Dynamics of SARS-CoV-2 IgG antibodies and neutralising antibodies in rheumatic and musculoskeletal disease patients with COVID-19

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Abstract

Objective

Rheumatic and musculoskeletal diseases (RMD) may exhibit different immune responses to novel coronavirus (COVID-19) infection compared to healthy individuals. While previous studies have primarily investigated changes in COVID-19-related antibodies post-vaccination for RMD patients, this study sought to explore the dynamics of SARS-CoV-2 IgG antibodies and neutralising antibodies (NAb) in RMD patients after COVID-19 infection.

Methods

In this longitudinal study, we monitored the SARS-CoV-2 IgG antibodies and NAb levels in RMD patients and healthy controls (HC) at 60 and 90 days post-COVID-19 infection. Chemiluminescent immunoassay was used to detect the levels of novel coronavirus-specific IgG (anti-S1/S2 IgG) antibodies and NAb.

Results

A total of 292 RMD patients and 104 HC were enrolled in the study. At both the 60-day and 90-day post-COVID-19 infection, RMD patients exhibited significantly lower levels of anti-S1/S2 IgG and NAb than those in the HC group ($p<0.001$). The anti-S1/S2 IgG antibody levels remained relatively stable, while the NAb levels in RMD patients could vary greatly between the 60th and 90th days. A logistic regression analysis revealed that the prior administration of glucocorticoids (GC), immunosuppressants, and b/tsDMARDs stood out as independent risk factors associated with reduced anti-S1/S2 IgG and NAb levels, irrespective of the specific RMD subtypes.

Conclusion

GC and anti-rheumatic medications can potentially alter the production of specific antibodies, especially NAb, in RMD patients post-COVID-19 infection. These findings emphasise the importance of continuous monitoring for NAb fluctuations in RMD patients following a COVID-19 infection.

Key words

COVID-19, rheumatic and musculoskeletal diseases, anti-S1/S2 IgG, neutralising antibodies

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Competing interests: none declared.

Introduction

Rheumatic and musculoskeletal diseases (RMD) comprise a diverse set of conditions affecting hundreds of millions of people globally. RMD are characterised by chronic inflammation that targets the musculoskeletal system, blood vessels, and other tissues. Although the exact mechanisms underlying RMD are not fully understood, a complex interplay of genetic, immune, and environmental factors is critical in disrupting immune tolerance to self-antigens (1).

Infections have been implicated in the pathogenesis of RMD. Infections can contribute to the onset and worsening of certain RMD by activating the immune system through a mechanism known as molecular mimicry (2). Additionally, deficiencies in the function or quality of specific immune cells, such as Treg cells, can impair the body's defence against viral and bacterial pathogens (1). Furthermore, medications commonly used to manage RMD, such as cytokine-targeted therapies and monoclonal antibodies, may elevate the risk of infections (3).

Since the outbreak of the novel coronavirus (COVID-19) in 2019, millions of people have been infected, triggering a global health crisis (4). COVID-19 pandemic also has posed significant challenges to patients with RMD. Due to pre-existing conditions and ongoing medications, the immune response of RMD patients to COVID-19 may diverge from that of the general population. Most studies supported an increased risk and poor outcomes of SARS-CoV-2 infection in patients with RMD. The individual risk factors associated with poor outcomes included gender male, older age and therapies (glucocorticoids, sulfasalazine, immunosuppressants and rituximab) (5). Therefore, understanding the difference of immune response in these patients following COVID-19 infection is essential for crafting personalised treatment plans and risk assessments.

One crucial element of immune response to viral infections is the production of specific antibodies, particularly IgG antibodies. These antibodies serve as important indicators of immune health and offer enduring protection during recovery (6). Neutralising antibodies (NAb), in contrast, directly counteract viral infection and are vital for containing and controlling viral spread (7). The presence of both types of antibodies is considered a key indicator of an effective immune response against viral infections.

In the case of COVID-19, much research has been conducted on the production of specific IgG antibodies within the general population (8-13). However, among RMD patients, most studies have concentrated on antibody levels post-vaccination. These results are inconsistent due to variations in the types of vaccines used and their efficacy. The question remains whether RMD patients are more susceptible to lower antibody levels following COVID-19 infection, possibly due to immune dysfunction or the use of immune-suppressing drugs (14-15).

We carried out a longitudinal study to monitor changes in SARS-CoV-2 IgG antibodies and NAb levels of RMD patients post 60 days and 90 days of COVID-19 infection in RMD patients. We aim to address two key questions: first, whether the specific type of RMD influences the production of IgG antibodies and NAb; and second, whether the use of glucocorticoids (GC) or immunosuppressive medications affects the production of COVID-19-related antibodies.

Patients and methods

Study design and population

This study enrolled COVID-19 patients who had a pre-existing RMD from December, 2022 to April, 2023. Diagnosis of COVID-19 was based on a polymerase chain reaction test or an antigen test for SARS-CoV-2 from saliva, or nasopharyngeal swabs. Initially, an electronic message about this study was sent to the RMD patients who were followed up regularly in our department, informing them that they should visit our outpatient department approximately 60 days after diagnosed with COVID-19 to participate in this study. Healthy volunteers with SARS-CoV-2 infection served as the healthy control group (HC). The study protocols and the consent forms were approved by the

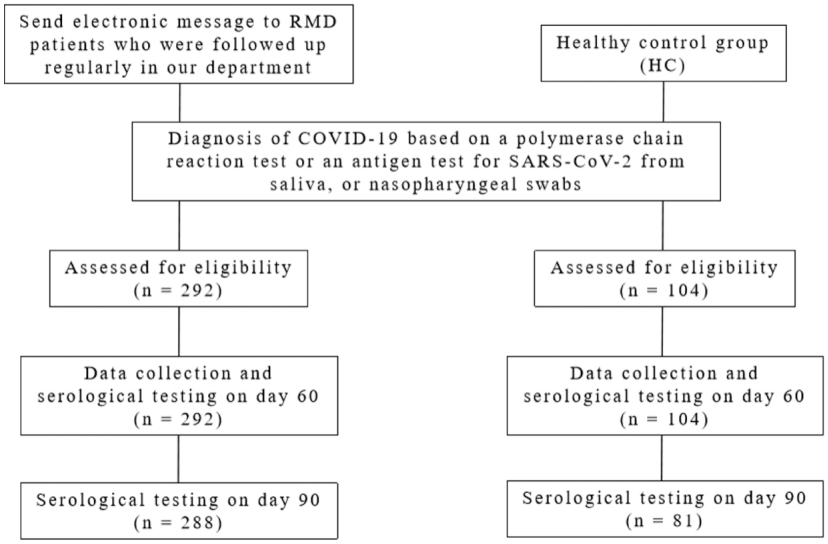

Fig. 1. Study flowchart.

Table I. Demographic and clinical characteristics of the study participants.

	RMD (n=292)	HC (n=104)	*p*-value
Demographics			
Age (years)	45.5 (32.0-57.0)	44.5 (32.0-57.8)	0.931
Male sex, n (%)	26 (8.9)	5 (4.8)	0.182
RMD	-	-	
RA, n (%)	66 (22.6)	-	-
SLE, n (%)	129 (44.2)	-	-
pSS, n (%)	39 (13.4)	-	-
IIM, n (%)	6 (2.1)	-	-
Primary vasculitis, n (%)	8 (2.7)	-	-
UCTD, n (%)	18 (6.2)	-	-
Other RMDs, n (%)	26 (8.9)	-	-
Disease duration, years	5.0 (3.0-10.0)	-	-
Therapy before infection			
Prednisone, n (%)	129 (44.2)	-	-
Dose, mean ± SD mg	13.4 ± 16.3		
Prednisone ≥10mg/day (3 months), n (%)	62 (21.2)	-	-
Hydroxychloroquine, n (%)	136 (47.6)	-	-
Immunosuppressive drugs, n (%)	136 (47.6)	-	-
Methotrexate, n (%)	33 (11.3)	-	-
Leflunomide, n (%)	25 (8.6)	-	-
Iguratimod, n (%)	16 (5.5)		
Mycophenolate mofetil, n (%)	44 (15.1)	-	-
Azathioprine, n (%)	7 (2.4)	-	-
Calcineurin inhibitors, n (%)	27 (9.2)	-	-
Cyclophosphamide, n (%)	7 (2.4)	-	-
b/tsDMRMDs, n (%)	22 (7.5)	-	-
Belimumab, n (%)	10 (3.4)	-	-
Rituximab, n (%)	3 (1.0)	-	-
JAK inhibitor, n (%)	8 (2.7)	-	-
anti-TNF, n (%)	2 (0.7)	-	-
Therapy after infection			
Prednisone, n (%)	28 (9.5)	-	-
Immunosuppressants, n (%)	14 (4.8)	-	-
b/tsDMARDs, n (%)	5 (1.7)	-	-

RMD: rheumatic and musculoskeletal diseases; HC: healthy control; RA: rheumatoid arthritis; SLE: systemic erythematosus lupus; pSS: primary Sjogren syndrome; IIM: idiopathic inflammatory myopathy; UCTD: undifferentiated connective tissue disease; b/tsDMARDs: biologic/targeted synthetic DMARDs; JAK: Janus Kinase; anti-TNF: tumour necrosis factor inhibitors.

Research Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, (approval no: 2023-SR-334) and written informed consent was obtained from all participants. The following items were recorded: demographic data, symptoms, clinical course and treatment of COVID-19 infection, disease activity and medication of RMD before and after COVID-19 infection. Immunosuppressants and b/tsDMARDs except cyclophosphamide and rituximab were defined as stable taken for more than one month before COVID-19 or the detection of antibodies, while cyclophosphamide or rituximab were defined as the use within 6 months of COVID-19 or antibody testing.

Serological testing

Peripheral blood was collected on day 60 and day 90 after COVID-19 infection from RMD and HC. Blood samples were collected, centrifuged and plasma was stored at -80°C. Human IgG antibodies against the SARS-CoV-2 spike (S) 1 and S2 proteins and the NAb targeting RBD were measured using the iFLASH-2019-nCoV IgG CLIA assay and iFLASH-2019-nCoV NAb CLIA assay (Shenzhen YHLO Biotech Co. Ltd.) The two assays were performed following the manufacturer's instructions. Seropositivity was defined as anti-SARS-CoV-2 (S1/S2) IgG or NAb of more than 10.0 UA/mL according to the manufacturer's guide.

Statistical analyses

Continuous variables were expressed as medians (IQR) and categorical variables were expressed as number and percentage (%). Anti-S1/S2 IgG and NAb levels between different groups were compared by chi-square tests for categorical variables, and by Mann-Whitney tests for continuous variables. Multivariable linear regression analyses and logistic regression analyses to assess the association of relevant variables with IgG and NAb antibody titres. A *p*-value less than 0.05 was considered as significant. Statistical analyses were made with IBM SPSS Statistics (v. 27.0), and graphical presentation of the data was made using GraphPad Prism (v. 9.1.0).

Results

Patient population

A total of 292 RMD patients and 104 HC participated in the study, with measurements taken on day 60 and day 90 (288 RMD patients and 81 HC) post-COVID-19 infection. The patient demographics for both groups on day 60 are provided in Table I. Systemic lupus erythematosus (SLE, 44.2%), rheumatoid arthritis (RA, 22.6%), and primary Sjögren's syndrome (pSS, 13.4%) were the most commonly represented RMDs. Other RMDs such as ankylosing spondylitis, psoriatic arthritis, and systemic sclerosis were also included. The mean duration of disease among RMD patients was 5.0 years (IQR 3.0–10.0 years). Table I lists the specific medications used. Prior to the onset of COVID-19, 40–50% of RMD patients were on treatments such as GC, hydroxychloroquine (HCQ), and immunosuppressive drugs (ISDs), while 5–10% started these treatments after exhibiting COVID-19 symptoms.

Serum anti-S1/S2 IgG and NAb levels were decreased in RMD patients

On day 60 post-infection, the median level of anti-S1/S2 IgG was significantly decreased in the RMD group [119.1 (IQR 19.7–318.9) AU/mL] as compared with those in the HC group [345.5 (IQR 257.4–389.6) AU/mL] (p<0.0001). The trend persisted on day 90, as shown in Figure 2A. The level of anti-S1/S2 in RMD patients [82.9 (IQR 11.8–285.3) AU/mL] was lower than those in HC [374.9 (IQR 269.4–422.6) AU/mL] (p<0.0001) (Fig. 2A).

A similar pattern emerged for serum NAb levels, with RMD patients showing significantly lower levels on both days 60 [17.9 (IQR 5.5–413.3) AU/ml *vs*. 558.0 (IQR 82.5–887.8) AU/ml, RMD *vs*. HC, p<.0001] and days 90 [14.6 (IQR 2.6–292.5) AU/ml *vs*. 545.0 (IQR 95.3–891.0) AU/ml, RMD *vs*. HC, p<0.0001] (Fig. 2B). Additionally, the seropositivity rates for both anti-S1/S2 IgG and NAb were remarkably lower in the RMD patients (Supplementary Table S1).

We further investigated changes in anti-S1/S2 IgG and NAb levels between days 60 and 90 in both HC and RMD groups. No significant fluctuation in anti-S1/S2 IgG levels was noted both in HC and RMD, However, NAb levels in the RMD group significantly declined on day 90 compared to day 60 (p<0.001), but there were no significant changes in the HC group (p>0.05) (Fig. 2C-D).

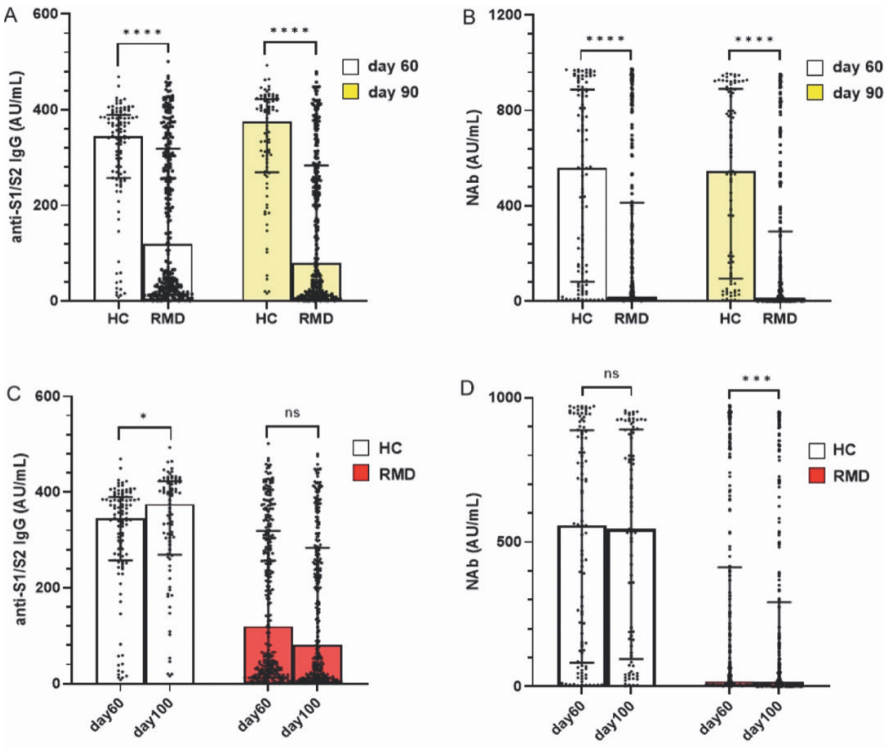

Fig. 2. Antibody response to COVID-19 infection in RMD patients and HC on day 60 and day 90.
A: The titres of anti-S1/S2 IgG in all RMD patients and HC on day 60 (RMD: n=292; HC: n=104) and day 90 (RMD: n=288; HC: n=81).
B: The titres of NAb in all RMD patients and HC on day 60 (RMD: n=292; HC: n=104) and day 90 (RMD: n=288; HC: n=81).
C: The levels of anti-S1/S2 IgG on day 60 compared with day 90 in HC and RMD groups.
D: The difference of NAb titres between day 60 and day 90 in both HC and RMD groups.
ns: not signifcant; *p<0.05, ***p<0.001, ****p<0.0001.

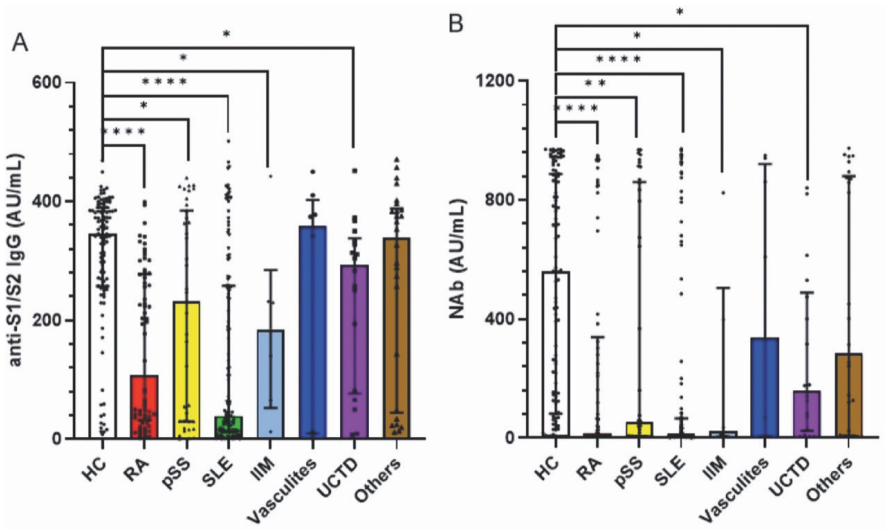

Fig. 3. The impact of RMD subtype on anti-S1/S2 IgG or NAb production.
A: The titres of anti-S1/S2 IgG in different subtypes of RMD patients compared with HC on day 60.
B: The titres of NAb in different subtypes of RMD patients compared with HC on day 60.
*p<0.05, **p<0.01, ****p<0.0001.

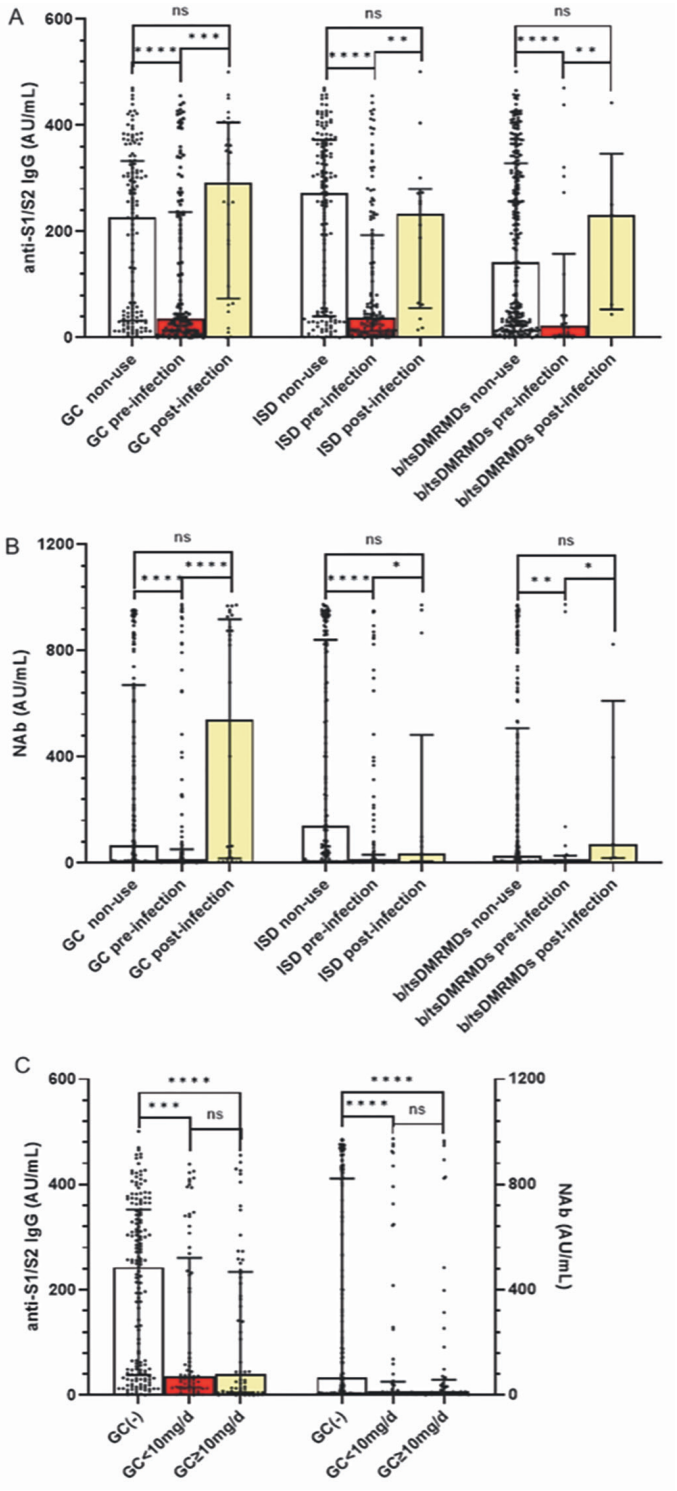

Fig. 4. Comparison of anti-S1/S2 IgG or NAb titres among RMD patients with different therapy.
A: The titres of anti-S1/S2 IgG in RMD patients with different therapy.
B: The level of NAb in RMD patients with different therapy.
C: Comparison of anti-S1/S2 IgG or NAb titres among RMD patients with different dosage of the GC groups. ns: not significant.
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

The impact of RMD subtypes on anti-S1/S2 IgG or NAb production

We next explored how different RMD subtypes influenced antibody production on day 60 following COVID-19 infection. Almost all RMD subgroups, except for vasculitis, exhibited significantly reduced levels of both anti-S1/S2 IgG and NAb when compared to the HC group (Fig. 3; Suppl. Table S2). Among these RMD subtypes, SLE patients had the lowest anti-S1/S2 IgG levels; however, these levels were not statistically different from other RMD subtypes. Conversely, NAb titres were significantly lower in SLE patients compared to other subtypes such as RA, pSS, UCTD (p<0.05).

The impact of anti-rheumatic medication on anti-S1/S2 IgG and NAb levels in RMD patients

In a subsequent analysis, we examined the effect of medications on antibody production among RMD patients. We categorised RMD patients into three groups based on medication history: non-users, those who initiated medications before contracting COVID-19, and those who started medications post-infection. Our data showed that RMD patients who had been treated with GC, ISDs, or b/tsDMARDs before COVID-19 infection had significantly lower levels of anti-S1/S2 IgG and NAb on day 60 compared to those who had not used these medications or started post-infection (p<0.001) (Fig. 4A-B; Suppl. Table S3). Similar results were found in the analysis of antibody production on day 90 (data not shown).

To further investigate the role of GC dosage on antibody levels, we collected data on GC usage among RMD patients in the three months prior to their COVID-19 infection. Patients were divided into three categories based on their average daily dosage: non-users, those using less than 10 mg/d, and those using 10mg/d or more. Our findings indicated that patients in both the lower (<10mg/d) and higher dosage (≥10mg/d) groups had significantly lower anti-S1/S2 IgG and NAb levels compared to non-users (p<0.001). However, there was no significant difference in antibody levels between the two GC dosage groups (p>0.05) (Fig. 4C; Suppl. Table S3).

Risk factors were associated with lower anti-S1/S2 IgG or NAb levels in RMD patients

To pinpoint factors that predict lower levels of anti-S1/S2 IgG and NAb levels in RMD patients post-COVID-19,

Table II. Multivariate analysis of the titres of anti-S1/S2 IgG or NAb.

		Regression coefficient	Standard error	t-statistics	*p*-value	95% CI
anti-S1/S2 IgG titre (AU/mL)	MMF	-74.24	27.93	-2.66	0.008	-129.23, -19.25
	CNIs	-98.28	31.72	-0.18	0.002	-160.73, -35.83
	MTX	-94.59	29.63	-0.19	0.002	-152.91, -26.28
NAb titre (AU/mL)	GC	-109.48	51.60	-2.12	0.035	-211.06, - 7.89
	CNIs	-163.15	73.60	-2.22	0.027	-308.05, -18.25
	MTX	-178.54	68.704	-2.60	0.10	-313.80, -43.29

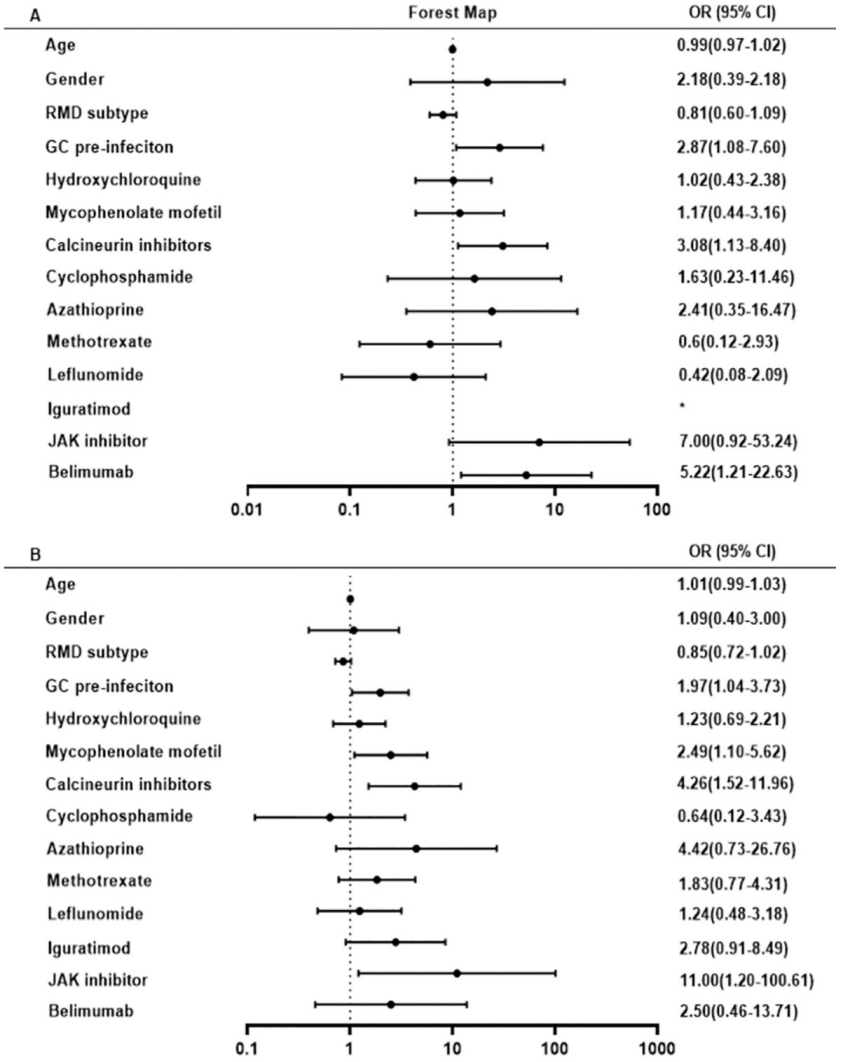

Fig. 5. Logistic regression analysis of the seropositivity of anti-S1/S2 IgG and NAb in RMD patients.
A: The analysis of the seropositivity of anti-S1/S2 IgG.
B: The analysis of the seropositivity of NAb.
*Model did not converge due to few outcomes.

we conducted a multivariate regression analysis. The analysis identified that the types of RMD, as well as the pre-infection use of GC and ISDs, were inversely correlated with anti-S1/S2 IgG levels ($p<0.05$) (Suppl. Table S4). However, for NAb levels, only the pre-infection use of GC and ISDs demonstrated a significant impact ($p<0.05$) (Suppl. Table S4).

In a subsequent step, we treated anti-S1/S2 IgG and NAb levels as dependent variables and examined various independent variables such as age, gender, disease types, and medication history. Our findings revealed that MMF, CNIs, and MTX were significantly correlated with anti-S1/S2 IgG levels, while GC, CNIs, and MTX exhibited a strong correlation with NAb levels ($p<0.05$) (Table II).

Finally, employing logistic regression, we further analysed factors that might be related to seropositivity. Notably, the pre-infection use of GC, CNIs, and belimumab stood out as significant risk factors for reduced anti-S1/S2 IgG levels ($p<0.05$; Fig. 4A). For NAb levels post-COVID-19, the pre-infection use of GC, MMF, CNIs, and JAK inhibitors were identified as substantial risk factors ($p<0.05$; Fig. 4B).

Discussion

Previously published studies primarily examined the changes in antibody production in RMD following SARS-CoV-2 vaccination (16-19). Differing from these, our 90-day longitudinal study analysed the dynamics of SARS-CoV-2 IgG antibodies and NAb responses post COVID-19 infection. We observed that RMD patients had significantly reduced levels of anti-S1/S2 IgG and NAb on both 60 and 90 days post-infection compared to healthy individuals. Notably, the NAb levels diminished more rapidly than the SARS-CoV-2 IgG antibodies. We identified multiple risk factors responsible for this decline. Particularly, prior use of GC, ISDs, and b/tsDMARDs emerged as significant contributors, regardless of the specific RMD types. Our findings emphasise the importance of preventing COVID-19 infection in RMD patients, and might have implications for longevity of immunity post COVID-19 infection.

In line with prior studies on antibody production in RMD post SARS-CoV-2 vaccination (16-19), our research indicated that using GC, ISDs, or b/tsDMARDs before infection impacted the humoral immune response in RMD patients. We first concentrated on the impact of GC on antibody production. Approximately 20% of our RMD patients had received ≥10mg/d GC doses before contracting COVID-19. Interestingly, even dosages <10mg/d were associated with significantly reduced specific IgG antibodies and NAb against COVID-19 in RMD patients. The effect of the du-

ration of GC therapy on antibodies was not explored in the present study. Given 123/129 of these patients were treated GC more than 3 months, it is evident that the duration of GC administration is a critical risk factor.

Beyond GC, our results identified various ISDs or b/tsDMARDs (including MTX, MMF, CNIs, belimumab, JAK inhibitors) as risk factors for diminished post-infection antibody levels. This is in line with earlier evidence indicating that certain anti-rheumatic medications influence antibody production post SARS-CoV-2 vaccination in RMD patients. For instance, studies by Haberman's group revealed that both MTX and GC negatively impact the immune response to COVID-19 mRNA vaccines in patients with inflammatory diseases (20). Similarly, medications like MMF, rituximab, TNF inhibitors, and abatacept have reported adverse impacts on the SARS-CoV-2 antibody response (17-19, 21-23). Drugs such as glucocorticoids, immunosuppressants or b/tsDMARDs can inhibit the immune system through a variety of mechanisms. They can inhibit the production of inflammatory mediators, reduce the activity of antigen presenting cells, reduce the proliferation and activation of T cells, and interfere with the process of B cells producing specific antibodies. These may lead to the weakening or delay of antibody response to novel coronavirus infection. It has been shown that immunosuppressants were associated with reduced T-cell immunity against spike protein, suggesting that these treatments could impair humoral and cell-mediated response to COVID-19 vaccination (24). When the COVID-19 pandemic began, HCQ garnered substantial attention as a potential SARS-CoV-2 treatment. However, large-scale randomised trials found HCQ lacking in significant benefits for treating or preventing COVID-19 (25). Our data showed the use of HCQ had no significant effect on post-infection antibody production. Our data indicate no noticeable impact of HCQ on post-infection antibody production, supported by earlier findings (20-23, 26, 27).

When examining the dynamics of SARS-CoV-2 IgG and NAb post-infection in RMD patients, we observed a pronounced reduction in neutralising antibodies on day 90 compared to day 60, while anti-S1/S2 IgG levels showed no significant changes. Previous research also indicated a drop in IgG seropositivity by roughly 20% six months post-vaccination, with NAb seropositivity declining by 40% in both HC and RMD patients (14). It is possible that immunosuppressive medications can modify immune responses, particularly affecting neutralising antibody production or maintenance. Interestingly, some studies suggest that immunosuppressed individuals might experience extended SARS-CoV-2 shedding, facilitating viral mutation (28). Such evolving viral scenarios could potentially influence NAb dynamics. These NAb are essential in preventing viral entry into cells. One study estimated based on a predictive model that about 20% of NAb levels during recovery could provide 50% protection against symptomatic infection, and 3% of NAb levels could provide 50% protection against severe illness (29). Our data highlight the importance of continuous monitoring for shifts in neutralising antibodies in RMD patients after contracting COVID-19.

Our study also has some limitations. Firstly, our findings are derived exclusively from observational data, which implies that a direct cause-and-effect relationship cannot be conclusively determined. As such, while there is an observed correlation between drug use and reduced antibody levels, there could be other factors at play that we have not considered. Secondly, due to the limited sample size, we could not thoroughly analyse the relationship between various drugs and antibody responses across different autoimmune diseases. Thirdly, we did not collect and further analyse the severity of COVID-19 infection in these patients, because mild *versus* severe cases might differ in their antibody responses. Fourthly, data on antibody levels on day 30 post-infection were lacking and a longer follow-up period of antibody monitoring is required. Lastly, the antibody levels were not compared with the cellular and humoral immune responses, and serum cytokine levels at different time points.

In summary, our study results reveal a significant reduction in SARS-CoV-2 IgG antibodies and NAb levels post-COVID-19 infection in RMD patients. We also observed distinct variations in NAb dynamics among RMD patients with COVID-19. However, whether the RMD patients with reduced antibody titres will have a significantly higher risk of reinfection of COVID-19 remains to be explained by further research. Therefore, a large cohort and longitudinal studies are needed to understand the correlation between dynamic antibody level changes and long-term immunity in RMD patients.

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