BRIEF REPORT

Antibody responses after documented COVID-19 disease in patients with autoimmune rheumatic disease

Padmanabha Shenoy[1] · Sakir Ahmed[2] · K. C. Shanoj[1] · Veena Shenoy[3] · Deepak Damodaran[4] · Aparna R. Menon[1] · Bazil Alias[1] · SanjoSaijan[1] · Divya Devakumar[1] · A. S. Sageer Babu[1]

Abstract

Patients with autoimmune rheumatic diseases (AIRD) are suspected to have less robust immune responses during COVID-19 due to underlying immune dysfunction and the use of immune-suppressive drugs. Fifty consecutive patients with a diagnosis of AIRD on disease-modifying drugs were included at around 30 days after a confirmatory test for COVID-19. Fifty controls matched one to one for age, sex, and severity of COVID-19 were also included at around 30 days after testing positive for COVID-19. Antibody titers for anti-spike protein IgG and anti-nucleocapsid protein IgG were estimated. Cases (mean age 45.9 ± 13; 76% females) and controls (mean age 45.9 ± 13; 76% females) had similar proportion of comorbidities. Of the cases, 4 had moderate and 1 had severe COVID-19, while 3 and 1 of controls had moderate and severe COVID-19 respectively. Positivity of anti-N IgG was similar between patients (80%) and controls (90%) ($p = 0.26$). Similarly, anti-S IgG was positive in 82% of patients and 86% of controls ($p = 0.79$). Both the antibodies were negative in seven (14%) patients and five (10%) of controls ($p = 0.76$, Fischer exact test). Only anti-N IgG titers were lower in patients as compared to controls. In four patients with rheumatoid arthritis, two with spondyloarthritis and one with eosinophilic fasciitis both antibodies were not detectable. They did not differ from the rest of the cohort in clinical characteristics. The patients with AIRD had adequate protective antibody responses to COVID-19 at a median of 30 days post-infection. Thus, the presence of AIRD or the use of immunosuppressants does not seem to influence the development of humoral immune response against COVID-19.

Key Points
• *Patients with autoimmune rheumatic diseases (AIRD) are suspected to have less robust immune responses.*
• *In our cohort of 50 patients with AIRD with confirmed COVID-19, only seven did not have detectable protective antibodies at 30 days post infection.*
• *Patients with AIRD on immunosuppressants have adequate protective antibodies post COVID-19 disease, at rates similar to that in health controls.*

✉ Padmanabha Shenoy
drdpshenoy@gmail.com

1 Centre for Arthritis and Rheumatism Excellence (CARE), 16/554-2, NH 47, Nettoor, Kochi 682040, Kerala(32), India

2 Department of Clinical Immunology &Rheumatology Kalinga Institute of Medical Sciences (KIMS), KIIT University, Bhubaneswar, India

3 Department of Transfusion Medicine, Amrita Institute of Medical Science & Research Centre, Kochi, India

4 Department of Internal Medicine, Sree Sudheendra Medical Mission Hospital, Kochi, India

Introduction

The COVID-19 pandemic has raged throughout the world like wildfire and is re-emerging in parts of Europe currently. There has been much debate on whether patients with autoimmune rheumatic diseases (AIRD) have increased susceptibility to COVID-19 and possibly develop a more serious disease. Firstly, these patients have altered immune response that itself makes patients more susceptible to infectious diseases. Secondly, they are on various immunosuppressant drugs that might further alter protective immune responses.

Though the initial reports of the Global Rheumatology Alliance did not show increased hospitalizations in patients with AIRD [1], primary care databases have implied that patients having rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or psoriasis were at higher risk for death due to COVID-19 [2]. Also, these patients are much more likely to have various comorbidities that are independent risk factors for severe COVID-19 [3]. The use of immunosuppressant drugs in AIRD can have two opposite effects. First, these may blunt the immune response to COVID-19. Second, paradoxically, most of these have been proposed to be effective in severe COVID-19 [4].

Various initial reviews have found the evidence to be severely limited to whether the presence of AIRD or the use of immunosuppressants alters the immune response to COVID-19 [5]. The presences of protective antibodies seem to predict a robust immune response in COVID-19. It has been shown that antibodies against the spike protein (S) and nucleocapsid protein (N) of SARS-CoV-2 protect from reinfection and symptomatic disease [6]. The presence of other non-neutralizing antibodies is associated with more severe COVID-19 [7]. Previously, in a cohort of 13 patients with rheumatic diseases in Boston, USA, neutralizing antibodies were present in 10 (76.9%) [8].

Considering that these antibodies may predict an adequate immune response in patients with autoimmune rheumatic diseases while on immunosuppression, we set about to estimate the prevalence of these antibodies in our cohort of AIRD patients who had confirmed SARS-CoV-2 infection.

Methods

Patients with AIRD who were on disease-modifying anti-rheumatoid drugs (DMARD) or immunosuppressants for at least 3 months and had RT-PCR or rapid antigen (SD biosensor kit) positivity for SARS-CoV-2 on oropharyngeal or nasopharyngeal swab were included. A convenient sample of 50 consecutive AIRD cases who developed COVID-19 was taken along with 50 matched controls. Controls were those who had no known autoimmune disease and were not on any DMARDS or immunosuppressants and were documented to have COVID-19 either by antigen testing or RT-PCR positivity. Controls were matched one to one for age, sex, and COVID-19 severity. COVID-19 severity was assessed and classified as asymptomatic, mild, moderate, and severe as per National Institutes of Health's Coronavirus Disease (COVID-19) Treatment Guidelines.

Serum was collected at approximately 30 days after the RT-PCR confirmation for both cases and controls and kept at -80°. IgG Antibodies to both N protein antibodies were assessed using electrochemiluminescence immunoassay (ECLIA) with Elecsys® Anti-SARS-CoV-2 kits as per the

manufacturer's instructions. The presence of anti-SARS-CoV-2 IgG antibody directed against the S1 domain of the SARS-CoV-2 spike protein was done using the VITROS Anti-SARS-CoV-2 IgG Chemiluminescent assay (Ortho-Clinical Diagnostics, Rochester, NY, USA). The cut-off for the anti-N antibody and the anti-S antibody was determined as per cut-off controls provided by the manufacturer. OD values higher than that of the cut-off control were taken as positive, and those below were taken as negative. Antibody results are presented as ratios with the cut-off control.

Written consent had been obtained from all participants, and the study was cleared by the Institutional Ethics Committee of Sree Sudheendra Medical Mission Hospital.

All analysis was carried out in SPSS© version 23. Normality of data was checked via the Shapiro–Wilk test, and this was used to guide appropriate parametric or non-parametric tests. The Fisher exact test was used to compare proportions. The independent samples t-test was used to compare between means for parametric data.

Results

The characteristics of both patients and controls are depicted in Table 1. The most common underlying AIRD in our cohort were RA, SLE, and spondyloarthritis. Even the presence of comorbidities was balanced between cases and controls (this was not matched at control selection).

Anti-N IgG was positive in 40 (80%) patients and 45 (90%) controls (p = 0.26, Fischer exact test), while anti-S IgG was positive in 41 (82%) patients and 43 (86%) of controls (p = 0.79, Fischer exact test). Both neutralizing antibodies were negative in seven (14%) patients and five (10%) of controls (p = 0.76, Fischer exact test).The titers of anti-N IgG were lower in the patient group (p = 0.005, independent t-test) but not statistically different for the anti-S IgG.

Table 2 summarizes the characteristics of patients with dual antibody negativity. These consisted of four patients with RA, two with spondyloarthritis and one with eosinophilic fasciitis. In an analysis of the sub-groups receiving different immunosuppressant, the use of hydroxychloroquine was associated with higher antibody positivity, while the use of sulfasalazine was associated with lower antibody positivity.

Clinical characteristics were not significantly different between those positive and those negative for either anti-N IgG or anti-S IgG individually (data not shown).

Discussion

The present study shows that patients with AIRD, despite their underlying immune defects and current immunosuppression, mount adequate antibody responses similar to those of

Table 1 Characteristics of patients versus controls

	Patients	Control	Tests for differences
Age (years)	45.90 ± 13.03	45.88 ± 12.97	p = 0.99
Females, n (%)	38 (76%)	38 (76%)	p = 1
Obesity, n (%)	5 (10%)	2 (4%)	p = 0.43
Diabetes, n (%)	5(10%)	5(10%)	p = 1
Hypertension, n (%)	10 (20%)	7 (14%)	p = 0.42
Duration of antibody test [median (IQR)] in days	60 (60–66.25)	60(60–66)	p = 0.87
COVID-19 severity			
Asymptomatic	7 (14%)	7 (14%)	p = 0.98
Mild	38 (76%)	39 (78%)	
Moderate	4 (8%)	3(6%)	
Severe	1 (2%)	1(2%)	
Type of autoimmune disease	RA: 27 (54%) SLE: 7 (14%) SPA: 6 (12%) PSA: 3 (6%) Sarcoid: 2 (4%) Others: 5 (10%)		NA
Prednisolone equivalent dose of steroids [median (range)]	0 (0–10) mg		NA
Corticosteroid use	11 (22%)		
HCQ	33 (66%)		
MTX	15 (30%)		
Leflunomide	4 (8%)		
Sulfasalazine	17 (34%)		
Rituximab	6 (12%)		
Azathioprine	1 (2%)		
Mycophenolate	5 (10%		
Tacrolimus	2 (4%)		
Etanercept	1 (2%)		
N protein titer [median (IQR)]	13.45 (1.94, 26.90)	24.05 (7.26, 100.8)	p = *0.005**
S protein titer [median (IQR)]	6.21(2.49, 13)	8.29 (2.96, 12.02)	p = 0.85

Note: * *p*-values significant at <0.05

healthy controls. This is very reassuring considering that there was a widespread fear that patients on immunosuppressants may not mount an adequate immune response and may be vulnerable to reinfection with COVID-19. This supports the previously published reports that patients with AIRD per se do not have more severe COVID-19 or poorer outcomes [1, 9].

In our cohort, the majority of patients were asymptomatic or mildly symptomatic, while only 1 had a severe disease. The proportion seems to mirror the pattern of COVID-19 infections in the general population. These patients had not interrupted their treatment for AIRDs and thus possibly did not have any flares. We have previously described how we had switched to teleconsultation in the early stages of the disease when the first cases had been detected in our country [10]. Thus, the majority of our patients could maintain the continuity of care. This might be one reason that severe disease was uncommon in the cohort.

Secondly, patients with AIRDs have a higher prevalence of comorbidities that can lead to poorer outcomes during COVID-19 illness [3]. In our cohort, the number of comorbidities was limited. Third, relatively more asymptomatic/mildly symptomatic patients might have been detected as these patients are more likely to be concerned about their health. Patients on immunosuppressants are more likely to be tested for COVID-19 than ones not on them [11].

A higher proportion of females had the protective antibody. This is to be expected since females have more robust humoral immunity overall [12].

The majority of patients in this cohort were not on steroids, but almost all were receiving some form of immunosuppression. There was a wide variety of immunosuppressants used. It has been shown that besides the presence of comorbidities, higher mortality due to COVID-19 is associated with rheumatic disease activity and not the

Table 2 Comparison between patients having at least one antibody versus those having no protective antibody

	Protective antibody present (n=43)	No antibodies (n=7)	Tests of difference
Age in years	43.7 ± 9.8	46.2 ± 13.3	p=0.56
Females	36 (94.7%)	2 (5.3%)	p=*0.006**
Severity of COVID-19			p=0.29
Asymptomatic	6 (85.7%)	1 (14.3%)	
Mild	33 (86.8%)	5 (13.2%)	
Moderate	4 (100%)	0	
Severe	0	1 (100%)	
Hypertension	8 (80%)	2 (20%)	p=0.62
Diabetes mellitus	5 (83.3%)	1 (16.7%)	p>0.99
Hypothyroidism	1 (50%)	1 (50%)	NT
Dyslipidemia	36 (94.7%)	1 (5.3%)	NT
Diagnosis			
Rheumatoid arthritis	23 (85.2%)	4 (14.8%)	NT
Lupus	7 (100%)	0	
Spondyloarthritis	4 (66.7%)	2 (33.3%)	
Vasculitis	1(100%)	0	
Psoriatic arthritis	3 (100%)	0	
Sarcoidosis	2 (100%)	0	
Others	3 (75%)	1 (25%)	
Drug use			
Corticosteroid use	10 (90.9%)	1 (9.1%)	
HCQ	31 (93.9%)	2 (6.1%)	p=*0.037**
MTX	12 (80%)	3 (20%)	p=0.38
Leflunomide	3 (75%)	1 (25%)	p=0.46
Sulfasalazine	11 (64.7%)	6 (35.3%)	p=*0.004**
Rituximab	5 (83.3%)	1 (16.7%)	p>0.99
Azathioprine	1 (100%)	0	NT
Mycophenolate	5 (100%)	0	NT
Tacrolimus	2 (100%)	0	NT
Etanercept	1 (100%)	0	NT

NT not tested

Note: * *p*-values significant at <0.05

immunosuppressants used [9]. The BIOBADASER registry of the Spanish Society of Rheumatology has shown that even the use of biologicals does not seem to influence outcomes of COVID-19 [13].

The small number of patients did not allow sub-group analysis for the effects of many immunosuppressants. However, it was interesting to note that those on hydroxychloroquine seemed to have more robust antibody responses while sulfasalazine appears to dampen it. Though one should be wary of interpreting data from such small numbers, the statistics are robust, and the results are supported by the biology of the drugs. We could not find literature suggesting how hydroxychloroquine could increase antibody production. It accumulates in lysosomes and can modulate antigen presentation [14], but this needs further exploration.

Previous experiments have shown inhibitory effects on systemic antibody in patients receiving sulfasalazine [15]. Patients with known antibodies to various Enterobacteriaceae have been shown to have reduced titers post sulfasalazine use [16]. Vaccination in healthy individuals also has been shown to produce lower antibody titers [17]. Interestingly, patients who had received rituximab previously also seem to have an adequate antibody response, and this has been discussed in our previous short communication [18].

The significance of this study can be extrapolated for COVID-19 vaccination. If patients have an adequate response to natural infection in presence of RMDs and immunosuppressant drugs, they would likely have a similar response to vaccination also. This supports the various

rheumatology societies' guidelines that vaccination should be extended to all patients with AIRDs [19].

The limitations of this study include looking at only antibody responses, having a limited sample size and having a mixture of different AIRD patients on different drugs. Firstly, though only antibody responses were seen, this was non-inferior to that in controls. The anti-N and anti-S antibodies correlate with protection from severe COVID-19, and thus the majority of the patients are protected [20]. Moreover, the one to one matching of patients and controls eliminates a lot of confounders like age, sex, and severity of disease which may influence the humoral immune response.

Secondly, though we had only 50 patients in the cohort with different rheumatological diseases and different drugs, it is the largest cohort to date, to the best of our knowledge, of proven COVID-19 in rheumatic diseases to have their specific protective antibodies tested, and it was done within a pre-fixed narrow time frame of 2 months after testing positive for COVID-19. The previous study includes 13 patients who were tested at different times after COVID-19 infection (intra-quartile range: 60–146 days) [8].

Though patients were heterogeneous with different diagnoses, what was common was that all patients had a systemic inflammatory disease and were on some form of immunosuppression. Thus, this data has important implication for rheumatologists in a real-life scenario to provide them with the confidence that such patients still have adequate humoral immune responses to SARS-CoV-2 despite the immunosuppressant drugs.

Further work should include a follow-up of this cohort to see if the antibody titers persist in the same proportion as in healthy controls. Also, the possible effects of hydroxychloroquine at augmenting antibody production and that of inhibition by sulfasalazine need further validation. Whether these have clinical implications needs to be seen.

Thus, we could show that patients with AIRDs on various immunosuppressant drugs develop adequate protective antibody responses, non-inferior to those in controls. This is in line with existing data that the presence of AIRDs does not independently increase the risk for severe COVID-19 [3, 21]. It also has implications for vaccination against COVID-19.

Declarations

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