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Original article

Robust neutralizing antibody response to SARS-CoV-2 mRNA vaccination in adolescents and young adults with childhood-onset rheumatic diseases

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Abstract

Objectives. Immunogenicity to the SARS-CoV-2 mRNA vaccines in adolescents and young adults (AYA) with childhood-onset rheumatic diseases (cRD) is unknown. We aimed to evaluate the humoral immunogenicity and safety of the vaccines in our AYA with cRD.

Methods. A monocentric observational study with 159 AYA (50.3% female and 70.4% Chinese). Humoral immunogenicity was assessed at 2–3 and 4–6 weeks following first and second vaccination by cPass™ SARS-CoV-2 Neutralization Antibody Assay. Inhibition signal of $\geq$30% defined the cut-off for positive detection of the SARS-CoV-2 neutralizing antibodies. Vaccine safety and disease activity were assessed within 6 weeks after second vaccination.

Results. A total of 64.9% and 99.1% of 159 patients (median age: 16.9, IQR: 14.7–19.5) mounted positive SARS-CoV-2 neutralizing responses after first and second vaccination, respectively. Most patients (89.8%) had $\geq$90% inhibition signal after second vaccination. Methotrexate and mycophenolate mofetil increased the risk associated with negative cPass neutralization responses following the first vaccination. Holding both medications after each vaccination did not affect immunogenicity. There was no symptomatic COVID-19 infection. Local reaction remained the most common (23.3–25.2%) adverse event, without serious complication. Two and seven patients flared following the first and second vaccination, respectively. Subgroup analyses of the 12–18-year-old cohort did not show any differences in vaccine efficacy, predictors of poor response and general safety, but higher proportion of disease flares.

Conclusions. SARS-CoV-2 mRNA vaccines were efficacious after the two-dose regimen in almost all AYA with cRD without serious adverse event. The rate of disease flare observed is 4.4% after the second mRNA vaccine dose.

Key words: SARS-CoV-2 vaccine, COVID-19, childhood-onset rheumatic disease, vaccine efficacy, vaccine safety, paediatric age, paediatric rheumatology

CLINICAL SCIENCE

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Rheumatology key messages

- Almost all patients mounted adequate SARS-CoV-2 neutralizing antibodies following first and second SARS-CoV-2 mRNA vaccination.
- Methotrexate and mycophenolate-mofetil significantly dampened first vaccine immunogenicity but not at all after the second.
- No major adverse events were noted following vaccination, with 4% rate of disease flare.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], has led to significant mortality and disrupted the global socio-economic ecosystem [2]. The clinical spectrum of COVID-19 ranges from severe respiratory and multi-organ failure leading to death, to an asymptomatic or mild disease course [3–5]. Meta-analysis showed that individuals with rheumatic diseases (RD) do not appear to have more severe SARS-CoV-2 infection in terms of hospitalization, intensive care admission and mechanical ventilation [6]. Any increased COVID-19 severity in these patients found across studies is associated with the currently known risk factors of older age, male gender, high body mass index and the presence of comorbidities [7]. Although an increased COVID-19 mortality in individuals with RD is not consistently observed, an increased un-adjusted odds of mortality of 1.74 (95% CI of 1.08, 2.80) was found in a meta-analysis with 16 included studies [6]. Current available paediatric studies have reported hospitalization rates of 18–51.3% [8–11], but limited data exists regarding the clinical course of COVID-19 in children with RD. Although children have a lower COVID-19 mortality rate compared with adults [12, 13], the paediatric-specific occurrence of multisystem inflammatory syndrome in children [9] and known vulnerability to other severe infections from therapeutic immunomodulation [14] are concerns for a potentially more severe clinical course of COVID-19 in children with RD.

Henceforth, it is clinically prudent to protect this vulnerable population from the imminent and eventual exposure to SARS-CoV-2 as the virus becomes endemic [15]. Both the Pfizer-BioNTech and Moderna SARS-CoV-2 mRNA vaccines have demonstrated a noninferior immune response in non-diseased, healthy adolescents in comparison to young adults and should constitute part of the protective strategy [16, 17]. Notably, the Pfizer-BioNTech vaccine produced a higher 50% neutralizing titre in 12–15-year-olds when compared with 16–25-year-olds, which illustrates a robust immune response in the former and provides evidence for immunization in this age group [16]. However, there is no clear vaccination recommendation or guideline for children with RD due to the paucity of data on the impact of SARS-CoV-2 mRNA vaccines on childhood onset RD (cRD). Herein, we aim to comprehensively characterize the protective humoral neutralization response in our cohort of patients with cRD after inoculation with the COVID-19 mRNA vaccine, evaluate the impact of different immunomodulatory drugs as well as examine the safety profile of this two-dose immunization strategy.

Methods

Participants and study approval

Patients with cRD who are currently evaluated at the rheumatology clinics at KK Women's and Children's Hospital, Singapore and had received Pfizer-BioNTech or Moderna COVID-19 mRNA vaccines were recruited from 31 May 2021 to 20 September 2021. The vaccines were given to patients with inactive or low disease activity on stable immunosuppressive therapies. Written informed consent was obtained prior to the study initiation. The study was approved by SingHealth Centralized Institutional Review Board (CIRB: 2019–2961, 2019–2239). Blood samples were collected at 2–3 weeks after first vaccination and 4–6 weeks after the second. Blood samples were collected in serum separation blood collection tubes and centrifuged at 1300 *g* for 10 min before being aliquoted into cryovials and stored at –80°C until analysis.

Humoral immune response to mRNA vaccines

The frozen serum was thawed in batches for the experiment to assess the humoral immune response by the commercial blocking ELISA, cPass SARS-CoV-2 neutralization antibody detection kit (GenScript USA, Inc., NJ, USA) [18], according to the manufacturer's protocol. This validated kit, which has received the US Food and Drug Administration authorization [19], assays the magnitude of antibody-mediated blockage of angiotensin-converting enzyme 2 receptor protein and its interaction with the SARS-CoV-2 receptor binding domain (RBD) required for viral entry into susceptible cells [20]. In SARS-CoV-2 infection, the RBD-targeting neutralizing antibodies are immunodominant [21]. The percent signal inhibition was calculated as [1-(Optical Density of sample/Optical Density of negative control)]$\times$100%. An inhibition signal of $\geq$30% was used as the cut-off for positive detection of SARS-CoV-2 neutralizing antibodies [18]. At this recommended cut-off by the manufacturer, the cPass assay exhibits a 100% specificity and 96.1% sensitivity in COVID-19 patients at $>$14 days after SARS-CoV-2 PCR positive [22]. Additionally, this level

also correlates strongly with the current gold standard for SARS-CoV-2 neutralizing antibody characterization, the plaque reduction neutralization test of 90% ($PRNT_{90}$) [23].

Safety monitoring

Patients were contacted by phone on the day of first vaccination to 6 weeks after the second to complete a questionnaire regarding adverse events.

Statistical analysis

Non-parametric analyses were used to describe data and were shown as median (interquartile range, IQR) for continuous variables and percentages for categorical variables. Chi squared/Fisher's exact, Mann–Whitney *U*, and Kruskal–Wallis tests were applied to compare differences between groups where appropriate. Predictors of vaccine response were examined using logistic regression. Variables that gave $P < 0.1$ in the univariate logistic regression were entered into the multivariate logistic regression by a backward conditional method and confirmed with a forward conditional method yielding odd ratios (ORs) and 95% CI for predictor variables. All analyses were performed using SPSS, version 23.0 (IBM Corp., NY, USA) and GraphPad Prism V.7 (GraphPad Software, Inc., CA, USA) with statistical significance set at $P < 0.05$.

Results

The study cohort comprised 159 consecutive patients with cRD (50.3% female, 70.4% Chinese) with the median age at first vaccination of 16.9 years (IQR: 14.7–19.5) and disease duration of 5.0 years (IQR: 2.0–7.0), Table 1. Overall, 147 (92.5%) and 12 (7.5%) patients had Pfizer-BioNTech and Moderna SARS-CoV-2 mRNA vaccines, respectively. JIA composed the majority (56.6%) followed by SLE (18.2%), and Undifferentiated CTD (UCTD, 6.3%), Table 1. Other diagnoses that had four or less patients each are shown in Supplementary Table S1, available at *Rheumatology* online. Anti-tumour necrosis factor (aTNF) was the most common biological DMARD used. As for conventional DMARDs, HCQ, SSZ and MTX were among the most prescribed. All patients taking prednisolone had concomitant DMARDs. Of 43 patients taking HCQ, 11 of them were on monotherapy.

Humoral neutralization response to mRNA COVID-19 vaccine in patients with cRD

All patients received two doses of mRNA COVID-19 vaccine, 131 (82.4%) and 108 (67.9%) patients had serum samples available for the neutralization assays after their first and second vaccination, respectively, Tables 2 and 3. A total of 65% of our patients mounted an adequate neutralization response (signal inhibition ≥30%) after the first vaccination (Table 2). This figure increased to 99.1% after the second dose, Table 3. There is no difference between the patient group with no ($n = 51$) and those with post second vaccination antibody response assayed ($n = 108$) in their demographics, diagnosis, gender, age at vaccination, disease duration and medication used (Supplementary Table S2, available at *Rheumatology* online). The antibody response after the second dose in the patients with cRD is comparable to our age-matched healthy cohort ($n = 17$) with all having an adequate post second dose response (Supplementary Table S3, available at *Rheumatology* online). Moreover, 89.8% who had completed two doses developed over a 90% inhibition level, but only 3.8% of the singly vaccinated patients achieved the same, Table 2 and 3.

Forty-six patients did not have an adequate neutralization response after the first vaccination, Table 4. Most JIA patients (63.5%) had an adequate response ($P = 0.004$) but around one-third of cSLE patients did not ($P = 0.020$). Regarding medication, a significant proportion of patients taking MMF could not mount an adequate response relative to other medications. Patients taking MTX seem to have an inadequate response as well despite non-significance. In those with the

Table 1 Clinical characteristics and medications of patients in our cohort ($n = 159$)

Clinical characteristics	*n* (%)
Female	80 (50.3)
Race	
Chinese	112 (70.4)
Malay	18 (11.3)
Indian	13 (8.2)
Others	16 (10.1)
Age at first vaccination (years)[a]	16.9 (14.7–19.5)
Disease duration at first vaccination (years)[a]	5.0 (2.0–7.0)
Duration from first vaccination to sample collection (weeks)[a]	2.4 (2.1–2.7)
Duration from second vaccination to sample collection (weeks)[a]	4.6 (3.9–5.1)
Diagnosis	
JIA	90 (56.6)
SLE	29 (18.2)
Overlap syndrome	2 (1.3)
UCTD	10 (6.3)
JDM	8 (5.0)
Others	20 (12.6)
Medications	
Prednisolone	29 (18.2)
Methotrexate	41 (25.8)
Azathioprine	7 (4.4)
Mycophenolate mofetil	23 (14.5)
Sulfasalazine	42 (26.4)
Hydroxychloroquine	43 (27.0)
Anti-TNFα	49 (30.8)
Tocilizumab	4 (2.5)
Rituximab	1 (0.6)

[a]Median (IQR); UCTD: undifferentiated CTD; Others (details in Supplementary Table S1, available at *Rheumatology* online).

Table 2 cPass % inhibition data after first vaccination for our whole COVID-19 vaccine cohort

cPass	% inhibition
First vaccination with sample, $n = 131$ (82.4%)[a]	43.7 (17.1–71.8)
cPass1	*n* (%)
≥30%	85 (64.9)
≥50%	57 (43.5)
≥70%	34 (26.0)
≥90%	6 (3.8)

[a]Median (IQR), cPass1, percent signal inhibition after first COVID-19 mRNA vaccine dose.

Table 3 cPass % inhibition data after second vaccination for our whole COVID-19 vaccine cohort

cPass	% inhibition
Second vaccination with sample, $n = 108$ (67.9%)[a]	96.9 (95.4–97.3)
cPass2	
≥ 30%	107 (99.1)
≥ 50%	105 (97.2)
≥ 70%	104 (96.3)
≥ 90%	97 (89.8)

[a]Median (IQR), cPass2, percent signal inhibition after second COVID-19 mRNA vaccine dose.

neutralization responses characterized after the first COVID-19 vaccination, 16/23 (69.6%) of MMF and 34/36 (94.4%) of MTX taking patients held their respective medication after vaccination for a week. Interestingly, there were no significant differences in the proportion of patients whether they held their MTX or MMF as determined by their neutralization responses [cPass < 30% *vs* cPass ≥ 30%: MTX 17/17 *vs* 17/19 ($P = 0.487$) and MMF 12/17 *vs* 4/6 ($P = 1.000$)].

Effect of immunosuppressive treatments on the immunogenicity of the COVID-19 mRNA vaccine

We then performed univariate and multivariate logistic regression analyses to determine the relationship of a negative cPass result (signal inhibition <30%) after the first vaccine dose with gender, age, disease duration, diagnosis and medications (Supplementary Table S4, available at *Rheumatology* online). In the univariate analyses, SLE and treatment with MMF were associated with a lack of response while JIA and treatment with SSZ were associated with a positive neutralization response. Following multivariate logistic regression analysis to adjust for confounders, an increased risk of a negative cPass inhibition was found only with MTX (OR: 7.616, 95% CI: 2.668, 21.738, $P < 0.001$) and MMF (OR: 10.643, 95% CI: 3.043, 37.227, $P < 0.001$) treatments.

When the post first-dose cPass inhibition level was compared across different diseases, those with SLE (median: 22.4%, IQR: 4.4–48.8) had a significantly lower inhibition level compared with those with Enthesis-Related Arthritis (ERA) (median: 50.0%, IQR: 33.3–73.7), $P = 0.039$ (Supplementary Fig. S1A, available at *Rheumatology* online). However, no difference in the cPass inhibition level was observed across different diseases after the second vaccine dose (Supplementary Fig. S1B, available at *Rheumatology* online).

MTX decreased the first dose neutralization response in patients with JIA

In our JIA cohort, dichotomized by treatment with MTX, a significant reduction in the first dose inhibition level was observed in those taking MTX (median: 33.2%, IQR: 8.8–69.7) *vs* those not (median: 57.5%, IQR: 41.2–74.1), $P = 0.014$ (Fig. 1A and B). However, the JIA patients treated with MTX differed significantly from the non-treated group in their clinical characteristics: female and ERA predominance, age at first vaccination and disease duration (Fig. 1C). Hence, we proceeded to analyse the ERA subtype separately, the most common JIA subtype in our cohort (44 of 77, 62.0%), to control for these potential confounders (Supplementary Fig. S2A and B, available at *Rheumatology* online). Although a reduced inhibition level was still observed in the MTX (median: 34.5%, IQR: 23.5–77.7) *vs* non-MTX treated in the ERA subgroup (median: 56.1%, IQR: 40.6–73.0), it was not significant ($P = 0.182$). Additionally, no difference in the neutralization response was observed with age in the ERA group with no MTX treatment (Supplementary Fig. S2C, available at *Rheumatology* online).

MMF decreased the first dose neutralization response in patients with SLE

We carried out a subgroup analysis on all patients with cSLE stratified by MMF treatment comparing the percent signal inhibition (Fig. 2A and B). There was a significant reduction in the inhibition level after the first vaccination in those taking MMF (median: 6.3%, IQR: 2.6–40.4) *vs* those without (median: 44.5%, IQR: 27.3–79.2), $P = 0.010$. For those with first dose cPass result, the two groups stratified by MMF treatment were comparable in terms of age, gender, type of COVID-19 mRNA vaccine, disease duration and activity, and medications except for a higher number of patients on azathioprine therapy in those without MMF treatment ($P = 0.017$). The immunomodulatory effect of MMF on the neutralization was absent after the second dose. Only one 18-year-old clinically inactive lupus patient (on stable doses of MMF, HCQ, tacrolimus and not on steroids) failed to achieve the cPass assay cut-off (≥30%) assessed on day 39 after receiving the second Pfizer COVID-19 mRNA vaccine.

Safety of COVID-19 mRNA vaccine in patients with rheumatic diseases

As shown in Table 5, pain at injection site remained the most common adverse reaction, up to one-quarter of

TABLE 4 Clinical characteristics and medications of patients by the adequacy of neutralization response after the first COVID-19 vaccination ($n = 131$)

Parameters	Total	cPass1 < 30%	cPass1 > 30%	*P*-value
	n = 131	*n* = 46	*n* = 85	
Female	68	27 (58.7)	41 (48.2)	0.253
Race				0.732
Chinese	91	34 (73.9)	57 (67.1)	
Malay	16	4 (8.7)	12 (14.1)	
Indian	10	4 (8.7)	6 (7.1)	
Others	14	4 (8.7)	10 (11.7)	
Age at first vaccination (years)[a]		16.8 (14.4-19.0)	16.6 (14.7-18.9)	0.589
Duration of disease (years)[a]		4.0 (1.8-6.0)	4.0 (2.0-7.0)	0.369
Duration of sampling after first vaccination (weeks)[a]		2.4 (2.1-2.7)	2.6 (2.3-2.9)	0.046
Diagnosis				0.026
SLE	25	14 (30.4)	11 (12.9)	0.020
JIA	71	17 (37.0)	54 (63.5)	0.004
JDM	6	2 (4.3)	4 (4.7)	0.925
UCTD	10	2 (4.3)	8 (9.4)	0.493
Medication				
Prednisolone	26	12 (26.1)	14 (16.5)	0.188
MTX	36	17 (37.0)	19 (22.4)	0.074
HCQ	38	17 (37.0)	21 (24.7)	0.140
AZA	6	0	6 (7.1)	0.090
SSZ	33	5 (10.9)	28 (32.9)	0.005
MMF	23	17 (37.0)	6 (7.1)	<0.001
Anti-TNF α	40	13 (28.3)	27 (31.8)	0.678

[a]Median (IQR); UCTD, Undifferentiated CTD; cPass1, percent signal inhibition after first COVID-19 mRNA vaccine dose.

our 159 patients. There was no serious adverse reaction noted up to 6 weeks post-vaccination. Fever and arthralgia were more frequent after second vaccination as compared with headache, myalgia and local swelling for the first. Moreover, there was no symptomatic COVID-19 infection case observed.

Two patients (1.3%) experienced disease flare after first vaccination: a 20-year-old female with extended oligo-JIA, on infliximab for her arthritis and uveitis had both joint and eye disease flares, after 6 days, and a 16-year-old girl with UCTD had a flare of autoimmune haemolytic anaemia after 23 days. Seven patients (4.4%) reported disease flares after second vaccination with a median of 4.7 weeks (IQR: 2.1–6.0): five-JIA patients of which four were on MTX and two on SSZ with a median 4.7 weeks, IQR: 2.2–7.5, a 14-year-old Caucasian girl with juvenile dermatomyositis on weaning MTX 6 weeks after, and a 15-year-old Indian girl with overlap syndrome off medication developing myositis 2.14 weeks after.

Pfizer-BioNTech mRNA response in 12–18 years old patients with cRD

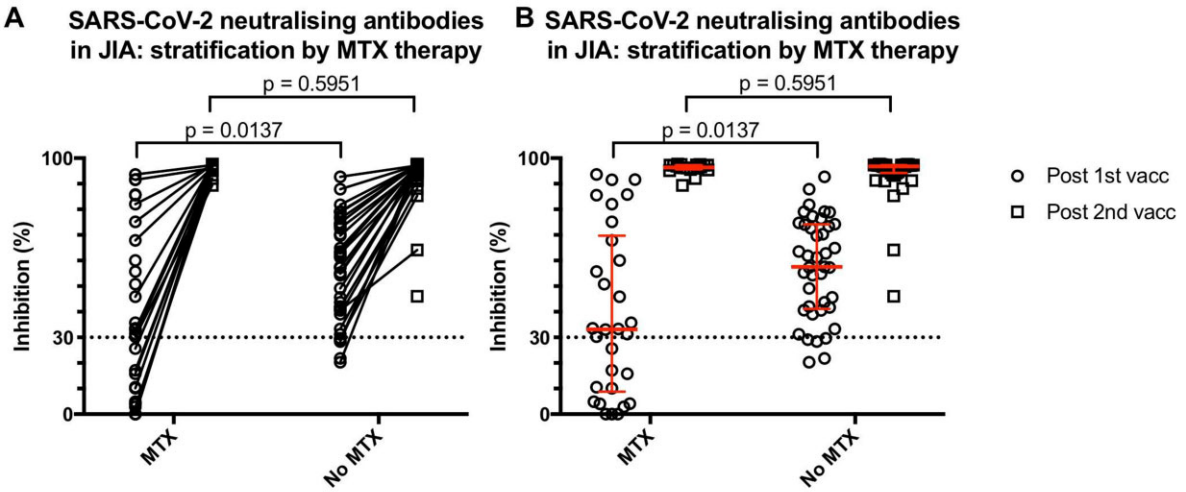
In our cohort, 95 (59.7%) were adolescents aged 12–18 [median age 15.2 (IQR: 14.1–16.6)]. This subgroup had similar disease distribution and medications used, proportion of patients with cPass neutralization response <30% inhibition (35.6%) and predictors for a negative cPass results including MTX (OR 4.800, 95% CI: 1.615, 14.263, $P = 0.005$) and MMF (OR 10.286, 95% CI: 2.681, 39.462, $P = 0.001$) (Supplementary Tables S5 and S6, available at *Rheumatology* online). The vaccine safety profile was comparable to the whole cohort report (Supplementary Table S7, available at *Rheumatology* online). Six out of seven patients who had flares of their underlying RDs after the second vaccination belonged to the adolescent subgroup, which constituted more than half of our study cohort (59.7%).

Discussion

In this study, we have demonstrated a robust humoral neutralization response against the SARS-CoV-2 spike RBD domain in 99.1% of adolescents and young adults (AYA) with cRD after two doses of the COVID-19 mRNA vaccine. Importantly, this positive cPass assay correlates strongly with a plaque reduction neutralization test of 90% ($PRNT_{90}$) [23], a current gold standard for SARS-CoV-2 neutralizing antibody characterization. Notably, we observe an attenuation of this neutralization response with MMF and MTX after the first dose, which is lost after completing the two-dose vaccine regime. Thus, we strongly recommend that any unnecessary delay in administering the second mRNA vaccine dose must be avoided in patients treated with either one of these two DMARDs.

While there are studies on the immune response of adult patients with RD after COVID-19 vaccination [24–

Fig. 1 Neutralization response after COVID-19 mRNA vaccination in patients with JIA



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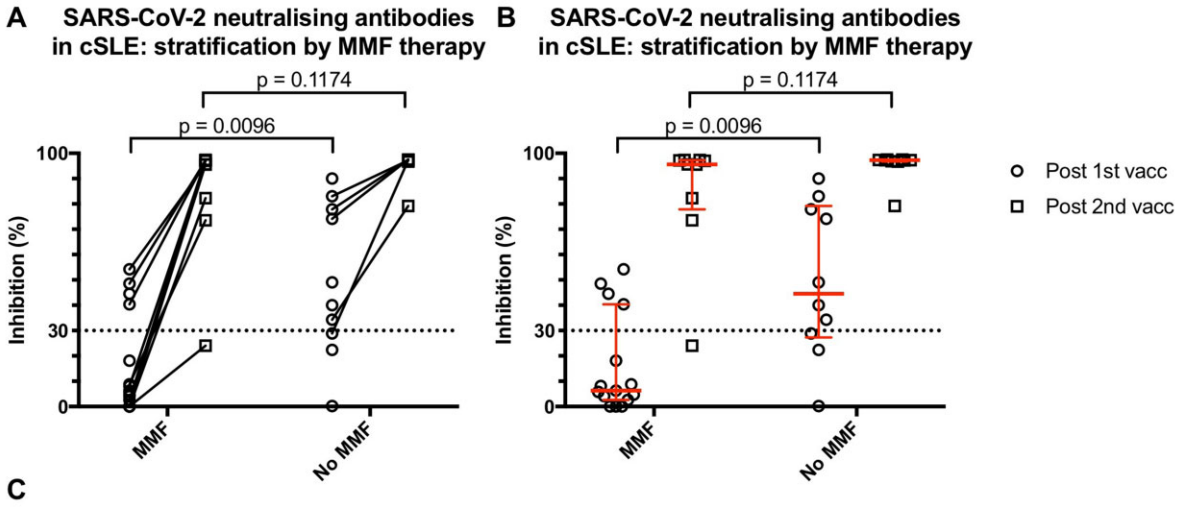
Clinical characteristics	MTX (n = 30)	No MTX (n = 41)	p
Female n (%)	18 (60.0)	11 (26.8)	0.0072
Pfizer mRNA vaccine, n (%)	29 (96.7)	39 (95.1)	>0.9999
JIA subtypes			
Systemic onset	3 (10.0)	3 (7.3)	0.6924
Oligo-persistent	2 (6.7)	2 (4.9)	>0.9999
Oligo-extended	3 (10.0)	2 (4.9)	0.6438
Polyarticular RF+	0 (0.0)	0 (0.0)	>0.9999
Polyarticular RF-	6 (20.0)	3 (7.3)	0.1540
Psoriatic	0 (0.0)	0 (0.0)	>0.9999
Enthesis related arthritis	14 (46.7)	30 (73.2)	0.0283
Undifferentiated	2 (6.7%)	1 (2.4)	0.5696
Age at 1st vaccination (years)#	15.75 (14.38-17.28)	17.9 (16.25-20.8)	0.0009
Disease duration at 1st vaccination (years)#	3.4 (1.075-6.5)	5.7 (2.8-9.15)	0.0391
Duration from 1st vaccination to sample collection (days)#	17.5 (15.75-19)	16 (15-19)	0.2993
Medications			
Sulfasalazine	10 (33.3)	22 (53.7)	0.0990
Anti-TNFα (Etanercept, Infliximab, Adalimumab, Golimumab)	13 (43.3)	19 (46.3)	0.8106
Tocilizumab	1 (3.3)	1 (2.4)	>0.999

(**A**) Symbols and lines plot of percent signal inhibition in patients with JIA stratified by MTX treatment. (**B**) Scatter plot with median and interquartile ranges of percent signal inhibition in patients with JIA stratified by MTX treatment. (**C**) Clinical characteristics of JIA patients with cPass inhibition results after first vaccine dose. vacc: COVID-19 mRNA vaccine dose. Statistical analysis by Fisher's exact or Mann–Whitney *U* tests. #Data presented as median (interquartile range).

33], data heterogeneity on the humoral response to COVID-19 vaccination in patients with autoimmune diseases exists and hinders the harmonization of these information for clinical applications. Differences in vaccine types, clinical cohorts, duration of observation for adverse effects, methods used and the timing of assessing the vaccination response (after first and/or second vaccination) contribute to this quandary. Additionally, the differences in analysing this humoral response either as a categorical (with seropositivity rate) or continuous (with measure of central tendency and spread) variable further compounds the difficulty for cross-study comparison and generalization. Bearing these issues in mind, we will highlight how our study builds upon this information with new insights.

Our study is the first to demonstrate a robust humoral neutralization response in a distinctively AYA with cRD who have completed COVID-19 mRNA vaccination. Notably, our cohort has a higher proportion (97 out of 108, 89.8%) with an inhibition level of $\geq$90% compared with an adult cohort (16 out of 22, 72.7%) that was similarly evaluated with the cPass assay at an earlier blood sampling timepoint of 7 days after the second vaccine dose [28]. Significantly, this is in spite of the lack of

Fig. 2 Neutralization response after COVID-19 mRNA vaccination in patients with SLE



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Clinical characteristics	MMF (n = 15)	No MMF (n = 10)	p
Female n (%)	12 (80.0)	7 (70.0)	0.6532
Pfizer mRNA vaccine, n (%)	14 (93.3)	9 (90.0)	>0.9999
Age at 1st vaccination (years)#	15.5 (14.3-18.2)	16.55 (15.55-18.73)	0.3669
Disease duration at 1st vaccination (years)#	3.6 (1.7 to 6.2)	5.2 (3.75-6.875)	0.1119
Duration from 1st vaccination to sample collection (days)#	18 (16 to 20)	18 (14-22.75)	0.8801
SLEDAI#	2 (0-2)	2 (0-2.5)	0.5776
Medications			
Prednisolone, n (%)	14 (93.3)	8 (80.0)	0.5435
Azathioprine, n (%)	0 (0.0)	4 (40.0)	0.0166
Hydroxychloroquine, n (%)	15 (100.0)	10 (100.0)	>0.9999

(**A**) Symbols and lines plot of percent signal inhibition in patients with SLE stratified by MMF treatment. (**B**) Scatter plot with median and interquartile ranges of percent signal inhibition in patients with SLE stratified by MMF treatment. (**C**) Clinical characteristics of lupus patients with cPass inhibition results after first vaccine dose. cSLE: childhood onset SLE; vacc: COVID-19 mRNA vaccine dose. Statistical analysis by Fisher's exact or Mann–Whitney *U* tests. #Data presented as median (interquartile range).

Table 5 Safety profiles of SARS-CoV-2 mRNA vaccine in our mRNA vaccine cohort

Clinical manifestations	After first vaccination, *n* (%)	After second vaccination, *n* (%)
Fever	2 (1.3)	8 (5.0)
Injection site reaction	40 (25.2)	37 (23.3)
Pain	38 (23.9)	37 (23.3)
Swelling	2 (1.3)	0 (0.0)
Erythema	0 (0.0)	0 (0.0)
Malaise	17 (10.7)	17 (10.7)
Arthralgia	1 (0.6)	2 (1.3)
Myalgia	4 (2.5)	2 (1.3)
Headache	9 (5.7)	3 (1.9)
Rash	1 (0.6)	1 (0.6)
Swollen lymph node	0 (0.0)	1 (0.6)

MMF and MTX use in the adult study [28], which would depress vaccine immune responses [34].

Second, we illustrated the suppressive effect of two DMARDs, MTX and MMF, on the neutralization response after the first vaccine dose. The immunomodulatory effect of MTX was exhibited via multivariate logistic regression analysis and stratified bivariate analysis in the JIA cohort, whereby the humoral neutralization response was evaluated as a dichotomous categorical and continuous variable, respectively. Although a lower inhibition was still observed in the MTX-treated group (median: 34.5%, IQR: 23.5–77.7 *vs* median: 56.1%, IQR: 40.6–73.0, $P = 0.182$) when ERA was considered separately, this effect is not statistically significant. This immunosuppressive effect of MTX after the first but not the second vaccine dose was similarly shown in adult patients with RD ($n = 574$) who had been inoculated mainly with the ChAdOx1 (AstraZeneca) adenoviral vaccine [29]. However, other studies involving adults with RD inoculated with the BNT162b2 (BioNTech) mRNA vaccine demonstrated a persistent attenuation of the humoral response by MTX even after completing the two-dose regime [25, 26].

For MMF, a 10-fold higher risk for a negative neutralization response (<30% inhibition) was also observed. Interestingly, this attenuation was only observed after the first dose but not with completion of COVID-19 vaccination. Contrastingly, in adults with RD, the suppressive effect of MMF persists even after the second vaccine dose with a lower seroconversion rate [25, 27, 32]. The persistence of MMF's suppressive effect may be attributed to variability in assays used and more importantly age, as a stronger immune response to COVID-19 mRNA vaccination has been reported in younger age groups [16]. This suggests that the American College of Rheumatology's recommendation of withholding DMARDs following each vaccine dose may not be relevant for youths with cRD given their robust humoral immune responses in spite of concurrent DMARD therapy [35]. Nevertheless, combinatorial DMARD therapy may dampen vaccine responses in AYA as evidenced by the young adult with cSLE in our cohort who was simultaneously receiving MMF, HCQ and tacrolimus and failed to achieve the cPass assay cut-off. Further validation of our observations in a larger cohort is required to elucidate the influence of DMARDs on vaccine responses.

The safety profiles of these mRNA vaccine in our youth cohort are comparable to those reported for healthy adolescents, for which pain at the injection site remains the commonest local reaction with few serious adverse reactions reported [16]. However, 1.3% of our patients flared after their first and 4.4% flared after their second vaccination. Younger patients (12–18 years old) may confer a higher risk of flare as six out of seven of patients who flared after second vaccination belonged to this subgroup.

The limitations of our study include a non-randomised design and the use of a surrogate virus neutralization test with a readout that measures the degree of antibody-mediated blockage of ACE2-spike protein–protein interaction instead of a quantifiable antibody titre [18]. However, unlike the time-consuming PRNT which requires a specialized biosafety level (BSL) 3 facility due to the use of live viruses, the quicker ELISA-based cPass assay can be done in a BSL2 laboratory and this greatly increases its accessibility [18]. The cPass surrogate viral neutralization test also gives results that are either similar or superior to the commercial kits that were utilized in the studies with rheumatic disease cohorts [23], specifically the SARS-CoV-2 ELISA kits from Abbott (Chicago, IL, US) [27], DiaSorin (Saluggia, Italy) [25], Euroimmun (Lübeck, Germany) [28, 32] and Roche Diagnostics (Basel, Switzerland) [24, 32]. It is crucial to emphasize that these assays, including cPass, provide a crude correlate for protection, an absolute protective threshold that confers definite protection against SARS-CoV-2 infection is still not known [36, 37]. Without a known protective threshold, there is no advantage of an antibody quantification over a viral neutralization assay like cPass, which measures the ability of the serum/plasma to inhibit a mechanistically important ACE2-SARS-CoV-2-spike protein–protein interaction.

In conclusion, we have shown that in AYA with cRD, a robust humoral neutralization response is elicited after two-dose COVID-19 mRNA vaccination with the main adverse effects being self-resolving localized injection site reaction with pain and swelling. By building upon current evidence from adult studies that support the efficacy and safety of the mRNA vaccine with insights from a well-characterised AYA cohort with cRD, our findings can further allay the fears due to lack of information that contributes to vaccine hesitancy [38, 39]. In addition, we have shown that the two-dose mRNA vaccine regimen is efficacious and adequate for AYA with cRD. Further studies looking at the humoral immune response decay rate is underway in this cohort. The results obtained will eventually assist in guiding the booster intervals after completing the two-dose vaccination regime.

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Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information, available at *Rheumatology* online. The data used to support the findings of this study are included within the article.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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