Response to SARS-CoV-2 vaccination in systemic autoimmune rheumatic disease depends on immunosuppressive regimen: a matched, prospective cohort study

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ABSTRACT

Objective To assess the humoral response to messenger RNA (mRNA) vaccine of patients with systemic autoimmune rheumatic disease (SARD) and the effect of immunosuppressive medication in a matched cohort study.

Methods Patients with SARD were enrolled and matched 1:1 for sex and age with healthy control (HC) subjects. Differences in humoral response to two doses of an mRNA vaccine in terms of seroconversion rate (SCR) and SARS-CoV-2 antibody level between the two groups and the impact of treatment within patients with SARD were assessed.

Results We enrolled 82 patients with SARD and 82 matched HC. SCR after the first dose was lower among the patient group than that of HC (65% compared with 100% in HC, p<0.0001) but levelled up after the second dose (94% vs 100%). After the second dose, SCR was lower for patients on combination disease-modifying antirheumatic drug (DMARD) therapy compared with all other groups (81% compared with 95% for monotherapy, p<0.01; 100% for both no DMARD therapy and HC, both p<0.0001). In addition, antibody levels after both doses were lower in patients compared with HC. We found that vaccination response was determined primarily by the number of DMARDs and/or glucocorticoids received, with patients receiving combination therapy (dual and triple therapy) showing the poorest response.

Conclusions Patients with SARD showed a good response after the second vaccination with an mRNA vaccine. However, the choice of immunosuppressive medication has a marked effect on both SCR and overall antibody level, and the number of different immunomodulatory therapies determines vaccination response.

INTRODUCTION

Systemic autoimmune rheumatic diseases (SARD) are among the most severe diseases affecting the musculoskeletal system. These diseases, particularly connective tissue diseases and systemic vasculitides, are characterised by specific or associated autoantibodies and are commonly treated with immunosuppressive therapies. Due to the underlying immune disorder, which often predisposes patients to infections, vaccinating such patients against infectious agents is of particular relevance. On top of this, the immunosuppressive medication poses an additional difficulty as it often interferes with vaccine efficiency, as shown in several studies.

The COVID-19 pandemic has challenged the global health system in a way not seen since the Spanish influenza pandemic of 1918–1920. After mounting concerns about the risk of severe COVID-19 in immunocompromised patients, both the European Alliance of Associations for Rheumatology (EULAR) and the American College of Rheumatology (ACR) published updated guidance on the use of COVID-19 vaccinations in patients with SARD.
Rheumatology (ACR) were quick to issue recommendations for vaccination against SARS-CoV-2. At the same time, there are scarce published data on the efficacy and safety of COVID-19 vaccines in patients with SARD. Vaccination efficacy has been demonstrated to be reduced in these patients compared with the general population, in particular for patients receiving B cell-depleting therapies, but also methotrexate. Targeted anticytokine treatments, although often analysed only after one immunisation, were not shown to have a significant effect on vaccine responsiveness. The objective of this study was to assess the response to vaccination with messenger RNA (mRNA) vaccines of patients with SARD in a matched control study after the first and second dose.

MATERIALS AND METHODS

Patients
We prospectively enrolled prospective patients with SARD (antineutrophil cytoplasmic antibody-associated vasculitis, dermatomyositis/polymyositis, mixed connective tissue disease/undifferentiated connective tissue disease, polymyalgia rheumatica/large-vessel vasculitis, primary Sjögren’s syndrome, systemic lupus erythematosus (SLE), systemic sclerosis) from the outpatient clinic of our department. Patients who received B cell-depleting therapy (rituximab) were excluded from the study. Individuals without known inflammatory rheumatic disease and no immunomodulatory therapy served as healthy controls (HC). Patients with a history of SARS-CoV-2 infection were excluded. All patients and HC were vaccinated twice with an mRNA vaccine. Serum samples were stored at the Biobank of the Medical University of Vienna, a centralised facility for preparing and storing biomaterial with certified quality management (International Standards Organization (ISO) 9001:2015). Antibodies against the SARS-CoV-2 receptor-binding domain (RBD) and the nucleocapsid protein were determined 2–3 weeks after the first vaccination and 4 weeks after the second vaccination. Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Anti-SARS-CoV-2 testing
The Elecsys Anti-SARS-CoV-2 S immunoassay was used for quantitative determination of antibodies to the RBD of the viral spike (S) protein. The quantitation range is between 0.4 and 2500.0 binding antibody units (BAU)/mL. Levels below the detection limit of 0.4 BAU/mL were manually set to 0. Previous SARS-CoV-2 infection was assessed by measuring nucleocapsid-specific antibodies with the qualitative Elecsys Anti-SARS-CoV-2 assay. Both tests were performed on cobas e801 analyser (Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Laboratory Medicine, Medical University of Vienna (certified according to ISO 9001:2015 and . according to ISO 15189:2012).

Statistical analysis
Patients with SARD and HC were gender-matched and age-matched in a 1:1 ratio and compared for differences in seroconversion rate (SCR) or anti-SARS-CoV-2 antibody level. According to the distribution, continuous variables were presented as mean with SD or median with IQR. SCRs were compared using Fisher’s exact test for multiple testing and antibody levels either by Mann-Whitney test or Kruskal-Wallis test followed by Dunn’s test to account for multiple comparison. To assess the effect of treatment on immune response, patients were grouped into four categories based on the current disease-modifying antirheumatic drug (DMARD) therapy: (1) no DMARD; (2) monotherapy consisting of a conventional synthetic DMARD (cSDMARD) or a biological/targeted synthetic DMARD (b/tsDMARD); (3) combination therapy consisting of two cSDMARDs, or cSDMARD(s) combined with a b/tsDMARD, or one DMARD combined with glucocorticoids (GC); and (4) triple therapy consisting of two DMARDs and GC. GC dose at the time of vaccinations was calculated and considered separately and GCs were regarded as DMARDs. In order to evaluate the effect of disease activity and comorbidities on SCR and anti-SARS-CoV-2 antibody level, we recorded antibodies against double-stranded DNA (dsDNA) and the proteolytic fragment of complement component 3 (C3c) levels at the two timepoints of vaccination and recorded the presence/absence of common comorbidities (hypertension, diabetes mellitus, hyperlipidaemia, thyroid disorders and chronic obstructive pulmonary disease) based on patient files. We then dichotomised the parameters based on the cut-off provided by the Department of Laboratory Medicine of the Medical University of Vienna and grouped the patients into dsDNA-negative and dsDNA-positive patients and those with normal or decreased C3c levels, and compared the two groups using Fisher’s exact test (SCR) and Mann-Whitney test followed by Dunn’s test (antibody levels) to account for multiple comparison. In univariate analyses, the association of anti-SARS-CoV-2 S level with patients’ demographic characteristics was investigated using Spearman’s correlation coefficient. GraphPad Prism (V.9.1.0) was used for statistical analysis and graphical presentation of the data.

RESULTS
We included a total of 82 patients with SARD and 82 age- and gender-matched and gender-matched HC in this study. The demographic characteristics and disease entities are shown in table 1. We obtained the serological data of 37 patients and 67 HC 2–3 weeks after the first immunisation and of 82 patients and 80 HC 3–6 weeks after the second immunisation; 37 patients and 66 HC had data on both timepoints.

Seroconversion after vaccination
In patients with SARD, SCR was significantly lower after the first vaccination (65% compared with 100% in HC, p<0.0001), but levelled up after the second dose of vaccination (94% vs 100%) of an mRNA vaccine (figure 1A). Of note, patients with SARD, independent of treatment regimen, had significantly lower SCR (77% for no DMARD therapy, 56% for monotherapy and 57% for combination therapy, all p<0.0001) after the first dose as compared with HC, with patients with SARD on monotherapy or combination therapy also having a significantly lower SCR as compared with those receiving no DMARD therapy (56% vs 77%, p=0.01; 57% vs 77%, p=0.01) (figure 1B). SCR after the second dose was significantly lower for patients on combination DMARD therapy compared with all other groups (81% compared with 95% for monotherapy, p=0.01; 100% for both no DMARD therapy and HC, both p<0.0001).

For evaluating the effects of disease activity on SCR, we examined the effect of dsDNA and C3c status in a subgroup of patients with SLE and found a significant reduction in patients who were dsDNA-positive (83% vs 50%, p=0.0006) or had decreased C3c levels (80% vs 60%, p=0.006) (online supplemental figure 1A,B). Patients with SARD with ≥1 common comorbidity had a reduced SCR as compared with those without such comorbidities after both the first vaccination (50% vs 88%, p=0.0003) and the second vaccination (88% vs 98%, p=0.03) (online supplemental figure 1C).
and the second dose (Spearman’s $r=-0.33$, 95% CI $-0.52$ to $-0.11$, $p=0.003$) of vaccination. In contrast, antibody levels did not correlate with age in patients with SARD, and neither were there significant differences between anti-SARS-CoV-2 S levels of male and female subjects in either group.

We next analysed the anti-SARS-CoV-2 S levels of patients according to the treatment the patients received. As we detected a significant inverse correlation of GC dose and anti-SARS-CoV-2 S level after the second vaccination (Spearman’s $r=-0.31$, 95% CI $-0.50$ to $-0.09$, $p=0.005$), we regarded GC as a class of DMARDs of their own. All patients receiving GC at the time of the first vaccination also received GC at the second vaccination. When we categorised our patients according to the number of individual DMARDs they received, we found a stepwise reduction of anti-SARS-CoV-2 S levels dependent on the number of DMARDs administered, with a significant decrease in patients treated with either a combination of two or three DMARDs (median 410 (IQR 38.7–701) BAU/mL and 2.2 (IQR 0–68.7) BAU/mL vs 1673 (IQR 915.5–2500) BAU/mL; $p=0.0003$ and $p=0.0001$) (online supplemental figure 2).

Analysing the effect of GC in more detail, patients treated with triple therapy (ie, two DMARDs and GC) had reduced anti-SARS-CoV-2 S levels when compared with HC both after the first vaccination (median 0 (IQR 0–0.9) BAU/mL vs 33.5 (IQR 13.2–189) BAU/mL, $p=0.005$) and the second vaccination (median 2.2 (IQR 0–68.7) BAU/mL vs 1673 (IQR 915.5–2500) BAU/mL, $p<0.0001$) (figure 2B,C). In addition to those on triple therapy, also patients on DMARD combination therapy and those receiving DMARD monotherapy and GC had lower anti-SARS-CoV-2 S levels as compared with HC after the second vaccination (median 410 (IQR 52.5–699) BAU/mL and 428.5 (IQR 5.8–809) BAU/mL vs 1673 (IQR 915.5–2500) BAU/mL; $p=0.05$ and $p=0.01$, respectively) (figure 2C).

Disease entities themselves were not associated with reduced anti-SARS-CoV-2 S levels after immunisation, except for patients with SLE (median 515 (IQR 20.2–2369) BAU/mL vs 1673 (IQR 915.5–2500) BAU/mL, $p=0.005$) (figure 3). However, also within this subgroup, vaccination response was determined by their treatment regimen. Anti-SARS-CoV-2 S levels in patients with SLE untreated or with DMARD monotherapy were not different from HC (median 2500 (IQR 2500–2500) BAU/mL

**Table 1 Study subject characteristics**

<table>
<thead>
<tr>
<th>SARD (n=82)</th>
<th>HC (n=82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>52.0 (±14.1)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>65 (79)</td>
</tr>
<tr>
<td>Systemic lupus erythematosus, n (%)</td>
<td>33 (40)</td>
</tr>
<tr>
<td>Systemic sclerosis, n (%)</td>
<td>13 (16)</td>
</tr>
<tr>
<td>Other connective tissue diseases*, n (%)</td>
<td>15 (18)</td>
</tr>
<tr>
<td>Vasculitis†, n (%)</td>
<td>17 (21)</td>
</tr>
<tr>
<td>Miscellaneous‡, n (%)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>csDMARD or btsDMARD monotherapy, n (%)</td>
<td>43 (52)</td>
</tr>
<tr>
<td>csDMARD and/or btsDMARD combination therapy§, n (%)</td>
<td>16 (20)</td>
</tr>
<tr>
<td>No therapy, n (%)</td>
<td>23 (28)</td>
</tr>
<tr>
<td>Methotrexate (monotherapy or combination), n (%)</td>
<td>13 (16)</td>
</tr>
<tr>
<td>Mycophenolate (monotherapy or combination), n (%)</td>
<td>28 (34)</td>
</tr>
<tr>
<td>Hydroxychloroquine (monotherapy or combination), n (%)</td>
<td>13 (10)</td>
</tr>
<tr>
<td>Azathioprine (monotherapy or combination), n (%)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Belimumab (monotherapy or combination), n (%)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Tocilizumab (monotherapy or combination), n (%)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Olumiant (monotherapy or combination), n (%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Glucocorticoid dose at first vaccination, mean (SD)</td>
<td>2.5 (±9.4)</td>
</tr>
<tr>
<td>Glucocorticoid dose at second vaccination, mean (SD)</td>
<td>2.2 (±9.2)</td>
</tr>
</tbody>
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*Dermatoisoty/polyisoty (n=4), mixed connective tissue disease (n=2), primary Sjögren’s syndrome (n=6) and undifferentiated connective tissue disease (n=3).
†Antineutrophil cytoplasmic antibody-associated vasculitis (n=3), Behcet’s disease (n=1), large-vessel vasculitis (n=3) and polyalgia rheumatica (n=10).
‡Adult-onset Still’s disease (n=1), immune deficiency (n=2) and sarcoidosis (n=1).
§Azathioprine+hydroxychloroquine (n=6), mycophenolate+hydroxychloroquine (n=5), azathioprine+belimumab (n=1), belimumab+hydroxychloroquine (n=1), mycophenolate+tocilizumab (n=1), azathioprine+belimumab+hydroxychloroquine (n=1), and mycophenolate+hydroxychloroquine+tocilizum (n=1).
btsDMARD, biological/targeted synthetic disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; HC, healthy control; mRNA, messenger RNA; SARD, systemic autoimmune rheumatic disease.

**Figure 1** Seroconversion rate after mRNA vaccine. (A) Seroconversion rate between the HC group and patients with SARD. (B) Seroconversion rate after the first and second vaccination dose between the HC group and patients according to therapy. *p≤0.05;**p≤0.01;***p≤0.005;****p≤0.0005. HC, healthy control; mRNA, messenger RNA; SARD, systemic autoimmune rheumatic disease.
and 1375.5 (IQR 408.3–2435) BAU/mL vs 1673 (IQR 915.5–2500) BAU/mL), whereas they were significantly reduced in those receiving two or more DMARDs (median 315.5 (IQR 41.6–561) BAU/mL for combination therapy and 1.89 (IQR 0–7.5) BAU/mL for triple therapy vs 1673 (IQR 915.5–2500) BAU/mL for HC; p=0.0025 and p<0.0001, respectively) (online supplemental figure 3).

In contrast, we detected no clear association of anti-SARS-CoV-2 S levels with serological markers of disease activity such as anti-dsDNA antibody status and C3c levels (online supplemental figure 4A,B). The levels of anti-SARS-CoV-2 S were similar between patients with SARD with or without common comorbidities (online supplemental figure 4C).

In an exploratory analysis, we determined the anti-SARS-CoV-2 S levels of patients according to individual medications after the second vaccination. Overall, patients on DMARD combination therapy had lower anti-SARS-CoV-2 S levels as compared with HC subjects, and this was particularly marked for those receiving mycophenolate mofetil (online supplemental figure 5).

**DISCUSSION**

Our study showed that in patients with SARD under immunosuppressive therapy, excluding those treated with B cell-depleting agents, vaccination response is very efficient, regardless of disease group, with an SCR approaching that of HC subjects after the second immunisation. Unlike other studies evaluating seroconversion and humoral response to vaccination in patients with autoimmune disease,8 11 14 we could show that especially combination of immunosuppressive therapies indeed lead to lower SCR and lower antibody levels after the second immunisation, highlighting the importance of choosing the optimal immunosuppressive treatment modality.

The current study suggests that most patients with SARD need both vaccinations to develop a substantial antibody response. This is in contrast to healthy individuals, all of whom developed antibody levels above the cut-off already after the first vaccination. It is necessary to note that evaluation of vaccination response in patients with SARD or autoimmune disorders needs to be done after the second vaccination dose, as SCR and antibody levels after the first immunisation are low and fail to predict immunisation success after both immunisations. The inverse correlation between age and antibody levels in healthy subjects is in line with previous observations.21

Although it is reassuring that patients with SARD had SCRs which were comparable with HC after both doses of an mRNA vaccine, the results of our study imply that the response of patients with SARD to single-dose vaccines needs to be cautiously evaluated. In addition, patients with SARD not receiving any DMARD therapy showed lower SCR after the first immunisation, suggesting that the reduced early antibody response might be attributed to the disease itself, urging a booster vaccination. In addition, it is interesting to note that while both mycophenolate mofetil and azathioprine, whose mode of action is believed to...
be similar, had an impact on vaccine efficiency, the effect of the former was much more robust. However, our study is not without limitations. In addition to the small sample size and incomplete antibody levels after the first vaccination in the patient group, we did not measure neutralising antibodies in our study. However, there is increasing evidence that levels of antibodies directed against the RBD domain of the S protein measured in our study are an excellent approximation of vaccine efficiency. As shown earlier, those specific binding antibodies were highly correlated with the presence of functional neutralising antibodies. We excluded patients receiving B cell-depleting therapies from our study, which were reported to have a significantly reduced SCR even after full immunisation. Finally, our cohort of patients with SARD encompasses a heterogeneous group of entities, yet we believe that these diseases nevertheless share common features, in particular systemic inflammation, multiorgan involvement and autoimmune features, which legitimise their evaluation as a single cohort. Indeed, with the exception of SLE, disease entities themselves were not associated with reduced anti-SARS-CoV-2 S levels after immunisation. This may be due in part to the large size of the SLE subgroup in relation to the entire cohort. Our results on reduced immunogenicity of SARS-CoV-2 vaccines in patients on DMARD therapies underline those of previously published studies. However, we find that the number of DMARDs patients receive importantly determines vaccine response, as anti-SARS-CoV-2 S levels drop lower with each additional DMARD. Patients receiving DMARD monotherapy overall show good vaccination efficiency, whereas those receiving two or more have substantially reduced anti-SARS-CoV-2 S levels. It is important to note that GCs, at least in our cohort, exert a significant effect despite the fact that the median dose of GC that our patients received was quite low. It does seem to potentiate the inhibitory effects of concomitant DMARDs, as patients on GC monotherapy did not show reduced anti-SARS-CoV-2 S levels. These data highlight the complexity or even unpredictability of humoral response when combination immunosuppressive therapies are employed.

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Contributors PM, ST, HB, SW, JS, DA and SB designed the study. PM, ST, TK, TN, JS, SB, ES, HH, HR and TP analysed the data. PM, ST, ES, HH, TP, JS, HB, DA, SW and SB interpreted the results. PM, ST, DA, JS, TK and SB wrote the paper. All authors revised the manuscript and were involved in editing or quality control. SB and PM had access to all the data and accept full responsibility for the work and conduct of the study and controlled the decision to publish.

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Patient and public involvement Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the Ethics Committee of the Medical University of Vienna (1291/2021; 559/2005; 1073/2021). Participants gave informed consent to participate in the study before taking part.

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