RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

Impact of different classes of immune-modulating treatments on B cell-related and T cell-related immune response before and after COVID-19 booster vaccination in patients with immune-mediated diseases and primary immunodeficiency: a cohort study

Michaela Koehm,[1,2,3] Maximilian Klippstein,[2,3] Stephanie Dauth,[2,3] Konstantin Hallmann,[2,3] Niko Kohmer,[4] Harald Burkhardt ,[1,2,3] Sandra Ciesek,[2,4,5] Gerd Geisslinger,[2,3,6] Holger F Rabenau,[4] Frank Behrens [1,2,3]

To cite: Koehm M, Klippstein M, Dauth S, *et al*. Impact of different classes of immune-modulating treatments on B cell-related and T cell-related immune response before and after COVID-19 booster vaccination in patients with immune-mediated diseases and primary immunodeficiency: a cohort study. *RMD Open* 2023;**9**:e003094. doi:10.1136/rmdopen-2023-003094

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/rmdopen-2023-003094).

Received 21 February 2023
Accepted 23 June 2023

For numbered affiliations see end of article.

Correspondence to
Dr Frank Behrens;
Frank.Behrens@itmp.fraunhofer.de

ABSTRACT

Objectives To evaluate the potential of immunosuppressed patients to mount B-cell and T-cell responses to COVID-19 booster vaccination (third vaccination).

Methods Patients with primary immunodeficiency (PID), immune-mediated inflammatory diseases (IMIDs) on CD20-depleting treatment with rituximab (RTX), or IMIDs treated with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) or biological disease-modifying antirheumatic drug (bDMARDs) were included and assessed before (baseline visit (BL)) and 2, 4 and 8 weeks after COVID-19 booster vaccination. Serum B-cell responses were assessed by antibody levels against SARS-CoV-2 spike protein (anti-spike IgG antibody (S-AB)) and a surrogate virus neutralisation test (sVNT). T-cell responses were assessed by an interferon gamma release assay (IGRA).

Results Fifty patients with PID (n=6), treated with RTX therapy (n=13), or treated with csDMARDs/bDMARDs (n=31) were included. At BL, anti-S-AB titres in PID and csDMARD/bDMARD-treated patients were low (although significantly higher than RTX patients); measures of B-cell-mediated response increased significantly after booster vaccination. In the RTX cohort, low BL anti-S-AB and sVNT values did not improve after booster vaccination, but patients had significantly elevated IGRA responses post booster vaccination compared with the other groups. csDMARD/bDMARD-treated patients showed the highest BL values in all three assays with greater increases in all parameters after booster vaccination compared with patients with PID.

Conclusion Patients with IMID on therapeutic B-cell depletion have low anti-S-AB and sVNT values before and after booster vaccination but show significantly higher levels of IGRA compared with other immunosuppressed patients, suggesting an underlying mechanism attempting to compensate compromised humoral immunity by upregulating T-cell responsiveness. PID appears to have a stronger impact on antiviral immune response than csDMARD/bDMARD treatment.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Protective immunity of patients with immune-mediated diseases is compromised by the underlying disease and the use of immune-modulating treatments.

⇒ The study of immune responses to COVID-19 vaccination is highly clinically relevant as patients with immune-mediated inflammatory diseases or immunodeficiency have an increased risk of a severe disease course of COVID-19.

⇒ Different laboratory assessments are available (eg, testing of antibodies, interferon gamma release assay and surrogate virus-neutralising tests), but little is known about their suitability for immune monitoring and guidance of booster or revaccination.

WHAT THIS STUDY ADDS

⇒ This study provides new information on B-cell and T-cell responses to COVID-19 vaccination and on infection rates following booster vaccination in patients with primary immunodeficiencies and immune-mediated diseases treated with immunomodulatory agents of different classes in a clinical routine care setting, including documentation of a compensatory T-cell response in patients undergoing B-cell suppressive therapy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results from this study provide insights on immunity provided by T cells in immune-deficient patients with impaired antibody responses and on how immune monitoring of vaccination responses by laboratory testing could be useful as a decision-making aid for COVID-19 management in immunosuppressed patients in routine care.

INTRODUCTION

COVID-19, caused by SARS-CoV-2, has become one of the most momentous and consequential pandemics in the history of infectious disease.[1] Fortunately, through enormous research efforts, effective vaccines have been developed within a short period. Their efficacy and safety during widespread use in the general population are supported by a growing number of studies proving the development of protection against infection and severe disease courses due to an induction of cellular immunity and the generation of specific antibodies against SARS-CoV-2.[2] Currently, six vaccines against COVID-19 are approved in Germany and Europe and are in clinical use.[3]

A prerequisite for an adequate response after vaccination is a competent immune system, the function of which, however, may be compromised by underlying diseases and treatments. Patients suffering from immune-mediated inflammatory diseases (IMIDs) with immunomodulatory treatment or from primary immunodeficiency (PID) have abnormal adaptive immune B-cell and T-cell responses, which may lead to an altered responsiveness to SARS-CoV-2 vaccines.[1 4–8] These impairments in immunological responses argue for close monitoring and support recommendations for high-priority booster vaccination to protect these specific patient groups from potentially fatal SARS-CoV-2 infection.[4 7] Beyond the type of IMID with its specific pathophysiological pathways, the immune modulatory regimen chosen for treatment is of particular importance when assessing the risk of a potentially insufficient immune response to vaccination in clinical routine care. Treatment regimens with the potential to suppress the humoral immune system, such as B cell-depleting therapies (anti-CD20; eg, rituximab (RTX)), oral/parenteral steroids and conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) or biological disease-modifying antirheumatic drugs (DMARDs), may compromise antibody-dependent protective immunity,[4–6 9–12] whereas abatacept is likely to preferentially impact T-cell responses.[10 13] Moreover, immune disorders are often associated with comorbid conditions that increase the likelihood of a more serious course of COVID-19.[4] These patients are therefore particularly likely to gain from a robust response to vaccination.[5] However, patients who had PID or were immunosuppressed were initially excluded from pivotal COVID-19 vaccine trials.[4 6 14] Thus, there are limited data available on the outcome of vaccination in terms of induced protective immunity to SARS-CoV-2 in these patient groups.[4]

Moreover, for any kind of vaccination, informative data derived from routine care are sparse. These data are critical to addressing questions concerning which laboratory tests are suitable to capture vaccination-induced immune responses and how to interpret assay results with regard to the assessment of an appropriate vaccine response to help guide management.[15] In the present study, performed in routine care, we determined B-cell and T-cell immune responses elicited by COVID-19 booster vaccination (third shot) in immunosuppressed patients. Laboratory assessments were performed in a stratified fashion to detect potential differences in immune responses in patient cohorts with distinct underlying clinical disease characteristics and therapies.

METHODS

Study design

The goal of this exploratory, investigator-initiated study (Relivienz) was to investigate whether suppressed immune function correlated with a compromised response to COVID-19 booster vaccination (third shot) as assessed by measures of T-cell and B-cell functions.

We recruited immunosuppressed patients with IMID through the outpatient clinic of the division of rheumatology at the University Hospital in Frankfurt. The study recruitment period was from 30 September 2021 to 31 January 2022.

We stratified the IMID cohort into three groups: patients with loss of B-cell function because of PID (eg, common variable immunodeficiency (CVID)), patients with B-cell depletion due to RTX therapy and patients with IMID treated with other immunosuppressive therapies (csDMARDs/bDMARDs). Assessments of the SARS-CoV-2 prebooster and postbooster vaccination immune response were performed in close collaboration with the Institute for Medical Virology of the University Hospital Frankfurt at a baseline visit (BL, before booster vaccination) and 2, 4 and 8 weeks after the booster vaccination, with a deviation of a maximum of 3 days (figure 1).

Study population

Adult patients (age ≥18 years) were included if they had completed the initial COVID-19 vaccination series (without a third (booster) vaccination) within the last 9 months, had an appointment for the third COVID-19 vaccination at their general practitioner or a vaccination center, and met at least one of the following criteria for immunosuppression: (1) diagnosis of PID by a rheumatologist at least 3 months prior to the first COVID-19 vaccination; (2) current treatment with RTX therapy for at least 3 months prior to the first COVID-19 vaccination; (3) current treatment with any csDMARD or bDMARD except RTX. A third vaccination (booster) had to be indicated according to the Standing Committee on Vaccination recommendation.

Outcomes

Clinical data collected at BL included demographic information, COVID-19 vaccination information, and disease, treatment and medical history. Furthermore, we screened every patient for a previous SARS-CoV-2 infection by measuring SARS-CoV-2-specific antinucleocapsid–protein IgG, which is only detectable on a resolved SARS-CoV-2 infection but not induced by the COVID-19 vaccines used in this study.

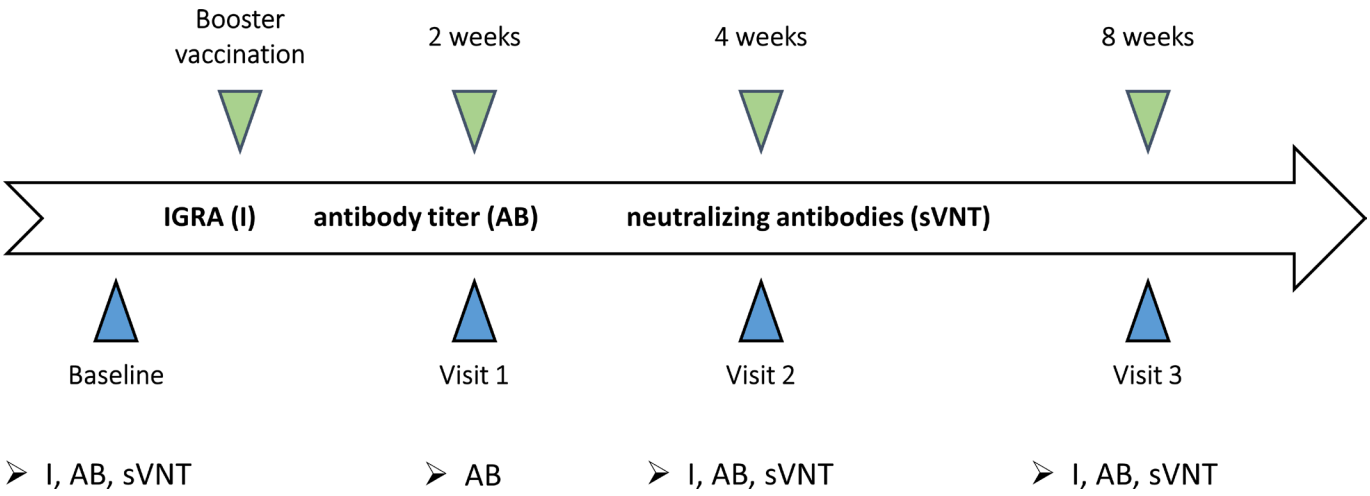

Figure 1 Study design and assays performed at each visit. AB, antibody titre; IGRA, interferon gamma release assay; sVNT, surrogate neutralisation test.

Laboratory assays were used to assess immune responses. All laboratory assays were conducted at BL and at each of the three postbooster vaccination visits and were performed according to the manufacturer's specifications. B-cell responses were evaluated by two different assays that measured (1) SARS-CoV-2-specific antispike IgG antibody (anti-S-AB) titres and (2) functional SARS-CoV-2-specific antibody neutralisation capacity. Anti-S-AB titres were determined using SARS-CoV-2 IgG on the Abbott Alinity i platform (Abbott GmbH, Wiesbaden, Germany). The positive response threshold was defined as ≥120 binding antibody units (BAU)/mL based on correlation with positivity in cell-based live SARS-CoV-2 (wild-type) neutralisation assays[16]; this clinical cut-off is much higher than the antibody titre of 8 BAU/mL listed in the manufacturer's information as the lowest detectable analytical threshold. Functional SARS-CoV-2 neutralisation was detected via a surrogate virus neutralisation test (sVNT) using the GenScript SARS-CoV-2 Surrogate Virus Neutralisation Test Kit (GenScript Biotech, Piscataway Township, USA), including the wild-type SARS-CoV-2 spike protein–receptor-binding domain. The positive response threshold was defined as neutralisation of at least 35% of viral components.

T-cell responses were evaluated by use of a SARS-CoV-2-specific interferon gamma release assay (IGRA) using Quan-T-Cell-ELISA (Euroimmun, Lübeck, Germany) on the EUROIMMUN Analyzer I (Euroimmun). The positive result threshold was defined as ≥200 milli-international units (mIU) of secreted interferon gamma (IFN-γ) per mL.

For the detection of nucleocapsid-specific SARS-CoV-2 antibodies to evaluate SARS-CoV-2 infection at baseline, the Abbott SARS-CoV-2 IgG II was used on the Abbott Alinity i platform (Abbott GmbH).

Statistical analysis

Because this was an exploratory study, it was not possible to adequately predict the effect size. Accordingly, sample size calculations were not performed. Sample sizes were based on the available patient population.

For all statistical analyses, we used the statistical programming language R V.4.2.0 and the integrated development environment RStudio V.2022.07.0 Build 548 with additional R packages (as described further).

For the primary analysis, we compared the absolute values of anti-S-AB levels (baseline, visit (V)1, V2 and V3), proportion of patients with a sVNT response, and proportion of patients with an IGRA response between visits (baseline, V2 and V3) and between groups (PMID, RTX and csDMARDs/bDMARDs) using descriptive and inferential statistics. Baseline characteristics were evaluated with descriptive statistics of demographic parameters and other parameters relevant to immune status, including the vaccination type for basic immunisation, the time from completed basic immunisation to BL, underlying IMID and the type of ongoing immunotherapy stratified by immunodeficiency group.

For inferential statistics, we used non-parametric approaches to account for deviations from the normal distribution assumption of many statistical procedures and tests indicated by the results of Q–Q plots (not shown) and the small sample sizes of the immunodeficiency groups. In the case of independent samples, we used Wilcoxon rank-sum tests with continuity correction to compare measurements (absolute values of anti-S-AB levels, sVNT response or IGRA response) of two immunodeficiency groups for a specific visit. For the comparison between two visits of one specific immunodeficiency group and thus dependent samples, we used Wilcoxon signed-rank tests with continuity correction. Both tests were conducted using the function 'wilcox.test' of the R-package 'stats', applying a continuity correction in the normal approximation for the p value and computing an exact p value and CIs. In the case of the Wilcoxon signed-rank test, we conducted a paired test as the measurements from two visits represent a

paired-sample design. A p value ≤0.05 was considered significant.

RESULTS

Baseline characteristics and patient disposition

Fifty patients were included in these analyses. Six had PID; 13 were patients with IMID treated with RTX therapy; and 31 were patients with IMID treated with csDMARDs/bDMARDs with low-dose steroids (<10 mg/day) except for 1 subject who received ≥10 mg/day. Thirty-three patients were female (66%) and 17 were male (34%) (table 1). The average age was 53 years. Most patients (42 (84%)) were vaccinated exclusively with mRNA vaccines (table 1). The exceptions were two patients in the RTX treatment cohort who received vector vaccines only (one (2%) Astra Zeneca, one (2%) Johnson & Johnson/Janssen) and five patients in the csDMARD/bDMARD group (10%) who received the AstraZeneca vaccine for the first vaccination and subsequently received an mRNA vaccine for the second vaccination. Data concerning type of initial immunisation were missing for one patient in the RTX cohort (2%). For the booster vaccination, only mRNA vaccines (Moderna or BioNTech) were approved in Germany and accordingly administered to the study cohort. The average time from last vaccination until BL (before booster vaccination) was 186 days for PID, 176 days for RTX and 179 days for csDMARD-treated/bDMARD-treated patients (table 1). None of the patients had a history of SARS-CoV-2 infection in their medical history. This was confirmed by a failure to detect any titres of SARS-CoV-2-specific antinucleocapsid protein antibodies at baseline.

The 50 patients had 18 different diagnoses of underlying disease conditions (table 1). Among six patients with PID, four suffered from CVID (67%). Rheumatoid arthritis (RA, 38%) and granulomatosis with polyangiitis (38%) were the most common diagnoses in patients treated with RTX. Among patients treated with cs/bDMARDs, psoriatic arthritis (29%) and RA (19%) were the most frequent diagnoses.

All patients with PID were receiving IgG substitution therapy (66% intravenously and 33% subcutaneously). The average timing of administration of IgG therapy with respect to the third SARS-CoV-2 vaccination was 12 days before and 20 days after vaccination. In the RTX treatment group, four patients (30%) received RTX as monotherapy and nine patients (70%) were on a combination of RTX with another immunomodulating agent, mostly low-dose prednisolone. The timing of the last RTX therapy was 1 year before booster vaccination in three patients (23%), half a year in one patient (8%), 4–6 months in three (23%) patients and 2–4 months in six patients (46%). Patients treated with csDMARDs/bDMARDs were on bDMARDs only (n=13 (42%)), bDMARDs combined with csDMARDs and low-dose steroids (n=13 (42%)), or csDMARDs and low-dose steroids without any biological agent (n=5 (16%)). Interleukin inhibitors were the most common class of bDMARD (n=12 (39%)) followed by tumour necrosis factor inhibitors (n=11 (35%)). Four patients were on abatacept therapy.

Measurement of B-cell counts was not a part of the study protocol and therefore was not performed in a standardised way or at a uniform point in time with respect to the screening visit, so the data are not conclusive. Within the PID group, the mean B-cell count was 201/µL; measurements were taken between 3 months and 2 years before the screening visit. In the RTX treatment group, B-cell counts were documented in the period before application of RTX (within 6 months) in nine patients with a mean value of 9 cells/µL. In patients in the csDMARD/bDMARD groups, B-cell counts were not documented.

Of the 50 patients, 8 (16%) missed at least one visit (figure 2). Reasons were lack of compliance to take part at the determined study visits in five cases, SARS-CoV-2 infections in two cases and one patient receiving a monoclonal antibody therapy against SARS-CoV-2 (see further), making it unreasonable to evaluate further results. The other 42 (84%) patients completed all scheduled visits, resulting in a total adherence rate of 186 out of 200 planned visits for the entire cohort (93%).

One patient received monoclonal antibody therapy against SARS-CoV-2 during the study period due to lack of an immune response to vaccination before and 2 weeks after booster vaccination. After transfusion of antibodies, the patient had the highest anti-S-AB titres among all participating patients (11 360 BAU/mL) with 97% neutralised virus components on the sVNT assay. IGRA measurement failed. Results obtained from this patient after receipt of the monoclonal antibodies (post visit 1) were excluded from the analysis.

Immunoassay performance

Anti-S-AB and sVNT were measured at every visit without any test failure. In contrast, IGRA had a considerable test failure rate (26 tests), leading to evaluable results in 112 of the total 138 measured IGRA at BL, V2 and V3 (86%). Test failure reasons were inconsistent controls for assay performance (21 positive results in a negative control (81%), 4 negative results in a positive control (15%) and one sample that could not be evaluated due to other reasons (4%)). Despite the small group size of patients treated with RTX (n=13) or with abatacept therapy (n=4), the highest failure rates of IGRA test performance were detected among those patients (11 tests among patients with RTX therapy (42% of failures) and 8 tests among patients with abatacept therapy (31% of failures)). The other seven test failures (27% of failures) were evenly distributed among the remaining 26 patients.

Humoral immune responses

At BL, the majority of patients had an anti-S-AB titre below the defined threshold of 120 BAU/mL. The PID group had a median of 75 BAU/mL, and csDMARD/bDMARD-treated patients had a median of 84 BAU/

Table 1 Baseline characteristics

	PID (N=6)	RTX (N=13)	csDMARD/bDMARD (N=31)
Sex, n (%)			
Female	1 (17)	12 (92)	20 (65)
Male	5 (83)	1 (8%)	11 (35%)
Mean age (years) (SD)	42 (8.80)	56 (14.50)	53 (12.97)
Mean BMI (kg/m²) (SD)	25 (5.45)	30 (8.24)	25 (5.72)
Smoking status, n (%)			
Non-smoker	3 (50)	9 (69)	12 (39)
Ex-smoker	2 (33)	4 (31)	11 (35)
Smoker	1 (17)	–	8 (26)
Vaccination type for initial immunisation, n (%)			
mRNA	5 (83)	10 (77)	27 (87)
mRNA⁺vector	1 (17)	–	4 (13)
Vector	–	2 (15)	–
Unknown	–	1 (8)	–
Mean time from completed basic immunisation to BL (days) (SD)	186 (33)	176 (43)	179 (27)
Underlying IMID, n (%)			
Agammaglublinaemia	1 (17)	–	–
CVID	4 (67)	–	–
SCID	1 (17)	–	–
Antisynthetase syndrome	–	1 (8)	–
GPA	–	5 (38)	–
Interstitial lung disease	–	1 (8)	1 (3)
MPA	–	1 (8)	–
RA	–	5 (38)	6 (19)
axSpA	–	–	2 (6)
Juvenile arthritis	–	–	1 (3)
IgA vasculitis	–	–	1 (3)
Adult-onset Still's disease	–	–	4 (13)
Polymyalgia rheumatica	–	–	2 (6)
PsA	–	–	9 (29)
Giant cell arteritis	–	–	1 (3)
Sarcoidosis	–	–	2 (6)
SLE	–	–	1 (3)
Systemic sclerosis	–	–	1 (3)
Ongoing immunotherapy			
IgG intravenous	4 (67)	–	–
IgG subcutaneous	2 (33)	–	–
RTX	–	13 (100)	–
csDMARD	–	5 (38)	14 (45)
Low-dose steroids (<10 mg/day)	–	8 (61)	9 (29)
High-dose steroids (≥10 mg/day)	–	–	1 (3)
TNF inhibitors	–	–	11 (35)
Abatacept	–	–	4 (13)
IL-1 inhibitors	–	–	4 (13)

Continued

Table 1 Continued

	PID (N=6)	RTX (N=13)	csDMARD/bDMARD (N=31)
IL-6 inhibitors			1 (3)
IL-17 inhibitors			4 (13)
IL-12/23 inhibitors			3 (10)

axSpA, axial spondyloarthritis; bDMARD, biological disease-modifying antirheumatic drug; BL, baseline; BMI, body mass index; csDMARD, conventional synthetic disease-modifying antirheumatic drug; CVID, common variable immunodeficiency; GPA, granulomatosis with polyangiitis; IL, interleukin; IMID, immune-mediated inflammatory disease; MPA, microscopic polyangiitis; PID, primary immunodeficiency; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RTX, rituximab; SCID, severe combined immunodeficiency; SLE, systemic lupus erythematosus; TNF, tumour necrosis factor.

mL. Among patients treated with RTX, the median was 0 BAU/mL, and the titre remained at 0 BAU/mL after booster vaccination (table 2 and figure 3). In contrast, PID and csDMARD-treated/bDMARD-treated patients exhibited an increase in anti-S-AB titres 2 weeks after booster vaccination with median values of 286 BAU/mL in the PID group (p=0.059 for change from baseline) and 2712 BAU/mL in csDMARD-treated/bDMARD-treated patients (p<0.0001).

The anti-S-AB values of patients with PID were significantly higher 2 weeks after booster vaccination compared with patients treated with RTX (p=0.0081) but significantly lower compared with the csDMARD/bDMARD cohorts (p=0.0066). During the study period of 8 weeks, the anti-S-AB levels remained at the same level in patients with PID (median of 300 BAU/mL after 4 weeks and 297 BAU/mL after 8 weeks). In csDMARD-treated/bDMARD-treated patients, anti-S-AB levels decreased over the study period (median of 1670 BAU/mL after 4 weeks and 1179 BAU/mL after 8 weeks) (table 2 and figure 3). A healthy control group was not included in the study design. However, another study using the same test system examined the vaccination response in 445 healthy volunteers.[17] Two weeks after booster vaccination, mean anti-S-AB values ranged between 3821 BAU/mL and 6045 BAU/mL, depending on the vaccine used,[17] and were thus higher than the mean values in the IMID groups observed here.

In agreement with the results for anti-S-AB titres, the sera of patients treated with RTX did not contain any noteworthy activity for virus neutralisation at baseline and also remained unaltered on vaccination (table 2 and

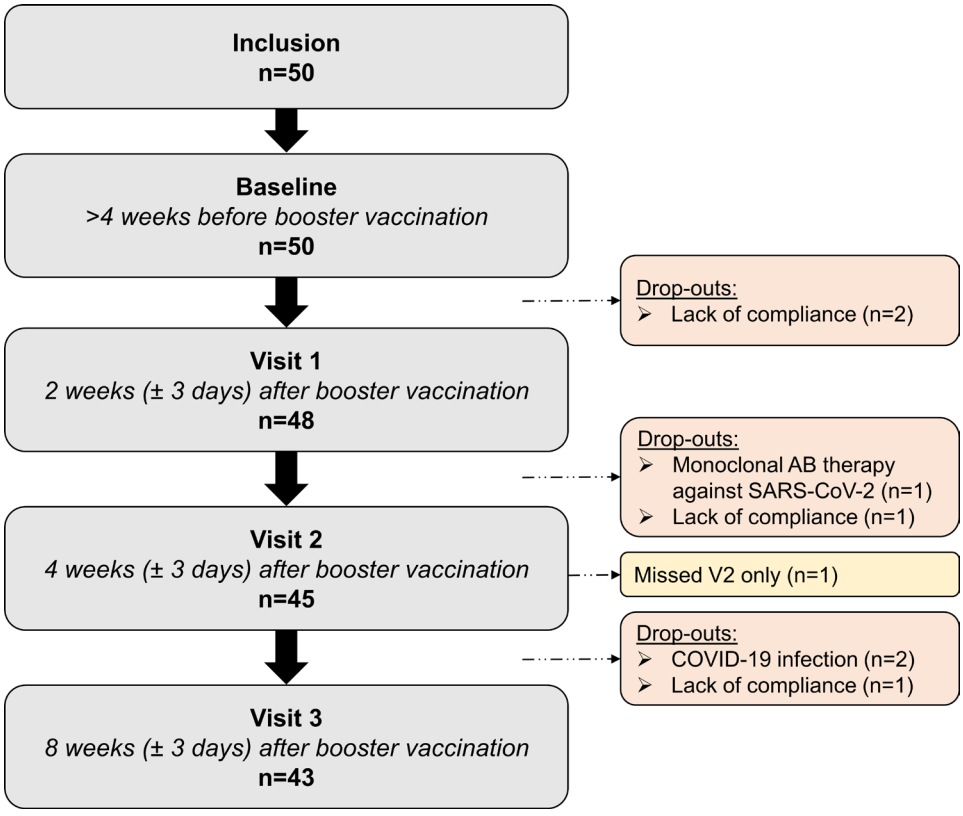

Figure 2 Patient distribution.

Table 2 Summary of humoral and cellular immunoassays in patients with IMID receiving COVID-19 booster vaccination

Assay	Time point				Key inferential statistics
	Baseline	Week 2	Week 4	Week 8	
Anti-S-AB, median (95% CI) BAU/mL					
PID	75 (–3 to 151)	286 (–236 to 807)	300 (–60 to 659)	297 (139 to 455)	At week 2, significantly higher than RTX (p=0.0081) but significantly lower than csDMARD/bDMARD (p=0.0066)
RTX	4 (–4 to 4)	0 (–9 to 9)	6 (–5 to 16)	0 (–10 to 10)	
csDMARD/bDMARD	84 (45 to 123)	2712 (1549 to 3830)	1670 (838 to 2502)	1179 (681 to 1678)	Significant increase between baseline and week 2 (p<0.001)
sVNT, median (95% CI) % of patients					
PID	53% (5 to 100)	ND	91% (62 to 119)	95% (89 to 101)	u
RTX	0% (0 to 0)	ND	0% (0 to 0)	0% (0 to 0)	
csDMARD/bDMARD	56% (33 to 79)	ND	96% (95 to 97)	97% (96 to 98)	Significant increase between baseline and week 4 (p<0.0001)
IGRA, median (95% CI) mIU/mL					
PID	215 (101 to 329)	ND	965 (163 to 1767)	664 (–1383 to 2710)	
RTX	1290 (511 to 2068)	ND	2555 (675 to 4435)	2404 (1794 to 3014)	Higher at baseline than the PID group (p=0.05) or csDMARD/bDMARD group (p=0.06)
csDMARD/bDMARD	331 (–53 to 715)	ND	1765 (1017 to 2512)	1308 (682 to 1934)	

BAU, binding antibody unit; bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; IGRA, interferon gamma release assay; MIU, micro international unit; ND, not determined; PID, primary immunodeficiency; RTX, rituximab; S-AB, spike IgG antibody; sVNT, surrogate virus neutralisation test.

figure 4). However, positive sVNTs at BL were exhibited in a substantial proportion of patients in the PID (median of 52.5%) and csDMARD/bDMARD cohorts (median of 56%). Booster vaccination increased the proportion of patients positive for sVNT in the PID (p=0.059) and csDMARD/bDMARD cohorts (p<0.0001), and these cohorts retained high levels at 8 weeks after vaccination (95% in the PID and 97% in the csDMARD/bDMARD cohorts). Although the anti-S-AB titre levels differed significantly between PID and csDMARD/bDMARD-treated patients, this difference was not reflected by relevant differences in sVNT assay results. In the previously mentioned study with a control group of 445 healthy volunteers, sVNT values at 4 weeks after booster vaccination were comparable to levels observed in patients in the PID and csDMARD/bDMARD groups.[17]

Except for RTX, no significant differences were observed in immune responses by therapeutic agent, including abatacept. With respect to RTX timing, the two patients who did not receive RTX for 1 year did not differ in their humoral immune response against SARS-CoV-2 compared with patients with the half-year time interval.

Covariate analyses of age and booster-type vaccine did not reveal any significant differences in response patterns related to these variables for humoral or cellular immunoassays.

Cellular immune responses

At BL, most patients in all groups exhibited a positive anti-SARS-CoV-2 T-cell response as determined by IGRA results (table 2 and figure 5). In contrast to the results of anti-S-AB and sVNT assessments, patients with RTX therapy exhibited the highest IFN-γ responses determined by IGRA, and these were higher than in the PID (p=0.05) or csDMARD/bDMARD cohorts (p=0.06). After booster vaccination, the IGRA values increased in all three groups. The highest median level was observed 4 weeks after booster vaccination among patients receiving RTX (2555 mIU/mL), followed by csDMARD-treated/bDMARD-treated patients (1764.5 mIU/mL) and patients with PID (965 mIU/mL). Eight weeks after booster vaccination, IGRA levels tended to slightly decrease but still remained far above the threshold defining positive responses. IGRA levels in the IMID groups were lower than the median IGRA values of 6492–14269 mIU/mL (depending on the vaccine) recorded in a study of healthy controls using the same test procedure.[18]

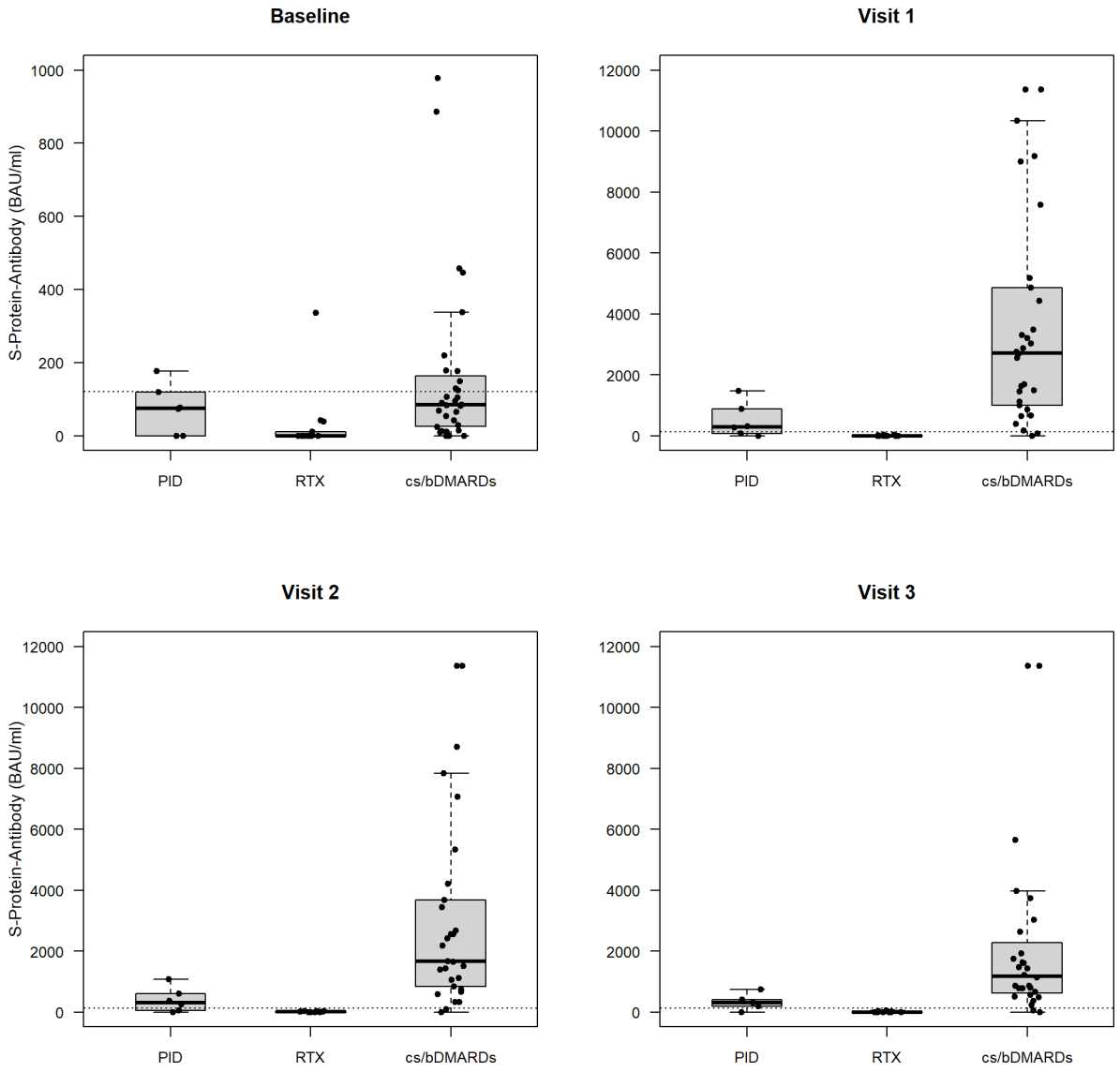

Figure 3 Levels of spike protein-specific antibodies (BAU/mL) by patient cohort and visit. Box plots show the range from the 25th to 75th percentiles, with the middle line indicating the median and the whiskers indicating minimum to maximum spread of data. The threshold for a positive test result is represented by the horizontal dashed line. The y axis shows antibody levels. Because antibody titres were significantly lower before the third vaccination, the scaling for baseline was adjusted to clarify the differences between the groups. Visit 1 was at 2 weeks, visit 2 at 4 weeks and visit 3 at 8 weeks. BAU, binding antibody unit; bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; PID, primary immunodeficiency; RTX, rituximab.

We further evaluated whether csDMARD versus bDMARD treatment influenced IGRA responses in the csDMARD/bDMARD cohorts. We did not detect any difference based on type of therapy between 13 patients on bDMARD monotherapy and 4 patients on csDMARD monotherapy (online supplemental figure 1), but analyses were complicated by patients treated with more than one drug class (eg, both a csDMARD and bDMARD) and by varying levels of glucocorticoid therapy among patients.

Patients with COVID-19 within the study period

Two patients acquired COVID-19 disease during the study period. Both patients had first symptoms in the time interval between 4 weeks and 8 weeks after the third vaccination. One was on secukinumab therapy and the other patient had PID. Both patients had mild symptoms and missed only the last visit (week 8) due to sickness. The patient on secukinumab therapy had especially strong positive results in all three parameters of SARS-CoV-2-specific immunity at BL. The patient with PID had anti-S-AB titres below the threshold at BL, but results from sVNT and IGRA testing were well within the positive range 4 weeks after booster vaccination.

DISCUSSION

Most of the immunosuppressed patients taking part in our study had a positive immune response to the SARS-CoV-2 booster vaccination on humoral, cellular or both levels.

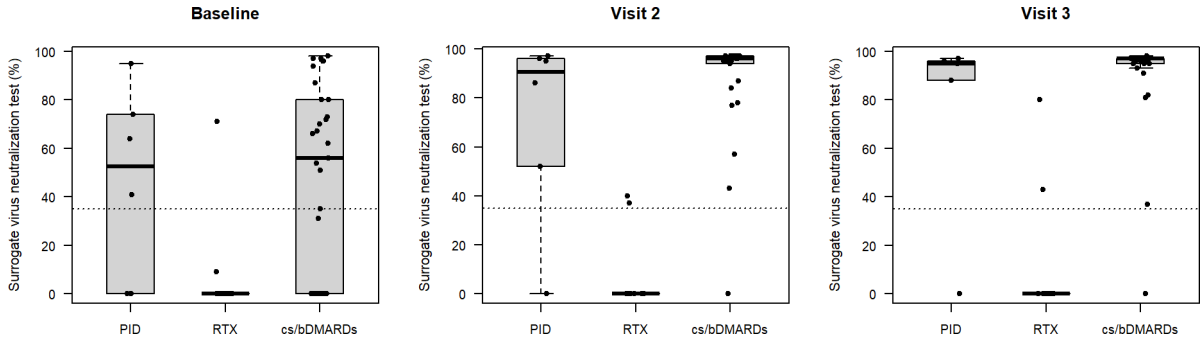

Figure 4 Levels of surrogate virus neutralisation test by patient cohort and visit. Box plots show the range from the 25th to 75th percentiles, with the middle line indicating the median and the whiskers indicating the minimum to maximum spread of data. The threshold for a positive test result is represented by the horizontal dashed line. The y axis shows the percentage of virus neutralisation. Visit 2 was at 4 weeks and visit 3 WAS 8 weeks. bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; PID, primary immunodeficiency; RTX, rituximab.

Patients on RTX therapy generally lacked adequate anti-S-AB titres and sVTN responses, even after booster vaccination. However, these B cell-depleted patients exhibited significantly higher levels of IFN-γ release responses detected by IGRA, indicating an increased activation of T cells, perhaps as a compensatory mechanism for the lack of humoral immune responses. Our study provides insights into immune responses in immunosuppressed patients following COVID-19 booster vaccination, including valuable comparative data on B-cell and T-cell immune responses in patients with immunosuppression mainly related to their primary disease and patients with immunosuppression potentially related both to IMID and to disease-related therapies.

Previous preliminary studies have suggested that vaccination against SARS-CoV-2 is safe and effective in patients with IMIDs.[1 4 7–12] Furer *et al*[9] showed that a two-dose regimen with the mRNA BNTb262 vaccine was effective in the majority of their patients with autoimmune inflammatory rheumatic disease (seropositivity of 86% vs 100% for controls) and had an acceptable safety profile. However, B cell-depleting substances are known to significantly impair antibody production,[19] including antibody responses to the initial SARS-CoV-2 vaccination series.[5 6 9 11 12 20–24] As might be expected, in the study by Furer *et al*,[9] immunogenicity was severely compromised by B cell-depleting therapies (mainly represented by RTX) (seropositivity of 41% for RTX monotherapy and 36% for RTX in combination with MTX). The time interval between RTX administration and vaccination played a critical role in predicting response to the vaccine.[9]

Similar results were published by Fabris *et al*[21] showing that patients with depleted B cells due to RTX or belimumab treatment have significantly lower anti-S-AB levels, but exhibit IFN-γ release levels in the range of healthy controls after SARS-CoV-2 vaccination. Other studies have also observed increased T-cell responses following SARS-CoV-2 vaccination in RTX-treated patients or patients receiving other anti-CD20 agents compared with patients with IMID treated with different therapies,[21 22 25 26] but some have reported similar T-cell responses.[27 28] Most of the cohorts in these studies have been small, so differences among studies may be influenced by the specific patients included in the cohorts. Our findings support the hypothesis of Jyssum *et al*,[22] who suggested that mRNA booster vaccination in RTX-treated patients might not lead to higher anti-S-AB concentration but could still induce an improved cellular immune response. A case

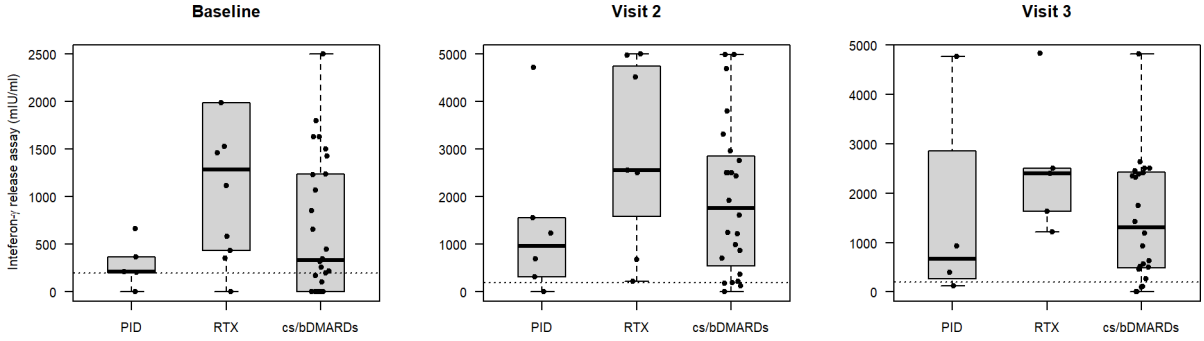

Figure 5 Levels of interferon gamma release assay by patient cohort and visit. Box plots show the range from the 25th to 75th percentiles, with the middle line indicating the median and the whiskers indicating minimum to maximum spread of data. The threshold for a positive test result is represented by the horizontal dashed line. The y axis shows the level of interferon released during the assay. bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; mIU, micro-international units; PID, primary immunodeficiency; RTX, rituximab.

series of RTX-treated rheumatology patients who developed COVID-19 following booster vaccination found that all patients had mild disease, despite very low anti-SARS-CoV-2 antibody levels, providing further support for this hypothesis.[24]

Even though patients on csDMARD/bDMARD therapy and patients suffering from PID exhibited low anti-S-AB titres at BL, the results of IGRA and sVNT remained above the threshold for positive responses in most patients, indicating a preserved responsiveness to SARS-CoV-2 prior to booster vaccination. The negative result of SARS-CoV-2-specific antinucleocapsid-AB testing in all patients demonstrates that none of the patients included in the study had acquired a SARS-CoV-2 infection before their booster vaccination, supporting the hypothesis that the vaccination-induced immune responses were sufficient to confer protective immunity for a relevant period of time. The average time between last vaccination and BL was around 6 months in both groups (186 days in the PID cohort and 179 days in the csDMARD/bDMARD cohorts).

After booster vaccination, all measured variables of adaptive immunity increased above the threshold for positivity, reaching higher levels among csDMARD-treated/bDMARD-treated patients with IMID compared with patients with PID. Hence, PID seems to have a stronger impact on antiviral immune response than immunosuppressive treatment. The patients with PID in our study cohort suffered from either CVID, severe combined immunodeficiency or agammaglobulinemia with a considerable heterogeneity in the associated degree of immunodeficiency. In general, the patients with PID were more severely immunocompromised compared with patients with IMID taking immunosuppressants; some patients with PID even required long-term antimicrobial prophylaxis.

Although Simon *et al*[4] concluded that reduced immune responses against SARS-CoV-2 in patients with IMID were mainly due to the impact of the underlying IMID-associated immune dysregulation and the disease activity itself rather than to concomitant treatment, other studies of humoral responses to COVID-19 vaccination in patients with rheumatic disease have found associations between therapy and immune response regardless of underlying diagnosis; specific agents associated with a reduced response included RTX, mycophenolate, methotrexate, tumour necrosis factor inhibitors, tocilizumab, Janus kinase inhibitors and abatacept.[10 12 29] RTX and abatacept appear to have a more profound effect on the COVID-19 antibody response than other immunosuppressive agents.[10 12 29 30] We did not observe an additional impact on vaccine response with abatacept, perhaps due to the small number of abatacept-treated patients in our study (n=4). Overall, our findings support the influence of both underlying diagnosis and specific therapeutic agents in determining immune responses to COVID-19 booster vaccination. It is important to note that the Simon *et al* study did not include patients with B cell-depleting therapy.[4] For B cell-depleting agents, the degree of B-cell recovery at the time of COVID-19 vaccination has been reported to correlate with the magnitude of induced humoral response to vaccination, similar to the observations for influenza vaccines in patients with RA treated with RTX.[31]

Although we did not evaluate the effect of COVID-19 booster vaccination on specific T-cell subpopulations, studies by others suggest that spike protein-specific Th1 cytokine responses are primarily mediated by $CD4^+$ cells and that this response is reduced in immunosuppressed patients with RA or multiple sclerosis compared with healthcare workers. In addition, reduced naïve $CD4^+$ T cells and increased effector memory cells were observed in immunosuppressed patients compared with control, perhaps due to chronic autoantigen exposure.[25 32]

After initial increases in immune responsiveness following booster vaccination, anti-S-AB titres and IGRA values subsequently slightly decreased over the period of 8 weeks. A monthly decay of antibody titre after initial SARS-CoV-2 mRNA vaccination is also detectable in healthy individuals, resulting in about 16% seronegative individuals 6 months after vaccination.[33]

Anti-S-AB and sVNT assays did not show test failures in any patient. IGRA, on the other hand, exhibited inconsistencies of assay performance at a substantial frequency (19%), especially due to failure rates of positive controls for T-cell stimulation in sera from RTX-treated and abatacept-treated patients. A case series of RTX-treated rheumatology patients also found a high rate of IGRA test failures due to an inadequate reaction to mitogen controls (2 of 4 (50%)).[24] This implies that in those patients, the T cells exhibit an increased activation threshold, rendering them unresponsive even to non-specific mitogenic T-cell stimulation. In the case of abatacept, its blocking effect on the costimulation of interferon-secreting cells might contribute to a state of reduced T-cell responsiveness. It is not clear why this reduced responsiveness would develop as a consequence of the depletion of $CD20^+$ cells, and therefore our results may reflect an induction of more complex disturbances in T-cell and B-cell interactions. It is possible that alternative COVID-specific interferon release assays may result in reduced failure rates.[34 35]

The vaccination-induced rise in SARS-CoV-2-specific antibodies remains not unexpectedly limited to increases beyond a certain level as observed for anti-S-AB titres in response to booster vaccination in PID and with csDMARD/bDMARD treatment. In general, increased anti-S-AB levels roughly correlated with the capability of neutralising viral components (sVNT), but interestingly, patients with PID exhibited a virus-neutralising capacity similar to csDMARD/bDMARD-treated patients despite considerably lower SARS-CoV-2-specific anti-S-AB titres. For B cell-depleted patients, our study results strongly support a recommendation to carry out both B-cell (anti-S-AB and sVNT) and T-cell (IGRA) response assays to determine the success of COVID-19 vaccination. Test failures, especially in RTX-treated or abatacept-treated

patients, however, might limit the usability of laboratory assays during routine clinical care.

During the 8 weeks of the study period, two patients were infected with SARS-CoV-2, even though both exhibited vigorous specific immune reactivities in our immunoassays after the booster vaccination. This finding illustrates the difficulty in judging at what threshold a respective test result represents a reliable marker for a sufficiently established protective immunity against SARS-CoV-2. However, both patients had a mild disease course, thereby suggesting that monitoring vaccine responses might help predict the likelihood of a severe course in case of a future infection. This knowledge could potentially inform management of suspected SARS-CoV-2 infections.

Study limitations include small numbers of patients in the three cohorts and multiple underlying IMIDs and immunomodulatory therapies, which may have influenced our findings. The only assay we had available for T-cell responses, IGRA, had a relatively high failure rate, particularly in RTX-treated or abatacept-treated patients. Our study covered the first 8 weeks following administration of a COVID-19 booster (third shot) but did not look at later timepoints. There was no control (non-IMID) group in our study, although others in our author group have used these methods to test healthy controls, and we have provided these data for comparison.[17 18] Additional studies have reported that patients with RA have reduced antibody and interferon responses compared with healthcare workers for both the initial vaccination series and for booster doses.[30 32] All booster shots administered were mRNA vaccines, as they were the only approved options for booster shots at the time the study was conducted. It is possible that other vaccine formulations, such as viral vectors, would have elicited different immune responses. In addition, the threshold of 120 BAU/mL for anti-S-AB corresponds to positivity in a cell-based live virus neutralisation assay using the SARS-CoV-2 wild type,[16] which does not necessarily reflect the neutralisation capacity against circulating variants of concern when the study was conducted. This limitation also applies to the positivity threshold used for the SARS-CoV-2 wild-type sVNT assay. For patients in the PID group, IgG immunotherapy could have potentially contained anti-SARS-CoV-2 antibodies that might have influenced assays of antibody levels or virus neutralisation. However, the low baseline levels of spike antibodies and virus neutralisation suggest that transfer of anti-SARS-CoV-2 antibodies in IgG, if any, did not have a notable impact on the humoral response in patients with PID. Although we conducted some analyses of potential confounding variables, including age and vaccine type, our confidence in these data were limited by the small size of the subgroups involved. In addition, it is possible that confounding variables not addressed by our analyses could have influenced our results.

In conclusion, our study found that B cell-level and T cell-level immunity in patients with PID or csDMARD/bDMARD treatment improved following COVID-19 booster vaccination. RTX-treated patients did not show improvements in B-cell responses following booster vaccination but had greater increases in T-cell immunity compared with other groups, supporting the existence of a compensatory mechanism for the impaired humoral response. Our findings provide insights into protection provided by T cells in immunosuppressed patients undergoing B cell-depleting therapy and suggest that laboratory testing may be valuable in guiding immune monitoring of vaccination responses as a decision-making aid for COVID-19 management in immunosuppressed patients in routine care.

Author affiliations
[1]Department of Rheumatology, Goethe University, Frankfurt am Main, Germany
[2]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Frankfurt am Main, Germany
[3]Fraunhofer Cluster of Excellence Immune-mediated Diseases CIMD, Frankfurt am Main, Germany
[4]Institute for Medical Virology, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt am Main, Germany
[5]Virology, German Centre for Infection Research, Frankfurt am Main, Germany
[6]Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology, Goethe University, Frankfurt am Main, Germany

Acknowledgements The authors sincerely thank the investigators, staff and patients involved in the Relivienz study. Some of the data reported here were presented at the Congress of German Society of Rheumatology in August/September 2022.

Contributors FB and MKo conceptualised the study and wrote the first draft of the study protocol. The protocol was finalised after being reviewed by FB, MKo, HB, SC, HFR, NK, SD and GG prior to submission to the Goethe University ethics committee. Recruitment and screening of study subjects was performed by MKI, MKo, and FB. Data were interpreted by MKo, SD, MKI, KH, NK, HFR, HB, GG, SC and FB. Statistics were obtained by KH. The manuscript was prepared by SD, MKI, MKo and FB with medical writing support provided by Sharon L. Cross, funded by Fraunhofer. MKo, MKI, SD, KH, NK, HFR, SC, HB, GG and FB reviewed and revised the manuscript. All authors approved the final manuscript. FB and MKo had access to data, accept full responsibility for the work and/or the conduct of the study, controlled the decision to publish, and act as guarantors for the study.

Funding This investigator-initiated study was sponsored by Fraunhofer ITMP, Frankfurt am Main, Germany, a non-profit organisation. Fraunhofer ITMP also funded writing/editorial support, provided by Sharon L. Cross, PhD (Mission Viejo, California, USA). The clinical research group Frankfurt was supported by the LOEWE-Center TMP of the state of Hesse (Germany), the ArthroMark Consortium, funded by German Federal Ministry of Education and Research (BMBF 01EC1401C Project 4), and the Fraunhofer Cluster of Excellence for Immune-Mediated Diseases CIMD.

Competing interests Fraunhofer ITMP, the sponsor of this study, supported MKo, MKI, SD, KH, HB, SC, GG and FB. NK received speaker's fees from Abbott Laboratories. HB received research support from the Fraunhofer Cluster of Excellence for Immune-mediated Diseases (a non-profit organisation). HFR has no conflicts of interest to declare.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the ethical committee of the University Hospital Frankfurt/Main (number 2021-368). The participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data from this study are available to researchers who provide a methodologically sound collaborative proposal. Proposals should be directed to the corresponding author.

ORCID iDs
Harald Burkhardt http://orcid.org/0000-0002-6261-3131
Frank Behrens http://orcid.org/0000-0001-8750-7186

REFERENCES

1 Kelly H, Sokola B, Abboud H. Safety and efficacy of COVID-19 vaccines in multiple sclerosis patients. *J Neuroimmunol* 2021;356:S0165-5728(21)00126-0.
2 Higdon MM, Wahl B, Jones CB, *et al*. A systematic review of Coronavirus disease 2019 vaccine efficacy and effectiveness against severe acute respiratory syndrome Coronavirus 2 infection and disease. *Open Forum Infect Dis* 2022;9:ofac138.
3 Paul-Ehrlich-Institut. COVID-19 vaccines. 2023. Available: https://www.pei.de/EN/medicinal-products/vaccines-human/covid-19/covid-19-list-1.html
4 Simon D, Tascilar K, Fagni F, *et al*. SARS-Cov-2 vaccination responses in untreated, conventionally treated and Anticytokine-treated patients with immune-mediated inflammatory diseases. *Ann Rheum Dis* 2021;80:1312–6.
5 Houot R, Levy R, Cartron G, *et al*. Could anti-Cd20 therapy jeopardise the efficacy of a SARS-Cov-2 vaccine? *Eur J Cancer* 2020;136:4–6.
6 Vijenthira A, Gong I, Betschel SD, *et al*. Vaccine response following anti-Cd20 therapy: a systematic review and meta-analysis of 905 patients. *Blood Adv* 2021;5:2624–43.
7 Landewé RBM, Kroon FPB, Alunno A, *et al*. EULAR recommendations for the management and vaccination of people with rheumatic and musculoskeletal diseases in the context of SARS-Cov-2. *Ann Rheum Dis* 2022;81:1628–39.
8 Shields AM, Faustini SE, Hill HJ, *et al*. SARS-Cov-2 vaccine responses in individuals with antibody deficiency: findings from the COV-AD study. *J Clin Immunol* 2022;42:923–34.
9 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;80:1330–8.
10 Saleem B, Ross RL, Bissell L-A, *et al*. Effectiveness of SARS-Cov-2 vaccination in patients with rheumatoid arthritis (RA) on Dmards: as determined by antibody and T cell responses. *RMD Open* 2022;8:e002050.
11 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:e002036.
12 Frommert LM, Arumahandi de Silva AN, Zernicke J, *et al*. Type of vaccine and immunosuppressive therapy but not diagnosis critically influence antibody response after COVID-19 vaccination in patients with rheumatic disease. *RMD Open* 2022;8:e002650.
13 Bonelli M, Scheinecker C. How does Abatacept really work in rheumatoid arthritis *Curr Opin Rheumatol* 2018;30:295–300.
14 Manansala M, Chopra A, Baughman RP, *et al*. COVID-19 and Sarcoidosis, readiness for vaccination: challenges and opportunities. *Front Med (Lausanne)* 2021;8:672028.
15 Bouwman W, Verhaegh W, Holtzer L, *et al*. Measurement of cellular immune response to viral infection and vaccination. *Front Immunol* 2020;11:575074.
16 Kohmer N, Rühl C, Ciesek S, *et al*. Utility of different Surrogate enzyme-linked immunosorbent assays (sELISAs) for detection of SARS-Cov-2 neutralizing antibodies. *J Clin Med* 2021;10:2128.
17 Kohmer N, Stein S, Schenk B, *et al*. Heterologous prime-boost immunization with Chadox1-S and BNT162b2: Reactogenicity and Immunogenicity in a prospective cohort study. *Int J Infect Dis* 2023;128:166–75.
18 Huzly D, Panning M, Smely F, *et al*. Accuracy and real life performance of a novel interferon-Γ release assay for the detection of SARS-Cov2 specific T cell response. *J Clin Virol* 2022;148:S1386-6532(22)00034-8.
19 Kado R, Sanders G, McCune WJ. Suppression of normal immune responses after treatment with Rituximab. *Curr Opin Rheumatol* 2016;28:251–8.
20 Braun-Moscovici Y, Kaplan M, Braun M, *et al*. Disease activity and humoral response in patients with inflammatory rheumatic diseases after two doses of the Pfizer mRNA vaccine against SARS-Cov-2. *Ann Rheum Dis* 2021;80:1317–21.
21 Fabris M, De Marchi G, Domenis R, *et al*. High T-cell response rate after COVID-19 vaccination in Belimumab and Rituximab recipients. *J Autoimmun* 2022;129:S0896-8411(22)00035-X.
22 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* 2022;4:e177–87.
23 Firinu D, Fenu G, Sanna G, *et al*. Evaluation of humoral and cellular response to third dose of BNT162b2 mRNA COVID-19 vaccine in patients treated with B-cell Depleting therapy. *J Autoimmun* 2022;131:S0896-8411(22)00056-7.
24 Ten Hagen A, Habermann E, Hermann S, *et al*. Mild COVID-19 despite inadequate antibody response after repeated Vaccinations in rheumatic disease patients with Rituximab-induced B cell depletion: a case series. *RMD Open* 2022;8:e002567.
25 Aiello A, Coppola A, Ruggieri S, *et al*. Longitudinal Characterisation of B and T-cell immune responses after the booster dose of COVID-19 mRNA-vaccine in people with multiple sclerosis using different disease-modifying therapies. *J Neurol Neurosurg Psychiatry* 2023;94:290–9.
26 Tortorella C, Aiello A, Gasperini C, *et al*. Humoral- and T-cell-specific immune responses to SARS-Cov-2 mRNA vaccination in patients with MS using different disease-modifying therapies. *Neurology* 2022;98:e541–54.
27 Bitoun S, Henry J, Desjardins D, *et al*. Rituximab Imparis B cell response but not T cell response to COVID-19 vaccine in autoimmune diseases. *Arthritis Rheumatol* 2022;74:927–33.
28 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;80:1322–9.
29 Krasselt M, Wagner U, Nguyen P, *et al*. Humoral and cellular response to COVID-19 vaccination in patients with autoimmune inflammatory rheumatic diseases under real-life conditions. *Rheumatology (Oxford)* 2022;61:SI180–8.
30 Picchianti-Diamanti A, Aiello A, Laganà B, *et al*. Immunosuppressive therapies differently modulate Humoral- and T-cell-specific responses to COVID-19 mRNA vaccine in rheumatoid arthritis patients. *Front Immunol* 2021;12:740249.
31 Eisenberg RA, Jawad AF, Boyer J, *et al*. Rituximab-treated patients have a poor response to influenza vaccination. *J Clin Immunol* 2013;33:388–96.
32 Farroni C, Aiello A, Picchianti-Diamanti A, *et al*. Booster dose of SARS-Cov-2 messenger RNA vaccines strengthens the specific immune response of patients with rheumatoid arthritis: A prospective multicenter longitudinal study. *Int J Infect Dis* 2022;125:195–208.
33 Israel A, Shenhar Y, Green I, *et al*. n.d. Large-scale study of antibody titer decay following BNT162b2 mRNA vaccine or SARS-Cov-2 infection. *Vaccines*;10:64.
34 Aiello A, Coppola A, Vanini V, *et al*. Accuracy of Quantiferon SARS-Cov-2 research use only assay and characterization of the $Cd4^+$ and $Cd8^+$ T cell-SARS-Cov-2 response: comparison with a homemade interferon-Γ release assay. *Int J Infect Dis* 2022;122:841–9.
35 Goletti D, Petrone L, Manissero D, *et al*. The potential clinical utility of measuring severe acute respiratory syndrome Coronavirus 2-specific T-cell responses. *Clin Microbiol Infect* 2021;27:1784–9.