and the blood donors at baseline ($p<0.001$). The cases had a significant increase

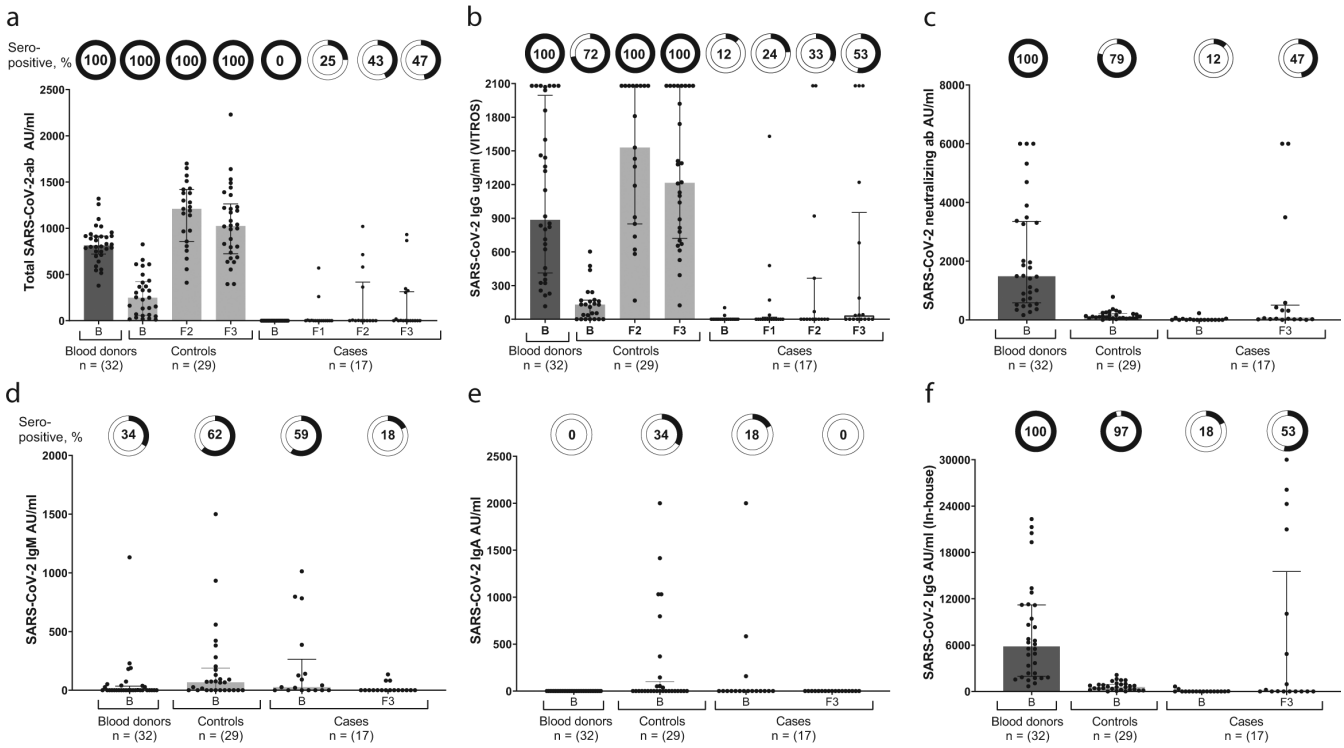

Fig. 1. Presence and levels of SARS-CoV-2 antibodies.
SARS-CoV-2 antibodies (abs) measured with six different assays after the initial 2-dose mRNA vaccination (B=baseline). For controls that all received a single booster mRNA dose, abs were additionally measured 2 and 6 weeks after boost (F2 and F3 under controls). For cases receiving full two-dose revaccination, abs were measured 2 weeks after the 3rd and 2 + 6 weeks after the 4th dose (marked F1, F2, and F3, respectively, under cases). Pie charts indicate the presence of measurable abs and neutralising abs inhibitory activity.

in ab-levels at follow-up compared to baseline (p=0.003), and ab-levels were at follow-up, no different from controls at baseline (p=0.50).

SARS-CoV-2 IgM antibodies
At baseline, fewer blood donors (34%, 11/34) compared to controls (62%, 10/29) and cases (59%, 14/17) had detectable SARS-CoV-2 IgM abs (both $p \leq 0.04$) (Fig. 1D). Significantly fewer cases were IgM-seropositive (18%, 3/17) at follow-up compared to baseline (p=0.04), which was mirrored by a similar significant decrease in IgM ab-levels (p=0.04).

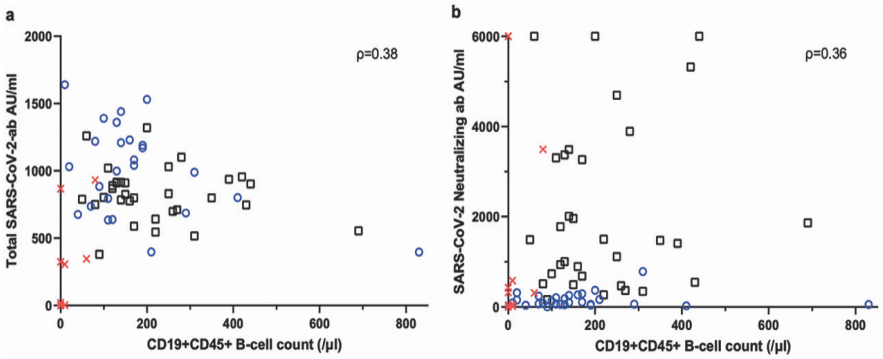

Fig. 2. Correlation between CD19+CD45+ B-cells pre-revaccination and antibody levels.
A: Total SARS-CoV-2 ab levels are measured 6 weeks after the last boost/revaccination for controls and cases.
B: Neutralising abs were not measured after boost for controls. Thus, only levels of pre-boost are given. Crosses = cases, circle s= controls, squares = blood donors. Correlation calculated by Spearman's rho.

SARS-CoV-2 IgA antibodies
None of the blood donors had measurable SARS-CoV-2 IgA abs at baseline, which was significantly lower than both controls (34%, 10/29) and cases (18%, 3/17) ($p \leq 0.04$) (Fig. 1E). This difference observed between controls and cases at baseline was not significant (p=0.32). All cases became SARS-CoV-2 IgA seronegative after revaccination.

B-cells
At baseline, all blood donors (n=32) and controls (n=27) had measurable B-cells, while this was the case for only 29% (5/17) of cases (Fisher's exact p<0.001). Blood donors had the highest levels of B-cells (median 170/µL(IQR 80–190)) followed by controls (130/µL (80–190)) and last the cases (0/µL(0-10) (all p<0.04). The number of B-cells correlated to both SARS-CoV-2 total abs (ϱ=0.38 p<0.001) and neutralising abs (ϱ=0.36, p=0.001) after revaccination (Fig. 2).

SARS-CoV-2 SPIKE-specific T-cells
There was no difference between the three groups at baseline regarding levels of measurable CD4+ and CD8+ T-cells or their percentage (all $p \geq 0.10$) (Fig. 3A). The number of cases with measurable SARS-CoV-2 specific T-

cells did not change significantly after revaccination. The proportion of cases with detectable CD4+ T cells increased from 69% to 88% (p=0.25), and for CD8+ T cells, the proportion decreased from 88% to 82% (p=1.00) (Fig. 3B). There was no correlation between change in CD4+ and CD8+ T cells for cases pre and post revaccination (p=0.90) (Suppl. Fig. S2). The percentage of SARS-CoV-2 specific CD8+ T-cells increased significantly from a median of 0.19% (0.06-0.40) to 1.00% (0.40–1.77) (p<0.001) after revaccination for cases, while CD4+ T-cells changed from 0.31% (0.11–0.51) to 0.51% (0.23–0.92) (p=0.43) (Fig. 3B). Descriptive statistics are reported in Supplementary Table S1.

Binomial presentation of the presence of B-cells, T-cells, and antibodies pre- and post-revaccination

Figure 4 is a binomial presentation of data generated on a patient level. The effect of revaccination is seen in the case-cohort, as revaccination was associated with an antibody class switch with a decrease in IgA, IgM, and increase in IgG post-revaccination (Fig. 4). Furthermore, 4 out of 5 with measurable B cells seroconverted, corresponding to 50% of the seroconverted cases.

Predictors of vaccine response

Univariate logistic regression analysis was performed to analyse if active RTX treatment, the presence of B-cells, or a positive T-cell response prior to revaccination predicted seroconversion of total SARS-CoV-2-abs in the patient cohort (Table II). We did not find a significant explanatory effect of either variable in the univariate logistic models.

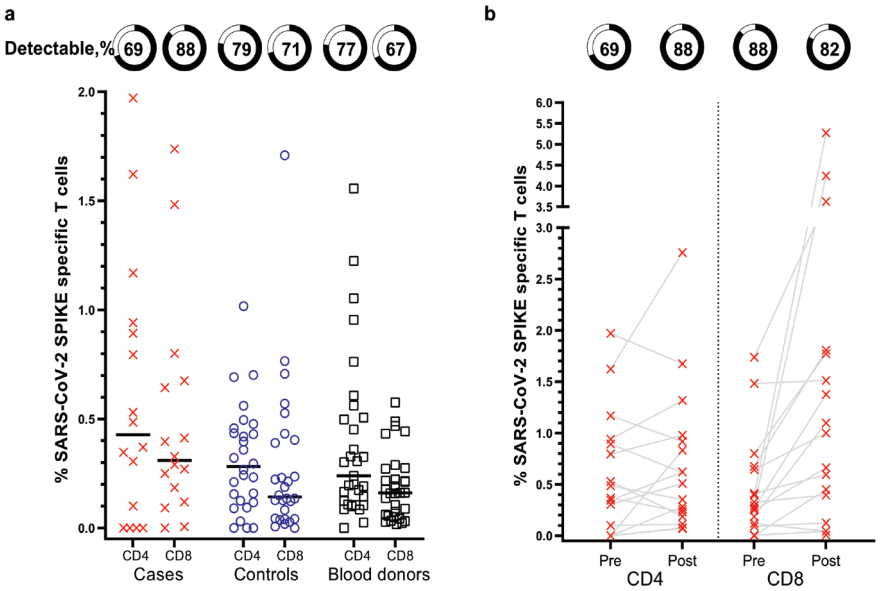

Fig. 3. SARS-CoV-2 SPIKE specific CD4+ and CD8+ T-cells in cases, controls, and blood donors.
A: Percentage of SARS-CoV-2 SPIKE specific CD4+ and CD8+ T-cells after initial two-dose COVID-19 mRNA, and pie charts indicate presence. Cases did not seroconvert after a two-dose COVID-19 mRNA vaccine and received a two-dose mRNA revaccination. Controls were cases that seroconverted after a two-dose mRNA COVID-19 vaccine. A detectable T-cell response was defined as CD4≥0.107% or CD8≥0.078%.
B: For cases, T-cell-response was measured before and after revaccination. T-cell-response in controls and blood donors was only measured once.

Table II. Univariate logistic regression analysis of total antibody response after revaccination in 17 COPANARD cases.

Univariate analysis	Odds ratio	95% CI	p
Presence of CD4 reactive T-cells	4.8	0.4-58.0	0.22
Presence of CD8 reactive T-cells	0.8	0.0-14.6	0.85
Presence of CD19-positive B-cells	10.2	0.7-97.0	0.10
Active rituximab treatment	0.48	0.1-4.0	0.49

Discussion

Patients with RD are at an increased risk of developing severe COVID-19 (6, 7). Vaccine responses are compromised in some RD patients, particularly patients receiving B-cell-depleting therapy (2-5, 20). Since these patients were not included in the initial studies examining the efficacy of COVID-19 mRNA vaccines (29, 30), there is an urgent need to understand humoral and cellular immune responses elicited by mRNA vaccines in RD. In this study, we investigated the effect of revaccination in initial vaccine non-responders. We examined humoural and T-cell responses induced by COVID-19 mRNA vaccines before and after revaccination.

We found that several patients with undetectable SARS-CoV2-spike-specific-IgG antibodies at baseline had detectable SARS-CoV-2-IgM antibodies. After revaccination, spike-specific-IgM became undetectable for most responders as observed (generally) in blood donors. IgM antibodies are produced early after immunisation before maturation of the class-switched, high-affinity IgG response associated with immunological memory and long-term immunity (31). An association between IgM and neutralising capacity after SARS-CoV-2 vaccination is reported for healthy individuals (32). We also observed a neutralising capacity in most IgM-spike-specific positive patients even for individuals without detectable spike-specific IgG. A subset of patients (n=2) initially exhibited positivity for IgM and demonstrated neutralising capacity, but tested negative for Total-Abs. We interpret this observation as a consequence of low, yet detectable, levels of IgM in the IgM assay, coupled with the Total-Abs assay's limited sensitivity in detecting low levels of IgM abs. It is yet unclear whether the level and neutralising capacities of the antibodies in the seroconverted patients can prevent infection or severe COVID-19. However, Ahmed *et al.* reported that rheumatic patients without breakthrough infections, on average, had a 40% higher plasma concentration of neutralising antibodies compared to rheumatic patients with breakthrough infections (17).

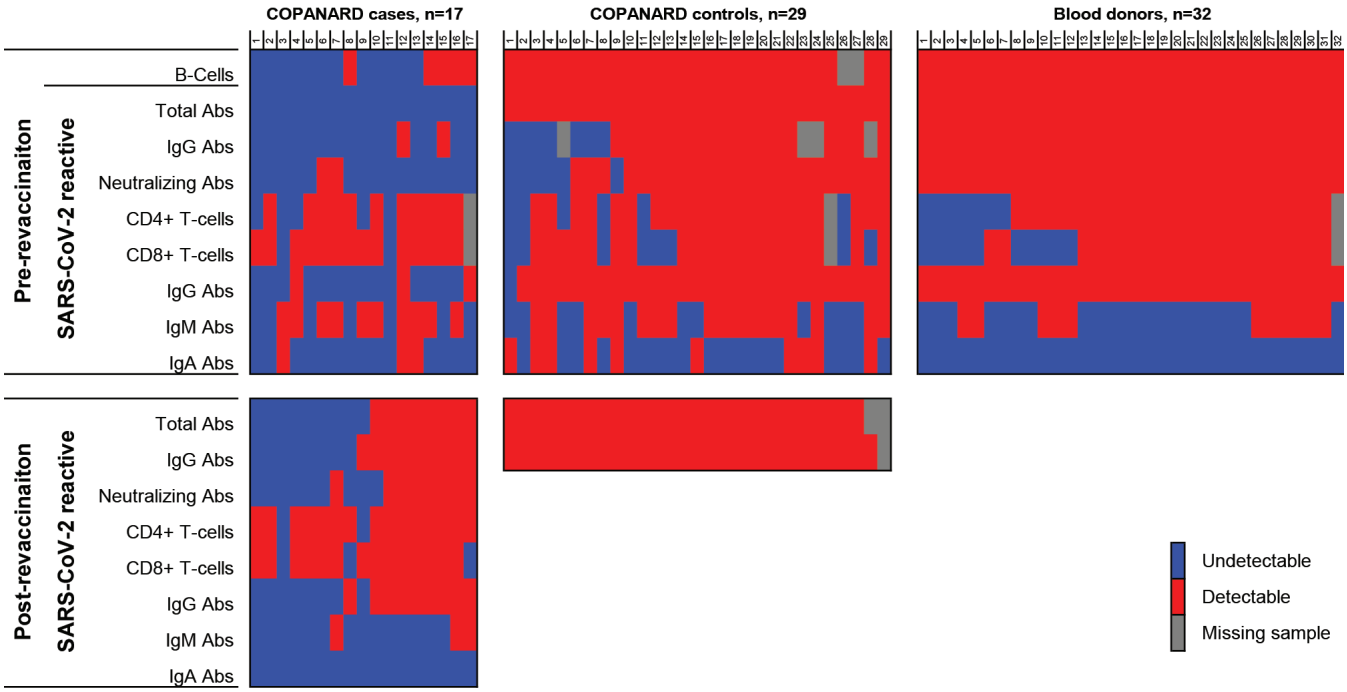

Fig. 4. Combined binomial presentation of the presence of B-cells, T-cells, and antibodies pre- and post-vaccination.
Combined presentation of CD19+CD45+ positive B-cells, detectable SARS-CoV-2 antibodies, neutralising antibodies, and SARS-CoV-2 SPIKE specific CD4/CD8 T-cells pre- and post-revaccination. Each individual is presented as a single column.

SARS-CoV-2-IgA antibodies were detected before vaccination in 10 out of 29 controls, 3 out of 17 cases, and none of the blood donors. The IgA response is typically moderate and is significantly influenced by waning immunity over time compared to the IgG response. Therefore, the time elapsed since the last vaccination is an important factor to consider when assessing the presence of IgA antibodies (33, 34). The blood donors, on average, received their primary vaccination 3 to five months before IgA was measured, which potentially explains the absence of detectable IgA antibodies in their blood. The cases exhibit an impaired immune response to vaccination compared to controls and blood donors. While the exact underlying reasons remain unclear, several possibilities can be speculated, including a potentially slower immune response, a more immature immune response, or the influence of immune-modulating therapies. However, we currently lack a definitive answer. Our data indicate that the initial non-responders have a more immature humoral response and that revaccination provides the "boost" for the immune system to mature, resulting in higher levels of specific IgG and neutralising abs and a humoral response more like the blood donors'.

Current guidelines from the ACR do not recommend performing antibody testing after vaccination (18), partly due to a lack of clinically meaningful cut-off values for available antibody tests. In a recent study, boosting RTX-treated RD patients who had not seroconverted after the first two doses yielded a serological response in only 16% of the patients (35). In contrast, 47% of our patients seroconverted after revaccination. Providing full revaccination thus seems beneficial to a significant fraction of patients who do not seroconvert after standard vaccination course. However, identifying routinely available predictors of a seropositive response to the COVID-19 vaccines in RD patients would assist in a more personalised vaccination approach.

We have previously shown that time since rituximab, and the presence of B-cells before the first two vaccinations were the primary determinants of seroconversion in rituximab-treated RD patients (5, 27). In the present study, we observed a significant correlation between B-cell levels and serological vaccine response. However, our regression model did not demonstrate an association between the presence of B-cells and seroconversion in the revaccinated population. Four of five patients without initial vaccine response, who had detectable B-cells before revaccination, developed a specific IgG response against SARS-CoV-2 after revaccination. The findings in the present study are likely biased by the small patient numbers in the revaccinated group. While the specificity remains high, the sensitivity of peripheral B cells in predicting seroconversion is not definitive. We observed that 4 out of 8 individuals who seroconverted for neutralising abs and 5 out of 9 individuals who seroconverted for specific IgG did not exhibit measurable B-cells prior to revaccination. These findings align with our previous findings, where 31 out of 44 patients who seroconverted were B-cell negative (27). It is possible that this seroconversion is attributable to the presence of tissue-resident memory B cells that are not measured in the blood (36).

Measurement of detectable peripheral B cells does seem to be a solid predictor of seroconversion (27, 37, 38). However, routine measurement might not be available in daily practice. A recent study suggested an algorithm for predicting the

likelihood of seroconversion in B-cell depleted patients, combining the level of total IgG prior to last RTX treatment, number of RTX treatments, and interval from RTX treatment to vaccination, yielding a sensitivity of 90.5%, specificity of 59.3% (39). This could be a reasonable approach, particularly where B-cell measurements are unavailable, but has to be validated in prospective cohorts.

As B cells play an important role in the maturation of T cells, the question was whether B-cell-depleted patients would be able to mount a functional T cell response to COVID-19 vaccines. T-cell reactivity and T-cell memory could represent important mechanisms for long-lasting vaccine-induced protection.

The percentage and function of specific T cells are primarily sustained after treatment with B-cell depletion. However, an increase in memory and loss of terminally differentiated CD4+ T cells have been described (40, 41). We did not observe any difference in SARS-CoV-2 specific T-cell response at baseline between patients, controls, and blood donors.

In patients with RDs, breakthrough COVID-19 is associated with seronegativity after vaccination (17) why T-cell-immunity alone is unlikely to prevent COVID-19 infection. However, T-cell response could be effective in reducing COVID-19 severity. Data from larger breakthrough cohorts are needed to establish the role of T cells in preventing severe diseases in vaccinated rheumatic patients.

In the current study, the majority of patients initially received two vaccinations with Pfizer/BioNTech, whereas the revaccination was two doses of Moderna. Each dose of the Pfizer/BioNTech vaccine contains 30 µg of mRNA compared to 100 µg in Moderna, which could significantly affect humoral response (42). A recent study demonstrated an improved response of a fourth dose Pfizer/BioNTech in RD patients with an initial poor response to inactivated vaccine (Sinovac-CoronaVac) (43). Elucidating the role of potential cross-platform revaccination (or booster) when primary vaccination is unsuccessful could prove relevant for initial vaccine non-responders.

The limitations of this study include the small sample size of the revaccinated patient group. Our cohort consisted of patients with RA or SLE who had been part of the COPANARD study (19). In the COPANARD cohort, thirty patients had undetectable SARS-CoV-2 antibodies after the first two-dose mRNA vaccine. We did not characterise SARS-CoV-2 positive memory B- and T-cells. Distinguishing COVID-19 specific B cells into different phenotypes, *e.g.* naive (CD27-IgD+), memory, and plasmablasts could potentially elucidate which subtypes that predicts vaccine response. Further, we did not have information on disease activity at the time of vaccination. Disease activity might influence the vaccine response. We also did not correlate our findings with clinical protection as the study was not designed with sufficiently long follow-up time to measure efficacy.

The strengths of our study are the prospective design and the ability to evaluate revaccination response in a well-characterised cohort using highly relevant controls and the extensive characterisation of the immunological vaccine response before and after revaccination. In conclusion, forty-seven percent of initial non-responders were able to seroconvert after two-dose revaccination. However, plasma concentrations of the antibodies against SARS-COV-2 and the levels of neutralising capacity remained significantly lower than in immunocompetent blood donors. Our study suggests that patients with RDs who did not mount a detectable serological response to a COVID-19 mRNA vaccine have a T cell response similar to immunocompetent controls. Future studies should establish the antibody levels that identify RD patients without sufficient protection against SARS-CoV-2 infection.

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