FINAL REPORT

Vaccine response against SARS-CoV-2 in patients with primary Sjögren's syndrome

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Summary

The primary objective of this study was to assess the antibody based immune response after nCoV-19 vaccination in patients with primary Sjögren's syndrome (pSS) compared to healthy controls. The primary endpoint was the absolute difference in blood antibody titers (IgM+IgG) against SARS-CoV-2 on day 28 after the second vaccination (first vaccination for Ad.26.COV2.S (Janssen)) between patients with pSS and healthy controls. Secondary objectives were safety evaluation, monitoring of pSS disease activity after vaccination and in-depth assessment of the immune response. Between 31 May and 30 July 2021, 104 subjects were enrolled in this study. The trial was completed on 23 August 2022. The main results have been published open-access. In conclusion, our study data suggest that the antibody based immune response after nCoV-19 vaccination in pSS patients is similar to healthy controls. Also, numbers and severity of adverse events were similar. Two serious adverse events occurred in two pSS patients during follow-up. All SAEs have been resolved and no deaths occurred.

Introduction

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Patients with primary Sjögren's syndrome (pSS) worry about the effectiveness and possible side effects of COVID-19 vaccination, in particular flaring of the disease.[1] Certain factors may contribute to their concerns, such as an imbalance of the immune system (e.g. lymphocytopenia), severe organ manifestations or in some cases use of immunosuppressive drugs.[2] Most previous studies investigated COVID-19 vaccination response in patients with various rheumatic diseases who were on immunosuppressive drugs.[3–5] However, only few pSS patients were included in these studies and little is known about COVID-19 vaccination responses in pSS patients without immunosuppressive drugs, which is the case for the majority of this patient population.

pSS is a systemic, auto-immune disease, which is generally characterized by an over-active immune system, illustrated by B-cell hyperactivity.[6] Previous studies showed that untreated pSS patients produced higher influenza-specific antibody levels after influenza vaccination compared to healthy controls (HC).[7,8] Influenza vaccinations also resulted in concomitant elevation of anti-EBV and auto-antibody levels, an indication of polyclonal B-cell activation.[7,8] Additionally, influenza-specific antibody levels were associated with higher steady-state interferon (IFN) signatures in monocytes.[7] Potentially, the toll-like receptor-7 (TLR-7)/type-I IFN pathway is an important driver of polyclonal B-cell activation in pSS.[9] A type-I IFN signature is present in approximately 55-80% of pSS patients, and has been associated with anti-SSA positivity and higher haematological and biological activity.[9–11] Furthermore, TLR-7 is overexpressed in pSS patients.[12] For SARS-CoV-2 host defence, this pathway seems particularly important, as illustrated by a study showing that four young males with live-threatening COVID-19 infection, without pre-existing medical conditions, had a loss-of-function TLR-7 variant on their X-chromosome and functional defects in type-I and type-II IFN responses.[13]

Based on these findings, we hypothesized that pSS patients may develop a stronger humoral response to COVID-19 vaccination. On the other hand, possible vaccination-induced polyclonal B-cell activation might lead to more side effects, or patients may experience an increase in disease activity after vaccination. Therefore, the primary objectives of this study were to evaluate anti-SARS-CoV-2 antibody responses in serum and adverse events (AEs) after COVID-19 vaccination in pSS patients compared to HC. A secondary objective was to evaluate disease activity after vaccination in pSS patients. Cellular response, anti-SARS-CoV-2 antibody levels in saliva and auto-antibody levels in serum were also measured.

Methods

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Study design

This study is a prospective, single-centre, longitudinal cohort study conducted in the pSS expertise centre at the University Medical Centre Groningen (UMCG; Groningen, Netherlands). Ethical approval was obtained from the institutional review board (METc 2021.084). All participants provided written informed consent.

pSS patients and female HC were included in a 2:1 ratio. Inclusion criteria were age of 18-75 years and exclusion criteria were a PCR-confirmed SARS-CoV-2 infection (current/previous) and pregnancy. pSS patients had to fulfil the ACR/EULAR classification criteria for pSS[14] and additional exclusion criteria were current use of conventional or biological DMARDs (except hydroxychloroquine) or prednisone >10 mg/day, and previous use of DMARDs ≤6 months before inclusion (rituximab ≤12 months). For HC, additional exclusion criteria were presence of auto-immune or mixed connective tissue diseases, confirmed infectious, inflammatory or malignant disease, and use of immunosuppressive medication.

Participants received COVID-19 vaccinations following the Dutch vaccination programme. Vaccine types included in the vaccination programme were BNT162b2 (Pfizer-BioNtech), ChAdOx1 nCoV-19 (AstraZeneca), mRNA-1273 (Moderna) or Ad.26.COV2.S (Janssen). Participants received two doses of the Pfizer-BioNtech, AstraZeneca or Moderna vaccine, or one dose of the Janssen vaccine. At the start of this study (March 2021), the time interval between the doses were 6 weeks for Pfizer-BioNtech, 12 weeks for AstraZeneca and 4 weeks for Moderna, but this varied during this study.

Study measurements

Baseline demographics were collected from electronic patient files for pSS patients and from questionnaires for HC. Blood was collected by a finger prick at home. Spike 1 (S1)-receptor binding domain (RBD)-neutralizing IgG antibodies were measured using Siemens Healthineers antibody assay (Labonovum, Limmen, the Netherlands)[15]. A level of >2500 AU/ml was considered positive. Total serum levels of IgG were also measured. The finger prick was collected before the first vaccination, to confirm that participants did not have a previous SARS-CoV-2 infection, and 28 days after the final vaccination.

Incidence and severity of (serious) AEs were self-reported 7 days after each vaccination. Frequencies of AEs were also compared to a general population cohort of the Dutch pharmacovigilance centre Lareb.[16] For patient-reported disease activity, EULAR Sjögren's Syndrome Patient-Reported Index (ESSPRI) and patient Global Disease Activity (GDA) were collected at baseline and 28 days after complete vaccination. Systemic disease activity measured with EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) was retrieved from medical records ≤1 year before the first vaccination and ≤6 months after the first vaccination.

Subgroup measurements

All participants (pSS and HC) were invited to donate additional blood and/or saliva samples. This was not mandatory for participation in this study. Additional blood samples were drawn before vaccination and 7 days after the second vaccination stimulated whole saliva (paraffin-chewing; 5 minutes) was collected before vaccination and 28 days after complete vaccination.

To validate the finger prick antibody levels, anti-S1 and anti-RBD IgG levels were measured in post-vaccination blood samples using the Multiplex Immuno assay (MIA) at the National Institute for Public Health and the Environment (RIVM)[17].
In the pre- and post-vaccination blood samples, frequencies of SARS-CoV-2 spike-specific T-cells were assessed by IFN-γ enzyme-linked immune absorbent spot (ELIspot) assay[18]. To calculate spike-specific T-cell response, the average spot-forming cell (SFC) count of the negative control was subtracted from summed average of the S1 and S2 SFC counts. Change (Δ) from pre- to post-vaccination spike-specific SFC counts was calculated. Responders were defined as patients who had at least a two-fold increase in post-vaccination compared to pre-vaccination SFC counts and SFC counts of ≥50/10^6 cells in the post-vaccination sample.
In pSS patients, anti-SSA antibody levels (Ro52 and Ro60) were measured in pre- and post-vaccination blood samples by an in-house enzyme-linked immunosorbent assay (ELISA)[19].
In the saliva samples, anti-S1 and anti-RBD IgG and IgA antibody levels were measured using a fluorescent-bead-based MIA[20].

Statistical analyses

Descriptive statistics were used for baseline characteristics and safety outcomes. Results were expressed as number (%), mean±SD or median (IQR) for respectively categorical, normally or non-normally distributed data. Differences between groups were tested using Chi-Square test or Fisher's exact test for categorical data, independent samples t-test for normally distributed data and Mann-Whitney U-test for non-normally distributed data. For changes within patients over time paired t-test was used for normally distributed data and Wilcoxon signed rank test for non-normally distributed data. For correlations, Spearman's correlation test was used. Univariate linear regression was performed with SARS-CoV-2 antibody level as dependent variable and pSS/HC group as independent variable. Multivariate linear regression was used to correct for age as potential confounder. SARS-CoV-2 antibody levels were log-transformed to obtain a normal distribution of residuals in the linear regression models. Within the pSS group, univariate linear regression was used to explore associations between SARS-CoV-2 antibody levels and baseline characteristics. Main analyses were split for the separate vaccine types. P-values <0.05 were considered statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23.0.

Main results

**Partly published (Verstappen GM et al., RMD Open 2022;8:e002265. doi:10.1136/rmdopen-2022-002265 & Steinmetz TD et al., Clin Exp Rheumatol. 2022 Sep 5. doi:10.55563/clinexprheumatol/1iaqnu)*

1. Levels of S1-RBD-neutralizing IgG antibodies after nCoV-19 vaccination were not significantly different in pSS patients compared with healthy controls (Figure 1).

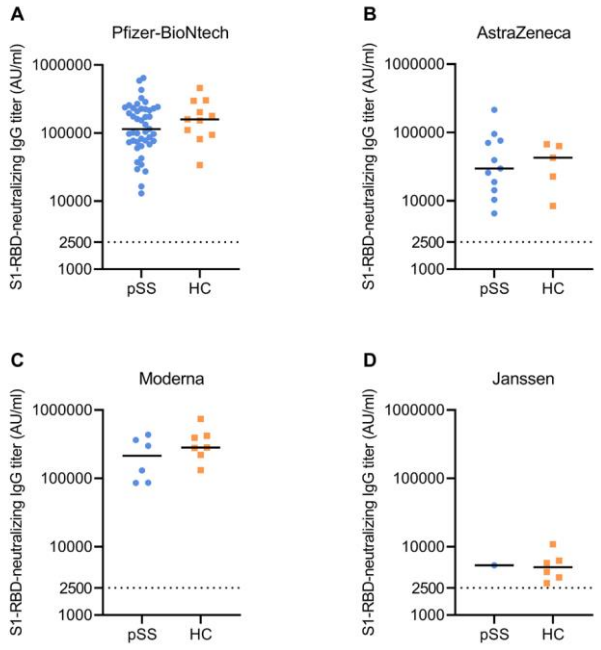

Figure 1. S1-RBD-neutralizing IgG antibody levels after vaccination. Finger prick SARS-CoV-2 antibody levels (S1-RBD neutralising IgG) of participants who received (A) Pfizer-BioNtech (B) AstraZeneca (C) Moderna or (D) Janssen. An antibody level of ≥2500 AU/mL was considered positive, indicated with the dashed line. HC, healthy controls; pSS, primary Sjögren's syndrome; RBD, receptor binding domain; S1, spike 1 This Figure was published in RMD Open 2022;8:e002265. doi:10.1136/rmdopen-2022-002265.

2. No significant differences in adverse event (AE) rates were seen between pSS patients and healthy individuals.

Taking all vaccine types together, *frequencies* of (systemic) AEs were comparable between patients with pSS and HC (for details, we refer to Verstappen GM et al., RMD Open 2022;8:e002265). After the second vaccination, arthralgia and myalgia were not more frequent, but more *severe* in patients with pSS compared with HC (p=0.024 and p=0.016, respectively). Analyses of AEs split for the separate vaccine types also revealed no significant differences in frequencies of AEs. The frequency of systemic AEs in the pSS and HC groups was comparable to a general population cohort of the Dutch pharmacovigilance centre Lareb.[16] Two serious AE (SAE) occurred in two pSS patients during follow-up. The first SAE occurred in patient who was later excluded because of a screening failure (use of a conventional DMARD at the time of inclusion). This patient (female, 51 years old) visited the hospital Emergency department for thoracic pain, 10 days after the second vaccination with BNT162b2 (Pfizer-BioNtech). There were no indications for acute underlying cardiac or pulmonal pathology and the patient was discharged on the same day. A second pSS patient (male, 66 years old) who was vaccinated with AZD1222 (AstraZeneca) had an SAE five months after the second vaccine dose. The SAE

was a visit to the Emergency department and hospitalization because of cholecystitis acuta, treated with a laparoscopic cholecystectomy.

3. Patient-reported symptoms, measured by ESSPRI, remain stable during one year of follow-up post-vaccination (Table 1). Data are shown for the total group (all types of nCoV-19 vaccines).

Patient participants received ESSPRI questionnaires at different time points during follow-up. Overall, patient-reported outcomes remained stable during follow-up (Table 1). At day 360, the total ESSPRI score was significantly higher compared to baseline, but the absolute difference is not considered clinically relevant an may be due to natural disease progression.

Table 1: One year follow-up of patient-reported symptoms of pSS patients after COVID-19 vaccination

	Baseline	Day 28	Day 90	Day 180	Day 360
ESSPRI total score					
Total	6.0 (4.7-7.0) (n=67)	5.7 (3.8-7.0) (n=64)	5.7 (4.3-7.3) (n=63)	5.8 (4.6-7.3) (n=66)	6.3 (5.0-7.7) (n=59)
		p=0.155	p=0.275	p=0.803	p=0.004
ESSPRI dryness					
Total	7 (5-8) (n=67)	6 (4-7) (n=66)	6 (5-7) (n=64)	6 (5-7) (n=67)	7 (5-8) (n=60)
		0.053	0.228	0.184	0.055
ESSPRI fatigue					
Total	6 (5-8) (n=67)	7 (4-7) (n=65)	7 (4-8) (n=63)	6 (5-7) (n=66)	7 (5-8) (n=59)
		0.643	0.795	0.607	0.217
ESSPRI pain					
Total	6 (4-7) (n=67)	5 (3-7) (n=65)	6 (3-8) (n=64)	6 (4-8) (n=67)	7 (5-8) (n=60)
		0.167	0.736	0.358	0.055
Patient GDA					
Total	6 (5-7) (n=67)	6 (4-7) (n=66)	6 (5-7) (n=64)	6 (5-7) (n=67)	6 (5-8) (n=59)
		0.202	0.488	0.851	0.485

Data presented as median (IQR)

P-values are compared to baseline values (using Wilcoxon signed rank test)

Patient-reported symptoms were collected via questionnaires at 28, 90, 180 and 360 days after the second vaccination (first for Janssen)

4. Relatively low incidence of COVID-19 infections in pSS patients during one year of follow-up (Table 2). Data are shown for the total group (all types of nCoV-19 vaccines).

During one year of follow-up, 23% of pSS patients and 72% of healthy controls reported a COVID-19 infection confirmed by PCR or self-test (Table 2). None of the participants was admitted to the hospital because of severe COVID-19 illness. During COVID-19 infection, pSS patients tended to experience more symptoms, including a higher frequency of dyspnea, loss of smell or taste, headache and arthralgia (Table 2).

Table 2: Incidence of COVID-19 infections after COVID-19 vaccination during one year of follow-up in pSS patients and healthy controls (HC)

	TOTAL	
	pSS (n=67)	HC (n=33)
Positive COVID-19 test since last questionnaire		
Day 28	0/66	0/30
Day 90	1/66 (2%)	0/29
Day 180	0/66	1/29 (3%)
Day 360	13/62 (21%)	17/26 (69%)
Total number of reported COVID-19 infections (confirmed with test)	14	18
Hospital admissions due to COVID-19 infections	0	0
Symptoms during COVID-19 infection	n=14	n=18
Cold	12 (86%)	18 (100%)
Fever	5 (36%)	8 (57%)
Dyspnea	10 (71%)	4 (22%)
Coughing	11 (79%)	16 (89%)
Fatigue	13 (93%)	17 (94%)
Loss of smell or taste	9 (64%)	1 (1%)
Headache	13 (93%)	12 (67%)
Skin abnormalities	1 (7%)	0
Arthralgia	10 (71%)	6 (33%)

5. Both pSS patients and controls show RBD-specific T- and B-lymphocytes in their blood after vaccination, while no major changes in the overall lymphocyte compartment were induced by vaccination.

For detailed results, we refer to the following publications: Verstappen GM et al., RMD Open 2022;8:e002265. doi:10.1136/rmdopen-2022-002265 & Steinmetz TD et al., Clin Exp Rheumatol. 2022 Sep 5. doi:10.55563/clinexprheumatol/1iaqnu).

6. Levels of autoantibodies in serum do not change after vaccination.

Since influenza vaccination seems to increase serum levels of autoantibodies in pSS patients[7-8], we investigated serum levels of anti-SSA/Ro, anti-SSB/La, and rheumatoid factor at day 7 after the second vaccination. Because we could not collect enough serum from the fingerprick samples for autoantibody measurements, we only included patients who donated extra blood samples for in-depth measurement of the cellular immune response (n=26). Because of the smaller sample size, all nCoV-19 vaccine groups were combined for autoantibody analysis. For detailed results, we refer to the following publications: Verstappen GM et al., RMD Open 2022;8:e002265. doi:10.1136/rmdopen-2022-002265 & Steinmetz TD et al., Clin Exp Rheumatol. 2022 Sep 5. doi:10.55563/clinexprheumatol/1iaqnu). In summary, no changes in autoantibody levels were observed after nCoV-19 vaccination.

Conclusion

In conclusion, COVID-19 vaccination led to similar anti-SARS-CoV-2 antibody levels and T-cell responses in patients with pSS without immunosuppressives compared with HC, providing evidence that COVID-19 vaccination is effective in patients with pSS. Furthermore, side effects were comparable between patients with pSS and HC, and no increase in disease activity was seen, indicating that COVID-19 vaccination is safe for patients with pSS. A main limitation of this study is that four different vaccine types were included, which were administered according to the national vaccination programme. This led to an unequal distribution of participants within the distinct vaccination groups. Relatively more participants in the HC group received the Moderna or Janssen vaccine, compared with patients with pSS. Other limitations are single-centre recruitment and a relatively small sample size, especially for certain vaccine types. Because of the small HC group, not all subgroup measurements could be statistically compared with patients with pSS. Nonetheless, our finding that nCoV-19 vaccines in general appear to be effective and safe in pSS is an important and reassuring message for patients and provide arguments that patients with pSS may also benefit from future booster vaccinations.

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